DESIGN AND SYNTHESIS OF STEROL-POLYAMIDES, STEROL-POLYAMINES AND BILE ACID BISTRIAZOLES: A NEW CLASS OF ANTIMICROBIALS

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

BY
Mr. SUDHIR N. BAVIKAR

Dr. BRAJA G. HAZRA
(RESEARCH GUIDE)

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

DESIGN AND SYNTHESIS OF STEROL-POLYAMIDES, STEROL-POLYAMINES AND BILE ACID BISTRIAZOLES: A NEW CLASS OF ANTIMICROBIALS

THESIS

SUBMITTED TO THE

UNIVERSITY OF PUNE

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

BY

Mr. SUDHIR NARAYANRAO BAVIKAR

DIVISION OF ORGANIC CHEMISTRY

NATIONAL CHEMICAL LABORATORY

PUNE 411 008, INDIA

Dedicated to my Family....

राष्ट्रीय रासायनिक प्रयोगशाला
(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद)
डॉ. होमी भाभा मार्ग पुणे - 411 008. भारत
NATIONAL CHEMICAL LABORATORY
(Council of Scientific & Industrial Research)
Dr. Homi Bhabha Road, Pune - 411 008. India.

Dr. Braja G. Hazra
Emeritus Scientist
Division of Organic Chemistry

Phone: +91-20-25898164
Fax: +91-20-25902629
E-mail: bg.hazra@ncl.res.in
brajagopal2002@yahoo.co.in
Website: http://www.ncl-india.org

November 03, 2009

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "*Design and Synthesis of Sterol-polyamides, Sterol-polyamines and Bile acid Bistriazoles: A New Class of Antimicrobials.*" which is being submitted to the *University of Pune* for the award of *Doctor of Philosophy* in *Chemistry* by ***Mr. Sudhir Narayanrao Bavikar*** *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.

Dr. Braja G. Hazra
Research Guide
Division of Organic Chemistry
National Chemical Laboratory
Pune 411008, India

National Chemical Laboratory, Pune (India).

CANDIDATE'S DECLARATION

I hereby declare that the thesis entitled "***Design and Synthesis of Sterol-polyamides, Sterol-polyamines and Bile acid Bistriazoles: A New Class of Antimicrobials***" 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, 2004 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.

Sudhir N. Bavikar **November 2009**
Senior Research Fellow
Division of Organic Chemistry
National Chemical Laboratory
Pune 411008, India

Acknowledgement

It gives me immense pleasure to express my deep sense of gratitude to my research supervisor and mentor Dr. Braja Gopal Hazra for his splendid guidance, invaluable suggestions, inspiration and personal freedom rendered to me during my research period. I will be always indebt to him for his constant support, perceptive criticisms and encouragement. His endless enthusiasm for science, ethics and receptive attitude will always remain a source of inspiration for me.

I owe my special thanks to Dr. Mrs. Vandana S. Pore, Dr. M. S. Shashidhar and Dr. H. V. Thulasiram for their kind help, encouragement, valuable guidance and moral support during the completion of this investigation.

It is my privilege to thank to Dr. Ganesh Pandey, Head, Division of Organic Chemistry and Dr. Dilip D. Dhavale for their valuable guidance and fruitful discussion during the course of this work. I extend my gratitude to Dr. K. N. Ganesh (Former Head, OCS Division), Dr. P. L. Joshi, Dr. N. N. Joshi, Dr. M. N. Deshmukh, Dr. N. P. Argade, Dr. M. Muthukrishnan and Dr. Mrs. S. Hazra for their help and encouragement during the course of this work.

Help rendered by the members of IR, microanalysis, mass spectroscopy, NMR group and library staff members is also acknowledged. I also thank Mr. Pawar, Khandekar, Moreji and other OCD staff members for their timely help through out this work. I take this opportunity to thank Dr. Samit Chattopadhyay and Mr. Sreenath Kadreppa of National Centre for Cell Science, Pune and Dr. Mukund V. Deshpande and Mr. Fazal Shirazi of Biochemical Sciences Division, NCL, Pune for providing the biological data.

I feel great sense of gratitude Professor Dr. Babasaheb P. Bandgar, Dr. Vinod T. Kambli and Late H. N. Suresh for their inspirational teaching and discipline. They have introduced me to this fascinating subject and inspired me to move towards the research during my M. Sc. studies.

I am very much indebted to my senior colleagues Bapu, Dr. Deepak, Namdev and Dr. Nilkanth for their useful initial training, advices, true help, love and care. I would specially like to thank Namdev for endlessly correcting drafts of my thesis. My special thanks to lab friends Dr. Susmita, Patwa, Dr. Khirud, Sachin, Pankaj, Swati, Prabhakar, Deepti, Ashwini, Manish, Saikat, Atul, Rincy, Trushna, Nilofer, Devdutta and Krithika for helping me in various capacities throughout my work and maintaining a warm and cheerful atmosphere in the lab. The warm memories of my days in Laboratory No. 201 and 289 of NCL will haunt me forever.

I would like to extend my thanks to NCL friends, Dr. Rameshwar, Dr. Vinod, Nagendra, Dr. Kulbhushan, Dr. Shriram, Dr. Amol, Pandu, Awadut (Maharaj), Sharad, Jogdand, Dr. Muklesh, Mehraj, Umesh, Ankush, Satish, Ajay, Pinak, Aba, Suleman, Manmath, Omprakash, Ravi, Sutar, Kishor, Manash, Shailesh, Murali, Madhuri, Rajendra, Bharat, Alson, Majid, Pushpesh, Shijo, Arun, Deepak, Gitali for making my stay at NCL, Pune very comfortable and memorable one.

I have been fortunate to have friendship with people like Kishan, Ganesh, Anil, Ravibhushan, Kiran, Namdev, Barure, Pramod (Guru), Santosh which has stood the test of time and I am grateful to them for always encouraging me in whatever I choose to do.

It has been a difficult task to capture and express my feelings for my family members. I have no words to express my sense of gratitude to my father Baba (Narayanrao) and mother AAI (Sudha) for their continuous showering of boundless affection on me and supporting me in whatever I chose or did. It is my parent's prayer, constant struggle and relentless hard work to overcome the odds of life, which has inspired me to pursue life with a greater optimism. This Ph. D. thesis is a result of the extraordinary wills, efforts and sacrifices of my parents. I would like to thank Brother Narhari, and all family members for enormous support in materializing this work into a reality.

I can't find the right words for the one person whom I love most, my wife Ashwini. She has experienced all my ups and down. Without her patience, love and endless support this thesis wouldn't have been written. Together with our beloved daughter Aditi we are waiting for the next perfect wave. We are curious where it is going to take us. I thank "Aditi" for making our life more beautiful than it was. My successes are dedicated to them now and always.

I take this opportunity to thank each and every person who have helped and supported me throughout my education period.

I thank Director, National Chemical Laboratory, Pune for providing all necessary infrastructural facilities to complete my work successfully. I am also thankful to University Grant Commission (UGC), New Delhi for the financial assistance in the form of fellowship. Finally, my acknowledgement would not be completed without thanking the God Ganesha, for giving me the strength and the determination to overcome the hardship faced in my life.

Sudhir

CONTENTS

GENERAL REMARKS

- Independent reference and compound numbering have been employed for abstract, as well as each chapter (Chapter 1-3) and each section (Section A and B).
- 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 dichloromethane-methanol or 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 50.32 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 100.61 MHz for ^{13}C NMR) and DRX 500 (500.13 MHz for ^{1}H NMR and 125.76 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. High resolution mass spectra were obtained on a Kratos MS-80 spectrometer .
- 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
CSA	Cationic Steroid Antibiotics
CSD	Cambridge Structural Database
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
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
FIC	Fractional Inhibition Concentration

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
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)
Mp	Melting Point
MS	Mass Spectrum
MS 4Å	Molecular Sieves (4Å)
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Microwave

MsCl	Mesyl Chloride
NCCLS	National Committee for Clinical Laboratory Standard
NCIM	National Collection of Industrial Micro-Organisms
NMR	Nuclear Magnetic Resonance
NHS	*N*-Hydroxysuccinimide
OSu	*N*-Hydroxysuccinimide Ester
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
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

Research Student:	Sudhir N. Bavikar
Research Guide:	Dr. Braja G. Hazra
Title of the Thesis:	"Design and Synthesis of Sterol-polyamides, Sterol-polyamines and Bile acid Bistriazoles: A New Class of Antimicrobials."
Ref. No. :	EI/209/Ph.D./2007 Dated: January 23, 2007
Date of Registration:	06.03.2006
Place of Work:	Division of Organic Chemistry, National Chemical Laboratory, Pune 411 008.

The thesis entitled **"Design and Synthesis of Sterol-polyamides, Sterol-polyamines and Bile acid Bistriazoles: A New Class of Antimicrobials."** has been divided into three chapters.

Chapter 1: Design Synthesis and Bioevolution of Novel Steroid Polyamine Conjugates

Chapter 2, Section A: Design, Synthesis and Bioevaluation of Novel Steroid Polypeptide Conjugates

Chapter 2, Section B: Pd Catalyzed One-pot Chemoselective Protocol for the Preparation of Carboxamides Directly from Azides

Chapter 3: Cu(I) Catalyzed Alkyne-Azide "Click" Cycloaddition: Efficient Synthesis and Bioevaluation of Bile Acid Bistriazole in the Presence of Base

---

Chapter 1: Design, Synthesis and Bioevaluation of Novel Steroid Polyamine Conjugates.

Nature continues to be the main source of inspiration for synthetic chemists in their quest to make novel conjugates, which can have different physical, biological and medicinal properties. Steroids 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 cholic acid and deoxycholic 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 **1** and squalamine **2** in the cyclic form (Figure 1). Squalamine **2** is the first natural steroid-polyamine conjugate isolated from stomach extract of the dogfish shark and it represents a new class of naturally occurring antibiotic of animal origin.[3] It contains a cholestane ring system with 5α-hydrido, 7α-hydroxy, 3β-spermidinyl and 24 (R)-sulphate group. Squalamine possess broad-spectrum antibiotic activity, extremely active against Gram-negative and Gram-positive bacteria. It is fungicidal and induces osmotic lysis of protozoa. The mode of action of squalamine might be through membrane disruption and squalamine achieves this by acting as an ionophore.[4]

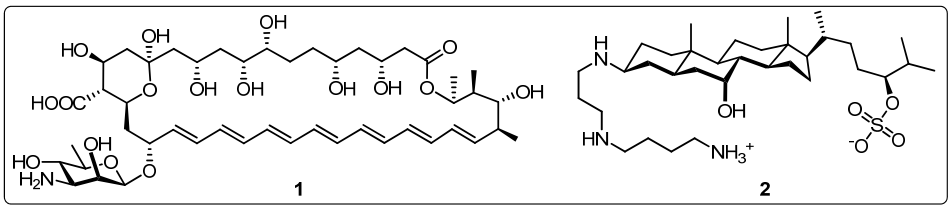


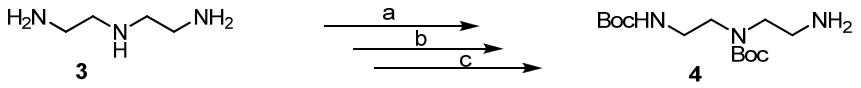
Figure 1

The fact that inadequate amounts of squalamine was available for bioactivity and mechanistic studies, coupled with clear need for the preparation of analogs prompted several groups to undertake the synthesis of squalamine analogs. There are several reports in the literature[4-10] for the synthesis of squalamine analogs from different steroids. A variety of squlamine analogs with different polyamines, sulfate, hydroxy groups at different positions of steroids have been synthesised. Some of these analogues showed similar anti-bacterial activity to the parent compound squalamine **2**. Variations in the structure of the analogues led to changes in the spectrum of activity against a variety of bacteria and yeasts.

Accordingly we have chosen (i) cholic acid as starting material for the synthesis of new analogues of squalamine as rigid and long hydrophobic unit. (ii) Spermine and spermidine side chin is replaced by simple diethylenetriamine or triethylenetetramine as flexible

hydrophilic chain. (iii) Sulphate, hydroxy and amine group are to be attached at different positions as a pendant polar group. This chapter provides a detailed description of the approaches in which we planned to synthesize novel steroid polyamine conjugates.

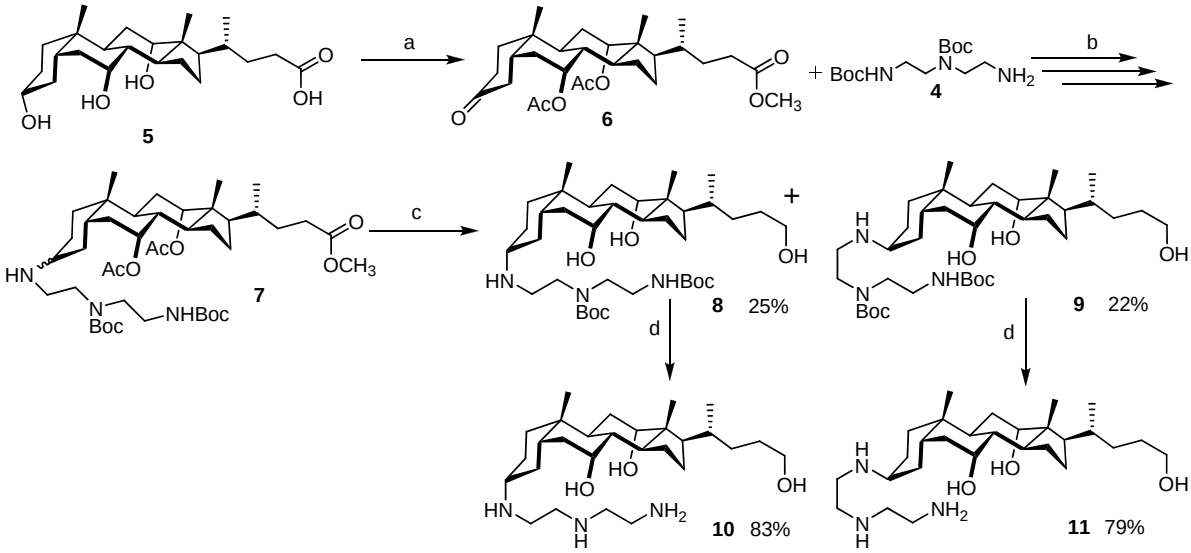
Accordingly synthesis of polyamine chain was carried out using diethylenetriamine **3**. One of the primary amino functionality of diethylenetriamine **3** was selectively protected using methyl trifluroacetate[11] to afford monotrifluroacetamide which was not isolated and to this solution the remaining free amines were protected with ditert-butyldicarbonate to afford bis Boc protected trifluroacetamide (Scheme 1). Selective deprotection of the bis Boc trifluroacetamide was carried out by increasing the pH of the solution above 11 with concentrated aqueous ammonia to afford compound **4** with over all yield 70%.



Scheme 1: Reagents and conditions (a) CF_3COOCH_3, CH_3OH, -70-0 °C, 8 h; (b) Boc anhydride, 0-25 °C; (c) NH_4OH, 16 h.

Cholic acid **5** on treatment with *p*TSA/CH_3OH gave methyl cholate in 91% yield, which on acetylation afforded the triacetate in 92% yield. Selective C-3 acetate hydrolysis of triacetate with Na_2CO_3 in CH_3OH for 7 h, followed by Jones oxidation furnished 3-oxo compound **6** in 71% yield in two steps (Scheme 2). Reductive amination of compound **6** and *N*-Boc protected polyamine **4** using sodium cyanoborohydride[10] gave epimeric mixture of polyamine **7** at C-3 in 68% yield. LAH reduction of compound **7** afforded C-7, C-12 and C-24 triol with an epimeric mix of polyamine at C-3. This mixture is separated by silica gel flash column chromatography to furnish compounds **8** and **9** in 25% and 22% yield respectively. N-Boc deprotection of each of the pure compounds **8** and **9** were carried with dry CH_3OH/HCl to furnish the corresponding trihydrochlorides. These hydrochlorides were adsorbed on silica gel and eluted with CH_3OH-NH_4OH-

CH_2Cl_2 to gave pure polyamine **10** and **11** in 83% and 79% yield. Priliminary bioevaluation study was carried out with compounds **10** and **11**.



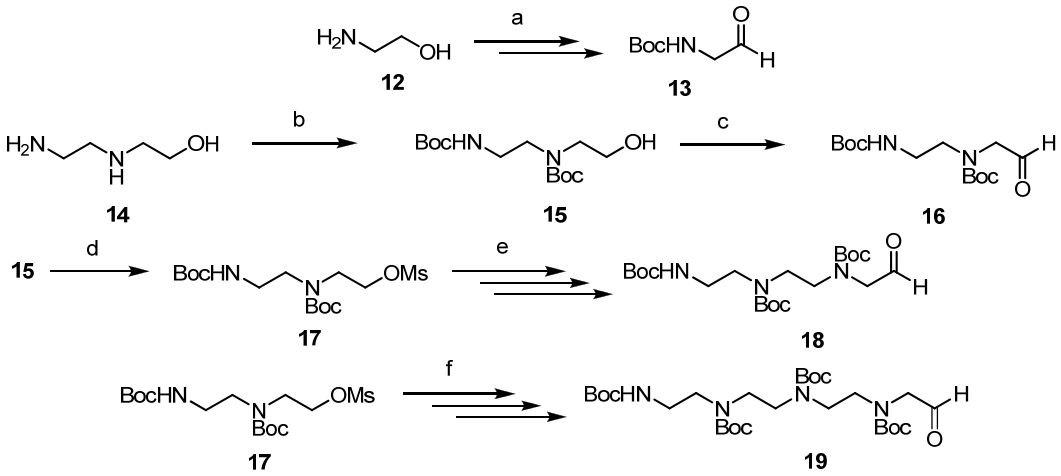
Scheme 2: Reagents and conditions (a) (i) *p*TSA, MeOH, 27 °C, 12 h, 91%; (ii) Ac_2O (5 eq.), DMAP, Et_3N, DCM, 25 °C, 9 h, 92%; (iii) Na_2CO_3, MeOH; (iv) H_2SO_4+CrO_3, Acetone; (b) $Na(CN)BH_3$, AcOH, MeOH/DCM, 0-27 °C, 14 h, 68%; (c) LAH, THF, 25 °C, 1 h ; (d) MeOH, HCl, 1 h.

Zone of inhibition shown by compound **10** and **11** indicates these compound are reasonably good antifungal agents with respect to standard drug Amphotericin-B **1**.

Analogs prepared by above synthetic path have certain limitation such as a) Synthetic route depicted in the above scheme has less overall yields b) Separation and purification of both diasteriomers **10** and **11** is difficult because of highly polar nature c) To produce a library of compounds with variable amphiphilicity, need simplified synthetic procedure and hassle free purification of compounds.

With this in view we synthesize polyamine at C-3 position following different approach. The *N*-Boc protected aminoaldehydes **13** and **16** were synthesized starting from the commercially available amino alcohols **12** and **14**, respectively (Scheme 3). Long chain *N*-Boc protected polyaminoaldehyde **18** and **19** were synthesized from compound **15**.

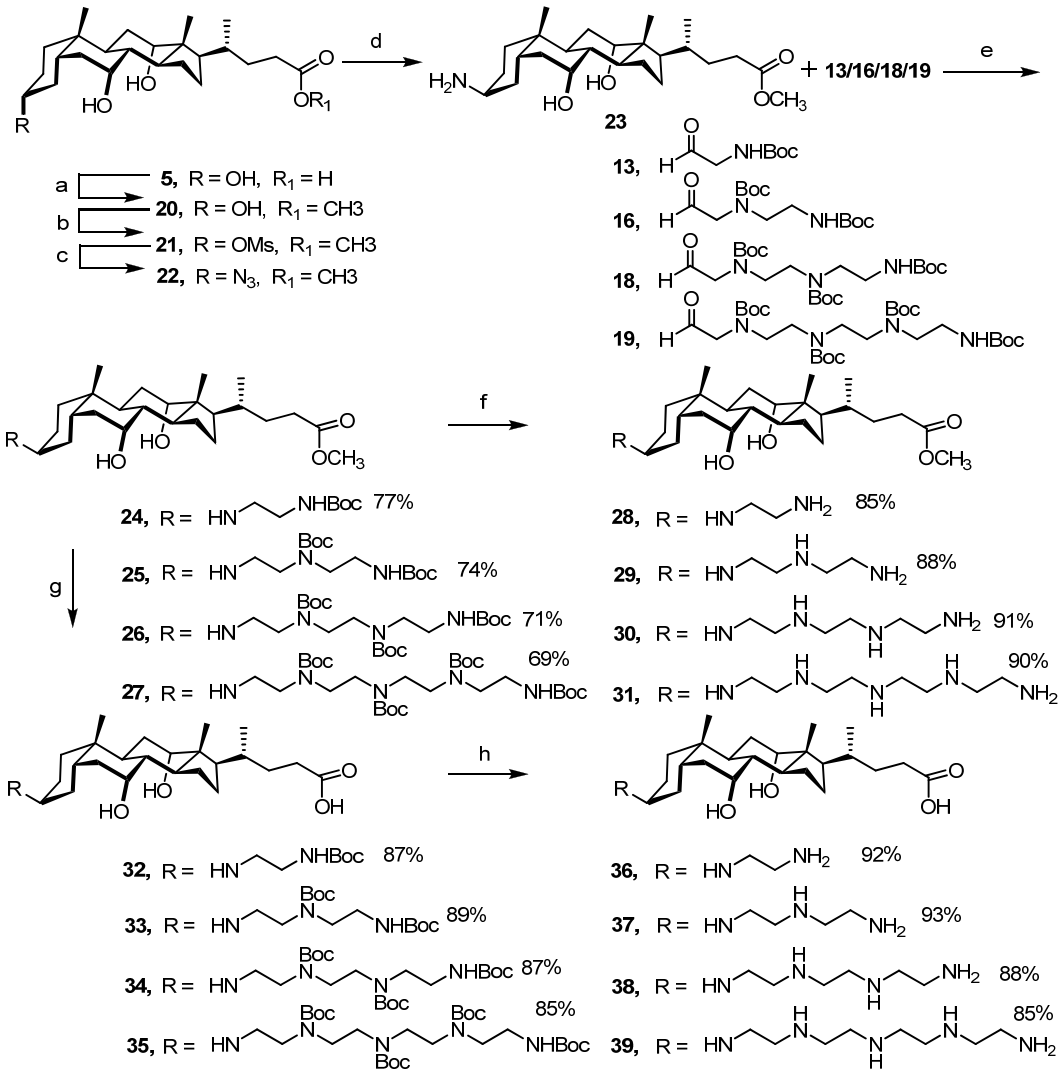
Primary alcohol group of compound **15** was mesylated using methanesulfonyl chloride to give compound **17**. Compound **17** on nucleophlic substitution using ethanol amine **12** at 80 °C and *N*-(2-Aminoethyl) ethanolamine **14** at 90 °C respectively, furnished secondary amines, which on Boc protection followed by oxidation of the terminal hydroxy functionality using IBX (2-Iodoxybenzoic acid) in DMSO to afford *N*-Boc protected amino aldehydes **18** and **19**.



Scheme 3: Reagents and conditions **(**a) (i) Boc anhydride, 1N NaOH, dioxane: water, 0-25 °C, 30 min 98%; (ii) IBX, DMSO, 25 °C, 4 h, 77 %; (b) Boc anhydride, 1N NaOH, dioxane: water, 0-25 °C, 30 min, 97%; (c) IBX, DMSO, 25 °C, 4 h; (d) MsCl, , DCM, 0 °C; (e) (i) Ethanol amine, 80 °C, 7 h; (ii) Boc anhydride, Et_3N, DCM, 0-25 °C, 3 h, 87%; (iii) IBX, DMSO, 26 °C, 4 h; (f) (i) *N*-(2-Aminoethyl) ethanolamine, 90 °C, 6 h; (ii) Boc anhydride, Et_3N, DCM, 3 h, 83%; (iii) IBX, DMSO, 26 °C, 6 h.

3β-Amino methyl cholate **23** was synthesized from cholic acid **5** in four steps in a straightforward manner with an overall yield of 77% (Scheme 4).[12] Reductive amination of 3β-amino methyl cholate **23** with *N*-Boc protected aldehydes **13**, **16**, **18** and **19** under mild condition using $Na(CN)BH_3$ and AcOH in DCM/CH_3OH afforded the *N*-Boc protected 3β-polyamino compounds **24-27** in 69-77% yields. Subsequent hydrolysis of the C-24 methyl ester functionality in compounds **24-27** with 2M, LiOH in CH_3OH for 12 h provided the corresponding acids **32-35** in 85-89% yield. Cleavage of the Boc protecting groups in compounds **24-27** and **32-35** was accomplished with 2M HCl in

Et_2O to afford free amino compounds **28-31** and **36-39** in yields ranging from 85-93%, respectively.



Scheme 4: Reagents and conditions (a) MeOH, PTSA, 28 °C, 24 h, 96%; (b) MsCl, TEA, CH_2Cl_2, 0 °C,10 min; (c) NaN_3, DMF, 60 °C, 8 h, 94%; (d) H_2, Pd-C, MeOH, 6 h, 87%; (e) $Na(CN)BH_3$, AcOH pH 5-6, DCM/MeOH, 25 °C 10-12 h, 69-77%; (f) 2M HCl in Et_2O, 0-25 °C, 1 h, 85-91%; (g) 2M LiOH, MeOH, 25 °C, 12 h, 85-89%; (h) 2M HCl in Et_2O, 0-25 °C, 1 h, 85-93%.

Cholic acid **5** and all the newly synthesized conjugate molecules **28-31** and **36-39** exhibited significant antifungal and antibacterial activity against all the tested strains.

Chapter 2, Section A: Design, Synthesis and Bioevaluation of Novel Steroid Polypeptide Conjugates.

The class of membrane-disrupting drugs is ideal as antimicrobial agents because microbes are unlikely to develop resistance to them.[13,14] As the outer membrane or cell wall of microbes provides a protective barrier against many types of antibiotics,[15] amphipathic molecules that can act synergistically with various hydrophobic antibiotics as outer membrane permeabilizers may represent a new class of antibiotic agents. Naturally occurring steroid-amino acid conjugates such as bufotoxin 40 and polymastiamide-A 41, exhibit *in vitro* antimicrobial activity.[16] Several peptides have been identified that increase the permeability of the outer membranes of Gram-negative bacteria and sensitize these organisms to hydrophobic antibiotics.[17] The best studied of these peptides are the polymyxin B (PMB) 42 derivatives (Figure 2).

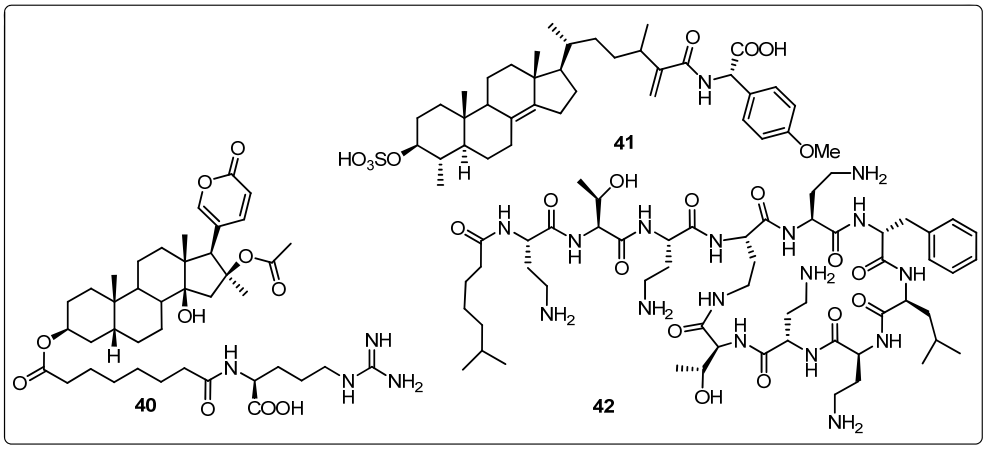
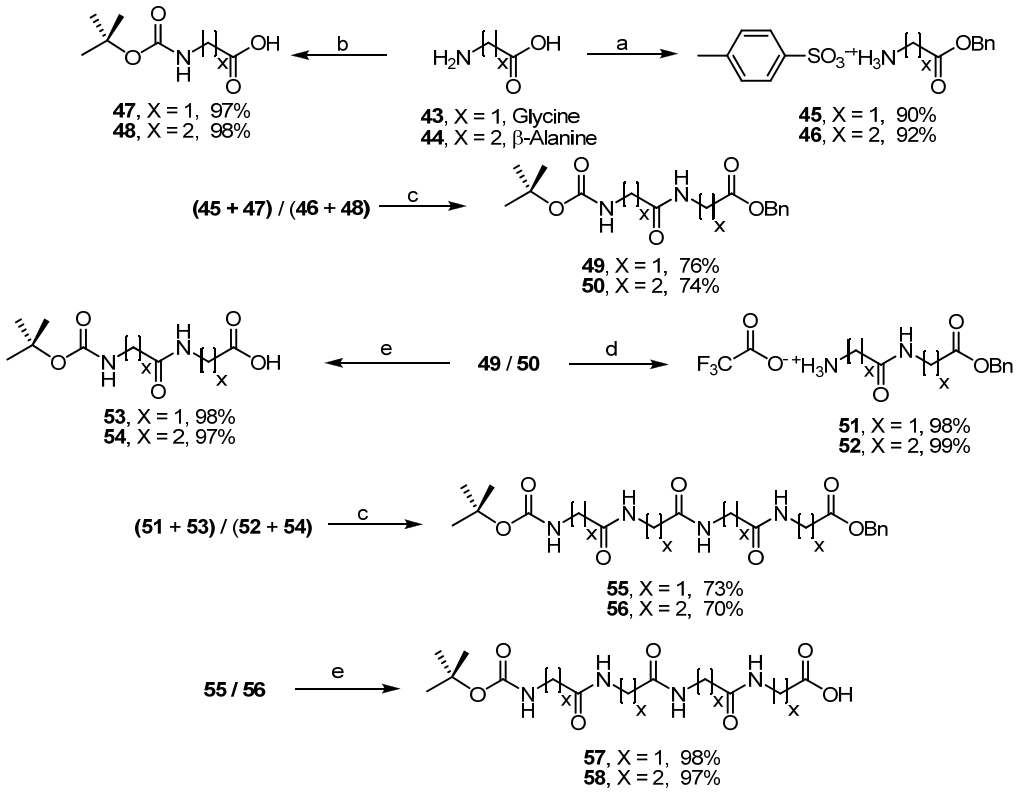


Figure 2

With this in view, novel cholic acid-tetrapeptide conjugates of glycine and β-alanine were synthesized and tested against a wide variety of microorganisms. This chapter provides a detailed description of the our approach which we planned to synthesize novel bile acid-polyamide conjugates as PMB mimic. A convergent approach followed for the synthesis of the desired targets. Following the literature procedures,[18] suitably protected monomers

45, **47** and **46**, **48** were synthesized from glycine **43** and β-alanine **44**, respectively (Scheme 5).

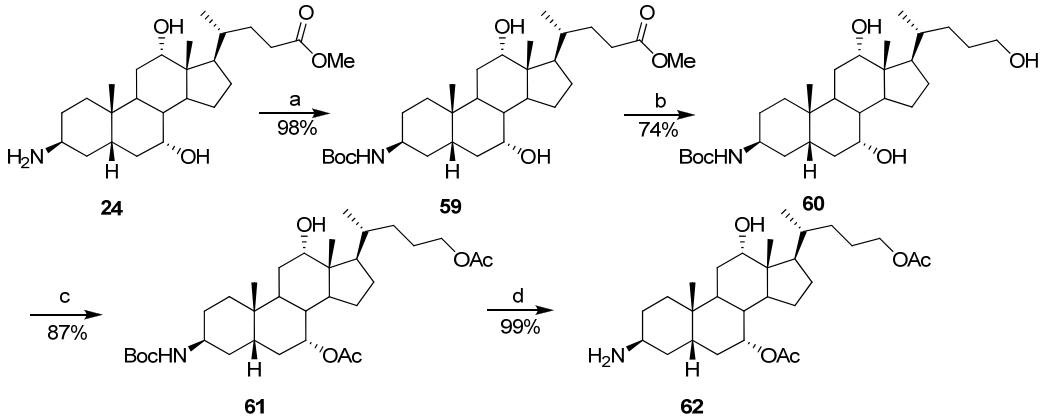
The stepwise elongation was performed in DCM using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) activation to furnish Boc-$(Gly)_2$-OBn **49** in 76% yield and Boc-$(\beta\text{-Ala})_2$-OBn **50** in 74% yield (Scheme 5).



Scheme 5: Reagents and conditions (a) Benzyl alcohol, *p*TSA, toluene, reflux, 4 h; (b) Boc anhydride, 1N NaOH, dioxane:water, 0-25 °C, 30 min; (c) EDCI, HOBt, Et_3N, DCM, 0-25 °C, 6 h; (d) TFA, DCM, 0-25 °C, 2 h; (e) H_2, Pd-C, MeOH, 25 °C, 1 h; (f) 2M HCl:Et_2O, 0-25 °C, 1.5h.

Removal of the *tert*-butoxycarbonyl (Boc) group from peptides **49** and **50** was performed in trifluoroacetic acid (TFA)/DCM, to afford compounds **51** and **52** in quantitative yield. On the other hand, catalytic hydrogenolysis of the benzyl (Bn) group from peptides **49** and **50**, furnished compounds **53** and **54** in excellent yields. Compounds **55** and **56** were obtained by fragment condensation of **51** with **53** and **52** with **54** in DMF using EDCI activation in the presence of 1-hydroxybenzotriazole (HOBt) as catalyst. These

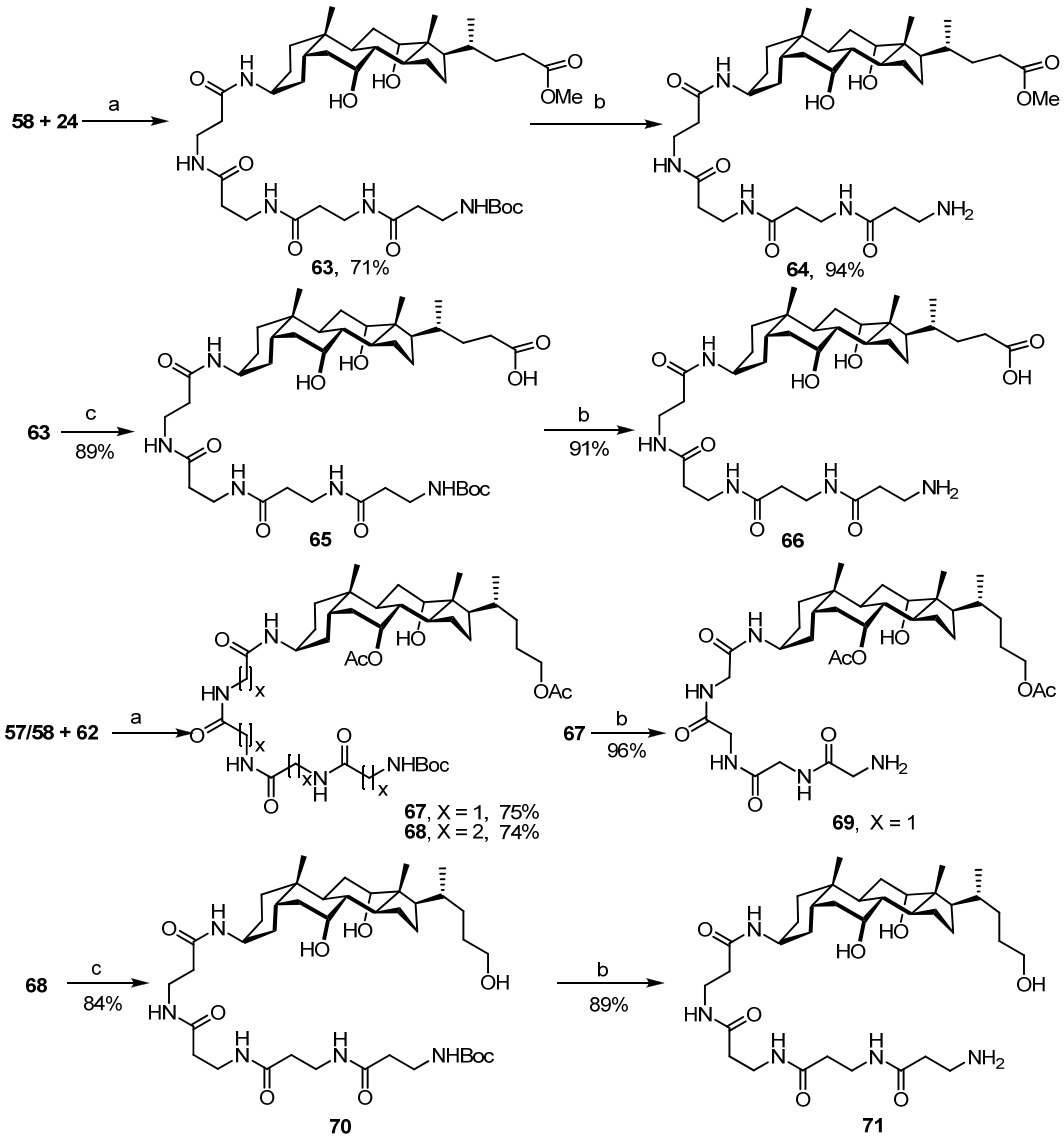
tetrapeptides **55** and **56** were purified by column chromatography to furnish white solids in 73 and 70% yields, respectively. The Benzyl groups of Boc-$(Gly)_4$-OBn **55** and Boc-$(\beta\text{-Ala})_4$-OBn **56** were removed by a similar catalytic hydrogenation reaction to furnish compounds **57** and **58** in 98 and 97% yield, respectively. Tetrapeptide **58** was attached at the 3β-position of modified cholic acid as follows. The amine functionality in compound **24** was protected using Boc anhydride to afford compound **59** in excellent yield (Scheme 6).



Scheme 6: Reagents and conditions **(**a) Boc anhydride, Et_3N, dioxane:water, 25 °C 6 h; (b) LAH, THF, 0-25 °C, 1 h; (c) Ac_2O, DMAP, Et_3N, DCM, 25 °C, 8 h; (d) TFA, DCM, 0-25 °C, 2 h.

Reduction of the methyl ester of compound **59** was carried out using LAH to afford C-24 hydroxy compound **60** in 74% yield. Acylation of the C-7 and C-24 hydroxyl groups of compound **60** was carried out using acetic anhydride and a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) to furnish compound **61** in 87% yield. The protected C-3β amino functionality of compound **61** was unmasked using TFA to afford compound **62** in almost quantitative yield. Coupling of 3β-amino cholic acid intermediates **24** with Boc-$(\beta\text{-Ala})_4$-OH **58** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **63** in 71% yield (Scheme 7). Similarly coupling of 3β-amino cholic acid derivative **62** with Boc-$(Gly)_4$-OH **57** and Boc-$(\beta\text{-Ala})_4$-OH **58** furnished compounds **67**, **68** in 75 and 74% yield, respectively. Subsequent hydrolysis of the methyl ester

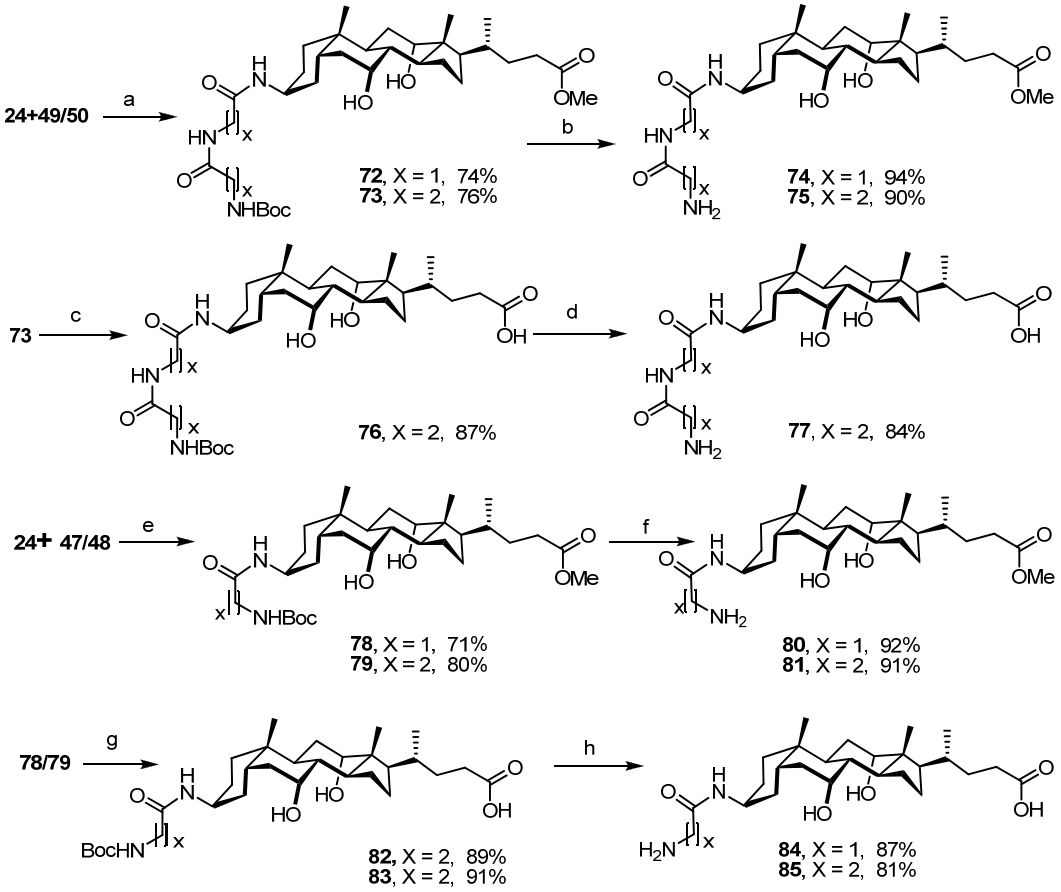
functionality in compound **63** and C-7 and C-24 acetates in compound **68** (LiOH 2M, CH_3OH) and aqueous work-up provided the corresponding acid **65**, and hydroxy compound **70** in 89 and 84% yield, respectively. Cleavage of the Boc group in compounds **63**, **65**, **67** and **70** was accomplished with 2M HCl:Et_2O to afford free amino compounds **64**, **66**, **69** and **71** respectively in yields ranging from 89-96%.



Scheme 7: Reagents and conditions **(**a) EDCI, HOBt, Et_3N, DMF, 0-25 °C, 6 h; (b) 2M HCl:Et_2O, 0-25 °C, 1.5 h; (c) 2M LiOH, MeOH, 25 °C, 12 h.

Tetrapeptides **55-58**, cholic acid **24**, and cholic acid-tetrapeptide conjugates **63-71** were examined for *in vitro* antifungal as well as antibacterial activity. These compounds were also tested for their ability to permeabilize the outer membrane of Gram-negative bacteria

such as *E. coli* causing sensitization to hydrophobic antibiotics that inefficiently cross the outer membrane. We also demonstrated such permeabilization by cholic acid derivatives with *C. albicans*, a pathogenic fungus.



Scheme 8: Reagents and conditions (a) EDCI, HOBt, Et_3N, DMF, 0-27 °C, 7-8 h,74% and 76%; (b) 2M HCl in Et_2O, 0-25 °C, 1 h, 90% and 94%; (c) 2M LiOH, MeOH, 28 °C, 10 h, 87%; (d) 2M HCl in Et_2O, 0-28 °C, 1 h, 84%; (e) EDCI, HOBt, Et_3N, DMF, 0-27 °C, 7 h,71% and 80%; (f) 2M HCl in Et_2O, 0-28 °C, 1 h, 92% and 91%; (g) 2M LiOH, MeOH, 27 °C, 9 h, 89 and 91%; (h) 2M HCl in Et_2O, 0-28 °C, 1 h, 87% and 81%.

This chapter also provides detailed description of synthesis of 3β-cholic acid-dipeptide and monopeptide conjugates of glycine and β-alanine and study their antimicrobial activity. A dipeptide and monopeptide segment of glycine and β-alanine has been synthesized using classical solution phase synthesis. Coupling of 3β-amino cholic acid intermediates **24** with Boc-$(Gly)_2$-OH **49** and Boc-$(\beta\text{-Ala})_2$-OH **50** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **72** and **73** in 74% and 76%

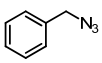
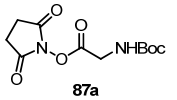
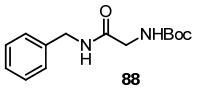
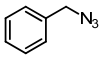
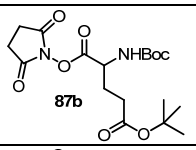
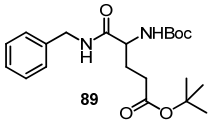
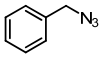
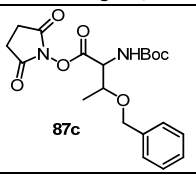
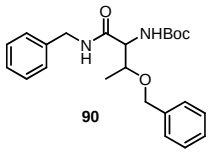
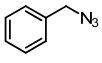
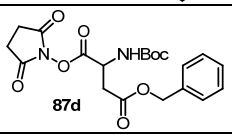
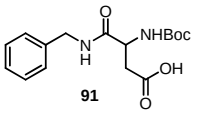
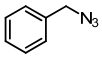
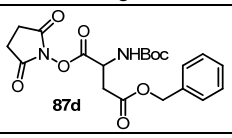
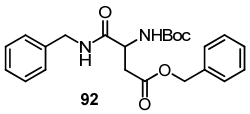
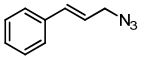
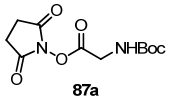
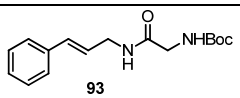
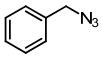
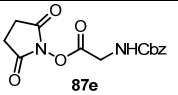
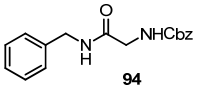
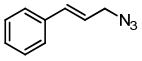
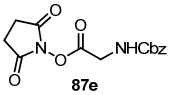
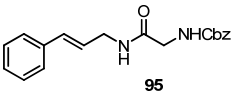
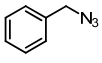
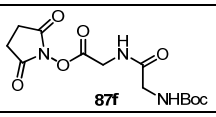
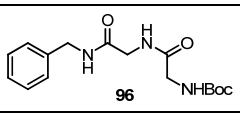
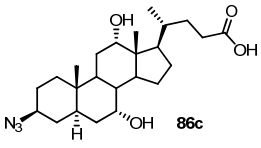
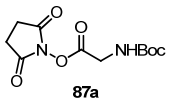
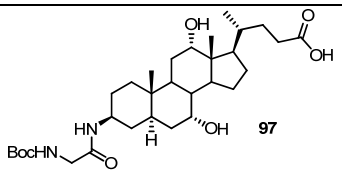
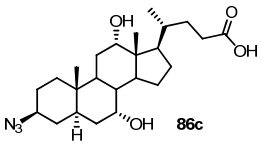
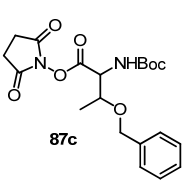
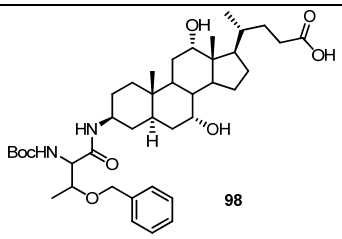
yield, respectively (Scheme 8). Subsequent hydrolysis of the C-24 methyl ester functionality in compound **73** (LiOH 2M, CH_3OH) provided the corresponding acid **76** in 87% yield. Cleavage of the Boc group in compounds **72**, **73** and **76** was accomplished with 2M HCl in Et_2O to afford free amino compounds **74**, **75** and **77** in 94%, 90% and 84% yields, respectively.

In the similar way coupling of 3β-amino cholic acid intermediates **24** with Boc-(Gly)-OH **47** and Boc-(β-Ala)-OH **48** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **78** and **79** in 71% and 80% yield, respectively. Subsequent hydrolysis of the C-24 methyl ester functionality in compound **78** and **79** using aq.LiOH 2M, CH_3OH provided the corresponding acid **82** and **83** in 89% and 91% yield, respectively. Cleavage of the Boc group in compounds **78**, **79**, **82** and **83** was accomplished with 2M HCl in Et_2O to afford free amino compounds **80**, **81**, **84** and **85** respectively in yields ranging from 81-92%. These newly synthesized compounds **72-85** were evaluated *in vitro* for their antifungal and antibacterial activity. Most of the compounds exhibited antifungal as well as antibacterial activity against all the tested fungal and bacterial strains.

Chapter 2, Section B: Pd Catalyzed One-pot Chemoselective Protocol for the Preparation of Carboxamides Directly from Azides

The conversion of azides to amines is an important transformation in organic synthesis.[19] The amines are further converted to the corresponding carboxamides which is the most common functionality in many biological interesting molecules. Several methods have been developed for the direct amidation of carboxylic acids and amines.[20] To overcome the situation where free amines can not be used because of structural wavering, azides have been used to directly form amide bonds.

Table 1

Entry	Azide Component	NHS ester Component	Product	Catalyst[†]	Yield
1	 **86a**	 **87a**	 **88**	A B	89/86[‡] 91
2	 **86a**	 **87b**	 **89**	A B	85/83[‡] 87
3	 **86a**	 **87c**	 **90**	A B	82/82[‡] 81
4	 **86a**	 **87d**	 **91**	A	86/79[‡]
5	 **86a**	 **87d**	 **92**	B	81
6	 **86b**	 **87a**	 **93**	A B	85 84
7	 **86a**	 **87e**	 **94**	A B	88 87
8	 **86b**	 **87e**	 **95**	A B	77 80
9	 **86a**	 **87f**	 **96**	A B	87 83
10	 **86c**	 **87a**	 **97**	B	76
11	 **86c**	 **87c**	 **98**	B	70

[†] **A**- Palladium, 5 wt. % on barium sulphate; **B**- Palladium, 5 wt. % on calcium carbonate.

[‡]Yields for the reactions carried out in THF (Other all yields are for the reaction carried out in EtOH).

Entry 1A, 2A, 3A was earlier reported in our laboratory.[26 b]

The classical method of conversion of azide to amides includes Staudinger-type ligation involving the acylation of an iminophosphorane,[21] the amidation of carboxylic acid with an azide in the presence of triallyl phosphine,[22] selenocaboxylate/azide amidation,[23] Williams thio acid/azide amidation,[24] new chemical ligation method in which phosphinobenzenethiol was used to

link a thioester and azide to form an amide bond.[25] The major drawback of these classical mothod involves with the overall yield and purification of products. As a part of an ongoing program on synthesis of biologically interesting bile acid-aminoacid conjugates,[26] we required an efficient one pot chemoselective and operationally very simple protocol for the synthesis of carboxamides directly from azides.

In this section, we have demonstrated a new robustic, efficient "one pot" chemoselective protocol for the preparation of carboxamides. In this practice carboxamides were obtained efficiently in high yields from azides on reaction with corresponding preformed activated carboxylic acids, in a single-step reductive transformation using hydrogen atmosphere (balloon) under $Pd/CaCO_3$ and/or $Pd/BaSO_4$ catalysis.

The simplicity, remarkable chemoselectivity and mildness of this catalytic transformation were exhaustively studied with compounds bearing different sensitive functional groups such as benzyl ethers (Entry 3 and 11), olefins (Entry 6 and 8) and benzyl carbamates (Entry 7 and 8). In particular, synthesis of compound **95** (Entry 8) was realized when azide bearing double bond **86b** and NHS ester bearing *N*-Cbz protecting group **87e** were exposed to the present modified protocol. As expected, azide possessing olefin functional group was smoothly converted into the corresponding carboxamides **95** in excellent yield without affecting the carbon-carbon double bonds and *N*-Cbz protecting group. The scope and generality of this protocol was utilized in our current research for the synthesis of

peptide **96** (Entry 9) and steroidal carboxamides **97** as well as **98** (Entry 10 and 11, respectively).

Chapter 3: Cu(I) Catalyzed Alkyne-Azide "Click" Cycloaddition: Efficient Synthesis and Bioevaluation of Bile Acid Bistriazole in the Presence of Base.

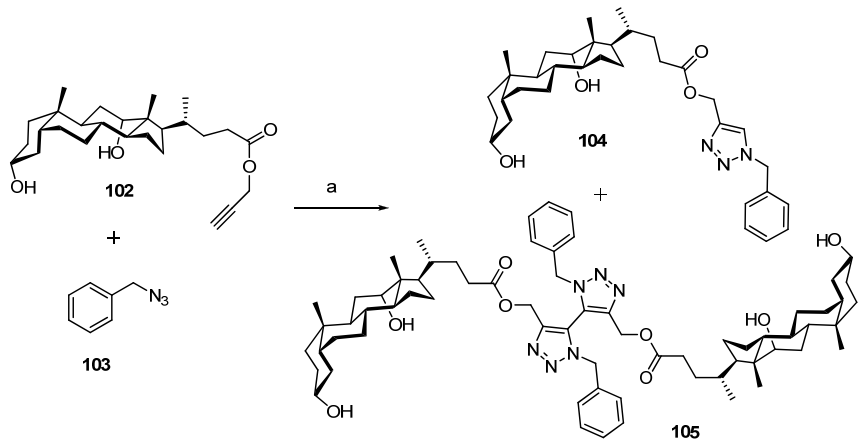
Currently, bile acids have attracted considerable interest for diverse significant applications, involved in pharmacology, asymmetric synthesis, molecular recognition and also polymeric materials[27] due to their inexpensive availability, the rigid steroid frame works, unique facial amphiphilicity, and together with viability of being chemically modified on C-3, C-7, C-12 and C-24

The Huisgen 1,3-dipolar cycloaddition reaction[28,29] has gained considerable attention in recent year due to introduction of Cu(I) catalyst independently by Meldal,[30,] and Sharpless[31] leading to a major improvement in both rate and regioselectivity of the reaction (broadly known as Click reaction). The great success of this reaction rooted in the fact the mild reaction conditions, high yielding, simple reaction and purification performance, and exclusive formation of 1,4-disubstituted 1,2,3-triazole products. The reactants and products are stable to various solvents including water, have promoted it as a unique organic synthetic modular approach widely applied in biomedical science, organic synthesis, and material chemistry during the past several years.[32]

Usually the quantitative formation of 1,4-disubstited triazole is ensured through hydrolytic cleavage of the Cu(I)-bound end product of the catalytic cycle to regenerate active catalyst and liberate triazole. Sharpless and co-workers perceive minor amounts of undesired byproducts bis-triazols by oxidative coupling during development of the aqueous $CuSO_4$/Ascorbate Huisgen cycloaddition.[33] Without reporting their characterization, they attributed these byproducts to the direct use of Cu(I) species.

Recently very elegantly, Angell and Burgess[34] reported this oxidative coupling in the presence of base.

In this chapter herein we report the synthesis of bile acid oxidative dimers **105** and **108** which are the major products under basic conditions. In continuation of our work on bile acid dimers,[35] propargyl ester of deoxycholic acid **102** and benzyl azide **103** was chosen as a model alkyne and azide components respectively (Scheme 9).



Scheme 9: Reagent and condition (a) $CuSO_4.5H_2O$ (0.1 eq), Cu (powder) (1.0 eq.), Na_2CO_3 (1.5 eq), THF, 31 h, **104** (29%) and **105** (63%) (Entry 1, Table 2).

We varied the reaction conditions in attempts to optimize the yield of bistriazole **105**. Bistriazole **105** was a major product when $CuSO_4$, Cu (powder) and K_2CO_3 was used as base. The optimal base concentration was found to be 1.5M. We also used different Cu(I) sources in an attempt to optimize the yield of bistriazole **105**, such as CuI in combination with various organic as well as inorganic bases, but most of the attempts were not encouraging. The oxidative dimerisation methodology was extrapolated to different bases (both organic and inorganic) having variable concentrations for both copper catalyst ($CuSO_4$/Cu and CuI) and using THF or DMF as solvent are depicted in Table 2.

Our next target was to synthesize of bistriazole **108** having four bile acids units by using propargylic ester of deoxycholic acid **102** and C-24 azide of deoxycholic acid **106**

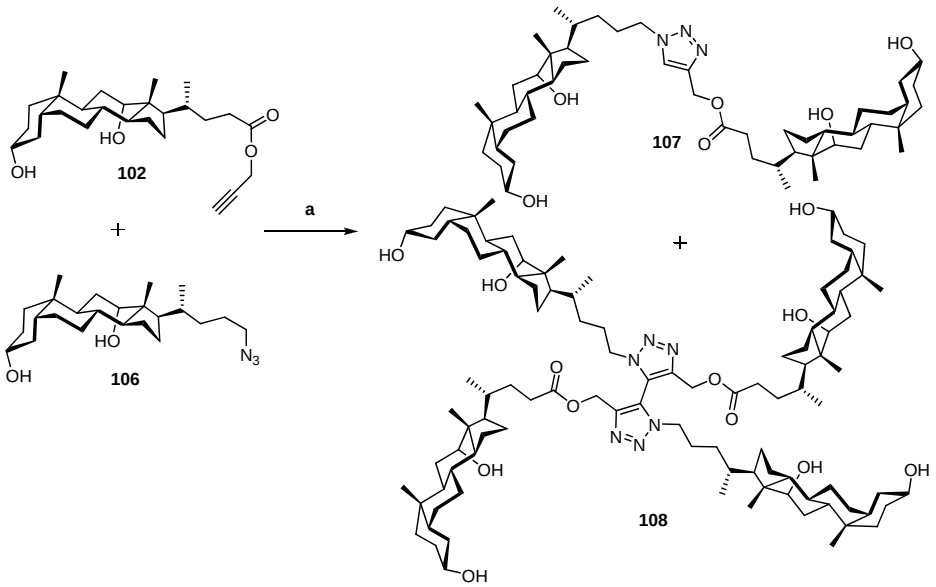
(Scheme 10). Attempts were made to increase the amounts of the bistriazole **108** by employing $CuSO_4$, CuI and by changing various inorganic bases as well as organic bases. Cycloaddition reaction of propargylic ester of deoxycholic acid **102** with C-24 azide of deoxycholic acid **106** using CuI and different nitrogen containing organic bases as well as inorganic carbonate bases mainly gives compound **107** (usual "click" 1,4 disubstuted triazole) and minor one was bistriazole **108**.

Table 2

Entry	Reagent used	Solvent	Base	Conc. of base	Time [h]	Product distribution	
						104[%]	**105**[%]
1	$CuSO_4$/Cu	THF	Na_2CO_3	1.5M	31	29	63
2	$CuSO_4$/Cu	THF	Na_2CO_3	2.0M	30	32	60
3	$CuSO_4$/Cu	THF	Na_2CO_3	3.0M	29	39	53
4	$CuSO_4$/Cu	THF	K_2CO_3	1.5M	28	29	68
5	$CuSO_4$/Cu	THF	K_2CO_3	2.0M	28	30	66
6	$CuSO_4$/Cu	THF	K_2CO_3	3.0M	27	32	60
7	$CuSO_4$/Cu	THF	$NaHCO_3$	1.5M	34	61	38
8	$CuSO_4$/Cu	THF	$NaHCO_3$	2.0M	30	60	35
9	$CuSO_4$/Cu	THF	$NaHCO_3$	3.0M	36	69	22
10	$CuSO_4$/Cu	DMF	Na_2CO_3	1.5M	26	48	43
11	$CuSO_4$/Cu	DMF	K_2CO_3	1.5M	27	51	41
12	$CuSO_4$/Cu	THF	DIPEA	2 eq.	29	83	-
13	$CuSO_4$/Cu	THF	DBU	2 eq.	31	91	-
14	$CuSO_4$/Cu	THF	TEA	2 eq.	26	90	-
15	CuI	DMF	DIPEA	2 eq.	13	67	19
16	CuI	DMF	DBU	2 eq.	14	69	21
17	CuI	DMF	TEA	2 eq.	12	68	24
18	CuI	THF	DIPEA	2 eq.	16	65	21
19	CuI	THF	DBU	2 eq.	16	69	18
20	CuI	THF	TEA	2 eq.	12	73	14
21	CuI	THF	Na_2CO_3	2 eq.	23	60	32
22	CuI	DMF	Na_2CO_3	2 eq.	23	63	35
23	CuI	THF	K_2CO_3	2 eq.	22	45	52
24	CuI	DMF	K_2CO_3	2 eq.	21	56	39

DIPEA- *N*,*N*-Diisopropylethylamine; DBU- 1,8-Diazabicycloundec-7-ene; TEA- triethylamine.

Significant amounts of oxidative dimerisation product bistriazole **108** was observed when we attempted coupling reaction with $CuSO_4$, Cu (powder), in the presence of 1.5M Na_2CO_3 and also using 2M K_2CO_3 (Scheme 10). The oxidative dimerisation methodology



Scheme 10: Reagents and conditions (a) $CuSO_4.5H_2O$ (0.1 eq), Cu (powder) (1.0 eq.), K_2CO_3 (2 M), THF, 12 h, **107** (19%) and **108** (72%) (Entry 5, Table 3).

Table 3

Entry	Reagent used	Solvent	Base	Conc. of base	Time [h]	Product distrubution	
						107 [%]	**108** [%]
1	$CuSO_4$/Cu	THF	Na_2CO_3	1.5 M	16	20	72
2	$CuSO_4$/Cu	THF	Na_2CO_3	2.0M	16	21	71
3	$CuSO_4$/Cu	THF	Na_2CO_3	3.0M	15	23	68
4	$CuSO_4$/Cu	THF	K_2CO_3	1.5M	13	23	71
5	$CuSO_4$/Cu	THF	K_2CO_3	2.0M	12	19	72
6	$CuSO_4$/Cu	THF	K_2CO_3	3.0M	12	27	63
7	$CuSO_4$/Cu	THF	$NaHCO_3$	1.5M	21	34	60
8	$CuSO_4$/Cu	THF	$NaHCO_3$	2.0M	19	38	53
9	$CuSO_4$/Cu	THF	$NaHCO_3$	3.0M	18	37	55
10	$CuSO_4$/Cu	DMF	Na_2CO_3	1.5M	16	41	53
11	$CuSO_4$/Cu	DMF	K_2CO_3	1.5M	17	28	64
12	$CuSO_4$/Cu	THF	DIPEA	2 eq.	20	78	14
13	$CuSO_4$/Cu	THF	DBU	2 eq.	21	83	7
14	$CuSO_4$/Cu	THF	TEA	2 eq.	19	80	9
15	CuI	DMF	DIPEA	2 eq.	13	54	37
16	CuI	DMF	DBU	2 eq.	14	60	30
17	CuI	DMF	TEA	2 eq.	13	54	32
18	CuI	THF	DIPEA	2 eq.	17	79	12
19	CuI	THF	DBU	2 eq.	15	68	23
20	CuI	THF	TEA	2 eq.	14	72	17
21	CuI	THF	Na_2CO_3	2 eq.	20	61	28
22	CuI	DMF	Na_2CO_3	2 eq.	20	57	40
23	CuI	THF	K_2CO_3	2 eq.	20	41	52
24	CuI	DMF	K_2CO_3	2 eq.	19	60	38

was extrapolated to different bases having variable concentrations for both copper catalyst ($CuSO_4$/Cu and CuI) and solvents are depicted in Table 3. These newly synthesized compounds **104-108** were evaluated *in vitro* for their antifungal and antibacterial activity. Most of the compounds exhibited antifungal as well as antibacterial activity.

References

1. (a) Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem*. **2004**, 3385; (b) Salunke, D. B.; Hazra, B. G.; Pore, V. S. *Curr. Med. Chem*. **2006**, *13*, 813.
2. Enhsen, A.; Kramer, W.; Wess, G. *Drug Discov. Today* **1998**, *3*, 409.
3. (a) Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N. Jr.; McCrimmon, D.; Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1354. (b) Wehrli, S. L.; Moore, K. S.; Roder, H.; Durell, S.; Zasloff, M. *Steroids* **1993**, *58*, 370.
4. Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6138.
5. Khabnadideh, S.; Tan, C. L.; Croft, S. L.; Kendrick, H.; Yardley, V.; Gilbert, I. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1237.
6. Kikuchi, K.; Bernard, E. M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 1433.
7. Jones, S. R.; Kinney, W. A.; Zhang, X.; Jones, L. M.; Selinsky, B. S. *Steroids* **1996**, *61*, 565.
8. Kim, H-S.; Choi, B-S.; Kwon, K-C.; Lee, S-O.; Kwak, H. J.; Lee, C. H. *Bioorg. Med. Chem.* **2000**, *8*, 2059.
9. Shu, Y.; Jones, S. R.; Kinney, W. A.; Selinsky, B. S. *Steroids* **2002**, *67*, 291.
10. Choucair, B.; Dherbomez, M.; Roussakis, C.; Kihel, L. E. *Tetrahedron* **2004**, *60*, 11477.
11. Geall, A. J.; Blagbrough, I. S. *Tetrahedron* **2000**, 56, 2249.

12. (a) Anelli, P. L.; Lattuada, L.; Uggeri, F. *Synth. Commun.* **1989**, *28*, 109; (b) Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178.

13. Naka, K.; Sadownik, A.; Regen, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2278.

14. Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961.

15. (a) Hancock, R. E. *Annu. Rev. Microbiol.* **1984**, *38*, 237; (b) Labischinski, G.; Bradaczek, H.; Naumann, D.; Rietschel, D. T.; Giesbrecht, P. *J. Bacteriol.* **1985**, *162*, 9; (c) Nikaido, H.; Vaara, M. *Microbiol. Rev.* **1985**, *49*, 1.

16. (a) Shimada, K.; Fujii, Y.; Mitsuishi, E.; Nambara, T. *Tetrahedron Lett.* **1974**, *15*, 467; (b) Kong, F.; Anderson, R. J. *J. Org. Chem.* **1993**, *58*, 6924.

17. (a) Vaara, M. *Microbiol. Rev.* **1992**, *56*, 395. (b) Rehman, A.; Li, C.; Budge, L. P.; Street, S. E.; Savage, P. B. *Tetrahedron Lett.* **1999**, *40*, 1865.

18. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, 2nd Edition; Springer-Verlag, New York, 1994.

19. Scriven, E. F. V.; Turanbull, K. *Chem. Rev.* **1988**, *88*, 297.

20. Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827-10852.

21. (a) Garcia, J.; Urpf, F.; Vilarrasa, J. *Tetrahedron Lett.* **1984**, *25*, 4841-4844; (b) Bosch, I.; Urpi, F.; Vilarrasa, J. *J. Chem. Soc., Chem. Commun.* **1995**, 91-92; (c) Maunier, V.; Boullanger, P.; Lafont, D. *J. Carbohydr. Chem.* **1997**, *16*, 231-235; (d) Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.* **2000**, *324*, 97-106; (e) Damkaci, F.; DeShong, P. *J. Am. Chem. Soc.* **2003**, *125*, 4408-4409; (f) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141-2143.

22. (a) Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869-870; (b) Mizuno, M.; Haneda, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284-290.

23. Wu, X.; Hu, L. *J. Org. Chem.* **2007**, *72*, 765-774 and the references cited therein.

24. Kolawski, R. V.; Shangguan, N.; Sauers, R. R.; Williams, L. J. *J. Am. Chem. Soc.* **2006**, *128*, 5695-5702.

25. Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939-1941.

26. (a) Bavikar, S. N.; Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J.; Shirazi, F.; Deshpande, M. V.; Srinath, K.; Chattopadhyay, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5512-5517; (b) Ph. D. Theisis submitted by Deepak B. Salunke to Univercity Pune, India in March 2008.

27. Zhang, Z.; Ju, Y.; Zhao, Y. *Chem. lett.* **2007**, *36*, 1450.

28. Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613.

29. Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1967**, *100*, 2494.

30. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

31. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, B. K. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.

32. a) W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun*. **2007**, *28*, 15. b) W. H. Binder, C. Kluger, *Curr. Org. Chem*. **2006,** *10*, 1791. c) Q. Wang, S. Chittaboina, H. N. Barnhill, *Lett. Org. Chem.* **2005**, *2*, 293.

33. Tornøe, C.W.; Meldal, M. *Chem. Rev*. **2008**, *108*, 2952.

34. Angell, Y.; Burgess, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 3649.

35. a) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Chavan, P. S.; Deashpande, M. V. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 2043.; b) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Deashpande, M. V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R. *Org. Biomol Chem.* **2008**, *6*, 3823.

CHAPTER - 1

Design, Synthesis and Bioevaluation of Novel Steroid Polyamine Conjugates

1.1. Abstract

A series of novel 3β-polyamino cholic acid conjugates have been synthesized. The introduction of polyamines —NH(-CH_2-CH_2-NH-)$_n$-H (n = 1, 2, 3 and 4) have been carried out by reductive amination of 3β-amino methyl cholate and *N*-Boc protected aminoaldehydes. These synthetic products have been investigated for their antifungal as well as antibacterial activity against a wide variety of microorganisms (Gram-negative bacteria, Gram-positive bacteria and fungi). Most of these compounds exhibit significant antimicrobial activity. Changing the identity of the polyamine has remarkable effect upon antimicrobial activity. This effect shown by our novel compounds is due to their inherent amphiphilicity.

1.2 Introduction

Steroids have become ideal synthons for the development of diverse conjugates due to their rigid framework, potential for varying levels of functionalization, broad biological activity profile, ability to penetrate the cell membranes and bind to specific hormonal receptors. The steroids form a group of structurally related compounds that are widely distributed in animals and plants. The medicinal chemistry of steroids covers a large and interesting series of structures and biological activities.[1] 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. However, the number of steroidal natural products is limited, whereas millions of hybrids as conjugates of steroids can be prepared. 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. The medicinal applications are based on the fact that the biological activity of the several new hybrids exceeds that of the parent compounds. The advantage of this concept over a combinatorial chemistry approach is the high diversity and the inherent biological properties of the steroid molecules.

1.3. Literature Survey on Steroid Polyamine Conjugates

As the outer membrane or cell wall of microbes provides a protective barrier against many types of antibiotics,[2] the class of membrane-disrupting drugs is ideal as antimicrobial agents because microbes are unlikely to develop resistance to them.[3] The sterol-polyamine conjugates as new class of antibiotics have attracted much interest in recent years, due to the emergence of penicillin-resistant *Staphylococci*, *Streptococcus pneumoniae* in hospitalized patients.[4]

A literature survey of antimicrobial steroids reveals that several amino cholesterol derivatives exhibit profound antimicrobial activity against Gram-positive, Gram-negative bacteria and yeast.[5] The *in vitro* antibacterial properties of bile acids against certain Gram-positive microorganisms are well known.[6] Consequently, the preparation of various bile acid-based aminosterols was reported with a view to examining their activity as antimicrobial agents. Cholic acid derivatives **1** and **2** with amine groups incorporated at the C-24 position were described to display only weak antimicrobial activity (Figure 1).[7]

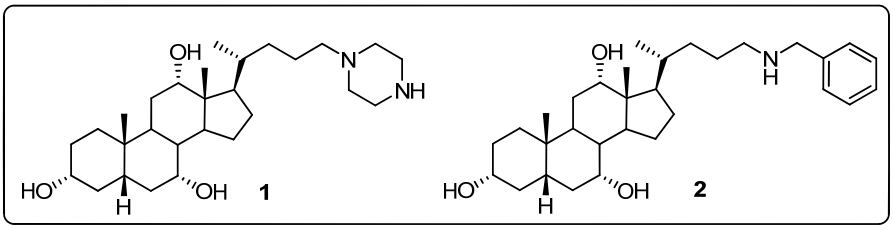


Figure 1

A recent approach to combat against pathogens is to introduce a polycationic chain onto a steroid scaffold. One such chimeric natural product namely squalamine **3** is the first sterol-polyamine conjugate that has been isolated from tissues of the dogfish shark, *Squalus acanthias* (Figure 2).[8]

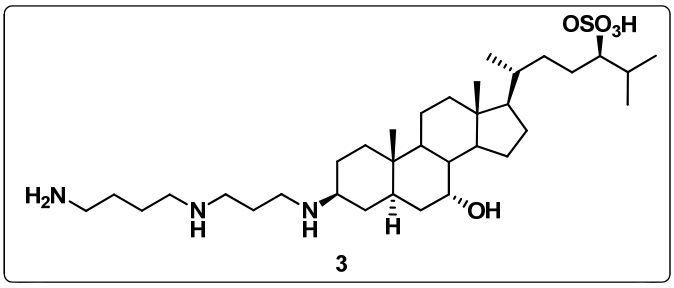


Figure 2

It contains a cholestane ring system with 5α-hydrido, 7α-hydroxy, 3β-spermidinyl and 24 (*R*)-sulphate group. This unusual natural product has attracted considerable attention because of its potent broad-spectrum antibiotic activity and extremely active against Gram-negative and Gram-positive bacteria. It is fungicidal and induces osmotic lysis of protozoa.[9] It also possesses anti-angiogenic and anti-tumor properties[10] and is in Phase-II

clinical trials for the treatment of solid tumors, including non small cell lung cancer, ovarian cancer, prostate and brain cancer. A mini review has appeared recently which summarizes and highlights the different advances in the understanding of the antimicrobial and antiangiogenic activity of squalamine.[10]Attempts to obtain large amounts of squalamine from the dogfish shark resulted in the discovery, isolation and characterization of family of novel aminosterols.[11]

Mechanism of action of Squalamine: No information exists at present regarding the molecular mechanism of the biological action of squalamine **3**. In common with the polyene antibiotic amphotericin B **4**, and also peptide antimicrobial agents having cationic residues and hydrophobic amino acids such as Polymixin B **5**, which consist basically a lipophilic half and a polar half, squalamine likewise may be depicted in a similar cyclic form **6** (Figure 3).

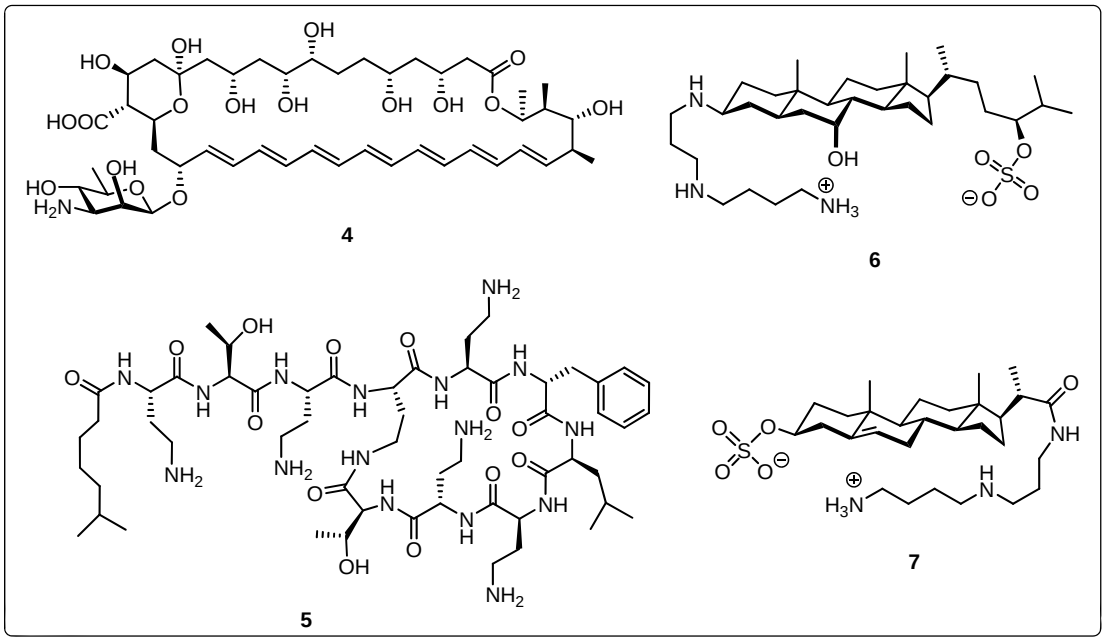
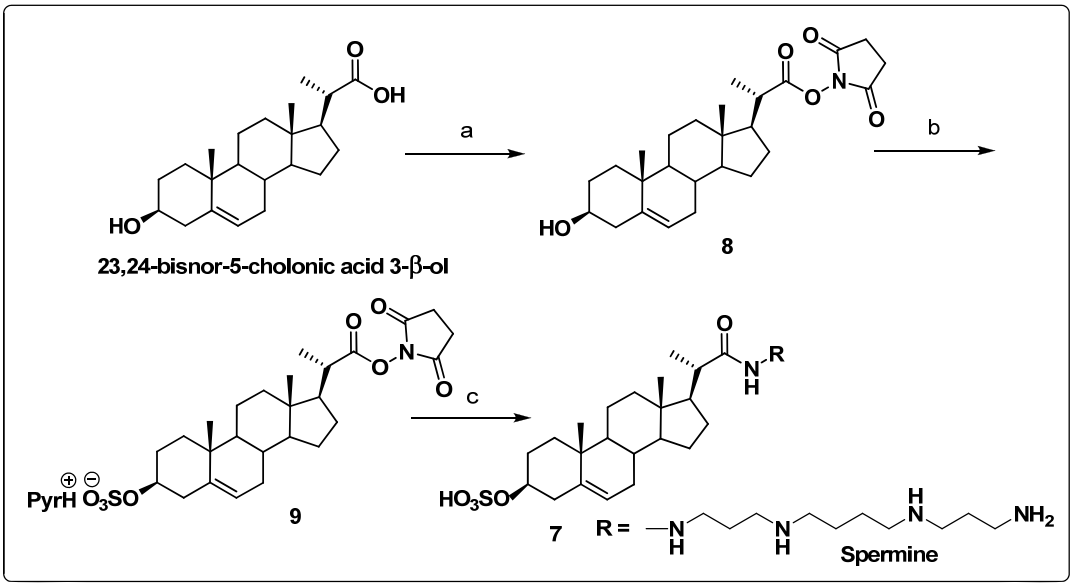


Figure 3

This salt-bridged cyclic form consists of an upper lipophilic sterol part and the lower hydrophilic part was the polyamine chain. The mode of action of squalamine might be

through membrane disruption and squalamine **3** achieves this by acting as an ionophore.[12] It has been shown experimentally that squalamine recognizes negatively charged phospholipid membranes.[13] The fact that the outermost layer leaflet of the plasma membrane of bacterial cells is negatively charged, while those of mammalian cells are electrically neutral suggests that sterol conjugate **3** may be able to selectively demolish the bacterial membranes.

Although, the majority of squalamine used in preclinical studies was obtained by extraction and purification of dogfish livers, this source was projected to be too costly and unreliable to provide adequate supplies for clinical trial. The fact that insufficient amounts of squalamine were available from natural recourses for mechanistic studies, coupled with clear need for the preparation of squlamine by chemical process prompted several groups to undertake the synthesis of squalamine.[14] A short review on synthesis of squalamine has been reported.[15] Formal synthesis of squalamine has been achieved in twelve steps from desmosterol with 7.4% overall yield by Takeuchi and co-workers.[16] Recently Zhou *et al.* have accomplished a concise and stereoselective synthesis of squalamine from easily available chenodeoxy cholanate.[17] While chemical process requires expensive starting material and numerous steps with low chemical yields, make such a route impractical for large scale preparation. These consequences prompted considerable impetus for devising fundamentally new approaches towards new drug design. Soon after the isolation of squalamine, Regen and co-workers reported[12a] the rapid construction of squalamine mimic **7** (Scheme 1). In this molecule they have exchanged the positions of pendant spermidine and sulfate groups on the A and D rings of a closely related sterol. Compound **7** not only mimics the structure of squalamine but also its extraordinary antimicrobial properties.



Scheme 1: Reagents and conditions (a) *N*-hydroxysuccinimide/DCC, THF; (b) $Pyr.SO_3/CHCl_3$; (c) spermine/DMF.

Furthermore, Gilbert *et al.* have investigated[18] the role of Boc, acetae and sulfate groups by preparing various simplified analogues of compound **7**. Several analogues showed significant *in vitro* activity against *T. brucei* and *L. donovani* and little activity against *T. cruzi*. He also proved presence of the Boc or acetate protecting groups had little effect on activity, whilst the presence of the sulphate group showed to decrease activity (Figure 4).

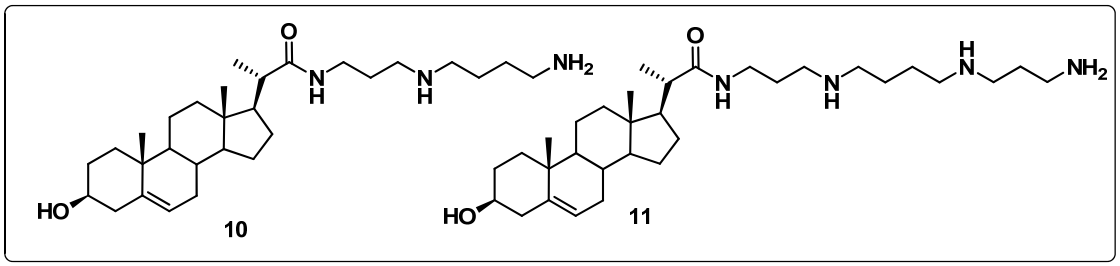


Figure 4

Bradly *et al.* have achieved synthesis of analogue **7** of squalamine by using safety catch linker on the solid-phase and also showed these linker are suitable for cleaving directly with in the biological system.[19] Armstrong and co-workers investigated the antimicrobial properties of bile acid based squalamine mimics by attaching polyamines at C-24 position of bile acid (Figure 5).[20]

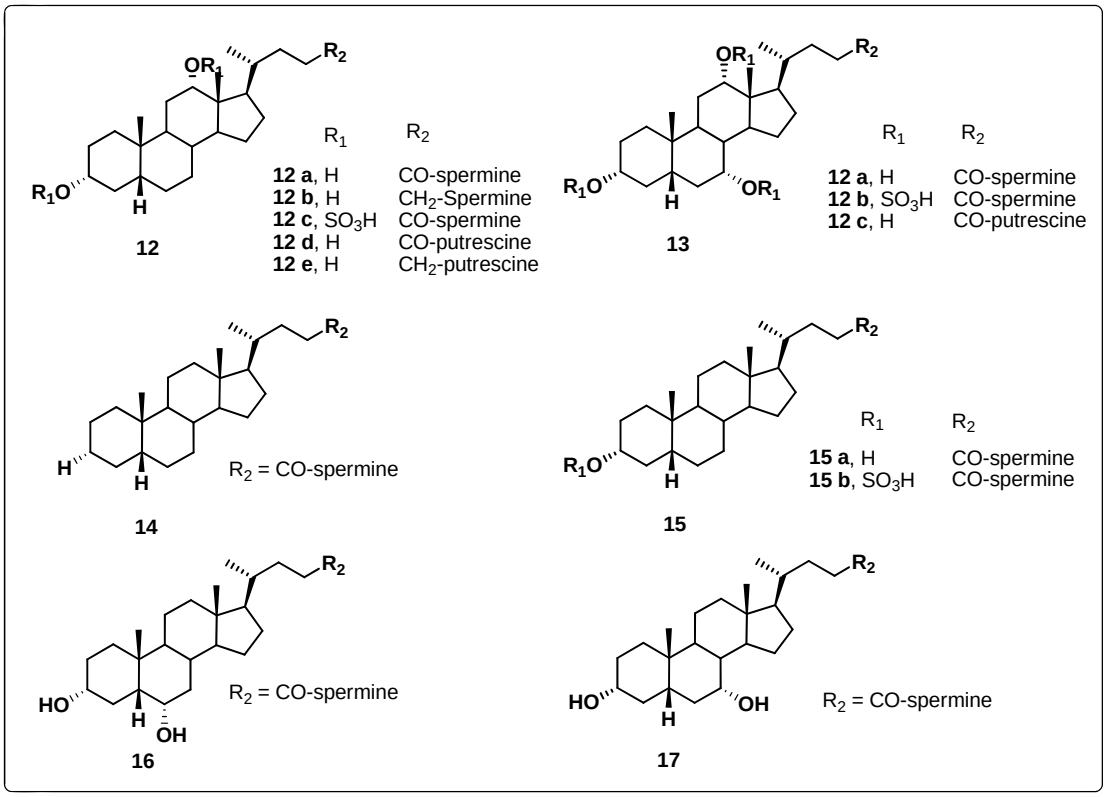


Figure 5

The mimics like **12** to **17** were prepared by linking putrescine, triethylenetetramine and spermine in the side chain (C-24 position) of different bile acids such as cholic acid, deoxycholic acid, lithocholic acid, ursocholanic acid, chenodeoxycholic acid and hyodeoxycholic acid. Some of these compounds have broad spectrum of antimicrobial activity and appear to act as potent membrane disrupting agents.

Jones *et al.* described the synthesis of new squalamine analogues, such as 6β-hydroxy-3-aminosterol **18** and **19** with *trans* A/B ring junction, from hyodeoxycholic acid.[21] Polyamines such as ethylene diamine, spermine were added to the 3-keto group by reductive amination yielding both 3α and 3β addition product (Figure 6). The synthetic products exhibited potent broad-spectrum antimicrobial activity similar to that of parent compound and demonstrate that changing the identity of the polyamine or stereochemistry of addition has little effect upon antimicrobial activity.

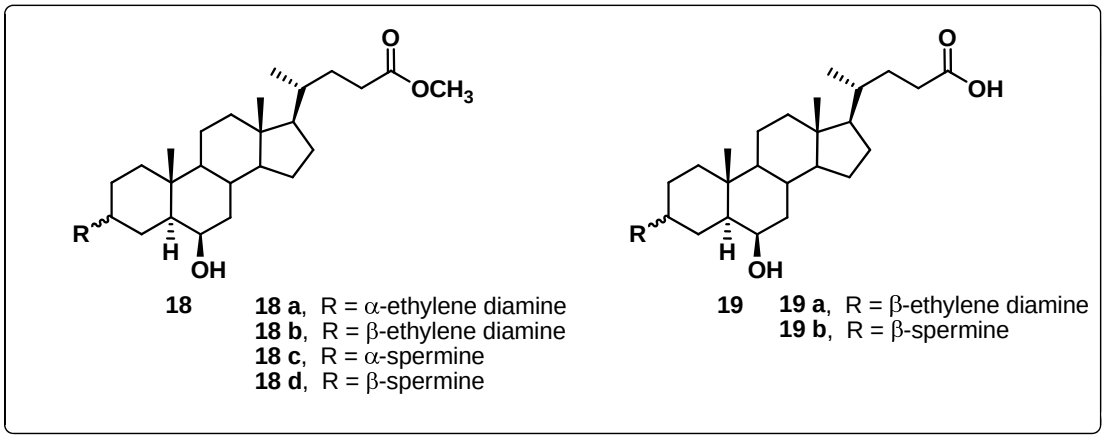


Figure 6

Kim and co-workers reported synthesis of squalamine analogue **20** having short side chain and sulphate group at C-22 and polyamine such as spermidine at C-3 positiom from the inexpensive 22-hydroxy-23,24-bisnorchola-4en-3-one.[22] This synthesized compound shows weaker antimicrobial activity. Also series of 7-fluoro-3-aminosteroids like **21** were synthesized and their *in vitro* antimicrobial activities were evaluated against Gram-positive and Gram-negative bacteria by Kim and co-workars. Antimicrobial result suggests that the nature and stereochemistry of functional groups exert a great influence on antimicrobial activity (Figure 7).

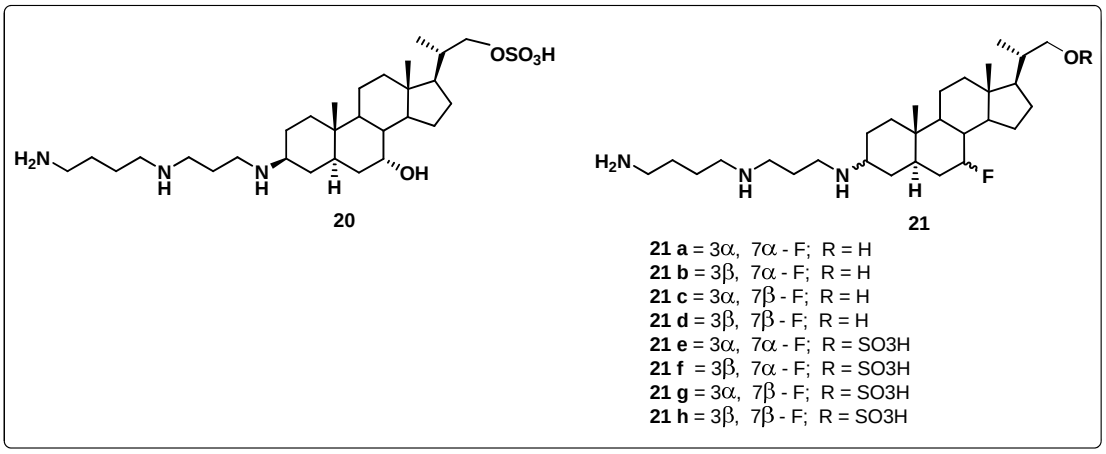


Figure 7

Selinsky *et al.* synthesized series of several new analogues e.g. **22** to **26** of MSI-1463 in which spermine is attched to C-3 position of stigmasterol and studied their antimicrobial activity (Figure 8).[23] All the analogues possess significant antimicrobial activity,

suggesting that C7 and C24 substituent of the aminosterols plays a minor role in the antimicrobial potency.

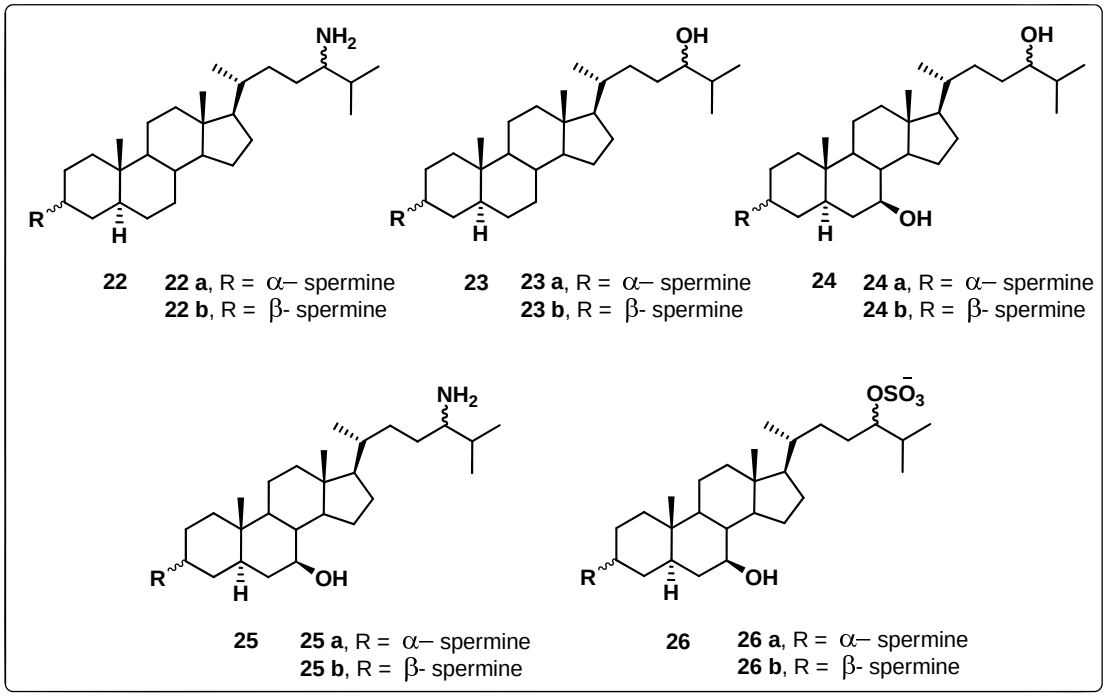


Figure 8

Stereoselective synthesis of squalamine desulfates analogues **27** to **30** in which spermidine chain is attached at 7 α, 7 β, and 6 β position to B ring of cholesterol skeleton were reported by Kihel and co-workers (Figure 9).[24] Some of these analogues showed comparable antibacterial and antifungal activities with respect to squalamine **3**, and were cytotoxic on a human non-small cell bronchopulmonary carcinoma line. Therefore, these molecules with antibiotic and cytotoxic activities are promising for immune-compramised patients in cancer chemotherapy.

Very recently Brunel and co-workers have synthesized new 7-polyaminosterol derivative like **31** employing titanium reductive amination reaction (Figure 10).[25] All the compounds present excellent activities against Gram-positive bacteria exhibiting similar results against *Staphylococcus aureus* and *Streptococcus faecalis* with minimum inhibitory concentrations (MICs) varying from 2.5 to 10 μg/mL. Numerous derivatives

also possess MICs against Gram-negative bacteria *Escherichia coli* (MICs varying from 2.5 to 10 μg/mL) suggesting that nature of the amino group attached to the sterol moiety plays an important role on the activities of such products.

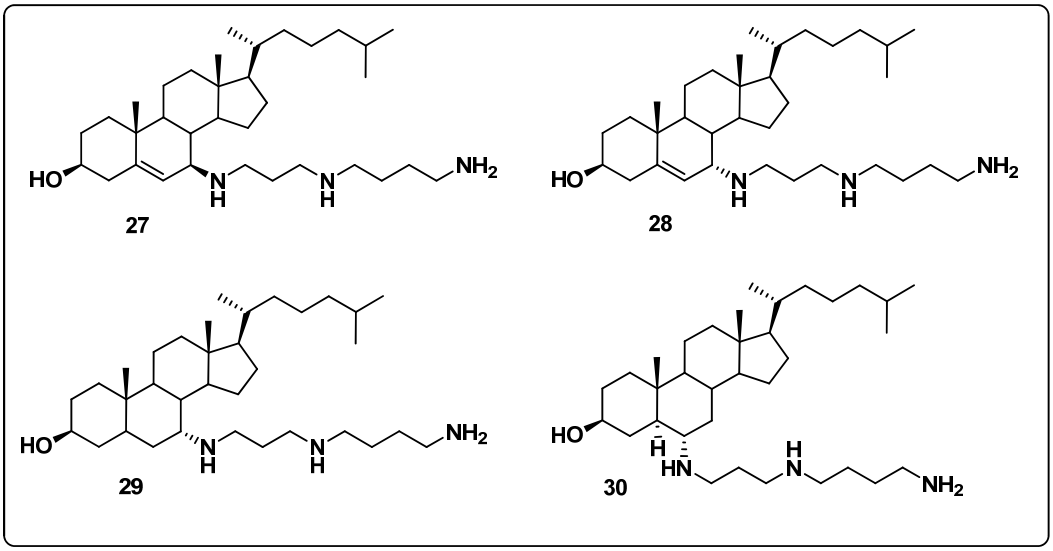


Figure 9

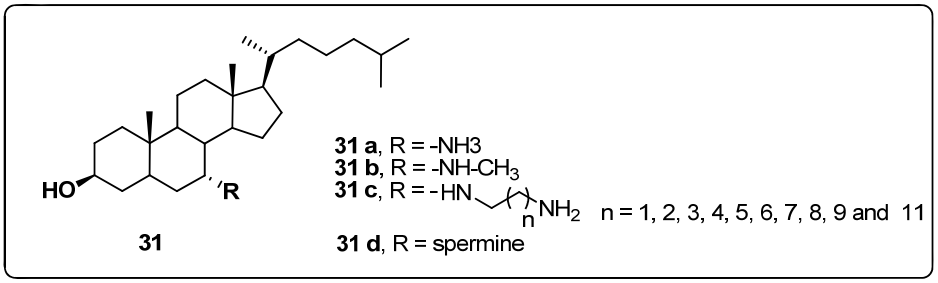


Figure 10

Savage *et al*. have designed a class of cationic steroid antibiotics (CSA) as steroid polyamine conjugates, with the intention of mimicking the antibacterial activities of polymyxin B (PMB) **5** (Figure 11).[26] These antibiotics display antibacterial activity comparable or superior to that of PMB against Gram-negative bacteria. PMB contains a lipophilic acyl chain and a heptapeptide ring that is responsible for lipopolysaccharide (LPS) binding. Resistance to PMB involves modification of LPS in the outer membranes of Gram-negative bacteria.[27] Since PMB is difficult to prepare and purify, simple molecules capable of associating with LPS and alter the permeability of Gram- negative

bacteria were designed.[28] In their approaches in designing PMB mimics modification of steroids with polyamines have been carried out.

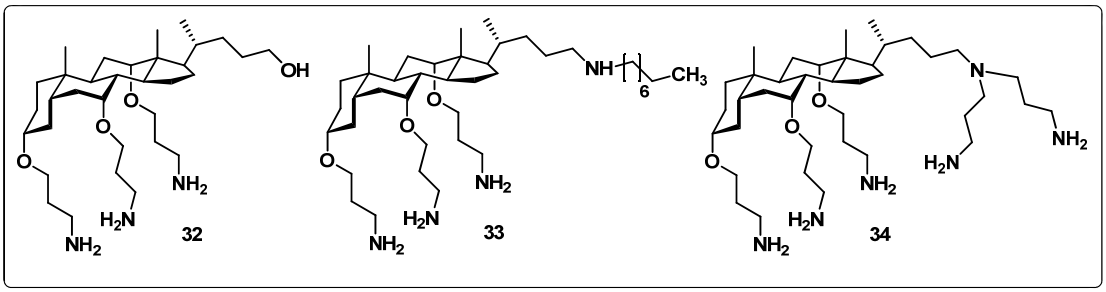


Figure 11

In PMB the amine groups on the macrocyclic ring are oriented on one face of the molecule but are segregated from the hydrophobic groups. Based on these observations PMB mimics **32** to **34** have been synthesized from cholic acid. In these molecules amino groups have been separated from the hydrophobic steroid moiety by ether linkage with stereochemically oriented oxygen atoms, which force the amine groups to occupy one face of the steroid. This allows the cholic acid derivatives **32**, **33** and **34** to exhibit facial amphiphilicity common to cationic peptide antibiotics. Compound **33** shows potent bactericidal activity against Gram-negative and Gram-positive bacteria while compound **32** that has no hydrophobic chain at C-24, does not show bactericidal activity against Gram-negative bacteria. This parallels to that of PMB and its derivatives.[29]

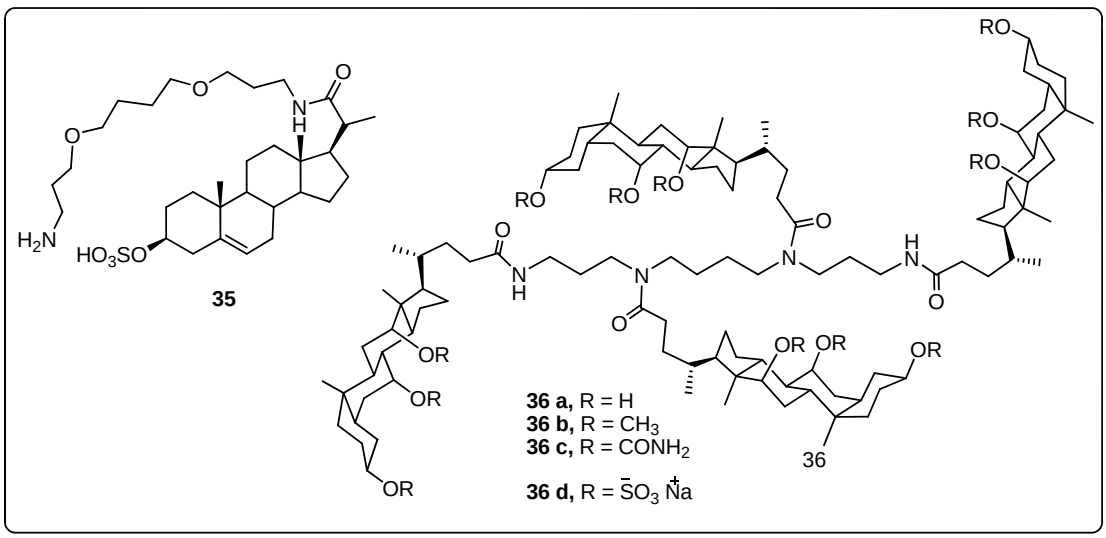


Figure 12

Regen and co-workers have described the utilization of bile acid-polyamine conjugates as synthetic ionophores and extremely useful leads in the process of drug discovery. Squalamine mimics **7** (Figure 3) and **35** (Figure 12) functions as ionophores and exhibit membrane selectivity based on surface charge.[30] It functions, as an ionophore by discharging a pH difference across the vesicle membrane. Compound **7** was found to be active for H^+/OH^- transport but not for Na^+ ion transport. Compound **35** showed[31] exactly the opposite properties to that of compound **7**. It is active for Na^+ ion transport but not for H^+/OH^- transport. Recently there are reports[32] on the synthesis of ion conductors **36 a** to **36 d** derived from spermine and cholic acid with varying degrees of facial amphiphilicity (Figure 12). These compounds promote the passive transport of Na^+ across phosphatidylcholine vesicles. However this transport activity decreases substantially as the thickness of the bilayer is increased. A dendritic approach to the construction of a homologous series of pore-forming amphiphiles has been reported[33] based on the use of spermidine, spermine, lysine, and cholic acid.

Amphotericin B (Amp B) **4** (Figure 3) is a widely used antifungal drug for systemic fungal infections.[34] It kills the cells by punching holes in the cell membranes. Drug resistance towards Amp B is extremely rare during its therapeutic use.[35] However, due to its high toxicity and apparent inability to be metabolized, there was a need of biodegradable alternatives with higher membrane selectivity. Regen and coworkers sought the simplest molecules that are capable of forming ion channels and synthesized[36] sterol-oligo conjugate **37** (Figure 13). The compound **37** obtained from 5-androstene-3β,17β-diol, exhibited significant ionophoric activity and is viable as a functional equivalent of Amp B. Davis and co-workers explored the use of steroid nucleus especially cholic acid in the area of anion recognition.[37] The biological and pharmacological

implication of synthetic channel forming molecules is currently a focal point of attention.[38]

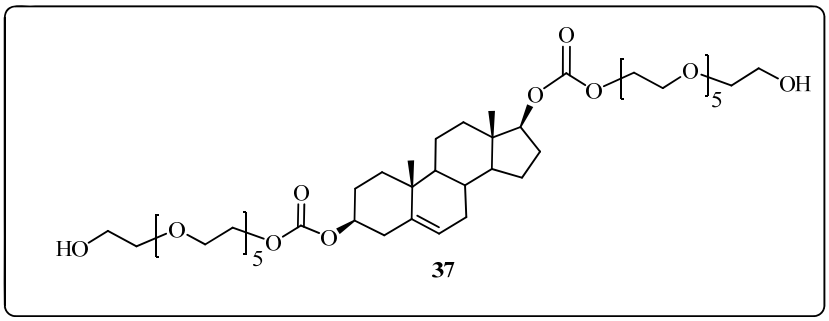


Figure 13

Steroid-polyamine conjugates are not only exploited for their antimicrobial or ionophoric properties but also for several other different properties.

Genzyme's GL-67 **38** (a spermidine bound through a carbamate functional group to a cholesteryl lipid moiety), bis(guanidium)-tren-cholesterol [BGTC] **39** (cationic cholesterol derivatives containing two guanidinium polar headgroups) and its spermidine analogue, are efficient for gene transfection *in vitro* and *in vivo*, transferring a luciferase reporter gene into primary human airway epithelial cells (Figure 14).[39]

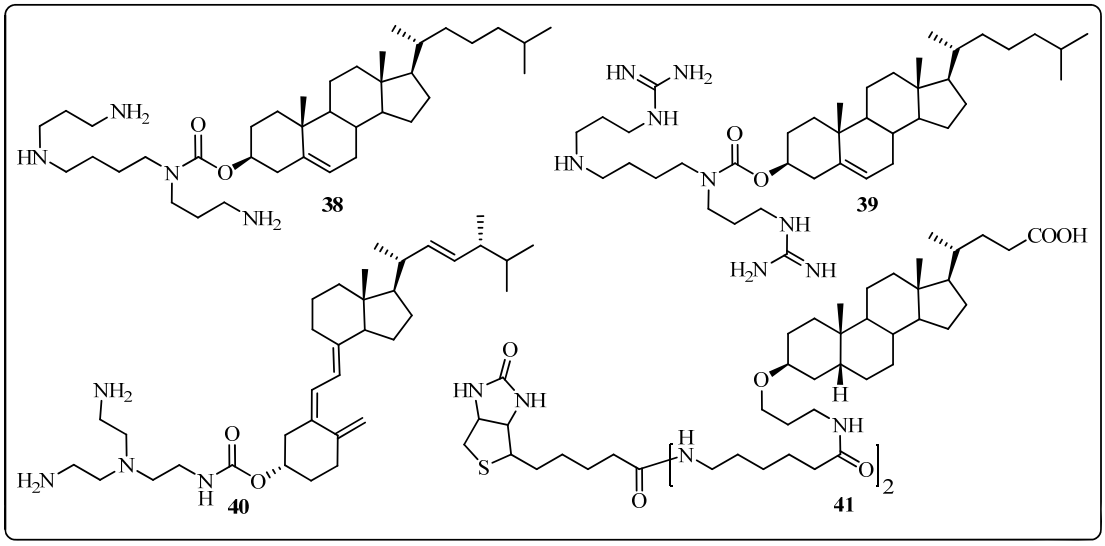


Figure 14

Vitamin D_2 (ergocalciferol) polyamine conjugate **40** is a steroid with an opened B-

ring, designed to probe the structure-activity effects on transfection of modifying the geometry within the hydrophobic steroid motif.[40] Sugawara *et al* have synthesized biotinylated lithocholic acid **41**. Compound **41** inhibited mammalian DNA polymerase α and β with dose-dependant manner.[41]

Ohwada[42] and Blaghrough[43] synthesized several steroid-polyamine conjugates e.g. **42** and **43** (Figure 15) consisting of a hydrophobic, structurally rigid steroids (lithocholic acid and cholestane), a flexible hydrophilic polyamines, the nitrogen atom of which can be protonated under physiological conditions, and a linker which connects the hydrophobic and hydrophilic unit.

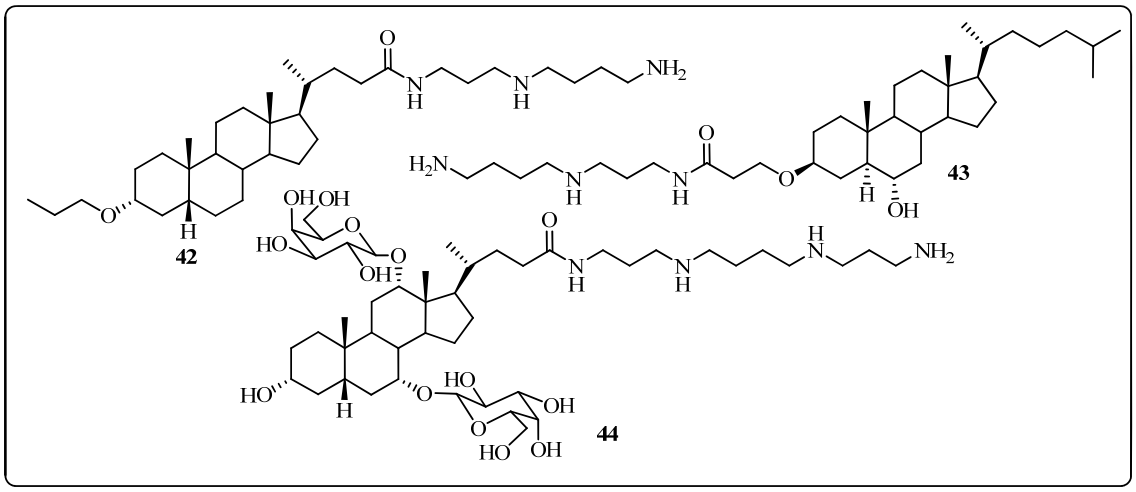


Figure 15

Ohwada studied the hemolytic activity of these conjugates towards the bovine erythrocytes and also found that the gene transfection activity of these steroid-polyamine conjugates is influenced by the polyamine chain length and steroid structure. Blaghrough *et al* demonstrated the use of these novel polyamine conjugates **42** and **43** for efficient DNA condensation and subsequent drug delivery. They have also designed and prepared novel fluorescent molecular probes as tool to throw light on the problematic steps in non-viral gene delivery, which still impede efficient gene therapy. Walker and co-workers have designed a promising class of compounds such as **44** for DNA transfection by

conjugating various polyamines to bile acid based amphiphiles.[44] Formulations containing these compounds were tested for their ability to facilitate the uptake of a β-galactosidase reporter plasmid into COS-7 cells.

1.4. Resulit and Discussion

A number of intuitive assumptions can be drawn from this wide-ranging sterol polyamine literature

a) Common elements required for the amphiphilicity is

- long and rigid hydrophobic unit
- flexible hydrophilic chain which is linked to hydrophobic unit
- pendant polar head group
- the precise structure of the polyamine is not important
- the sulfate groups can be replaced by a carboxylate or hydroxyl or even removed altogether
- the structure of the rigid hydrophobic unit i.e. steroid can also be varied

b) A common feature of these antimicrobials is the potential to exhibit facially amphiphilic conformation containing polar and hydrophobic surfaces.[45]

This type of amphiphilicity can be achieved by polyene macrolide antibiotics[46] such as amhotericine B **4** and also peptide antimicrobial[47] polymixin B **5** (Figure 1). Although the squalamine and PMB mimics are morphologically dissimilar they display similar activity.[48]

c) The overall amphiphilicity of the molecule plays a key role in determining the level of antimicrobial activity. A generic structure with fine-tuning of the molecular amphiphilicity may lead to novel molecules capable of selectively permeabilizing the microbial membranes.

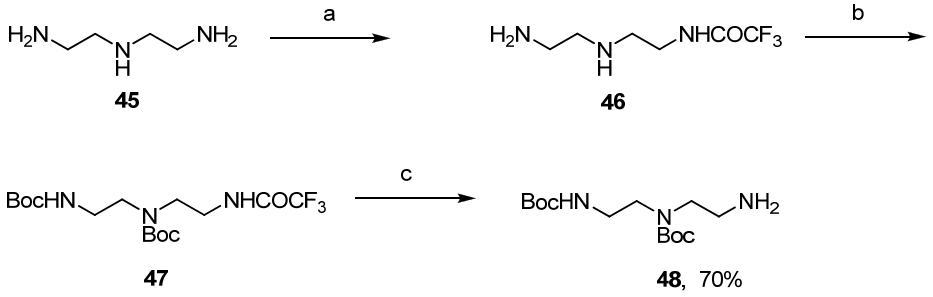
d) Variations in the structure of the analogues led to changes in the spectrum of activity against variety of bacteria and yeasts.

With this in view, novel cholic acid-polyamine conjugates were synthesized and tested against wide variety of microorganisms. Cholic acid has been chosen as starting material for the synthesis of new analogues as rigid and long hydrophobic unit because it attracted significant attention due to availability as well as the orientation of the hydroxyl groups that may be exploited in pedant-type receptors[49] and its natural facial amphiphatic nature.[50] It differs from the conventional head-to-tail amphiphiles because the polar and non-polar domains are separated along the longitudinal axis of the molecule, which gives rise to distinct polar and non-polar faces.[51] Also they are pharmacologically interesting as potential carriers of liver-specific drugs, absorption enhancers, and cholesterol lowering agents.[52] Polyamines are of considerable interest due to their advantages of low toxicity, low immunogenicity, controllable synthesis and defined molecular structure for pharmaceutical characterization.[53]

In our studies spermine and spermidine side chain has been replaced by simple polyamines such as ethylenediamine as flexible hydrophilic chains. These polyamines were introduced at the C-3 position of cholic acid to have a combination of hydrophilic functional moiety as well as hydrophobic carrier in the same molecule. Accordingly we have achieved the synthesis of novel C3-α and C3-β linear cholic acid-polyamine conjugates, wherein polyamines derived from diethylenetriamine were hooked at C-3 position of the modified cholic acid by reductive amination.

1.5. Chemistry

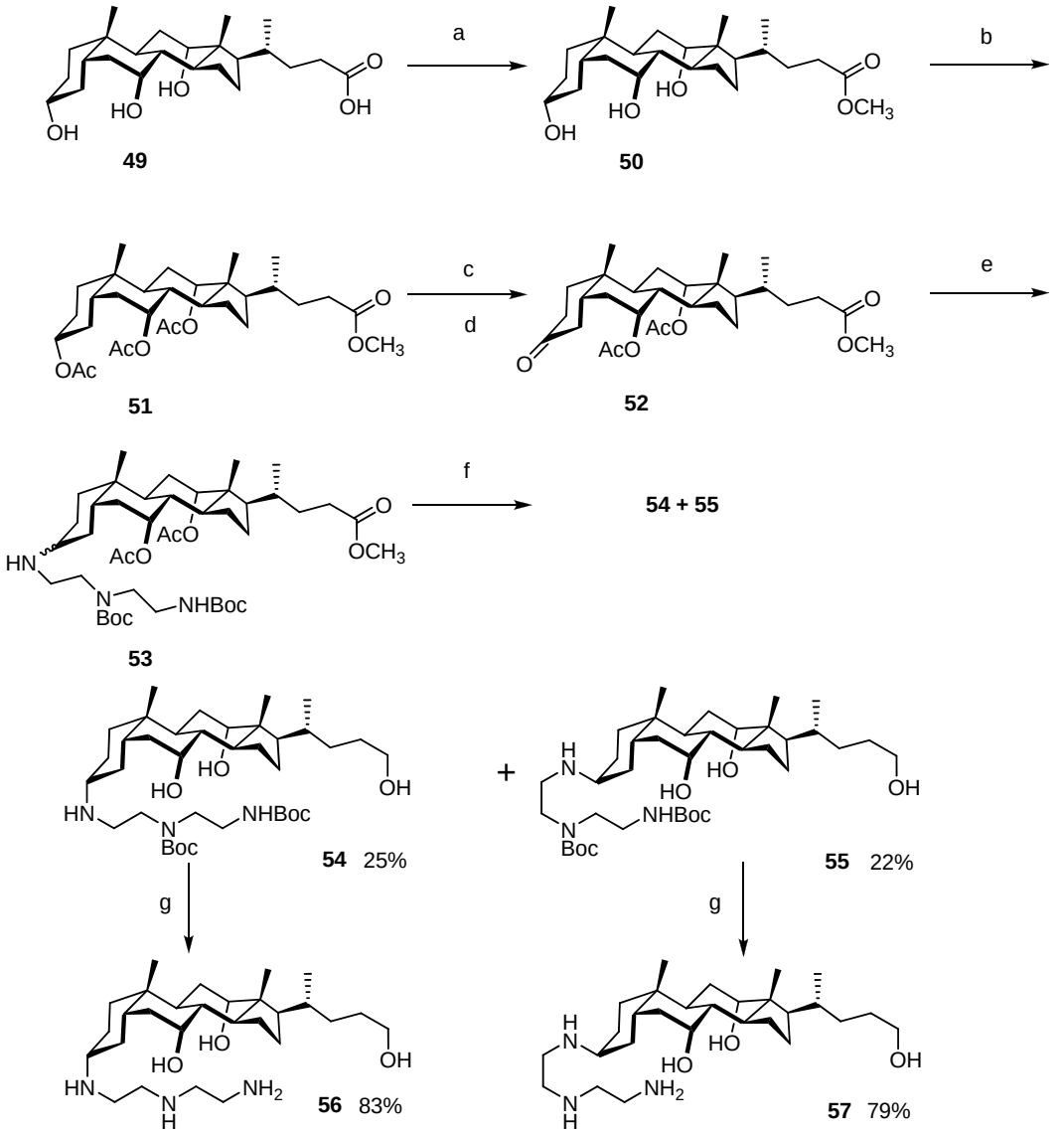
Synthesis of N-protected polyamine side chain: Synthesis of polyamine chain was carried out using diethylenetriamine **45**. One of the primary amino functionality of diethylenetriamine **45** was selectively protected using methyl trifluroacetate[54] to afford monotrifluroacetamide **46** (Scheme 2). Compound **46** was not isolated and to this solution the remaining free amines were protected with di-*tert*-butyldicarbonate to afford bis *N*-Boc protected compound **47**. Selective deprotection of the bis *N*-Boc trifluroacetamide **47** was carried out by increasing the pH of the solution above 11 with concentrated aqueous ammonia to afford triamine **48** with overall yield 70% in which one of the free primary amine is unmasked.



Scheme 2: Reagents and condition (a) CF_3COOCH_3, MeOH, -70-0 °C, 8 h; (b) Boc anhydride, Et_3N, 0 -25 °C, 6 h; (c) NH_4OH, 16 h, 70%.

Synthesis of new analogues of squalamine from cholic acid: Synthesis of the cholic acid intermediates, which are the building blocks for realization of the desired conjugates, is depicted in Scheme 3. Cholic acid **49** on treatment with MeOH/*p*-TSA gave methyl cholate **50** in 91% yield. Compound **50** on acetylation afforded the triacetate **51** in 92% yield. Selective hydrolysis of **51** with Na_2CO_3 in MeOH for 7 h, followed by Jones oxidation furnished 3-oxo compound **52** in 71% yield in two steps. Reductive amination of 3-oxo-7,12-diacetoxy methyl cholate **52** and *N*-Boc protected polyamine **48** using sodium cyanoborohydride[55] gave epimeric mixture of polyamine **53** at C-3 in 68% yield. LAH reduction of compound **53** afforded C-7, C-12 and C-24 triol with an epimeric mix

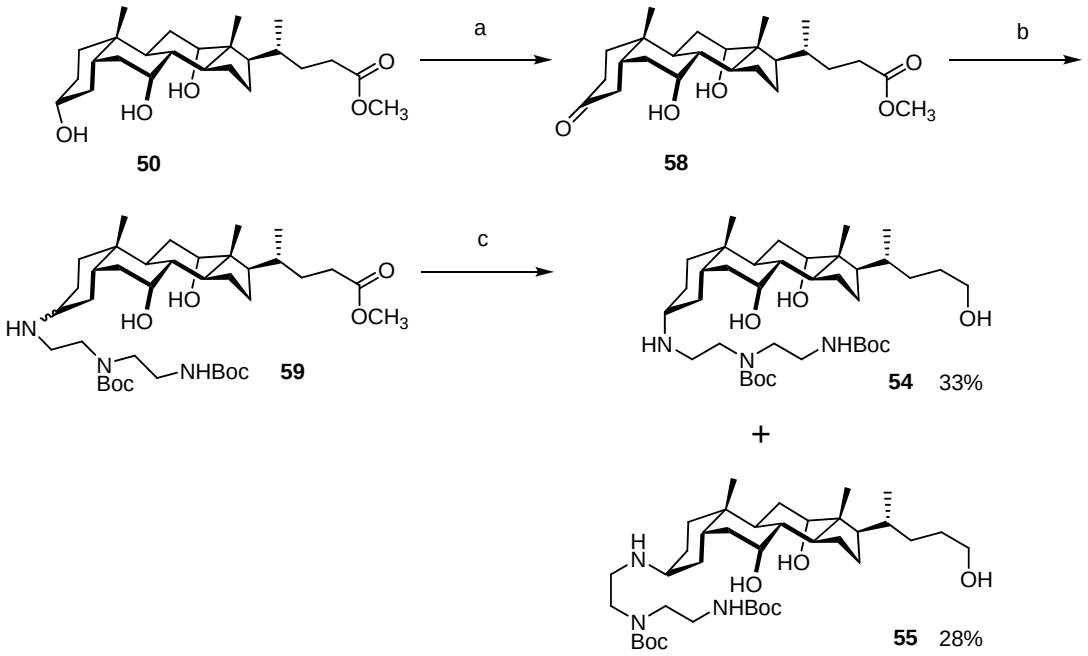
of polyamine at C-3. This mixture is separated by silica gel flash column chromatography to furnish compounds **54** and **55** in 25% and 22% yield respectively.



Scheme 3: Reagents and condition (a) *p*-TSA, MeOH, 27 °C, 12 h, 91%; (b) Ac_2O (5 eq.), DMAP, Et_3N, 25 °C, 9 h, 92%; (c) Na_2CO_3, (1.2 eq), MeOH, 7 h; (d) H_2SO_4+ CrO_3, Acetone, 71%; (e) Compound **48**, $NaBH_3(CN)$, AcOH, MeOH/DCM, 10-27 °C, 14 h, 68%; (f) LAH, THF, 25 ° C, 1 h; (g) MeOH, HCl, 1 h.

N-Boc deprotection of each of the pure compounds **54** and **55** were carried with dry MeOH/HCl to furnish the corresponding unmasked trihydrochlorides. These hydrochlorides were mixed with silica gel and the mixture was loaded in glass column. This was then eluted with MeOH-NH_4OH-DCM to gave pure polyamine **56** and **57** in 83% and 79% yield.

In an alternate route, 3-oxo methyl cholate **58** was obtained in 92% yield by selective oxidation of the 3α hydroxy of methyl cholate **50** with Ag_2CO_3 on celite[13] (Scheme 4). Reductive amination of compound **58** and *N*-Boc protected polyamine **48** using sodium cyanoborohydride gave epimeric mixture of polyamine **59** at C-3 in 61% yield. LAH reduction of compound **59** followed by silica gel flash column chromatographic separation to furnished compounds **54** and **55** in 33% and 28% yield respectively.



Scheme 4: Reagents and condition (a) $AgCO_3$ on Celite, toluene, Reflux, 5 h, 92%; (b) Compound **48**, $NaBH_3(CN)$, AcOH, MeOH/DCM, 10-27 °C, 12 h, 61%; (c) LAH, THF, 25 ° C, 1 h.

These polyamino alcohols **54** and **55** are synthesized in 4 steps from cholic acid with an overall yield of 13% and 11% respectively. Compound **54** and **55** prepared by this route are identical in all respect to the compounds prepared earlier from cholic acid (Scheme 3) in 6 steps with an over all yield of 10% and 9% respectively. These two novel bile acid-polyamine conjugates **56** and **57** were examined for preliminary bioevaluation at concentrations at 15, 30 and 45 μg/mL for the *in vitro* antifungal activity.

The preliminary antifungal activity of synthesized compound **56**, **57** was tested using NCL isolate fungal strains *Candida albicans* (human pathogens), *Benjaminiella poitrasii* (saprophytes), *Fusarium oxysporum* (plant pathogen). The zone of inhibition values were determined using Disc Diffusion technique. Amphotericin B was used as the reference antifungal agents. Biological data of the tested compounds **56** and **57** has been depicted in Table 1.

In the preliminary bioevaluation study, the zone of inhibition value for compound **56** was 1-2 mm at 30 μg/mL against *C. albicans* and *B. poitrasii* and 2-3 mm at 30 μg/mL against *F. oxysporum* having polyamine at C3-α position. Compound **57** in which polyamine at C3-β position also exhibited significant antifungal activity against *C. albicans* at 30 μg/mL (zone of inhibition value 2-3 mm) comparable to that of reference drug amphotericin B.

Table 1: Zone of inhibition in mm of synthesized compounds.

Strains	**Compound 56**		**Amp-B**	**Compound 57**		**Amp-B**
	30μg/ml	50μg/ml	15μg/ml	30μg/ml	50μg/ml	15μg/ml
C. albicans	1-2mm	2-3mm	3-4mm	2-3mm	3-4mm	4-5mm
B. poitrasii	1-2mm	2-3mm	4-5mm	--	1-2mm	5-6mm
F.oxysporoum	2-3mm	3-4mm	1-2mm	--	1-2mm	3-4mm

In spite of having antifungal potency, we found that analogues prepared by above synthetic path have certain limitation such as

a) Synthetic route depicted in the above scheme 3 and 4 has less overall yields

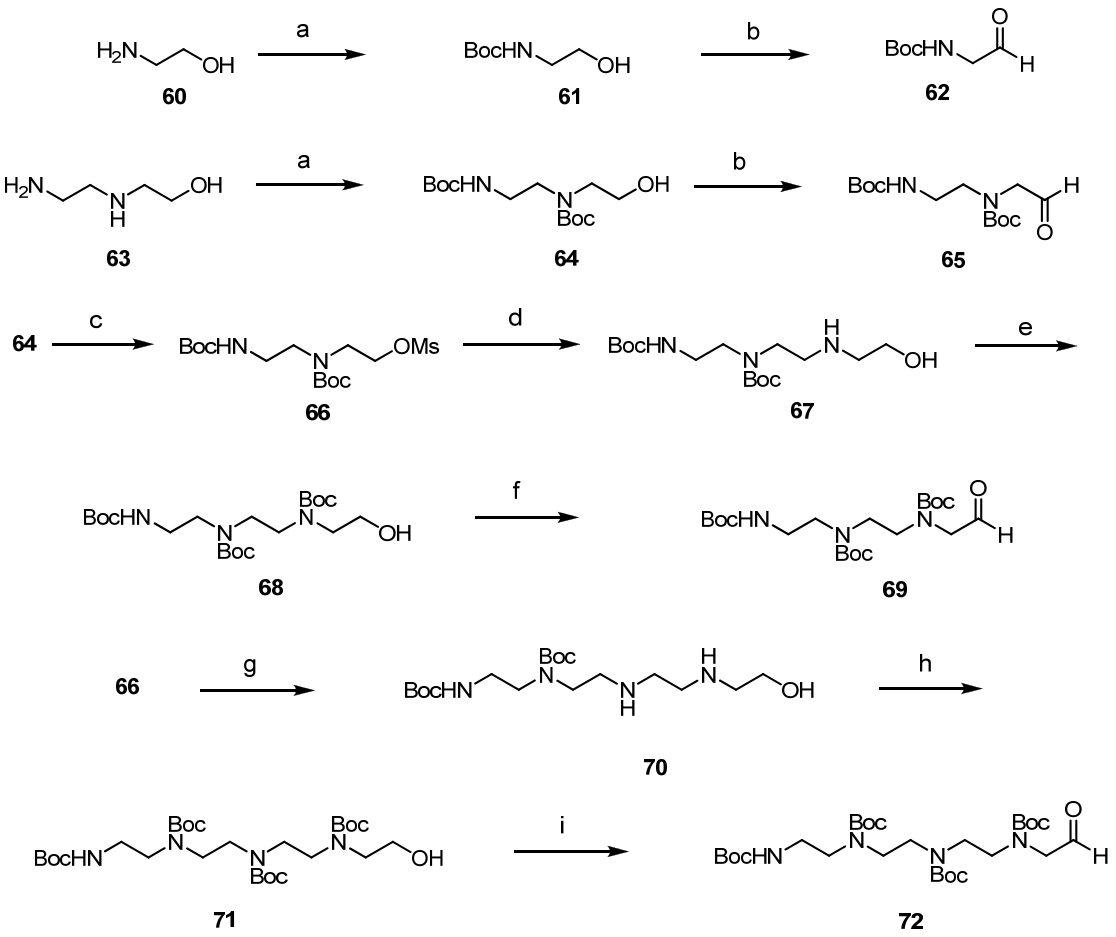
b) Separation and purification of both diasteriomers **56** and **57** is difficult because of highly polar nature

c) To produce a library of compounds with variable amphiphilicity, need simplified synthetic procedure and hassle free purification of compounds.

With this in view to overcome these drawbacks, we would like to synthesize polyamine at C-3 position following different approach. In this approach reductive amination has been carried out with the amine functionality at C-3 position of methyl cholate with *N*-Boc protected aminoaldehymodified **62**, **65**, **69** and **72**. Diethylenetriamine **48** side chain has been replaced by a series of simple polyamines such as —NH-(-CH_2-CH_2-NH-)$_n$-H (n = 1, 2, 3 and 4) as flexible hydrophilic chains. These polyamines were introduced at the 3β-position of cholic acid by reductive amination of *N*-Boc protected aminoaldehydes **62**, **65**, **69** and **72** and 3β-aminomethyl cholate **75** to have a combination of hydrophilic functional moiety as well as hydrophobic carrier in the same molecule. Along with this, we thought of an exact molecular amphiphilicity which is one of the important factor which accounts for the easy transport of molecules through the membranes. This has been achieved for the first time by utilizing fine-tuning approach of these synthesized conjugates. In continuation to our work on bile acid-conjugates,[47,57] we report herein the synthesis of novel cholic acid-polyamine conjugates **76**-**79** along with their fine-tuning counterpart **80**-**91** and their bioevalution against a wide variety of microorganisms.

Preparation of aminoaldehyde 62 and polyaminoaldehydes 65, 69, 72: We undertook the synthesis and evaluation of a new cholic acid-polyaminno conjugates by exploiting a convergent approach. Accordingly suitably protected aminoaldehyds **62** and **65** were synthesized starting from the commercially available ethanol amine **60** and of *N*-(2-aminoethyl) ethanolamine **63**, respectively (Scheme 5). The amine functionality in compounds **60** and **63** were protected using Boc anhydride to afford compounds **61** and **64**, respectively in excellent yield. Oxidation of the terminal hydroxyl functionality of

compounds **61** and **64** were carried out using IBX (2-Iodoxybenzoic acid) in DMSO to afford *N*-Boc protected aminoaldehydes **62** and **65**. In IR spectrum of compound **62** and **65** absorbance assigned to aldehyde carbonyl appeared at 1708 cm^{-1} and 1709 while primary hydroxy appeared at 3344 and 3341 cm^{-1} in compound **61** and **64**, respectively. Long chain *N*-Boc protected polyaminoaldehyde **69** and **72** were synthesized by exploiting stepwise homologation starting from the compound **64**.

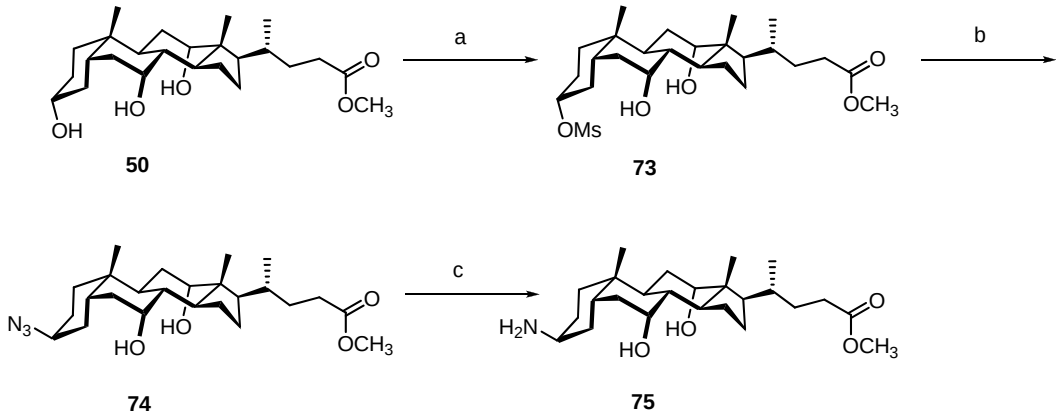


Scheme 5: Reagents and condition (a) Boc anhydride, 1N NaOH, dioxane:water, 0-25 °C, 30 min, 98% for **61** and 87% for **64**; (b) IBX, DMSO, 25 °C, 4 h; (c) MsCl, Et_3N, DCM, 0 °C; (d) ethanol amine, 80 °C, 7 h; (e) Boc anhydride, Et_3N, DCM, 0-25 °C, 3 h, 87%; (f) IBX, DMSO, 26 °C, 4 h; (g) *N*-(2-aminoethyl)ethanolamine, 90 °C, 6 h; (h) Boc anhydride, Et_3N, DCM, 3 h, 83%; (i) IBX, DMSO, 26 °C, 6 h.

Primary alcohol group of compound **64** was mesylated using methanesulfonyl chloride to give compound **66** which on treatment with ethanol amine **60** followed by Boc protection furnished compound **68** in 87% yield. Similarly reaction of mesylate **66** with **63** and

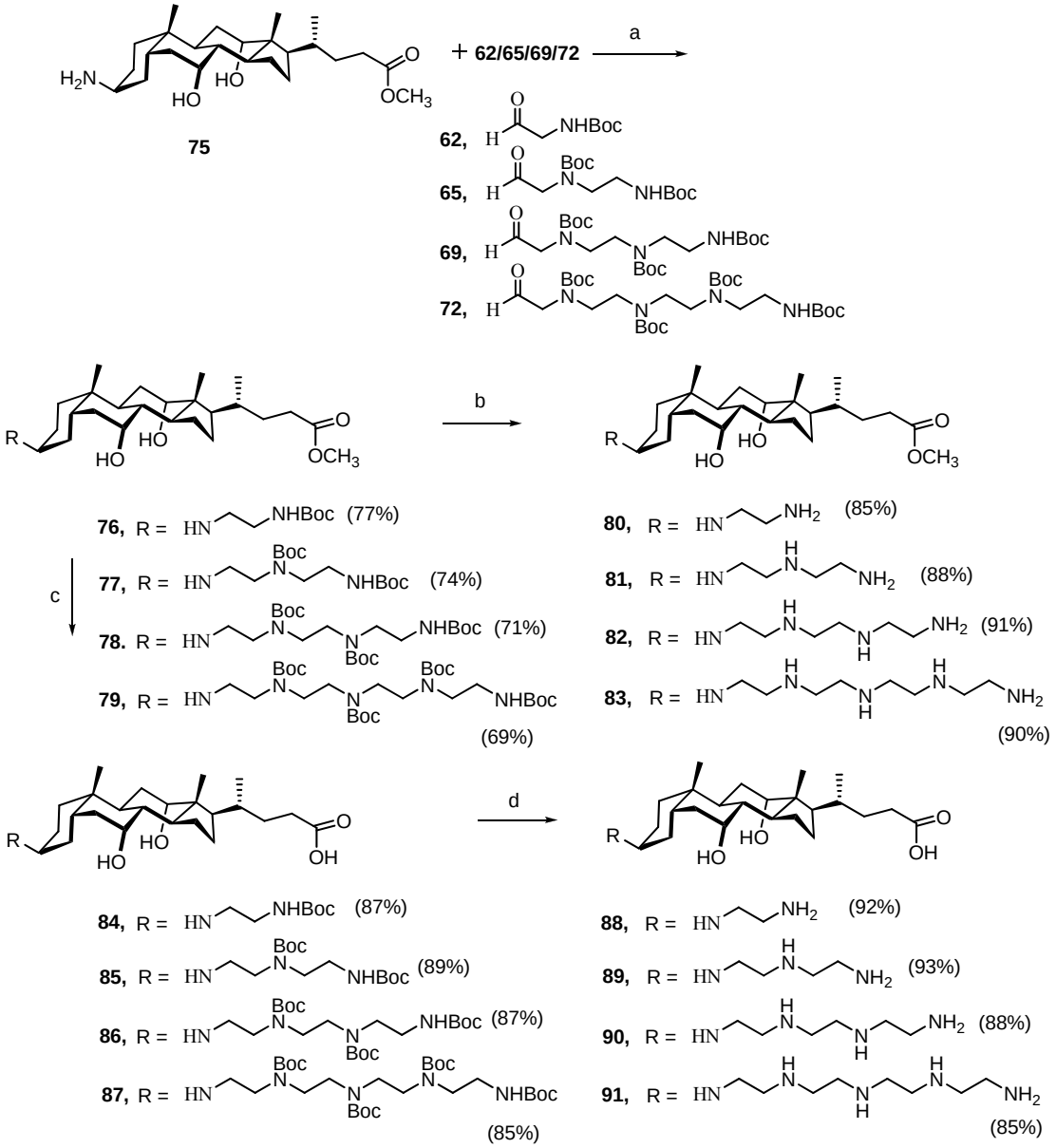
resulting secondary amine on *N*-Boc protection furnished compound **71** in 83% yield. Oxidation of the terminal hydroxy functionality of compounds **68** and **71** were carried out using IBX in DMSO to afford *N*-Boc protected aminoaldehydes **69** and **72**.

Synthesis of the cholic acid intermediates **75**, which are the building blocks for the realization of the desired conjugates, was synthesized from cholic acid **49** in four steps in a straightforward manner with an overall yield of 77%[45a] is depicted in Scheme 6. Methyl cholate **50** on treatment with methanesulfonyl chloride gave 3α-mesyl methyl cholate **73**. Compound **73** on nucleophilic displacement with sodium azide followed by reduction of 3β-azide **74** using hydrogen and Pd-C afforded the 3β-amino methyl cholate **75** in 87% yield.



Scheme 6: Reagents and condition (a) MsCl, Et_3N, DCM, 0 °C,10 min.; (b) NaN_3, DMF, 60 °C, 8 h, 94%; (c) H_2, Pd-C, CH_3OH, 6 h, 87%;

Coupling of 3β-amino cholic acid intermediates **75** with *N*-Boc protected amino aldehydes **62**, **65**, **69** and **72** by reductive amination under mild condition using $NaBH_3CN$ (saodium cyanoborohydride) and AcOH in DCM:MeOH provided the *N*-Boc protected 3β-polyamino compounds **76-79** in yields ranging from 69-77% (Scheme 7). Subsequent hydrolysis of the C-24 methyl ester functionality in compounds **76-79** with aqueous 2M, LiOH in methanol for 12 h afforded the corresponding acids **84-87** in yields ranging from 85-89%.



Scheme 7: Reagents and condition (a) $NaBH_3CN$, AcOH (*p*H 5-6), DCM:MeOH, 25 °C 10-12 h, 69-77%; (b) 2M HCl in Et_2O, 0-25 °C, 1 h, 85-91%; (c) 2M LiOH, CH_3OH, 25 °C, 12 h, 85-89%; (d) 2M HCl in Et_2O, 0-25 °C, 1 h, 85-93%.

Cleavage of the *N*-Boc protecting groups in compounds **76-79** and **84-87** was accomplished with 2M HCl in Et_2O to afford free amino compounds **80-83** and **88-91** in yields ranging from 85-93%. It is worth mentioning here that all the compounds **80-91** are new and characterized fully by IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

1.6. Bioevaluation Study

Cholic acid **49** and all the newly synthesized steroid-polyamine conjugates **76-91** were examined at concentrations from 1 to 128 μg/mL for the *in vitro* antifungal and antibacterial activity. The antifungal activity was tested using NCL isolate fungal strains *Candida albicans* and *Cryptococcous neoformans* (human pathogens), *Benjaminiella poitrasii* and *Yarrowia lipolytica* (saprophytes), *Fusarium oxysporum* (plant pathogen). The antibacterial activity was evaluated against *Escherichia coli* (NCIM 2574), and *Staphylococcus aureus* (NCIM 2122). The MIC values were determined using standard broth microdilution technique as described by NCCLS.[58] 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 has been depicted in Table 2 as MIC values.

In the preliminary bioevaluation, cholic acid **49** did not show any appreciable antifungal or antibacterial activity. The MIC value for compound **49** was >128 μg/mL. Compound **88** having free amine and free acid exhibited a significant antifungal activity against *C. albicans* at 6 μg/mL, *C. neoformans* at 16 μg/mL, *B. poitrasii* at 8 μg/mL comparable to that of reference drug amphotericin B and fluconazole. Also compound **88** exhibited a significant antibacterial activity against *E. coli* at 8 μg/mL comparable to that of reference drug tetracycline and erythromycin. With respect to antifungal and antibacterial activity, compound **88** was superior to fluconazole and erythromycin, respectively indicating that the presence of an acid and amine substituents are crucial. Compound **76** having masked amine and acid group showed moderate antifungal and antibacterial activity against *C. albicans*, *Y. lipolytica* and *S. aureus*. The growth inhibitory activity of compound **80** was more potent than amphotericin B and fluconazole against *C. neoformans.* Compound **84**

possessing free acid group was more potent against *C. neoformans, B. poitrasii* and *E. coli* than the reference antifungal drug (fluconazole) and antibacterial drug (tetracycline, erythromycin).

Table 2: Minimum inhibitory concentration (MIC) of synthesized compounds.

Sr. No.	Compound Number	Antimicrobial Activity MIC (μg/mL)						
		Fungal Strains					Bacterial Strains	
		A	***B***	***C***	***D***	***E***	***F***	***G***
1	**49**	>128	>128	128	>128	>128	>128	>128
2	**76**	28	64	64	32	32	128	16
3	**77**	26	32	14	16	48	64	8
4	**78**	128	32	64	24	88	64	64
5	**79**	128	16	>128	64	8	64	64
6	**80**	32	18	128	128	82	128	64
7	**81**	6	16	16	32	32	8	32
8	**82**	64	8	16	8	8	32	64
9	**83**	64	6	16	128	128	16	48
10	**84**	64	16	16	128	32	16	16
11	**85**	52	98	96	>128	72	32	64
12	**86**	128	8	64	128	10	128	32
13	**87**	16	16	32	32	24	64	64
14	**88**	6	16	8	>128	80	8	64
15	**89**	128	128	128	12	16	16	32
16	**90**	16	8	128	64	16	48	64
17	**91**	128	8	128	6	8	64	64
18	AmpB	2	16	16	16	16	NT	NT
19	Fluconazole	32	32	32	64	8	NT	NT
20	Tetracycline	NT	NT	NT	NT	NT	8	16
21	Erythromycin	NT	NT	NT	NT	NT	64	32

A: *C. albicans*; **B**: *C. neoformans*; **C**: *B. poitrasii*; **D**: *Y. lipolytica*; **E**: *F. oxysporum*; **F**: *E. coli*; **G**: *S. aureus*; NT - Not Tested.

Compound **81** having free amine exhibited potent antifungal activity against *C. albicans* at 6 μg/mL, *C. neoformans* at 16 μg/mL, *B. poitrasii* at 8 μg/mL comparable to amphotericin B and fluconazole. Also compound **81** exhibited significant antibacterial activity against *E. coli* at 8 μg/mL comparable to that of tetracycline and erythromycin. Surprisingly we found distinct antibacterial activity at 8 μg/mL against *S. aureus* for

compound **77** which lacks hydrophilic character. However, the compound **82** possessing free acid group showed significant inhibitory antifungal activity with MIC value of 12-16 μg/mL comparable to that of amphotericin B and fluconazole against *Y. lipolytica, F. oxysporum.* Compound **89** also showed significant antibacterial activity with MIC value of 16 and 32 μg/mL comparable to that of tetracycline and erythromycin against *E. coli* and *S. aureus*, respectively.

Compound **82** having free amine group exhibited a potent antifungal activity against *C. neoformans, Y. lipolytica*, *F. oxysporum* at 8 μg/mL and *B. poitrasii* at 16 μg/mL and good antibacterial activity was reported against *E. coli* at 32 μg/mL indicating that the presence of an amine substituent is crucial. Compound **90** having free amine and free acid exhibited a significant antifungal activity against *C. albicans* at 16 μg/mL, *C. neoformans* at 8 μg/mL. However, the compound **90** was less active against *B. poitrasii, Y. lipolytica*, *F. oxysporum*, *S. aureus* with MIC value of 64-128 μg/mL. Compound **86** having free acid and masked amine functionality exhibited a significant antifungal activity against *C. neoformans* at 8 μg/mL and *F. oxysporum* at 10 μg/mL. Compound **78** having masked amine and acid group was found to be less active against all antifungal and antibacterial strains.

Compound **91** having free amine and free acid exhibited a significant antifungal activity against *Y. lipolytica* at 6 μg/mL, *C. neoformans* and *F. oxysporum* at 8 μg/mL comparable to that of amphotericin B and fluconazole. However, compound **91** was not active against *C. albicans, B. poitrasii* and *S. aureus*. The growth inhibitory activity of compound **83** having free amine functionality was more potent than amphotericin B and fluconazole against *C. neoformans* at 6 μg/mL, *B. poitrasii* at 6 μg/mL and *E. coli* at 16 μg/mL.

Surprisingly, we found distinct antibacterial activity for compound **87** with MIC 16-32 μg/mL against all antifungal strains which lacks hydrophilic character. Compound **79** having masked amine and acid group showed significant antifungal activity against *F. oxysporum* at 8 μg/mL.

1.7. Conclusion

We have observed that, incorporation of linear polyamine fragment on the cholic acid skeleton, demonstrated potent antimicrobial activity against all the strains tested. In particular, compounds **81**, **82**, **84**, **88**, **90** and **91** showed significant antimicrobial activity with MIC value ranging from 6-16 μg/mL against fungal and bacterial strains. To conclude, we have demonstrated that molecular amphiphilicity is one of the important factors which accounts for the easy transport of molecules through the membranes, as a result a generic structure wherein fine-tuning of the molecular amphiphilicity is possible, have been designed utilizing amphiphilic nature of cholic acid.

1.8. Experimental Procedure

Compound 48: To a solution of diethylenetriamine **45** (0.54 mL, 5 mmol) in MeOH (70 mL) at -78 °C under nitrogen atmosphere methyl trifluroacetate[54] (0.50 ml, 5.1 mmol) was added drop wise over 30 min. Stirring was continued for further 30 min, then the temperature was increased to 0 °C for 8 h to afford predominantly the mono-trifluoroacetamide **46**. Without isolation, the remaining amino functional groups were quantitatively protected by drop wise addition of an excess of di-*tert*-butyldicarbonate (3.446 mL, 15 mmol) in methanol (20 mL) over 5 min. The reaction was then warmed to 25 °C and stirred for a further 6 h to afford the fully protected polyamine **47**, *R*f 0.6 (EtOAc). The trifluoroacetate protecting group was then removed in situ by increasing the pH of the solution to above 11 with conc. aq. ammonia and then stirring at 25 °C for 16 h. The solution was concentrated in *vacuo* and the product was further purified by flash chromatography on neutral alumina (DCM/MeOH, 99:1) to afford compound **48** (1.051 g, 70% yield, after three steps) as white, semisolid; Anal. Calcd. for $C_{14}H_{29}N_3O_4$: C, 55.44; H, 9.38; N, 14.03 Found: C, 55.42; H, 9.63; N, 13.85; IR ν_{max} (Nujol)/(cm^{-1}) 1679, 1689, 1699, 1703, 3348; δ_H ($CDCl_3$, 200 MHz) 1.19 (s, 2H), 1.42 (s, 9H), 1.42 (s, 9H), 1.45 (s, 9H), 2.83 (t, 2H, *J*=6.59, 12.71 Hz), 2.77-2.89 (m, 6H); MS (LCMS) *m/z* 303.9264$[M+1]^+$, 326.3907$[M+Na]^+$.

Compound 50: ***(Methyl 3α,7α,12α-trihydroxy-5β-cholan-24-oate)*** To a solution of cholic acid **49** (0.3 g, 0.74 mmol) in dry methanol (10 mL) was added *p*-TSA (0.03 g, 0.17 mmol). The mixture was allowed to stand at 28 °C for 24 hrs. Methanol was evaporated and the residue was extracted with DCM (3x50 mL). The organic extract was washed with cold water (2x10 mL), 10 % $NaHCO_3$ (2x10 mL), brine (2x10 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude

product. Purification by column chromatography on silica gel (5 %, $CH_3OH/CHCl_3$) afforded compound **50** (0.3 g, 98 %) as a white foamy solid; mp 157-158 °C; $[\alpha]_D^{28}$ + 31.33 (*c* 1.0, $CHCl_3$); Anal. Calcd. for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02 Found: C, 70.93; H, 10.36; IR ν_{max} (Nujol) 3670, 1739 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.65 (s, 3H), 0.86 (s, 3H), 0.95 (d, *J* = 6 Hz, 3H), 3.43 (m, 1H), 3.64 (s, 3H), 3.82 (bs, 1H), 3.94 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8, 17.7, 22.8, 23.6, 26.6, 27.9, 28.5, 31.2, 31.4, 31.5, 35.0, 35.2, 35.7, 35.8, 39.8, 39.9, 41.9, 42.0, 46.8, 47.3, 51.8, 68.8, 72.2, 73.4, 175.2.

Compound 51: ***(Methyl 3α,7α, 12α- triacetoxy-5β-cholan-24-oate)*** To a solution of methyl ester **50** (0.423 g, 1 mmol) in dry dichloromethane (10 mL), 4-dimethylaminopyridine (0.042 g, 10 mole%), triethylamine (0.694 mL, 5 mmol), acetic anhydride (0.47 ml, 5 mmol) were added and the reaction mixture was stirred at 30° C for 9 h. Reaction was quenched with cold water, and extracted with dichloromethane (2x25 mL). Organic extract was washed it with water, 5% $NaHCO_3$ (2x25 mL) and brine and dried on anhydrous Na_2SO_4. Solvent was removed under reduced pressure and the product was purified by column chromatography (20% ethylacetate/pet-ether) to give pure compound **51** (0.504 g, 92% yield) as white solid; IR (cm^{-1}): 1724, 1733; ^1H NMR (200 MHz, $CDCl_3$): δ 0.73 (s, 3H, CH_3-18), 0.81 (d, *J* = 6.1 Hz, 3H, CH_3-21), 0.92 (s, 3H, CH_3-19), 2.05 (s, 3H), 2.09 (s, 3H), 2.14 (s, 3H) 3.66 (s, 3H), 4.60 (m, 1H), 4.90 (bs, 1H), 5.08 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.9, 11.2, 21.1, 21.2, 21.3, 22.2, 22.5, 25.3, 26.6, 26.9, 28.6, 30.4, 30.5, 30.9, 34.0, 34.3, 34.3, 34.4, 37.4, 40.6, 43.1, 44.7, 47.0, 51.2, 70.4, 73.7, 75.0, 170.0, 170.1, 170.1, 174.1; MS (LCMS) *m/z*: 549.78 $(M+1)^+$, 571.75 $(M + Na)^+$.

Compound 52: ***(Methyl 3-keto-7α,12α-diacetoxy-5β-cholan-24-oate)*** To solution of compound **51** (0.548 g, 1 mmol) in methanol (10 mL) anhydrous Na_2CO_3 (0.127 g, 1.2

mmol) was added. The reaction mixture was stirred at 30° C for 7 h. Methanol was removed under reduced pressure, on rotavapour at low temperature. The residue was extracted with ethyl acetate (3x25 mL). Organic extract was washed with water and brine. It was dried over anhydrous Na_2SO_4 and concentrated to gave 3α-hydroxy,7α,12α-diacetoxy-methyl cholate as white solid which was used in next step without purification. To a solution of 3α-hydroxy,7α,12α-diacetoxy-methyl cholate in acetone (20 mL) Jones Reagent (5 mL) was added at 5-10 °C. The reaction mixture was stirred at the same temperature for 10 min. Methanol (5 mL) was added, the solvent was evaporated and the crude solid material was dissolved in EtOAc/H_2O (5:1) mixture (100 mL). The organic layer was washed with cold water (2x10 mL), 10% $NaHCO_3$ (2x10 mL), brine (2x10 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product which on further column chromatography purification on silica gel (2% MeOH/DCM) afforded pure product **52** (0.358 g, 71% yield) as a white solid; mp 203-205 ^oC; (Found: C, 68.82; H, 8.53 Anal. Calcd. for $C_{29}H_{44}O_7$: C, 69.02; H, 8.79); IR (cm^{-1}): 1701, 1724, 1733; ^1H NMR (200 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-18), 0.82 (d, J = 6.1 Hz, 3H, CH_3-21), 1.02 (s, 3H, CH_3-19), 2.07 (s, 3H), 2.11 (s, 3H), 2.98 (dd, J = 13.4 and 15.3 Hz, 1H), 3.66 (s, 3H), 4.99 (bs, 1H), 5.13 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.1, 17.4, 21.2, 21.3, 21.5, 22.6, 25.7, 27.0, 29.7, 30.6, 30.7, 30.8, 34.3, 34.4, 36.0, 36.5, 37.6, 42.0, 43.1, 44.4, 45.0, 47.2, 51.3, 70.4, 75.1, 170.0, 170.2, 174.3, 211.9; MS (LCMS) *m/z*: 505.61 $(M+ 1)^+$, 527.68 $(M+ Na)^+$.

Compound 53: Compound **52** (1 g, 1.98 mmol) and **48** (0.722 g, 2.37 mmol) were dissolved in dry DCM;MeOH (20 mL) under an argon atmosphere and the solution was cooled to 10 ºC. $NaBH_3CN$ (sodium cyanoborohydride) were added and stirring was continued for 30 min. The reaction mixture was allowed to warm to room temperature

and the *p*H was adjusted with acetic acid to 5-6. The reaction mixture was allowed to stirred for 14 h. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM (300 mL). The organic phase was treated with 0.1 N HCl (30 mL), 5% $NaHCO_3$ (30 mL) and washed successively water and brine, dried over anhydrous Na_2SO_4. The solvent was evaporated to afford epimeric mixture of polyamine at C-3 position, compound **53** (1.06 g, 68% yield) as a white powder; IR ν_{max} (Nujol)/(cm^{-1}) 1683, 1716, 1738, 3371; δ_H ($CDCl_3$, 200 MHz) 0.73 (s, 3H), 0.83 (d, 3H, *J* = 6 Hz), 0.92 (s, 3H), 1.42 (s, 9H), 1.46 (s, 9H), 2.60-2.95 (m, 3H), 3.30 (bs, 6H), 3.66 (s, 3H), 4.89 (bs, 1H), 5.09 (bs, 1H); MS (LCMS) *m/z* 815.3512 $[M+Na]^+$; MS (MALDI-TOF) *m/z* 791.7380 $[M]^+$.

Compound 54 and 55: To a 250 mL round bottom flask were added compound **53** (1.0 g, 1.26 mmol) in dry THF (100 mL) and $LiAlH_4$ (0.100 g, 3.78 mmol) at 0 ºC under nitrogen atmosphere. After stirring the reaction mixture for 1 h at 0 to 25 ºC, saturated aqueous Na_2SO_4 was introduced slowly. The reaction mixture was stirred for further 30 min. The solvent was removed at reduced pressure and the residue was extracted with ethyl acetate. The usual work up afforded crude product. This was further purified by column chromatography on silica gel (DCM/MeOH, 96:4) to afford compound **54** (0.214 g, 25%) and **55** (0.188 g, 22%) as a white solid.

Compound 54: Mp 141-144 ºC; $[\alpha]_D^{27}$ + 26.13 (*c* 1.13, $CHCl_3$); Anal. Calcd. for $C_{38}H_{69}NO_5$: C, 67.12; H, 10.23; N, 6.18 Found: C, 67.01; H, 10.35; N, 6.44; IR ν_{max} (Nujol)/(cm^{-1}) 1697, 3398; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.69 (s, 3H), 0.90 (s, 3H), 1.00 (d, 3H, *J* = 6.23 Hz), 1.42 (s, 18H), 3.10-3.40 (m, 7H), 3.60 (m, 3H), 3.85 (bs, 1H), 3.99 (bs, 1H), 5.12 (bs, 1H); ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 12.5, 17.7, 22.7, 23.1, 24.6, 26.5, 27.6, 28.2, 28.4x6, 29.4, 31.8, 33.1, 34.6, 35.5, 35.9, 38.7, 38.8, 39.4, 41.9, 42.0,

43.5, 44.1, 46.5, 47.4, 52.8, 63.2, 68.3, 73.1, 79.1, 156.2, 161.1; MS (LCMS) *m/z* 680.8154 $[M+1]^+$.

Compound 55: Mp 157-159 ºC; $[\alpha]_D^{27}$ + 20.96 (*c* 1.093, $CHCl_3$); Anal. Calcd. for $C_{38}H_{69}NO_5$: C, 67.12 Found: C, 67.23; H, 10.31; N, 6.39; H, 10.23; N, 6.18; IR ν_{max} (Nujol)/(cm^{-1}) 1693, 3412; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.69 (s, 3H), 0.93 (s, 3H), 1.01 (d, 3H, *J* = 6.45 Hz), 1.43 (s, 18H), 3.20-3.45 (m, 8H), 3.59 (t, 2H, J= 5.73 and 11.1Hz), 3.83 (bs, 2H), 3.97 (bs, 1H), 5.46 (bs, 1H); ^{13}C NMR ($CDCl_3$, 125.76 MHz) δ 12.4, 17.8, 22.3, 23.5, 23.8, 27.6, 28.2, 28.4x6, 29.4, 32.1, 32.2, 32.9, 34.6, 34.8, 35.6, 37.7, 38.5, 38.9, 41.2, 41.6, 43.0, 43.8, 46.3, 47.2, 49.2, 63.3, 68.4, 73.1, 78.9, 156.4, 162.1; MS (LCMS) *m/z* 680.8689 $[M+1]^+$.

Compound 56: Mp 136-138 ºC; $[\alpha]_D^{27}$ + 28.49 (*c* 0.754, MeOH); Anal. Calcd. for $C_{38}H_{69}NO_5$: C, 67.12; H, 10.23; N, 6.18 Found: C, 67.01; H, 10.35; N, 6.44; IR ν_{max} (Nujol)/(cm^{-1}) 1697, 3398; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.69 (s, 3H), 0.92 (s, 3H), 1.01 (d, 3H, *J* = 6.241 Hz), 3.14-3.38 (m, 7H), 3.64 (m, 3H), 3.83 (bs, 1H), 3.95 (bs, 1H), 5.39 (bs, 1H); MS (LCMS) *m/z* 480.5765 $[M+1]^+$.

Compound 57: Mp 128-131 ºC; $[\alpha]_D^{28}$ + 23.44 (*c* 0.796, MeOH); Anal. Calcd. for $C_{38}H_{69}NO_5$: C, 67.12; H, 10.23 Found: C, 67.23; H, 10.31; N, 6.39; N, 6.18; IR ν_{max} (Nujol)/(cm^{-1}) 1693, 3412; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.69 (s, 3H), 0.94 (s, 3H), 1.03 (d, 3H, *J* = 6.39 Hz), 3.24-3.40 (m, 8H), 3.60 (t, 2H, J= 5.81 and 12.3Hz), 3.82 (bs, 2H), 3.98 (bs, 1H), 5.62 (bs, 1H); MS (LCMS) *m/z* 480.4715 $[M+1]^+$.

Compound 58: (*Methyl 3-keto-7α,12α-dihydroxy-5β-cholan-24-oate)* Reaction was carried out in Dean Stark apparatus. To a solution of **50** (2 g, 4.926 mmol) in toluene (20 mL), well dried freshly prepared unhydrous Ag_2CO_3 on celite (5.96 g, 21 mmol) was

added. Reaction mixture was refluxed for 5-6 h. Reaction mixture was filtered through sintered funnel and the residue was washed with ethyl acetate. Filtrate was evaporated under reduced pressure to obtain crude product **58** which on further purification by column chromatography (1.5% Methanol:DCM) afforded pure product **58** (1.850 g) in 92% yield. [Preparation of the Ag_2CO_3 on celite: $AgNO_3$ (34 g) was dissolved in 200 mL distilled water, the purified celite, 30 g (Celite was washed with 10% methanolic HCl, then with distilled water to neutrality and dried in oven at 120 ^oC for 24 h) was added. The mixture was stirred for 10 to 15 min and $Na_2CO_3.10H_2O$, (30 g) dissolved in 300 mL distilled water was added. Stirring was continued for 10 min, yellow green precipitate was filtered and washed to neutrality with distilled water and dried for longer time. This reagent can be stored but preferably should be prepared prior to use[59]]. White solid, Yield: 92%; mp 170 ^oC (lit[60,61] 173-175 ^oC); Anal. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59 Found: C, 69.99; H, 9.43; IR (cm^{-1}): 1708, 1728; ^1H NMR (200 MHz, $CDCl_3$): δ 0.72 (s, 3H, CH_3-18), 0.97 (d, 3H, J = 6.1 Hz, CH_3-21), 0.99 (s, 3H, CH_3-19), 3.40 (dd, 1H, J = 13.5 Hz), 3.67 (s, 3H), 3.92 (bs, 1H), 4.02 (bs, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.4, 17.2, 21.5, 23.1, 26.9, 27.3, 28.4, 30.7, 31.0, 33.9, 34.8, 35.2, 36.5, 36.7, 39.3, 41.6, 42.9, 45.4, 46.5, 47.1, 51.5, 68.2, 72.8, 174.8, 213.5; MS (LCMS) *m/z*: 421.47 $(M + 1)^+$.

Compound 59: Compound **58** (1 g, 2.38 mmol) and **48** (0.872 g, 2.85 mmol) were dissolved in dry DCM:MeOH (20 mL) under an argon atmosphere and the solution was cooled to 10 ºC. $NaBH_3CN$ (sodium cyanoborohydride) were added and stirring was continued for 30 min. The reaction mixture was allowed to warm to room temperature and the *p*H was adjusted with acetic acid to 5-6. The reaction mixture was allowed to stirred for 12 h. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM (300 mL). The organic phase was treated with 0.1 N HCl (30 mL), 5%

$NaHCO_3$ (30mL) and washed successively with water and brine, dried over anhydrous Na_2SO_4. The solvent was evaporated to afford epimeric mixture of polyamine at C-3 position, compound **59** (1.03 g, 61% yield) as a white powder; IR ν_{max} (Nujol)/(cm^{-1}) 1687, 1717, 1737, 3335, 3470; δ_H ($CDCl_3$, 200 MHz) 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, 3H, J = 6.21 Hz), 1.42(s, 9H), 1.46 (s, 9H), 3.05-3.50 (m, 6H), 3.66 (bs, 6H), 3.84 (s, 1H), 3.96 (bs, 1H), 5.51 (bs, 1H); MS (LCMS) *m/z* 730.6129 $[M+Na]^+$; MS (MALDI-TOF) *m/z* 707.7441 $[M]^+$.

Compound 61: A solution of compound 60 (0.61g, 10 mmol) in a mixture of dioxane (20 mL), water (10 mL) and 1N NaOH (10 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butyl dicarbonate (Boc anhydride) (2.4 g, 11 mmol) was added and stirring was continued at room temperature for 30 min. The solution was concentrated under vacuum to about 10 to 15 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (30 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2-3. The usual work up procedure afforded crude product, which on further column chromatographic purification using silica gel afforded compound 61 (1.57 g, 98% yield) as a viscous, colorless oil; IR ν_{max} (Nujol)/(cm^{-1}) 1678, 2926, 3380 (br); δ_H ($CDCl_3$, 200 MHz) 1.41 (s, 9H), 3.31 (bs, 2H), 3.71 (bs, 2H,), 4.79 (bs, 1H); δ_C ($CDCl_3$, 50.32 MHz) 28.2x3, 42.8, 61.6, 79.3, 156.6; MS (LCMS) *m/z* 184.2113 $[M+Na]^+$.

Compound 62: Compound **61** (1.4 g, 8.68 mmol) and 2-Iodoxybenzoic acid (IBX) (5.6 g, 17.36 mmol) were dissolved in dry DMSO (25 mL) and the reaction mixture was allowed to warm to room temperature and was stirred for 4 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed successively with water, and brine, dried over anhydrous Na_2SO_4 and the solvent was

evaporated afforded crude product which was used without further purification. IR ν_{max} (Nujol)/(cm^{-1}) 1666, 1708, 2921.

Compound 64: Compound **64** was synthesized using *N*-(2-aminoethyl) ethanolamine **63** (1.5 g, 14.4 mmol) as per the procedure mentioned for compound **61**. Compound **64** (4.25 g, 87% yield) was obtained as viscous, colorless oil; IR ν_{max} (Nujol)/(cm^{-1}) 1672, 1778, 2968, 3351 (br); δ_H ($CDCl_3$, 200 MHz) 1.42 (s, 9H), 1.46 (s, 9H), 3.34 (bs, 6H), 3.72 (bs, 2H,), 4.94 (bs, 1H); δ_C ($CDCl_3$, 50.32 MHz) 28.2x6, 39.5, 48.3, 50.9, 61.3, 79.2, 80.1, 156.4, 156.5; MS (LCMS) *m/z* 305.2269 $[M+1]^+$.

Compound 65: Compound **65** was synthesized using Compound **64** as per the procedure mentioned for compound **62**. Compound **65** was obtained as crude product which was used without further purification. IR ν_{max} (Nujol)/(cm^{-1}) 1671, 1709, 1780.

Compound 66: To a reaction mixture of compound **64** (1.000 g, 3.28 mmol) in dry DCM (20 mL) was added triethylamine (0.68 mL, 4.92 mmol). Methane sulfonyl chloride (0.28 mL, 3.60 mmol in 5 mL DCM) was added drop wise in 10 min at 0 °C, and ice was added to the reaction mixture immediately after addition was complete. The reaction mixture was extracted with DCM. Organic layer was washed with NaHCO3, water, and brine. Solvent was evaporated under reduced pressure. The crude product obtained was used without further purification.

Compound 67: The compound **66** and ethanolamine **60** (3 mL) were heated at 80 °C for 7 h. The reaction mixture was cooled to room temperature, diluted with water (50 mL), and extracted with DCM (3x 100 mL). The organic portion was washed with brine, dried over Na_2SO_4, concentrated, and used in the next reaction without purification.

Compound 68: Di-*tert*-butyl dicarbonate (0.906 mL, 3.94 mmol) was added to the above crude product compound **67** and Et_3N (0.548 mL, 3.94 mmol) in DCM (10 mL) and the reaction mixture was stirred further at 23 °C for 3 h. The reaction mixture was washed with water, brine, dried over Na_2SO_4, and concentrated. The product was further purified by flash chromatography on Flash silica gel column (DCM/MeOH, 99:1) to afford compound **68** (1.277 g, 87% yield, after three steps) as colourless, viscous oils; Anal. Calcd. for $C_{21}H_{41}N_3O_7$: C, 56.35; H, 9.23; N, 9.39 Found: C, 56.57; H, 9.61; N, 9.11; IR ν_{max} (Nujol)/(cm^{-1}) 1687, 1704, 1780, 2967, 3340; δ_H ($CDCl_3$, 200 MHz) δ_H ($CDCl_3$, 200 MHz) 1.42 (s, 9H), 1.45 (s, 18H), 3.23-3.5 (m, 10H), 3.72 (bs, 2H,); δ_C ($CDCl_3$, 50.32 MHz) 28.3x9, 39.3, 45.8, 46.6, 47.8, 51.0, 62.1, 79.8, 80.2, 80.3, 156.0, 156.2, 156.7; MS (LCMS) *m/z* 448.4720$[M+1]^+$, 470.4547$[M+Na]^+$.

Compound 69: Compound **68** (0.200 g, 0.45 mmol) and 2-Iodoxybenzoic acid (IBX) (0.280 g, 0.9 mmol) were dissolved in dry DMSO (5 mL) and the reaction mixture was allowed to warm to room temperature and was stirred for 4 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed successively with water, and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated afforded crude product compound **69** which was used without further purification.

Compound 70: Compound **66** was synthesized using compound **64** (1.000 g, 3.28 mmol) as per the procedure mentioned above for compound **66**. The crude product compound **66** and *N*-(2-aminoethyl) ethanolamine **63** (4 mL) were heated at 90 °C for 6 h. The reaction mixture was cooled to room temperature, diluted with water (50 mL), and extracted with DCM (3X 100 mL). The organic portion was washed with brine, dried over Na_2SO_4, concentrated, and used in the next reaction without purification.

Compound 71: Di-*tert*-butyl dicarbonate (1.81 mL, 7.88 mmol) was added to the above crude product compound **70** and Et_3N (1.1 mL, 7.88 mmol) in $CHCl_3$ (20 mL) and the reaction mixture was stirred further at 26 °C for 3 h. The reaction mixture was washed with water, brine, dried over Na_2SO_4, and concentrated. The product was further purified by flash chromatography on Flash silica gel (DCM/MeOH, 98:2) to afford compound **71** (1.61 g, 83% yield, after three steps) as colorless, viscous oils; Anal. Calcd. for $C_{28}H_{54}N_4O_9$: C, 56.93; H, 9.21; N, 9.48 Found: C, 57.04; H, 9.47; N, 9.40; IR ν_{max} (Nujol)/(cm^{-1}) 1681, 1687, 1700, 1703, 2979, 3351; δ_H ($CDCl_3$, 200 MHz) 1.42 (s, 9H), 1.44 (s, 9H), 1.45 (s, 18H), 3.20-3.6 (m, 14H), 3.72 (bs, 2H,); δ_C ($CDCl_3$, 50.32 MHz) 28.3x12, 39.4, 45.5, 46.4, 46.6, 46.7, 51.5, 52.4, 61.9, 79.2, 79.8, 80.0, 80.1, 155.2, 155.6, 155.8, 156.7; MS (LCMS) *m/z* 591.5941$[M+1]^+$, 613.5807$[M+Na]^+$.

Compound 72: Compound **71** (0.200 g, 0.338 mmol) and 2-Iodoxybenzoic acid (IBX) (0.190 g, 0.677 mmol) were dissolved in dry DMSO (5 mL) and the reaction mixture was stirred for 6 h at 25 °C. The solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL). The organic phase was washed successively with water, and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated afforded crude product Compound **72** which was used without further purification. IR ν_{max} (Nujol)/(cm^{-1}) 1670, 1687, 1709, 2981.

Compound 73: (*Methyl 3α-mesyl-7α,12α-dihydroxy-5β-cholane-24-oate*) To a solution of **50** (1.0 g 2.4 mmol) in dry DCM (20 mL) was added Et_3N (0.36 mL, 2.6 mmol) at 0 °C. Methane sulfonyl chloride (MsCl) (0.2 mL, 2.6 mmol) was added drop wise in 10 min at 0 °C, and ice was added to the reaction mixture immediately after addition was complete. The reaction mixture was extracted with DCM. Organic layer was washed with $NaHCO_3$, water, and brine. Solvent was evaporated under reduced pressure to obtain

crude product as a white solid which was generally used in the next step without further purification. Mp. 83-85 ºC; $[\alpha]_D^{25}$ +29.98 ($CHCl_3$, *c* 0.9); Anal. Calcd. for $C_{26}H_{44}O_7S$: C, 62.37; H, 8.86; S, 6.40 Found: C, 62.23; H, 8.92; S, 6.23; IR ν_{max} (Nujol) 3460, 1728 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, 3H, *J* = 6.06 Hz), 2.99 (s, 3H), 3.67 (s, 3H), 3.88 (bs, 1H), 4.00 (bs, 1H), 4.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.2, 22.1, 23.0, 26.3, 27.4, 27.8, 28.0, 30.7, 30.9, 34.1, 34.4, 34.7, 35.1, 35.9, 38.7, 39.3, 41.3, 41.6, 46.4, 47.0, 51.4, 68.0, 72.8, 82.9, 174.7; MS (LCMS *m/z* 501.07 $[M+H]^+$, 523.17 $[M+Na]^+$.

Compound 74: (*Methyl 3β-azido-7α,12α-dihydroxy-5β-cholane-24-oate*) To a solution of **73** (0.5 g, 1.0 mmol) in dry DMF (10 mL) solid sodium azide (0.325 g, 5.0 mmol) was added and stirring was continued at 60 °C for 8 h and allowed to cool to room temperature. It was then poured in to ice cold water (30 mL) and extracted with EtOAc (3×50 mL). The organic extract was washed with cold water (3×50 mL), brine (25 mL) and was dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product **74**. Purification by column chromatography on silica gel (10 % EtOAc/PE) produced compound **74** (0.402 g, 94% yield) as a white solid; mp = 169-170 ºC (lit.[63] 157 ºC); $[\alpha]_D^{28}$ + 22.45 (MeOH, *c* 1.16), (lit.[63] + 23.7); (Found: C, 67.21; H, 9.18; N, 9.31 Anal. Calcd. for $C_{25}H_{41}N_3O_4$: C, 67.08; H, 9.23; N, 9.39); IR ν_{max} (Nujol) 1728, 2098, 3439 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.70 (s, 3H), 0.93 (s, 3H), 0.97 (d, 3H, *J* = 6.06 Hz), 3.86-3.89 (bs, 2H), 3.99 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.2, 22.7, 23.2, 24.5, 26.0, 27.4, 28.3, 30.4, 30.8, 31.0, 33.0, 34.2, 35.1, 35.2, 36.7, 39.3, 41.7, 46.5, 47.2, 51.5, 58.7, 68.4, 70.0, 174.7; MS (LCMS) *m/z* 448.24 $[M+H]^+$, 470.22 $[M+Na]^+$.

Compound 75: (*Methyl 3β-amino-7α,12α-dihydroxy-5β-cholane-24-oate*)

Compound **74** (0.25 g, 0.54 mmol) in MeOH (15 mL) was hydrogenated at 28 °C and 40 psi pressure using 10 % Pd/C (25 mg) for 4 h. After filtration of the catalyst and evaporation of the solvent, afforded compound **75** (0.225 g, 87 %) as a white solid, mp 227–230 °C dec. (lit.[62] 225-230 ºC dec.): $[\alpha]_D^{25}$ + 27.12 (MeOH, c 1.08); Anal calcd for $C_{25}H_{43}NO_4$: C, 71.22; H, 10.28; N, 3.32 Found: C, 71.41; H, 9.98; N, 3.01; IR ν_{max} (Nujol) 1732, 3439 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.68 (s, 3H), 0.94 (s, 3H), 0.99 (d, 3H, *J* = 6.0 Hz), 3.29 (bs, 1H), 3.66 (s, 3H), 3.84 (bs, 1H), 3.97 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.2, 22.8, 23.3, 25.7, 27.4, 27.5, 28.4, 29.8, 30.8, 31.1, 34.7, 35.3, 35.4, 35.9, 39.3, 41.5, 46.3, 46.4 (2C), 47.1, 51.4, 68.2, 73.0, 174.8; MS (LCMS) *m/z* 422.2417 $[M+H]^+$, 444.5211 $[M+Na]^+$.

{***Modified high yield protocol:*** Overnight stirring of cholic acid **49** in dry methanol using a catalytic amount of *p*-TSA followed by selective mesylation, nucleophilic displacement with sodium azide and hydrogenation of azido functionality using Pd-C furnished 3β-amino methyl cholate **75** as a white solid with an overall yield of 77 % in four steps and using only one final chromatographic purification.}

Compound 76: Compound **75** (1 g, 2.37 mmol) and **62** (0.414 g, 2.61 mmol) were dissolved in dry DCM;MeOH (20 mL) under an argon atmosphere and the solution was cooled to 10 ºC. $NaBH_3CN$ (sodium cyanoborohydride) were added and stirring was continued for 30 min. The reaction mixture was allowed to warm to room temperature and the *p*H was adjusted with acetic acid to 5-6. The reaction mixture was allowed to stirred for 10 h. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM (300 mL). The organic phase was treated with 0.1 N HCl (30 mL), 5% $NaHCO_3$ (30mL) and washed successively water and brine, dried over anhydrous Na_2SO_4

and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 23:2) to afford compound **76** (1.01 g, 77%) as a white powder. mp 95-97 ºC; $[\alpha]_D^{26.4}$ + 20.70 (*c* 0.97, MeOH); Anal. Calcd. for $C_{32}H_{56}N_2O_6$ C, 67.87; H, 10.12; N, 4.80 Found: C, 68.05; H, 9.99; N, 4.96; IR ν_{max} (Nujol)/(cm^{-1}) 1672, 1705, 1714, 1737, 3386, 3502; δ_H ($CDCl_3$, 400 MHz) 0.68 (s, 3H), 0.96 (s, 3H), 0.98 (d, 3H, *J* = 5.91 Hz), 1.44 (s, 9H), 2.74 (m, 1H), 3.22 (bs, 2H), 3.40 (s, 1H), 3.48 (bs, 2H), 3.66 (s, 3H), 3.87 (bs, 1H), 4.0 (bs, 1H), 5.82 (bs, 1H); δ_C ($CDCl_3$, 100.61 MHz) 12.4, 17.2, 21.8, 22.3, 23.1, 26.1, 27.5, 28.3x3, 29.6, 30.1, 30.8, 31.1x2, 33.6, 35.1, 35.3, 36, 38.2, 39.2, 41.6, 46.3, 47.1, 48.3, 51.5, 55.7, 68.0, 72.9, 81.3, 159.0, 174.9; MS (LCMS) *m/z* 565.5936 $[M+Na]^+$; MS (MALDI-TOF) *m/z* 565.0380 $[M]^+$.

Compound 77: Compound **75** (1.0 g, 2.37 mmol) and **65** (0.814 g, 2.61 mmol) were coupled to furnish compound **24** following the same procedure as described for compound **76**. The residue was further purified by flash chromatography on silica gel (DCM/MeOH, 93:7) to afford compound **77** (1.223 g, 74%) as a white powder. mp 104-106 ºC; $[\alpha]_D^{26.1}$ + 24.89 (*c* 0.884, MeOH); Anal. Calcd. for $C_{39}H_{69}N_3O_8$ C, 66.38; H, 10.21; N, 5.78 Found: C, 66.16; H, 9.82; N, 5.94; IR ν_{max} (Nujol)/(cm^{-1}) 1681, 1697, 1712, 1731, 3380, 3488; δ_H ($CDCl_3$, 400 MHz) 0.69 (s, 3H), 0.96 (s, 3H), 0.99 (d, 3H, *J* = 6.53 Hz), 1.42 (s, 9H), 1.48 (s, 9H), 2.74 (m, 1H), 3.05-3.34 (m, 6H), 3.39 (m, 2H), 3.61 (s, 1H), 3.66 (s, 3H), 3.87 (bs, 1H), 4.0 (s, 1H), 4.99 (bs, 1H); δ_C ($CDCl_3$, 100.61 MHz) 12.5, 17.3, 21.7, 22.3, 23.1, 26.2, 27.5, 28.3x3, 28.4x3, 29.5, 30.1, 30.8, 31.0x2, 33.7, 35, 35.2, 35.9, 39.2, 39.6, 41.7, 46.4, 46.7, 47.0, 47.2, 50.1, 51.5, 55.9, 68.0, 72.8, 79.9, 82.2, 156.5, 158.3, 174.8; MS (LCMS) *m/z* 708.5852 $[M+1]^+$; MS(MALDI-TOF) *m/z* 707.8965 $[M]^+$, 708.8931 $[M+1]^+$.

Compound 78: Compound **75** (1.0 g, 2.37 mmol) and **69** (1.098 g, 2.61 mmol) were coupled to furnish compound **78** following the same procedure as described for compound **76**. The residue was further purified by flash chromatography on silica gel (DCM/MeOH, 93:7) to afford compound **78** (1.431 g, 71%) as a white powder. mp 111-114 ºC; $[\alpha]_D^{28.0}$ + 20.45 (*c* 0.88, MeOH); Anal. Calcd. for $C_{46}H_{82}N_4O_{10}$ C, 64.91; H, 9.71; N, 6.58 Found: C, 64.66; H, 9.86; N, 6.41; IR ν_{max} (Nujol)/(cm^{-1}) 1671, 1691, 1721, 1734, 3361, 3498; δ_H ($CDCl_3$, 500 MHz) 0.69 (s, 3H), 0.97 (d, 3H, *J* = 6.33 Hz), 1.00 (s, 3H), 1.43 (s, 9H), 1.45 (s, 9H), 1.47 (s, 9H), 2.79 (m, 1H), 3.07-3.51 (m, 12H), 3.62 (bs, 1H), 3.66 (s, 3H), 3.88 (bs, 1H), 4.0 (s, 1H); δ_C ($CDCl_3$, 125.76 MHz) 12.5, 17.3, 20.6, 21.8, 22.3, 23.0, 26.3, 27.7, 28.3x9, 29.5, 30.1, 30.8, 33.6, 35, 35.1, 35.9, 38.9, 39.2, 40.7, 41.7, 41.8, 45.6, 46.4, 46.8, 46.9, 47.2, 47.8, 51.5, 56.2, 67.9, 72.8, 79.5, 81.0, 82.2, 156.0, 156.1, 156.3, 174.7; MS (LCMS) *m/z* 852.0163 $[M+1]^+$.

Compound 79: Compound **75** (1.0 g, 2.37 mmol) and **72** (1.534 g, 2.61 mmol) were coupled to furnish compound **79** following the same procedure as described for compound **76**. The residue was further purified by flash chromatography on silica gel (DCM/MeOH, 93:7) to afford compound **79** (1.624 g, 69%) as a white powder. mp 113-115 ºC; $[\alpha]_D^{28.2}$ + 23.40 (*c* 0.94, MeOH); Anal. Calcd. for $C_{53}H_{95}N_5O_{12}$ C, 64.02; H, 9.63; N, 7.04 Found: 64.41; H, 9.44; N, 7.10; IR ν_{max} (Nujol)/(cm^{-1}) 1677, 1685, 1714, 1735, 3342, 3456; δ_H ($CDCl_3$, 500 MHz) 0.66 (s, 3H), 0.96 (d, 3H, *J* = 6.13 Hz), 0.97 (s, 3H), 1.41 (s, 9H), 1.44 (s, 27H), 2.71 (m, 1H), 3.00-3.51 (m, 16H), 3.59 (bs, 1H), 3.64 (s, 3H), 3.84 (bs, 1H), 3.97 (s, 1H); δ_C ($CDCl_3$, 125.76 MHz) 12.4, 17.2, 21.7, 22.2, 22.4, 23.1, 26.1, 26.3, 27.4, 28.3x12, 29.4, 29.5, 30.1, 34.9, 35.1, 39.2, 41.7, 44.8, 44.9, 45.1, 46.4x2, 47.2, 47.4, 47.6, 51.5, 67.9, 72.8, 79.1, 80.3, 80.4, 80.8, 155.2 155.9, 156, 156.1 174.7; MS (LCMS) *m/z* 995.2748 $[M+1]^+$.

General procedure for synthesis of compounds 84, 85, 86 and 87: LiOH (2M in H_2O, 1 mL) was added to a solution of compound **76** (0.141 g, 0.25 mmol) in methanol (5 mL). The mixture was stirred at room temperature for 12 h and the solvent was removed in *vacuo*. The white residue was dissolved in cold water. Citric acid (5%) was added until pH = 7-8. The residue was filtered, washed with water and diethyl ether and dried thoroughly under vacuum to afford compounds **84** (0.124 g, 87% yield) as a white solid.

Compounc 84: mp 164-165 ºC; $[\alpha]_D^{26.6}$ + 21.76 (*c* 1.195, MeOH); Anal. Calcd. for $C_{31}H_{54}N_2O_6$: C, 67.84; H, 9.91; N, 5.20 Found: C, 67.60; H, 9.88; N, 5.09; IR ν_{max} (Nujol)/(cm^{-1}) 1649, 1672, 1679, 1703, 1714, 3350, 3386; δ_H (CD_3OD, 500 MHz) 0.65 (s, 3H), 0.94 (s, 3H), 0.96 (d, 3H, *J* = 6.05 Hz), 1.39 (s, 9H), 2.74 (m, 1H), 3.05 (m, 2H), 3.28 (s, 1H), 3.32 (m, 2H), 3.74 (bs, 1H), 3.91 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.1, 17.7, 22.7, 23.0, 24.2, 27.8, 28.7x3, 29.7, 30.7, 30.8, 31.3, 33.0, 33.9, 35.0, 36.2, 37.0, 37.3, 38.3, 40.9, 43.0, 45.9, 47.5, 48.2, 56.5, 68.7, 73.8, 81.1, 159.4, 180.5; MS (MALDI-TOF) *m/z* 550.9230 $[M]^+$.

Compound 85: From compound **77** (0.177 g, 0.25 mmol); Yield (0.161 g, 89%); mp 139-140 ºC; $[\alpha]_D^{26.6}$ + 18.60 (*c* 1.075, MeOH); Anal. Calcd. for $C_{38}H_{67}N_3O_8$ C, 65.96; H, 10.02; N, 6.41 Found: C, 65.77; H, 9.73; N, 6.06; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1660, 1693, 1703, 1712, 3371; δ_H (CD_3OD, 500 MHz) 0.67 (s, 3H), 0.95 (s, 6H), 1.38 (s, 9H), 1.44 (s, 9H), 2.77 (m, 1H), 3.00-3.21 (m, 4H), 3.29 (m, 2H), 3.33 (bs, 1H), 3.51 (bs, 2H), 3.75 (bs, 1H), 3.92 (s, 1H); δ_C (CD_3OD, 125.76 MHz) 13.1, 17.8, 22.8, 23.0, 24.2, 27.8, 28.7x3, 28.9x3, 29.6, 30.9, 31.5, 33.3, 34.6, 35.0, 36.2, 37.1, 37.5, 40.0, 40.9 42.9, 46.1, 46.3, 46.7, 47.6, 48.2, 50.0, 56.6, 68.7, 73.8, 80.2, 82.5, 158.5, 158.8 181.3; MS (MALDI-TOF) *m/z* 693.9260 $[M]^+$.

Compound 86: From compound **78** (0.212 g, 0.25 mmol); Yield (0.182 g, 87%); mp 145-148 ºC; $[\alpha]_D^{28}$+ 21.93 (*c* 1.55, MeOH); Anal. Calcd. for $C_{45}H_{80}N_4O_{10}$ C, 64.56; H, 9.63; N, 6.69 Found: C, 64.69; H, 9.48; N, 6.22; IR ν_{max} (Nujol)/(cm^{-1}) 1685, 1699, 1711, 3460; δ_H (CD_3OD, 500 MHz) 0.63 (s, 3H), 0.92 (s, 3H), 0.94 (s, 3H), 1.34 (s, 9H), 1.38 (s, 9H), 1.39 (s, 9H), 2.72 (m, 1H), 2.90-3.40 (m, 12H), 3.48 (bs, 1H), 3.72 (bs, 1H), 3.89 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.7, 22.7, 23.0, 24.2, 27.7, 28.7x3, 28.9x6, 29.6, 30.9, 31.4, 33.1, 34.1, 35.0, 36.2, 37.1, 37.5, 40.9, 42.9, 45.7, 45.8, 46.0, 46.4, 47.4, 47.5, 47.9, 48.2, 56.6, 68.7, 73.8, 80.0, 81.5, 82.6, 157.3, 158.4, 158.6 180.8; MS (LCMS) *m/z* 838.2438 $[M+1]^+$.

Compound 87: From compound **79** (0.248 g, 0.25 mmol); Yield (0.208 g, 85%); mp 123-127 ºC; $[\alpha]_D^{28}$ + 21.81 (*c* 1.1, MeOH); Anal. Calcd. for $C_{52}H_{93}N_5O_{12}$ C, 63.71; H, 9.56; N, 7.14) Found: C, 63.85; H, 9.73; N, 7.47; IR ν_{max} (Nujol)/(cm^{-1}) 1681, 1693, 1716, 3392; δ_H (CD_3OD, 400 MHz) 0.65 (s, 3H), 0.93 (s, 3H), 0.95 (s, 3H), 1.35 (s, 9H), 1.39 (s, 18H), 1.42 (s, 9H), 2.71 (m, 1H), 3.01-3.42 (m, 16H), 3.49 (bs, 1H), 3.73 (bs, 1H), 3.90 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.7, 22.7, 23.1, 24.2, 27.7, 28.8x12, 29.6, 30.8, 31.4, 32.8, 33.2, 36.2, 36.9, 37.5, 40.9, 43.0, 45.3x2, 45.8, 45.9, 45.97, 46.0, 46.5, 47.5, 48.1, 48.4, 56.8, 68.6, 73.8, 80.0, 81.4, 81.6, 82.6, 157.2, 158.4, 158.4, 158.7, 179.7; MS (LCMS) *m/z* 981.3905 $[M+1]^+$.

General procedure for the syntheses of compounds 80, 81, 82 and 83 and also for Compounds 88, 89, 90 and 91: A solution of compound **76** (0.056 g, 0.1 mmol) in 2M HCl:Et_2O (5 mL) was stirred at 0 ºC for 30 min and additionally at 25 ºC for 1.5 h. The volatiles were removed under vacuum, and the residue was dried thoroughly to afford compound **80** (0.039 g, 85% yield).

Compound 80: $[\alpha]_D^{25}$ +28.89 (*c* 0.91 MeOH); Anal. Calcd. for $C_{27}H_{48}N_2O_4 \cdot HCl$ C, 64.19; H, 9.64; N, 5.84 Found: C, 64.11; H, 9.73; N, 5.75; IR ν_{max} (Nujol)/(cm^{-1}) 1718, 1732, 3417, 3446; δ_H (CD_3OD, 500 MHz) 0.69 (s, 3H), 0.98 (s, 3H), 0.99 (s, 3H), 2.84 (m, 1H), 3.33 (m, 4H), 3.44 (bs, 1H), 3.62 (s, 3H), 3.78 (bs, 1H), 3.95 (bs, 1H); δ_C (CD_3OD, 50.32 MHz) 12.9, 17.5, 22.5, 22.7, 24.1, 27.7, 28.7, 29.6, 30.5, 31.2, 31.8, 32.2, 34.8, 36.1, 36.8, 37.1, 37.2, 40.9, 43.0, 44.0, 47.5, 47.9, 52.0, 57.9, 68.6, 73.8, 176.5; MS (MALDI-TOF) *m/z*: 465.3312 $[M+1]^+$, 561.1602 $[M+Na]^+$.

Compound 81: From compound **77** (0.071 g, 0.1 mmol); Yield (0.045 g, 88% yield); $[\alpha]_D^{25.1}$ +21.25 (*c* 1.58, MeOH); Anal. Calcd. for $C_{29}H_{53}N_3O_4 \cdot 2HCl$: C, 59.71; H, 9.76; N, 7.33 Found: C, 59.98; H, 9.55; N, 7.24; IR ν_{max} (Nujol)/(cm^{-1}) 1720, 1739, 3394, 3448; δ_H (CD_3OD, 500 MHz) 0.72 (s, 3H), 1.1 (bs, 6H), 2.87 (m, 1H), 3.27-3.37 (m, 6H), 3.38-3.45 (m, 3H), 3.64 (s, 3H), 3.81 (bs, 1H), 3.97 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.5, 22.5, 22.6, 24.1, 27.7, 28.7, 29.6, 30.5, 31.2, 31.8, 32.2, 34.8, 36.1, 36.8, 37.1, 37.4, 40.8, 43.0, 43.6, 45.2, 46.3, 47.5, 47.9, 52.0, 58.0, 68.6, 73.8, 176.5; MS (MALDI-TOF) *m/z*: 507.9 $[M+1]^+$.

Compound 82: From compound **78** (0.100 g, 0.12 mmol); Yield (0.060 g, 91% yield); $[\alpha]_D^{28}$ +37.8 (*c* 0.9, MeOH); Anal. Calcd. for $C_{31}H_{58}N_4O_4 \cdot 3HCl$ C, 56.40; H, 9.31; N, 8.49 Found: C, 56.35; H, 9.58; N, 8.21; IR ν_{max} (Nujol)/(cm^{-1}) 1734, 3361, 3373, 3451; δ_H (CD_3OD, 500 MHz) 0.67 (s, 3H), 0.95 (bs, 6H), 2.80 (m, 1H), 3.16-3.48 (m, 15H), 3.59 (s, 3H), 3.75 (bs, 1H), 3.92 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.5, 22.6, 24.1, 27.8, 28.7, 29.6, 30.6, 31.2, 31.9, 32.2, 34.8, 36.1, 36.8, 37.2, 37.8, 40.9, 43.0, 43.8, 45.4, 46.5x2, 46.7, 47.5, 48.0, 52.0, 58.1, 68.6, 73.8, 176.5; MS (LCMS) *m/z*: 551.9096 [M+1]-$^+$.

Compound 83: From compound **79** (0.100 g, 0.10 mmol); Yield (0.053 g, 90% yield); $[\alpha]_D^{28.2}$+38 (*c* 1.00, MeOH); Anal. Calcd. for $C_{33}H_{63}N_5O_4 \cdot 4HCl$ C, 53.58; H, 9.13; N, 9.47 Found: C, 53.66; H, 9.47; N, 9.51; IR ν_{max} (Nujol)/(cm^{-1}) 1738, 3381, 3433; δ_H (CD_3OD, 400 MHz) 0.63 (s, 3H), 0.91 (bs, 6H), 2.77 (m, 1H), 3.32-3.52 (m, 17H), 3.55 (s, 3H), 3.72 (bs, 1H), 3.88 (bs, 1H); δ_C (CD_3OD, 100.61 MHz) 12.9, 17.5, 22.6, 24.1, 27.8, 28.7, 29.6, 30.6, 30.7, 31.2, 31.8, 32.2, 34.8, 36.1, 36.8, 37.2, 37.3, 40.9, 42.9, 43.4, 45.3, 46.0, 46.3x2, 47.5, 48.0, 52.0, 58.1, 68.6, 73.8, 176.5; MS (LCMS) *m/z*: 594.9000 $[M+1]^+$.

Compound 88: From compound **84** (0.055 g, 0.1 mmol); Yield (0.041 g, 92% yield); $[\alpha]_D^{26.6}$ +22.72 (*c* 0.88, MeOH); Anal. Calcd. for $C_{26}H_{46}N_2O_4 \cdot HCl$. C, 64.33; H, 9.96; N, 5.42 Found: C, 64.11; H, 9.73; N, 5.75; IR ν_{max} (Nujol)/(cm^{-1}) 1679, 1718, 3398; δ_H (CD_3OD, 500 MHz) 0.67 (s, 3H), 0.96 (d, 6H, *J* = 6.32 Hz), 2.79 (m, 1H), 3.11-3.50 (m, 5H), 3.76 (bs, 1H), 3.92 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13, 17.6, 22.6, 22.7, 24.1, 27.8, 28.7, 29.6, 30.7, 31.3, 31.8, 32.3, 34.9, 36.8, 37.2, 40.9, 43.0, 43.8 44.0, 47.5, 48.0, 57.9, 68.7, 73.8, 178.3; MS (MALDI-TOF) *m/z*: 451.3 $[M+1]^+$.

Compound 89: From compound **85** (0.069 g, 0.1 mmol); Yield (0.046 g, 93% yield); $[\alpha]_D^{26.4}$ +20.64 (*c* 0.775, MeOH); Anal. Calcd. for $C_{28}H_{51}N_3O_4 \cdot 2HCl$ C, 59.55; H, 9.76; N, 7.36 Found: C, 59.35; H, 9.43; N, 7.42; IR ν_{max} (Nujol)/(cm^{-1}) 1703, 1718, 3361, 3419; δ_H (CD_3OD, 500 MHz) 0.68 (s, 3H), 0.96 (s, 3H), 0.97 (d, 3H, *J* = 6.0 Hz), 2.80 (m, 1H), 2.91-3.23 (m, 9H), 3.81 (bs, 1H), 3.98(bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.6, 22.6, 22.7, 24.1, 27.9, 28.7, 29.7, 30.7, 31.3, 32.0, 32.3, 34.9, 36.1, 36.8, 37.1, 37.3, 40.8, 43.0, 43.6, 45.2, 46.3, 47.5, 48.1, 57.5, 68.7, 73.8, 178.2; MS (MALDI-TOF) *m/z*: 494.4 $[M+1]^+$.

Compound 90: From compound **86** (0.075 g, 0.089 mmol); Yield (0.042 g, 88% yield); $[\alpha]_D^{28.4}$ +24 (*c* 0.9, MeOH); Anal. Calcd. for $C_{28}H_{51}N_3O_4 \cdot 3HCl$ C, 55.76; H, 9.20; N, 8.67

Found: C, 55.88; H, 8.93; N, 8.59; IR ν_{max} (Nujol)/(cm^{-1}) 1707, 1720, 3378, 3455; δ_H (CD_3OD, 500 MHz) 0.66 (s, 3H), 0.93 (s, 3H), 0.96 (d, 3H, J = 6.59 Hz), 2.77 (m, 3H), 3.00-3.42 (m, 16H), 3.41 (bs, 1H), 3.74 (bs, 1H), 3.91(bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.6, 22.6, 27.8, 28.7, 29.6, 30.6, 31.2, 32.0, 32.3, 34.8, 36.1, 36.8, 37.2, 38.3, 40.9, 43.0, 43.9, 44.7, 45.3, 46.2, 46.5, 46.9, 47.5, 48.0, 58.0, 68.7, 73.8, 178.3; MS (LCMS) *m/z*: 537.8801 $[M+1]^+$.

Compound 91: From compound **87** (0.075 g, 0.076 mmol); Yield (0.038 g, 85% yield); $[\alpha]_D^{28.6}$ +20 (*c* 0.8, MeOH); Anal. Calcd. for $C_{28}H_{51}N_3O_4 \cdot 4HCl$ C, 52.96; H, 9.03; N, 9.65 Found: C, 53.06; H, 8.95; N, 9.75; IR ν_{max} (Nujol)/(cm^{-1}) 1706, 1715, 3386, 3443; δ_H (CD_3OD, 500 MHz) 0.64 (s, 3H), 0.92 (s, 3H), 0.94 (d, 3H, J = 6.60 Hz), 2.73 (m, 1H), 3.22-3.57 (m, 17H), 3.73 (bs, 1H), 3.90 (bs, 1H); δ_C (CD_3OD, 125.76 MHz) 13.0, 17.6, 22.7, 27.8, 28.7, 29.0, 29.6, 30.6, 31.2, 32.0, 32.3, 34.8, 36.1, 36.8, 37.2, 37.7, 40.9, 43.0, 43.5, 44.6, 45.2, 46.0, 46.3x2, 47.5, 48.0, 58.0, 68.7, 73.8, 178.2; MS (LCMS) *m/z*: 580.9363 $[M+1]^+$.

Antimicrobial Activity:

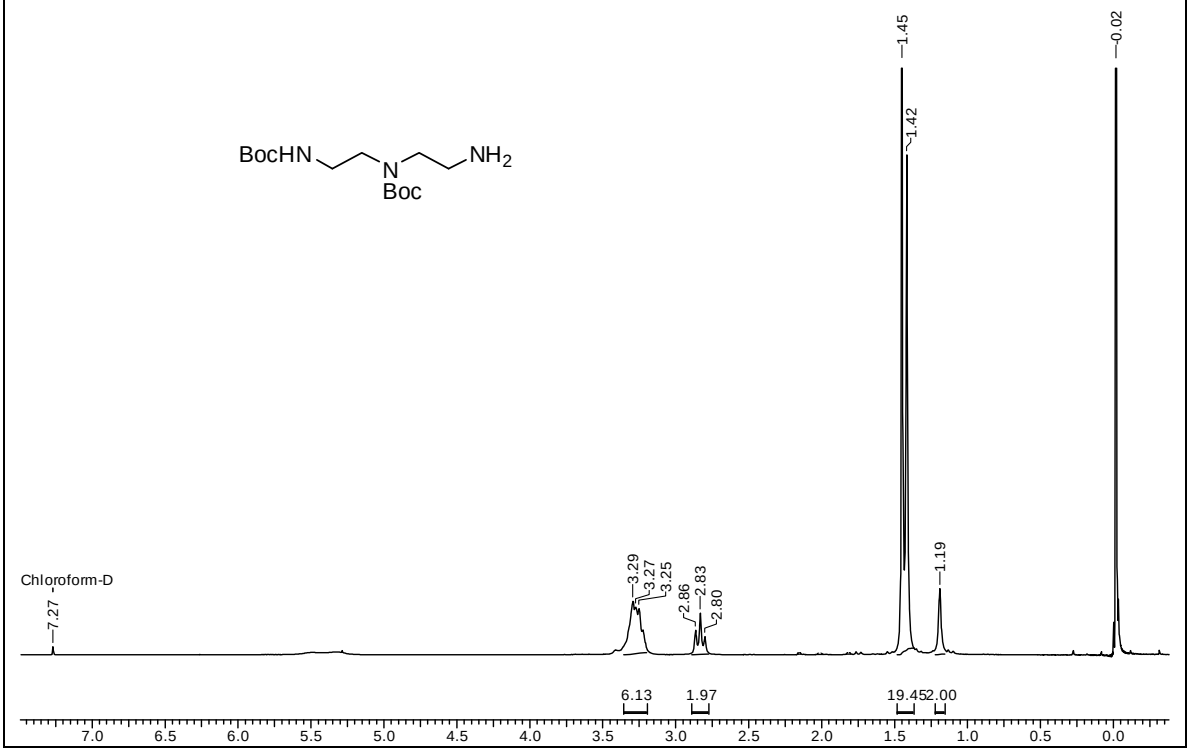
Materials and Methods. Human pathogens *C. albicans* and *C. neoformans*; saprophytes *B. poitrasii* and *Y. lipolytica* were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants. *F. oxysporum* (plant pathogen) was maintained on PDA (potato, 20%; dextrose, 2%) agar slants at 28 ^oC. *E. coli* (NCIM 2574) and *S. aureus* (NCIM 2122) were maintained on NA (beef extract, 0.3%; peptone, 0.5%; sodium chloride, 0.5%) 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. All the compounds were solubilized in DMSO, and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, fluconazole, tetracycline and erythromycin were also dissolved in DMSO, and were used as a positive control.

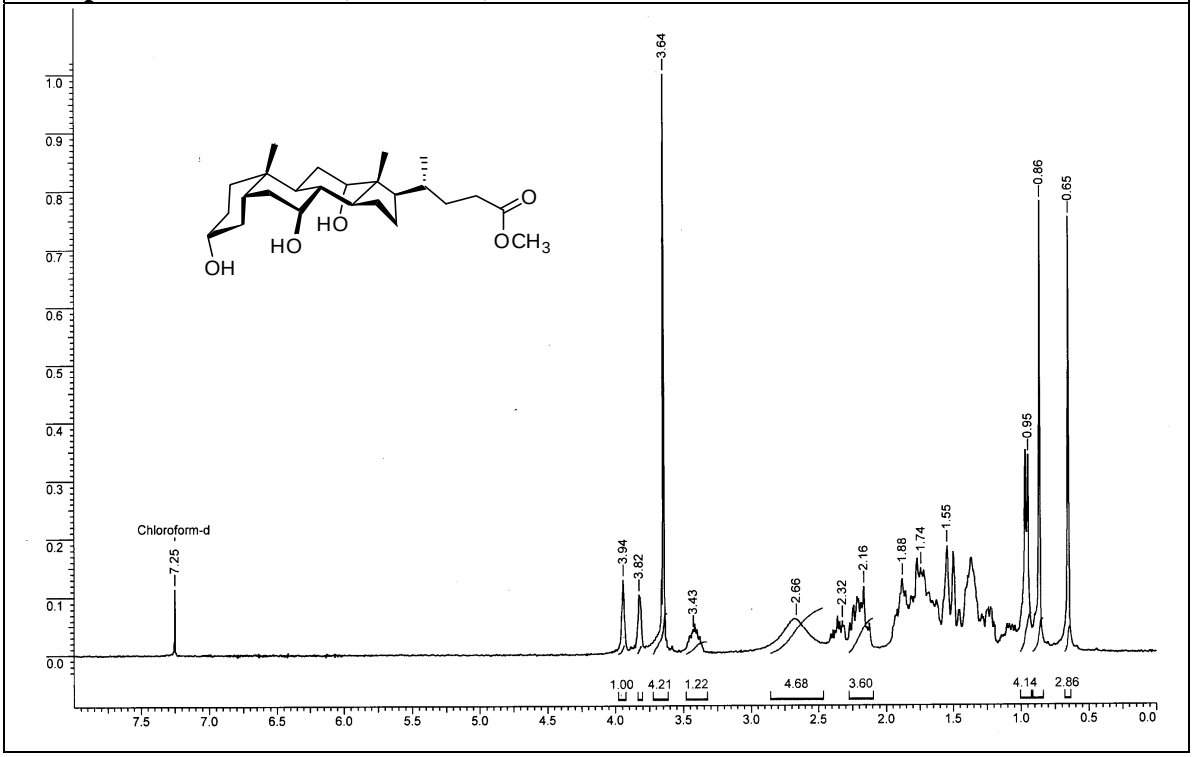
MIC and IC_{50} determination: In *vitro* antifungal and antibacterial activity of the newly synthesized compounds were studied against the fungal strains viz., *C. albicans*, *C. neoformans*, *B. poitrasii*, *Y. lipolytica, F. oxysporum* strains and bacterial strains *E. coli* (NCIM 2574), and *S. aureus* (NCIM 2122), respectively to find out MIC (Minimum Inhibitory Concentration) and IC_{50} (50%, Inhibition of Growth) values. Experiments were performed 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.[57] 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 for *B. poitrasii* (37 °C), 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.

1.9. Selected spectra

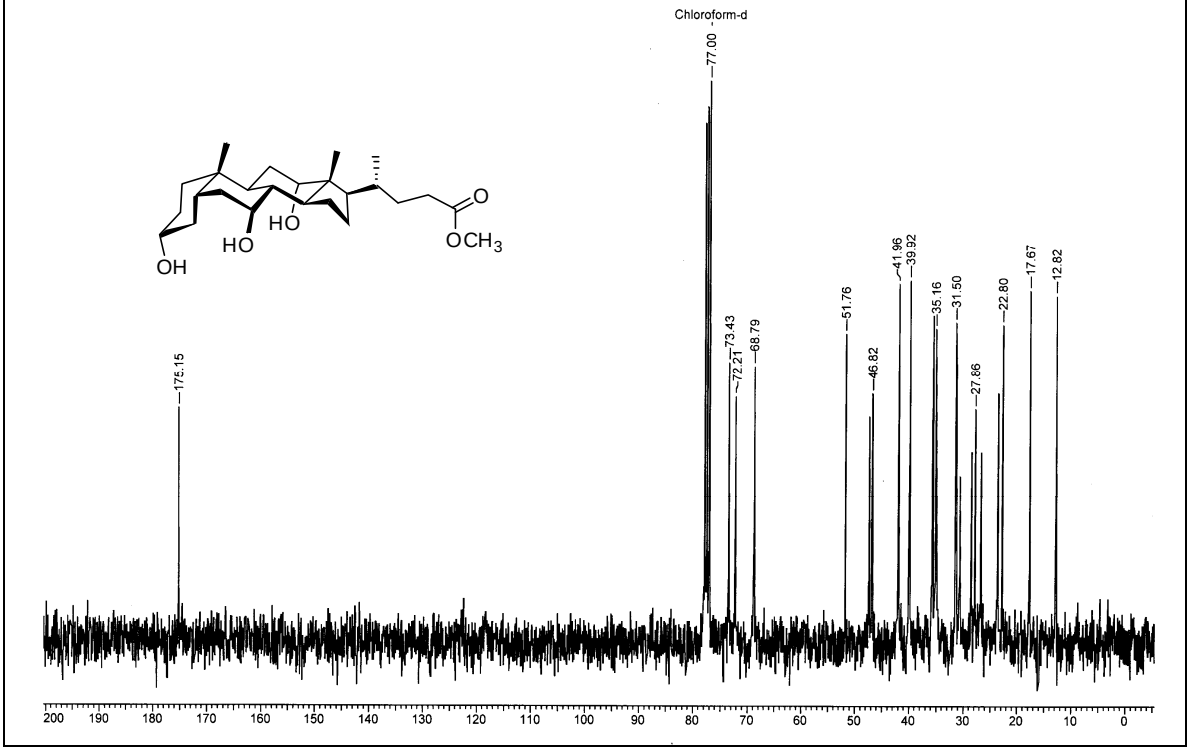
Compound 48: ^{1}H NMR, 200 MHz, $CDCl_3$



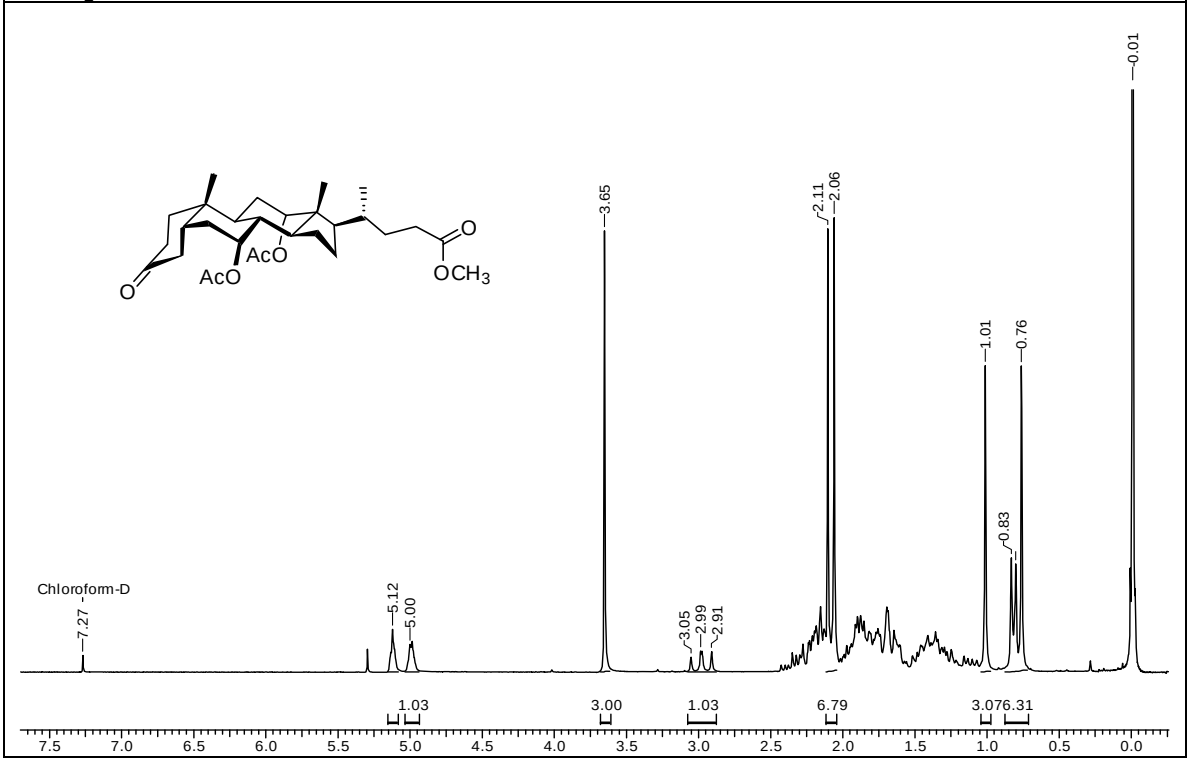
Compound 50: ^{1}H NMR, 200 MHz, $CDCl_3$



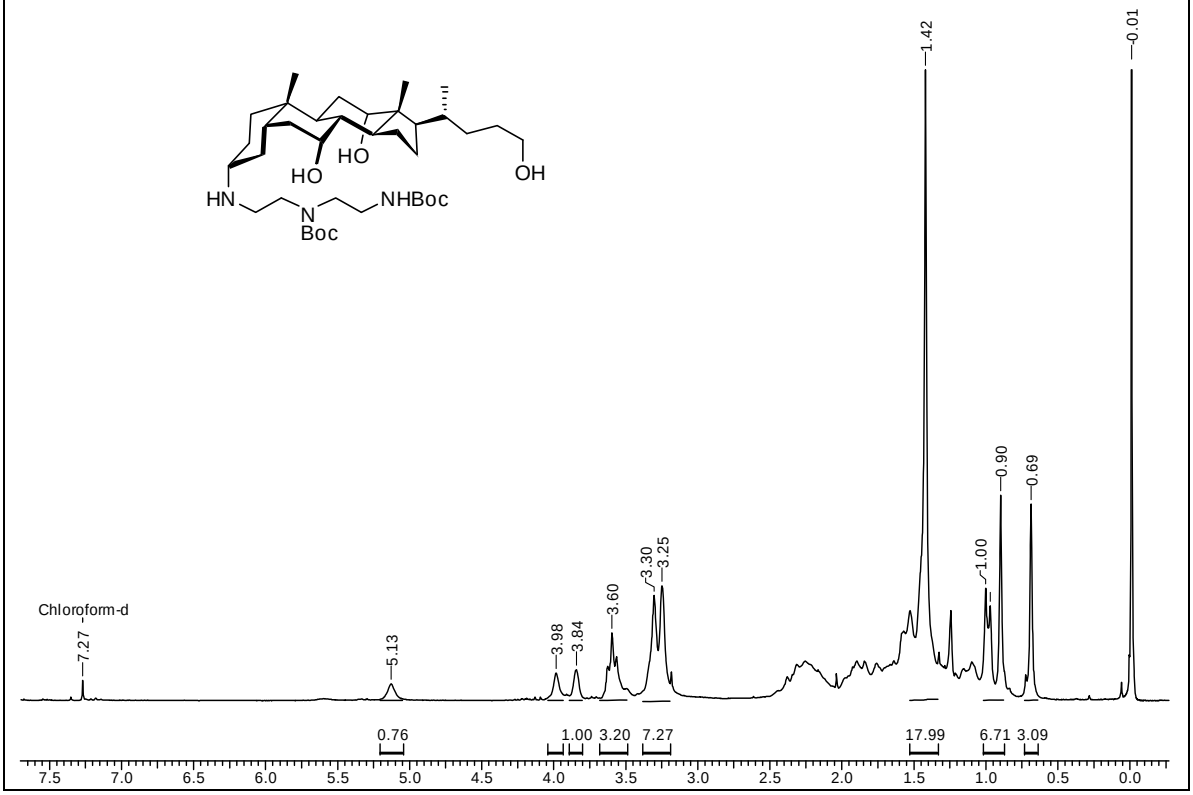
Compound 50: ^{13}C NMR, 50.32 MHz, $CDCl_3$



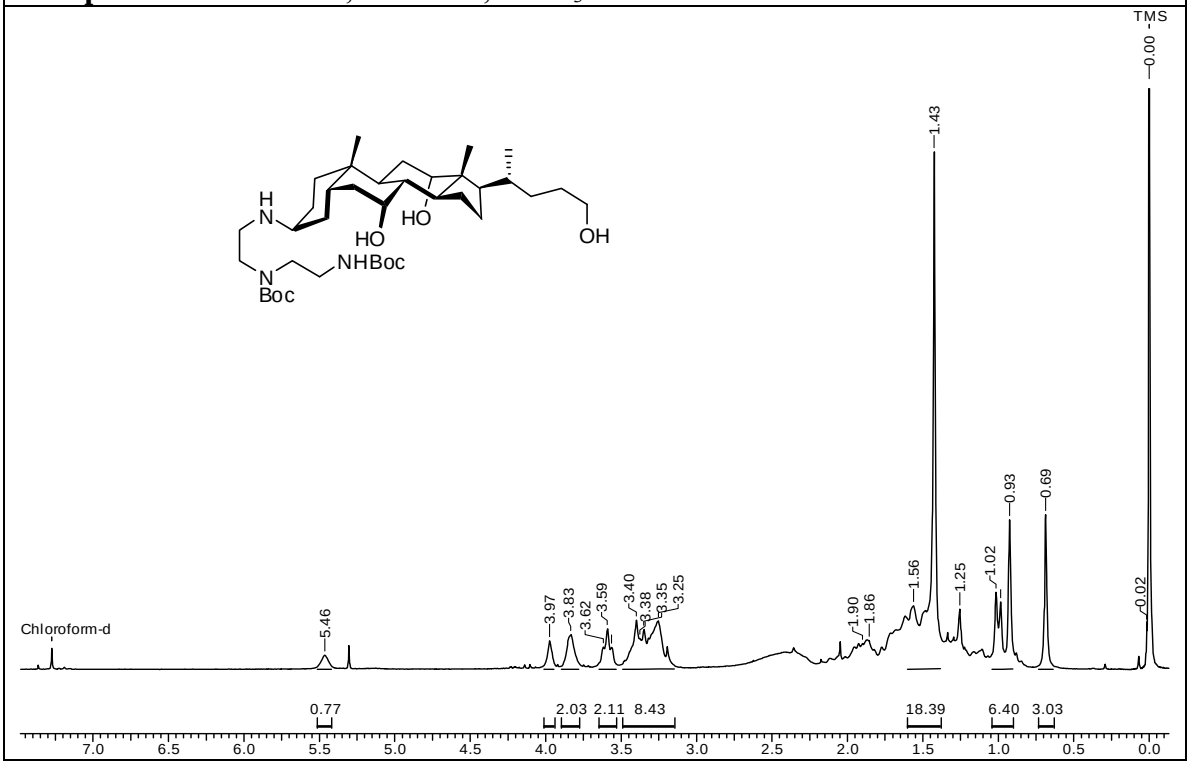
Compound 52: ^{1}H NMR, 200 MHz, $CDCl_3$



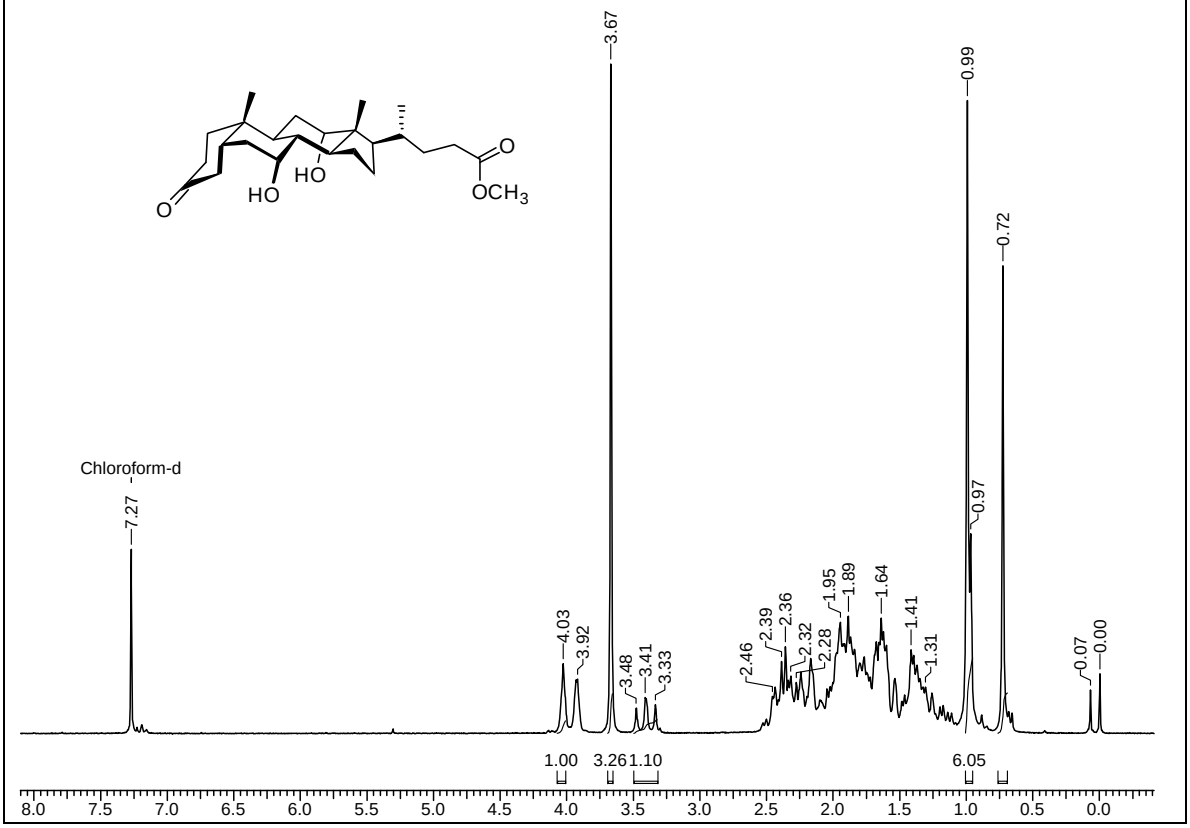
Compound 54: ^{1}H NMR, 400 MHz, $CDCl_3$



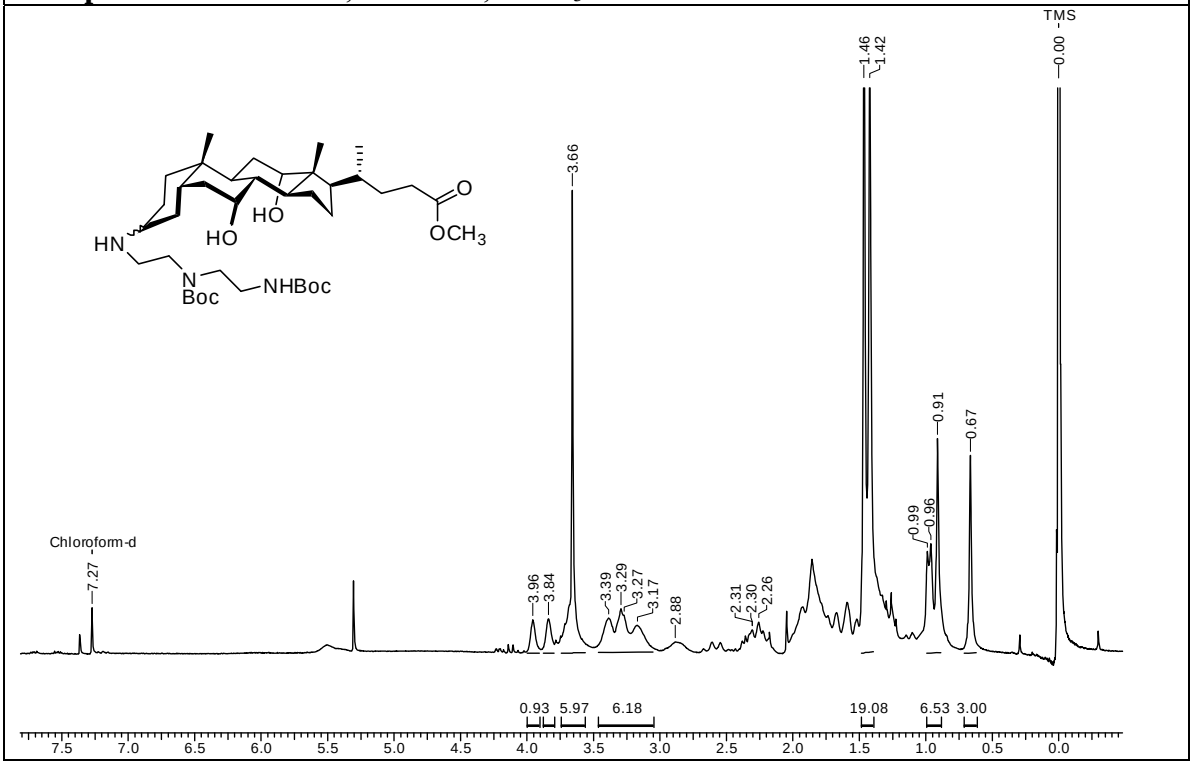
Compound 55: ^{1}H NMR, 400 MHz, $CDCl_3$



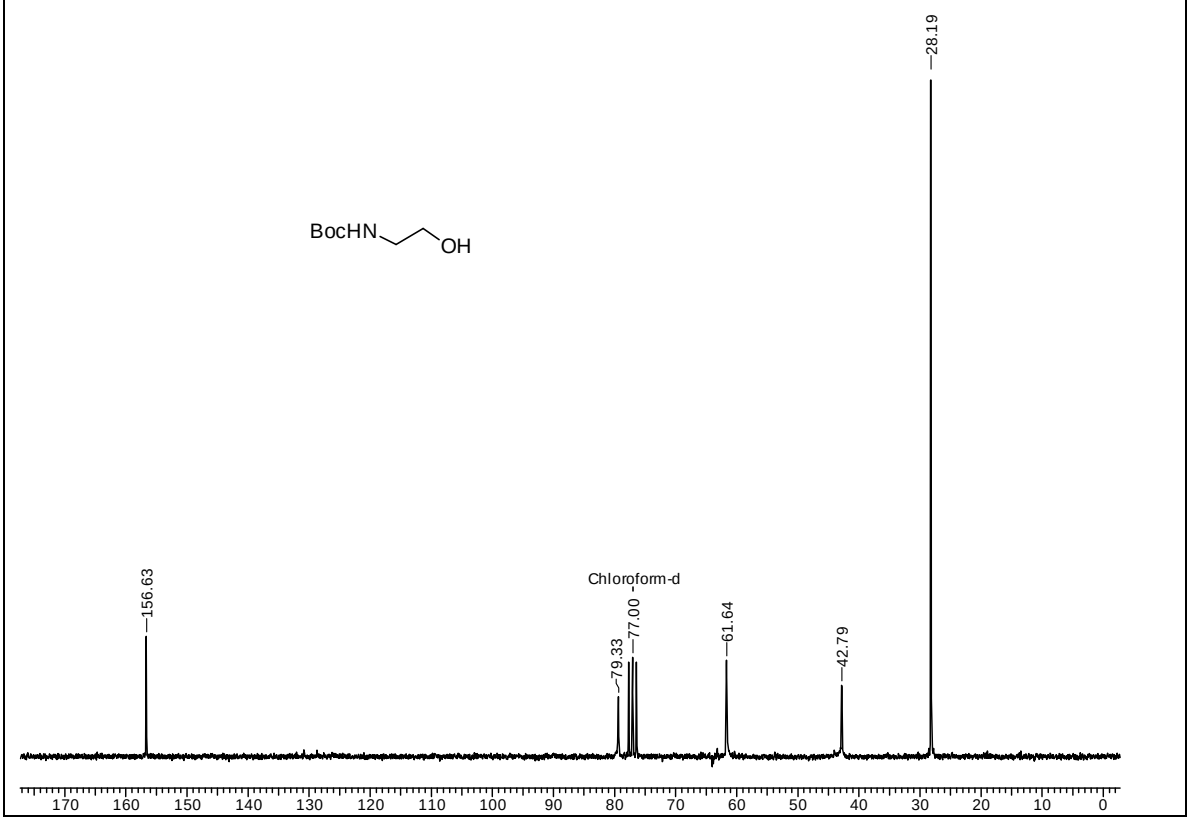
Compound 58: ^{1}H NMR, 200 MHz, $CDCl_3$



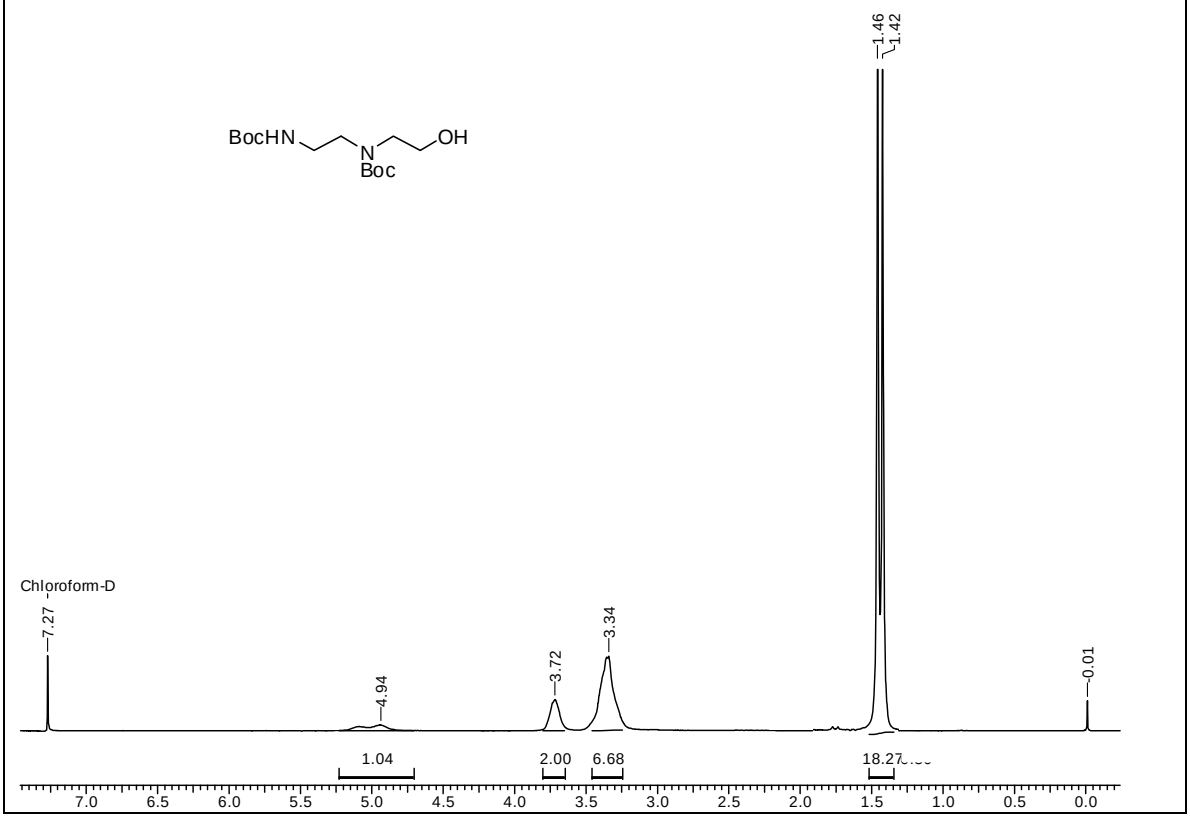
Compound 59: ^{1}H NMR, 200 MHz, $CDCl_3$



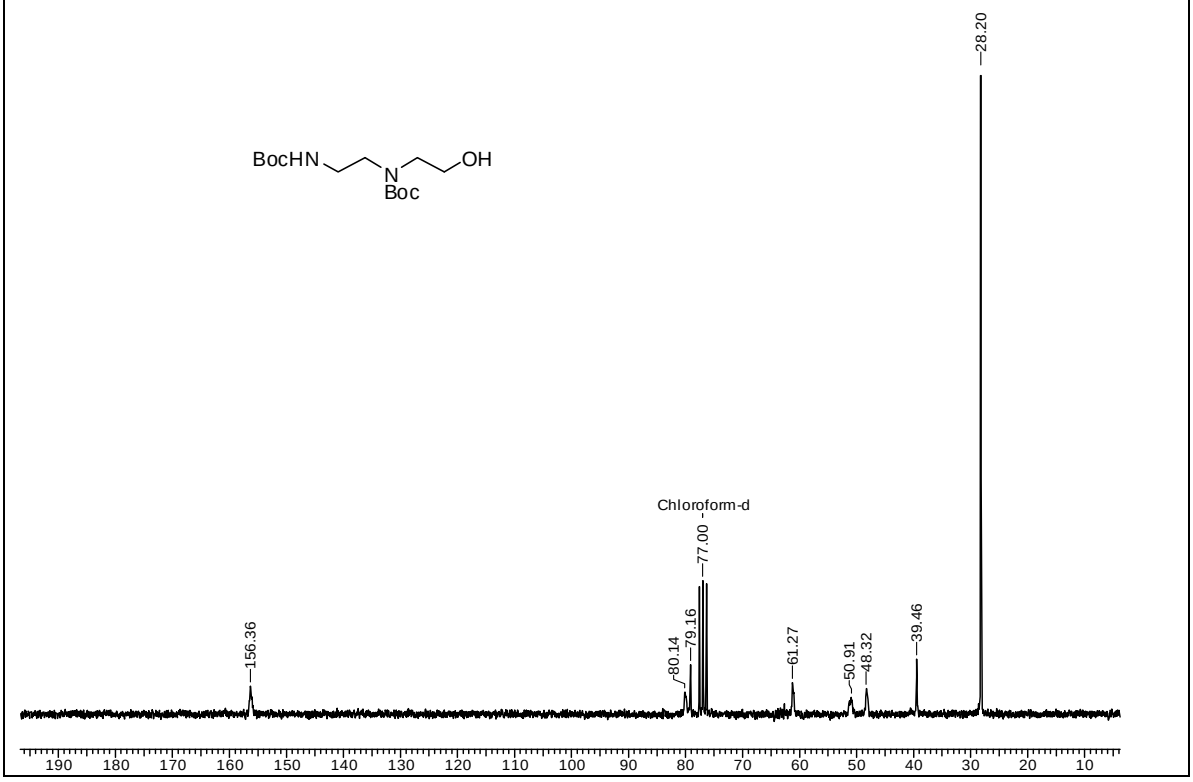
Compound 61: ^{13}C NMR, 50.32 MHz, $CDCl_3$



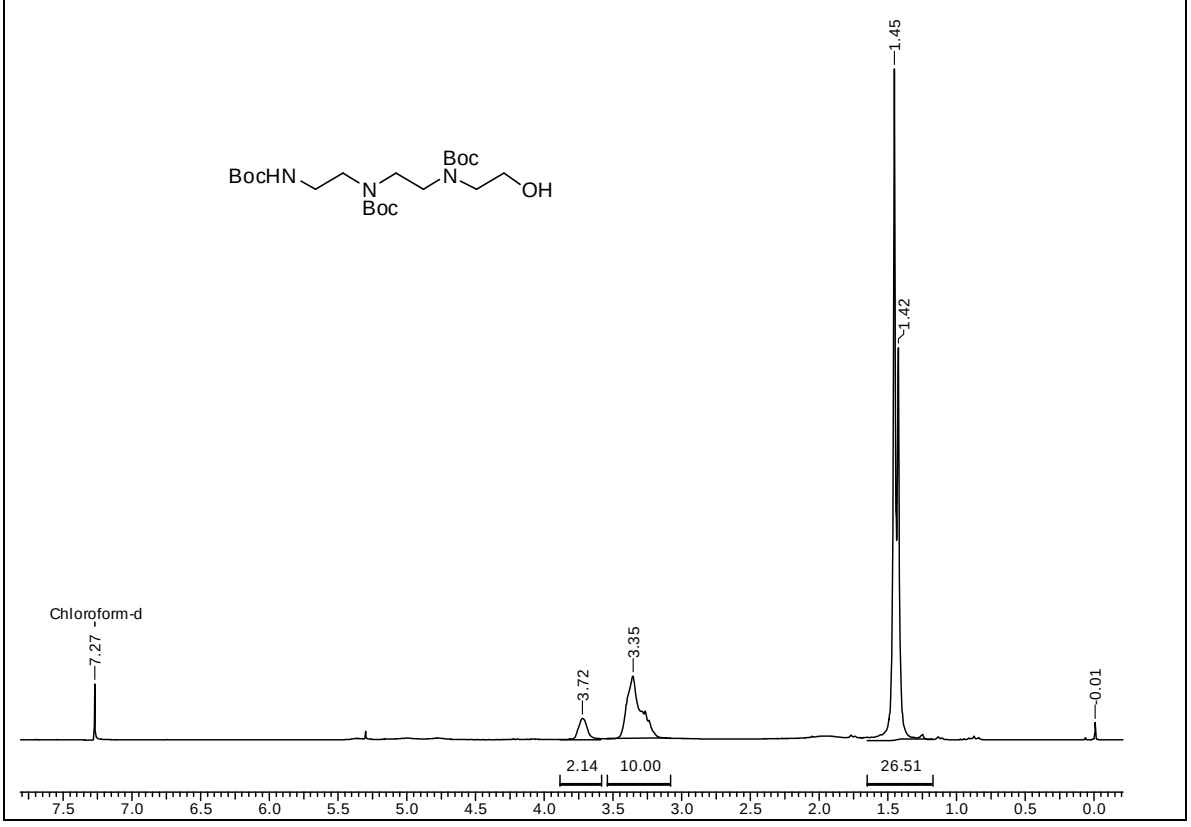
Compound 64: ^{1}H NMR, 200 MHz, $CDCl_3$



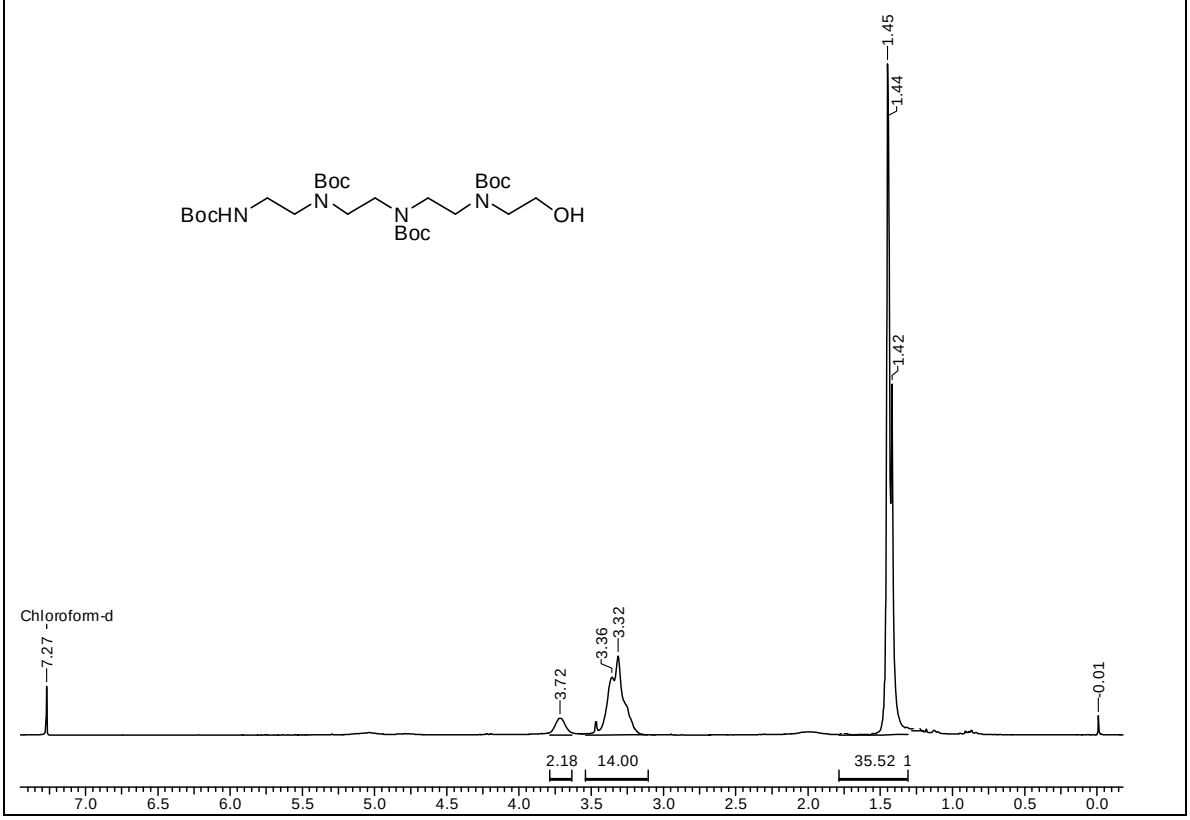
Compound 64: ^{13}C NMR, 50.32 MHz, $CDCl_3$



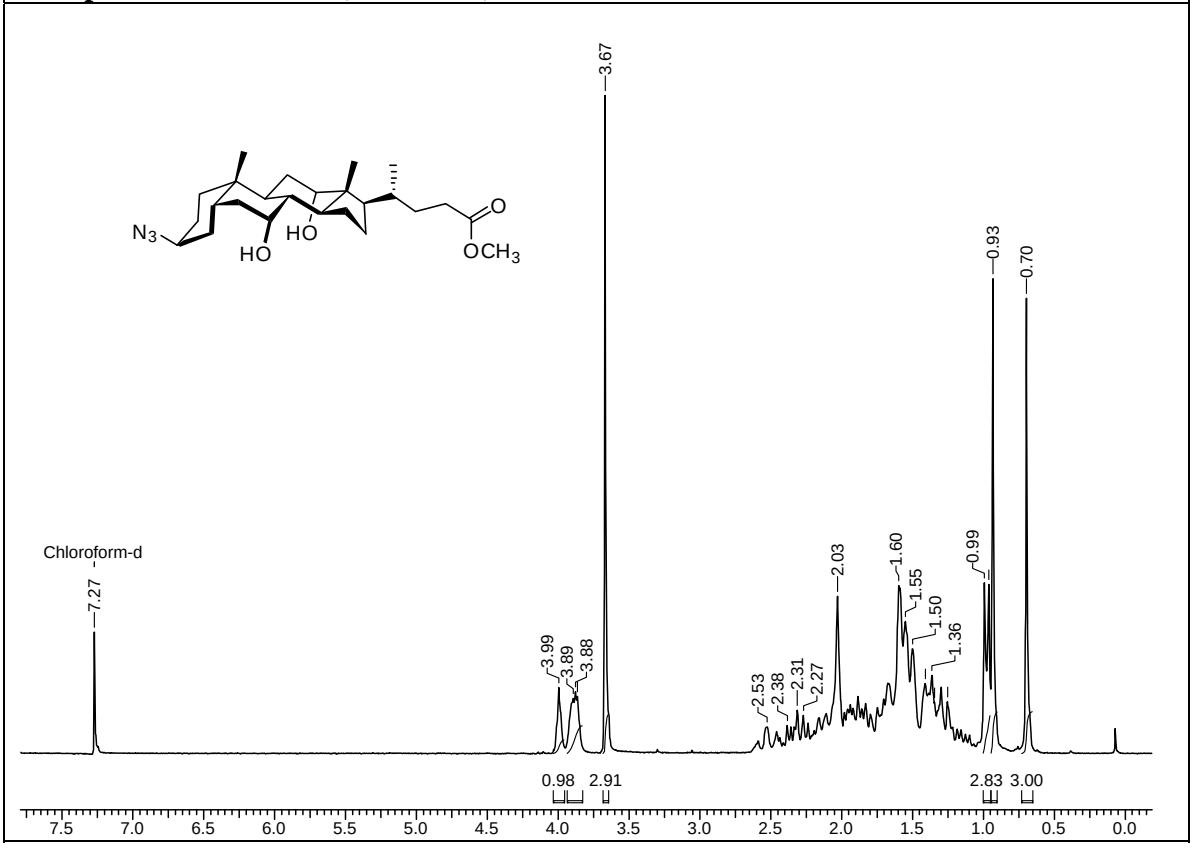
Compound 68: ^{1}H NMR, 200 MHz, $CDCl_3$



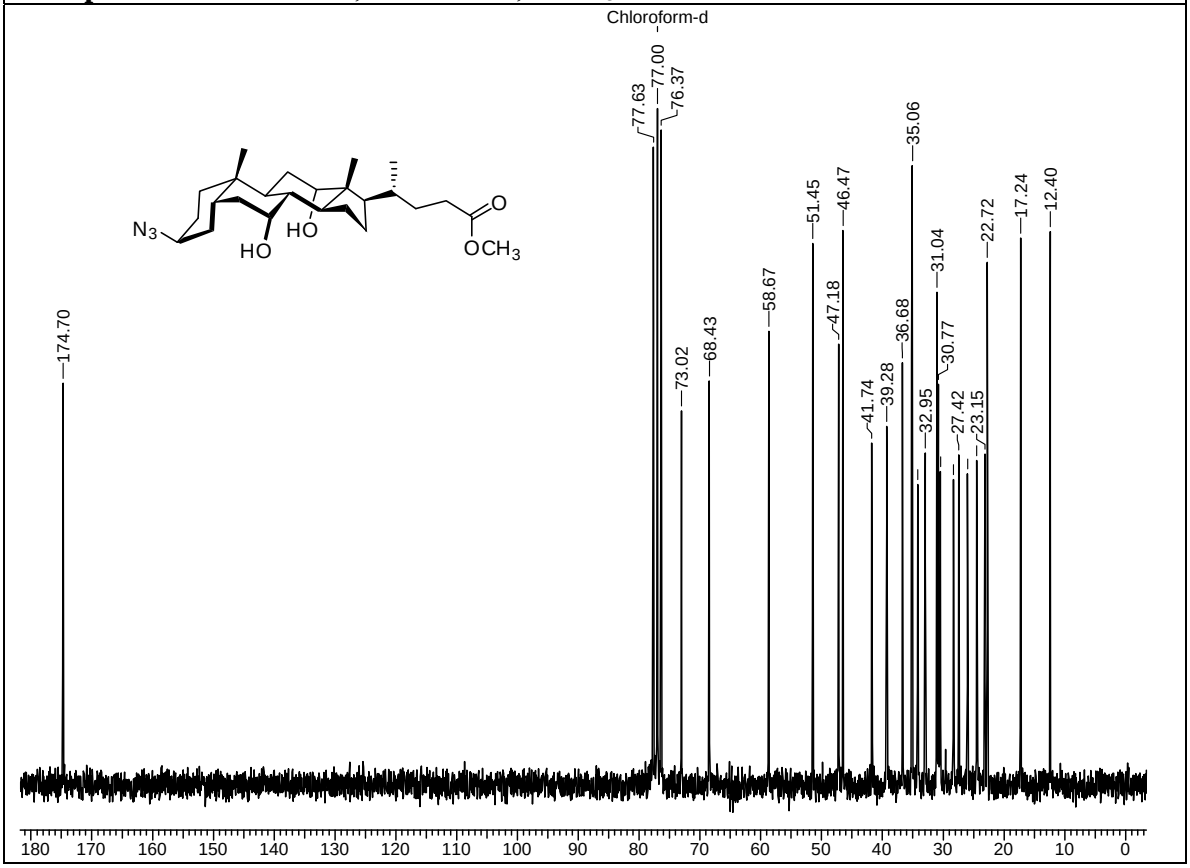
Compound 71: ^{1}H NMR, 400 MHz, $CDCl_3$



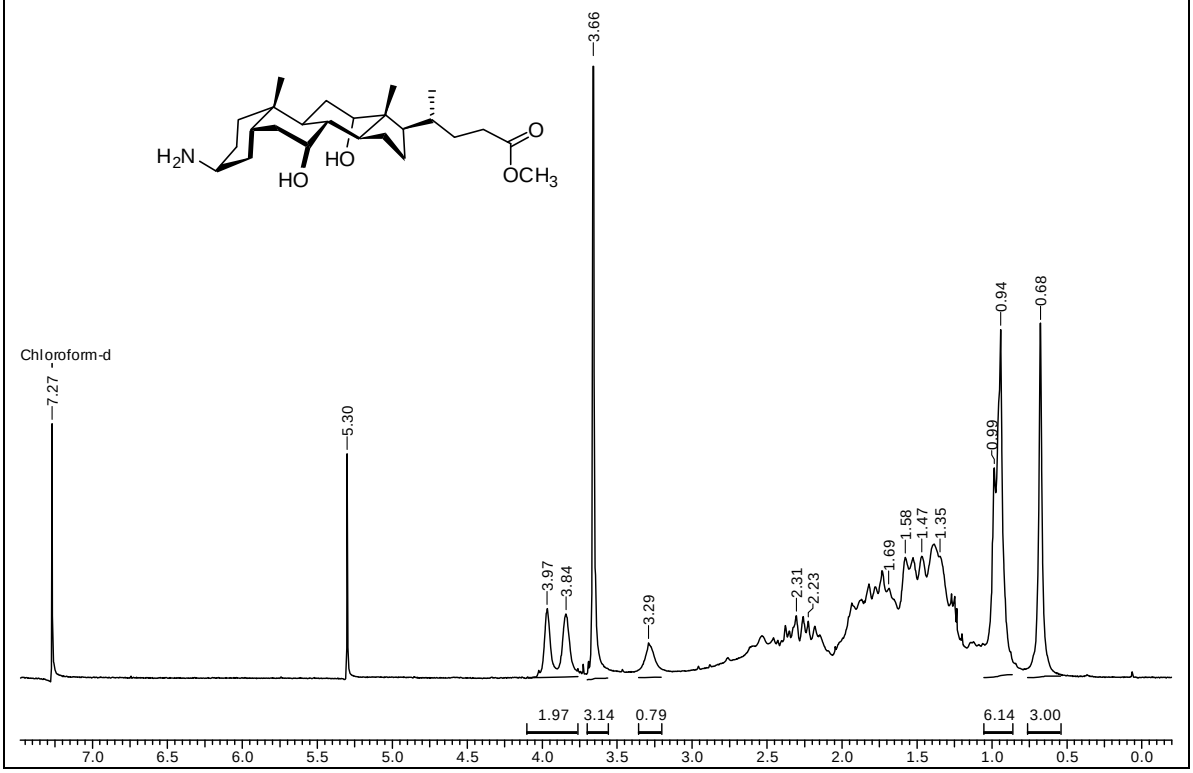
Compound 74: ^{1}H NMR, 200 MHz, $CDCl_3$



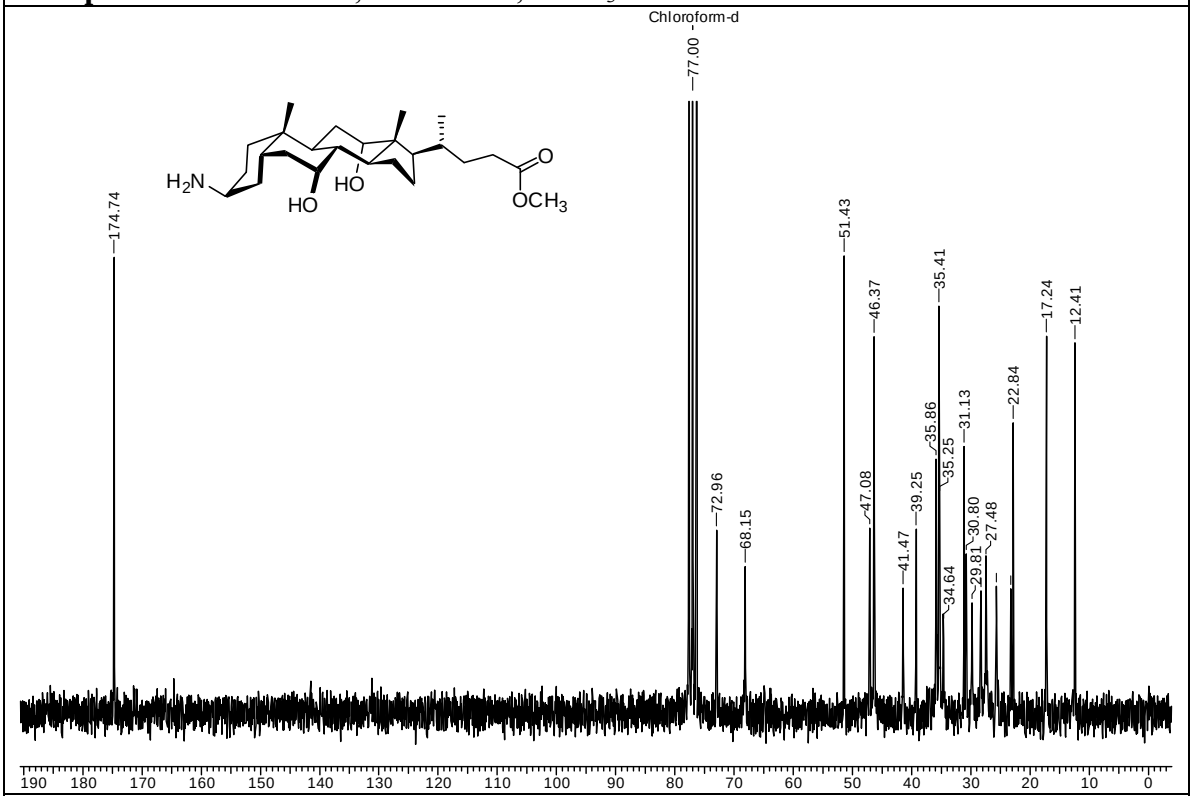
Compound 74: ^{13}C NMR, 50.32 MHz, $CDCl_3$



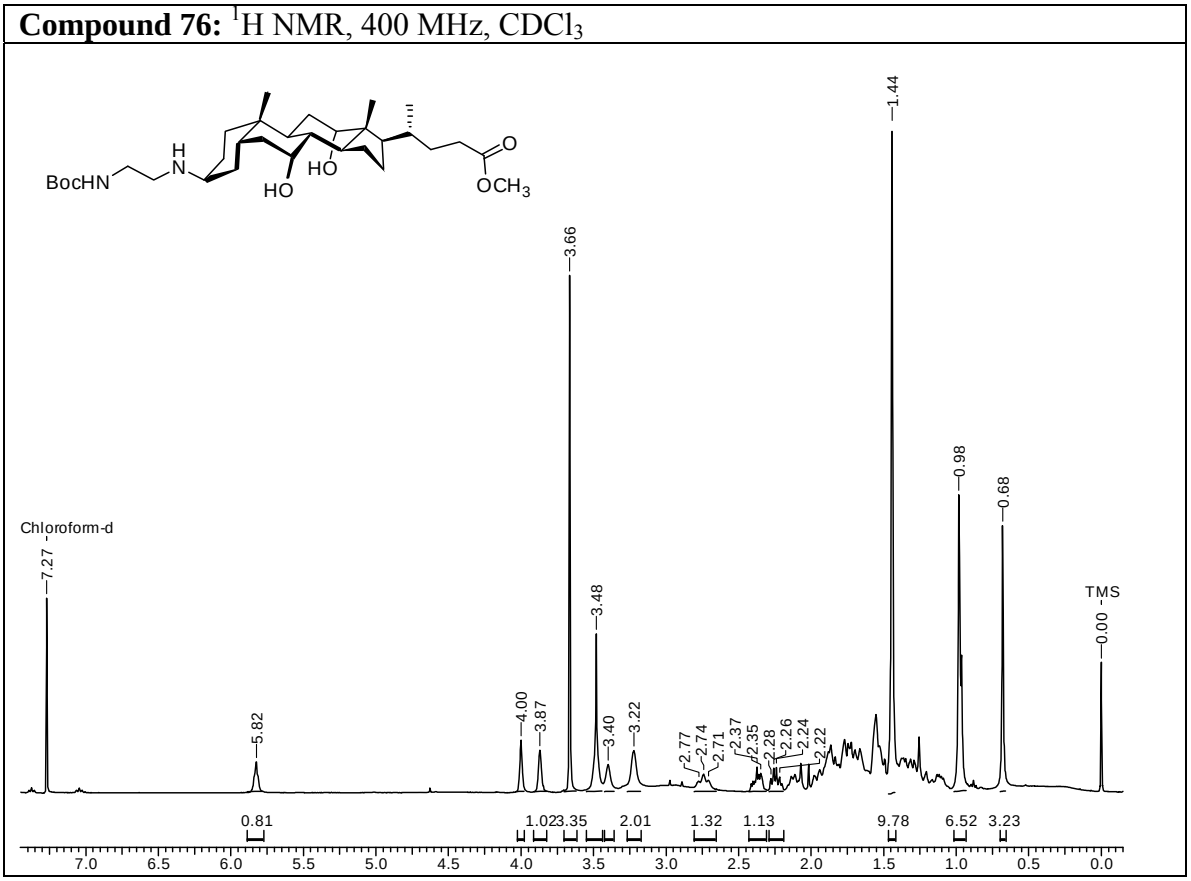
Compound 75: ^{1}H NMR, 200 MHz, $CDCl_3$



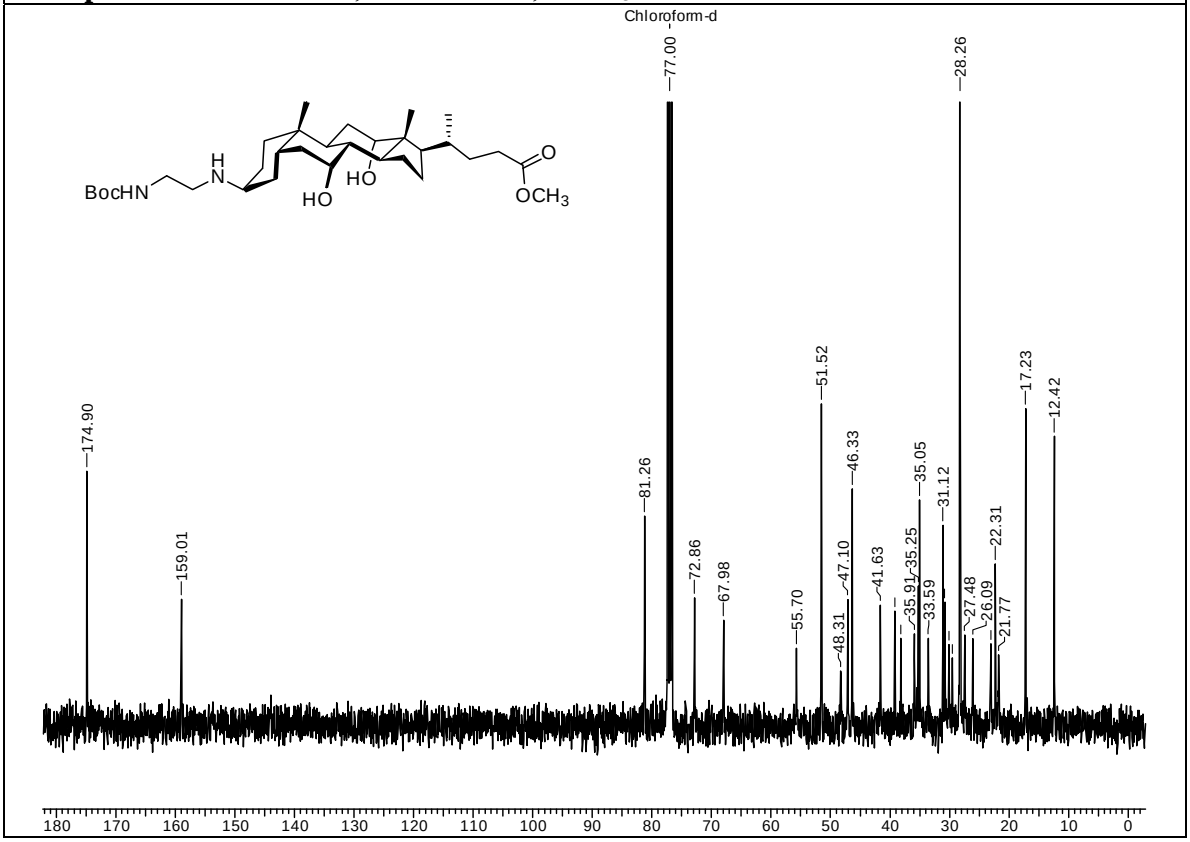
Compound 75: ^{13}C NMR, 50.32 MHz, $CDCl_3$



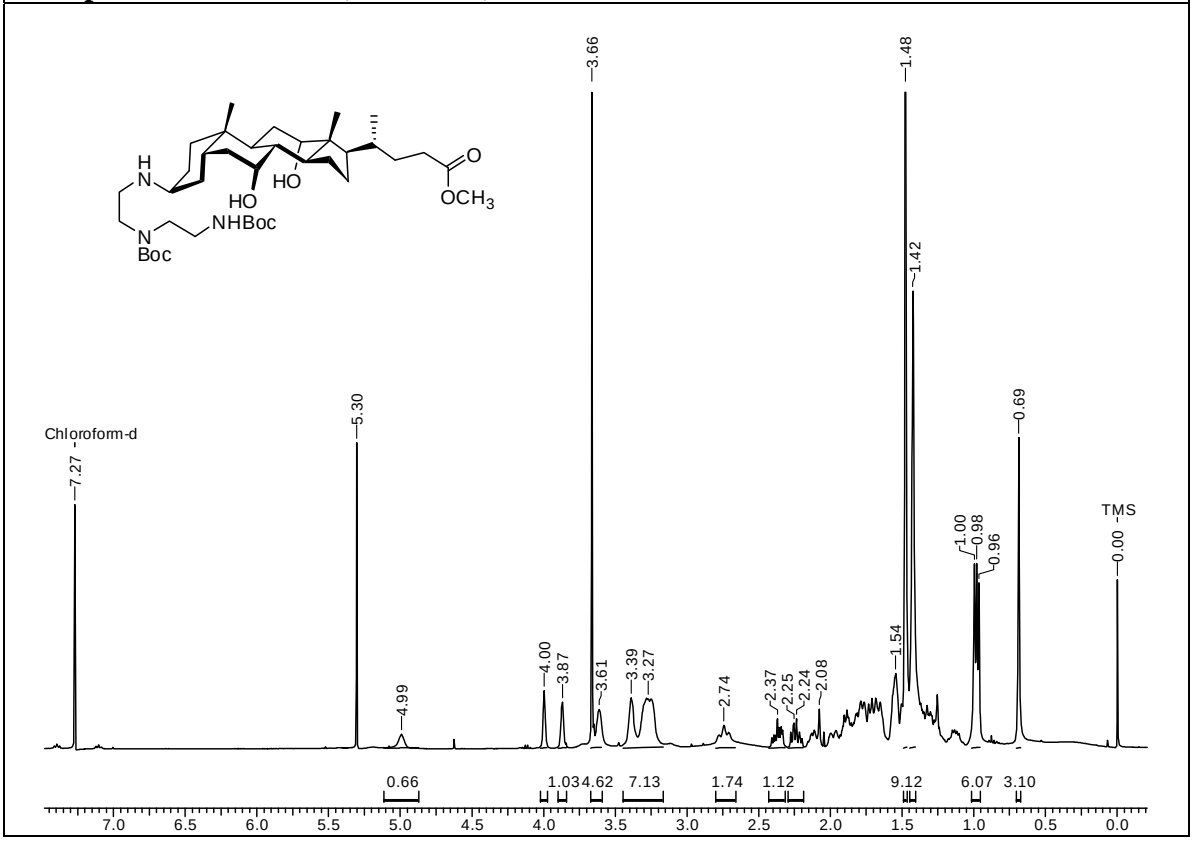
Compound 76: ^{1}H NMR, 400 MHz, $CDCl_3$



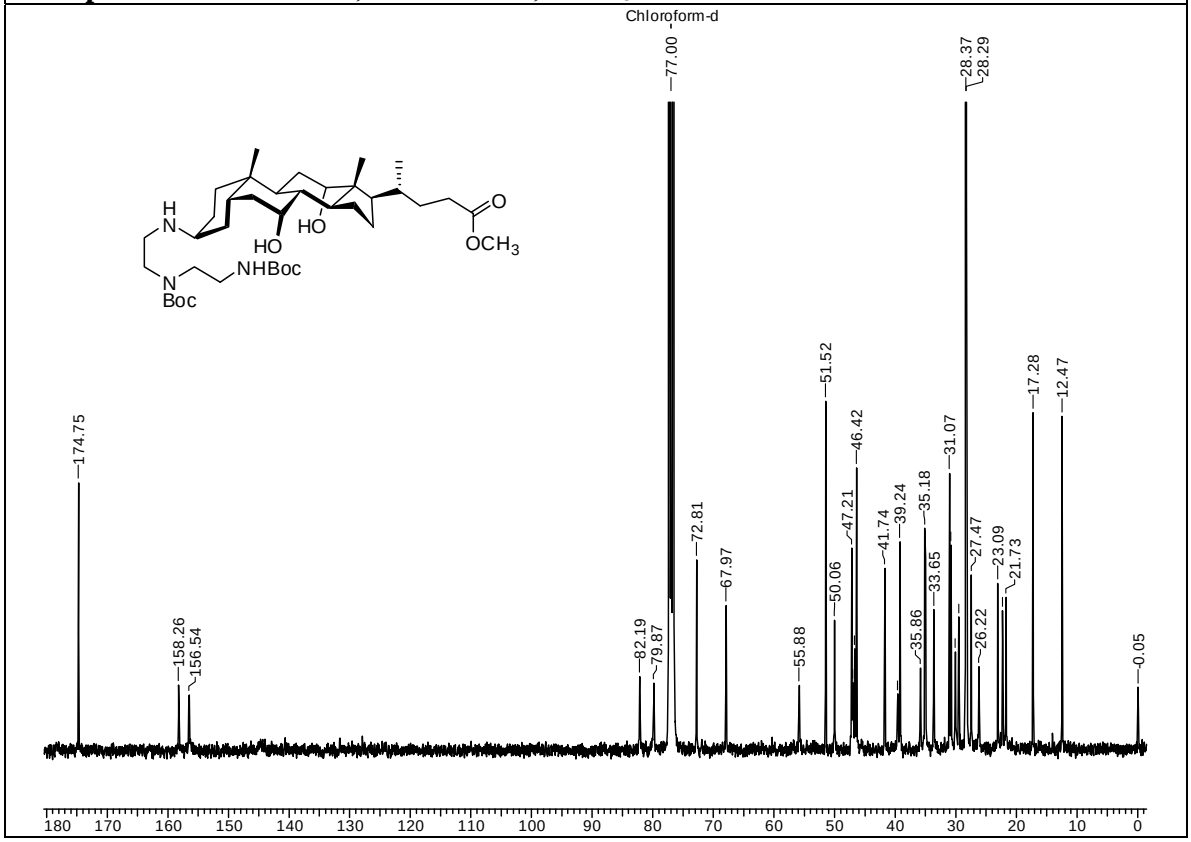
Compound 76: ^{13}C NMR, 100.61 MHz, $CDCl_3$



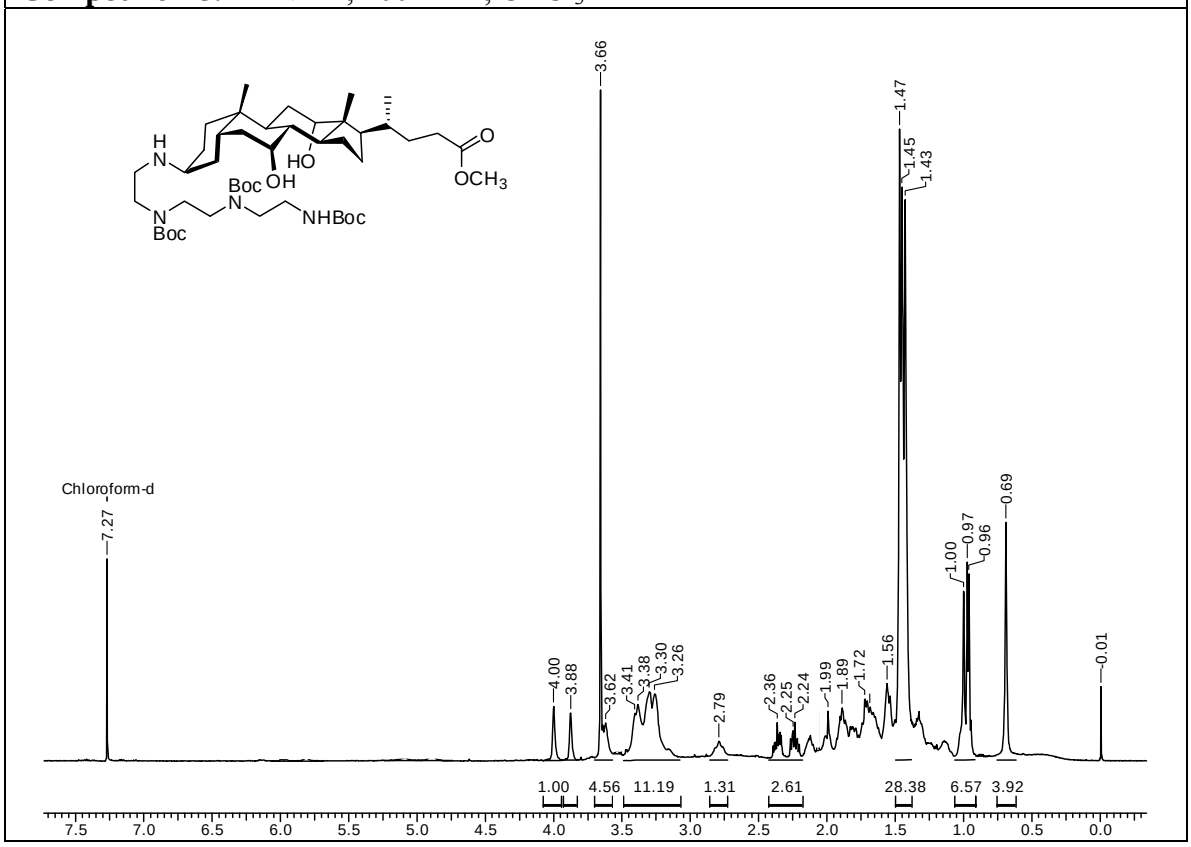
Compound 77: ^{1}H NMR, 400 MHz, $CDCl_3$



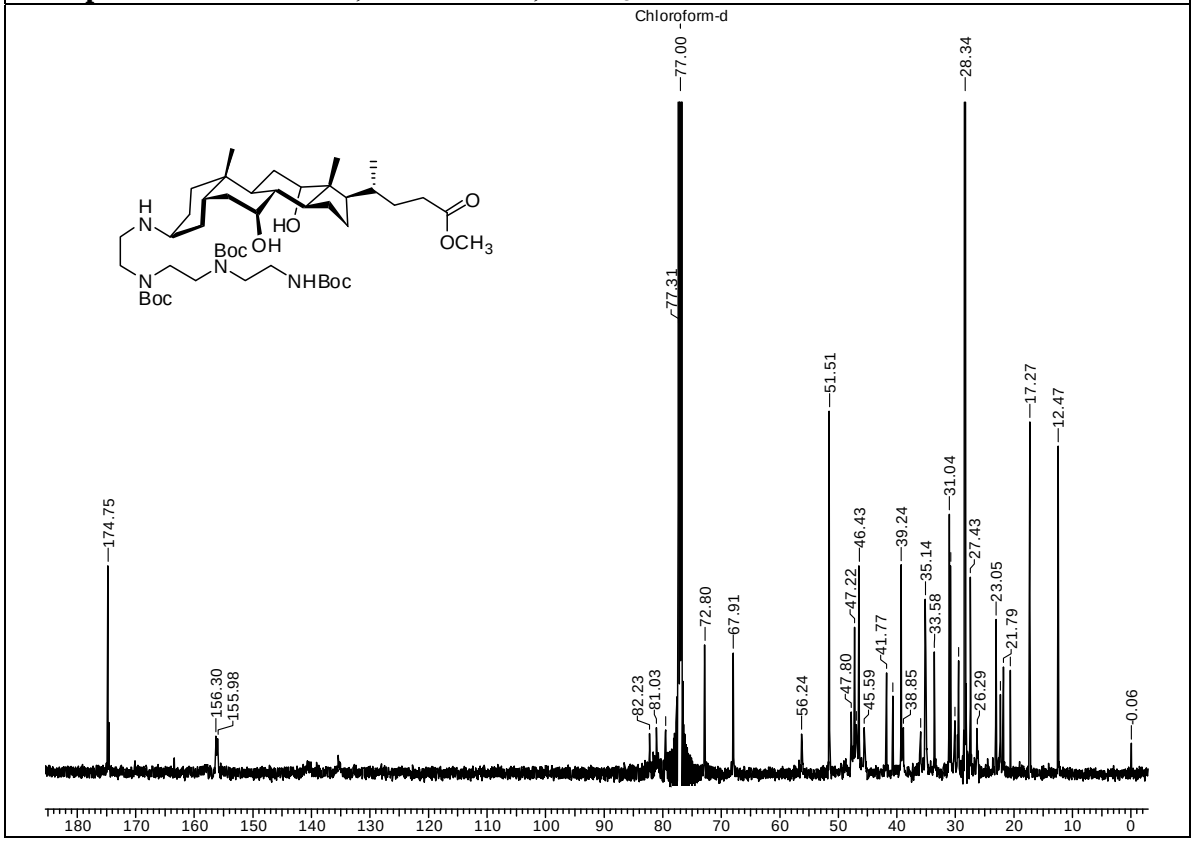
Compound 77: ^{13}C NMR, 100.61 MHz, $CDCl_3$



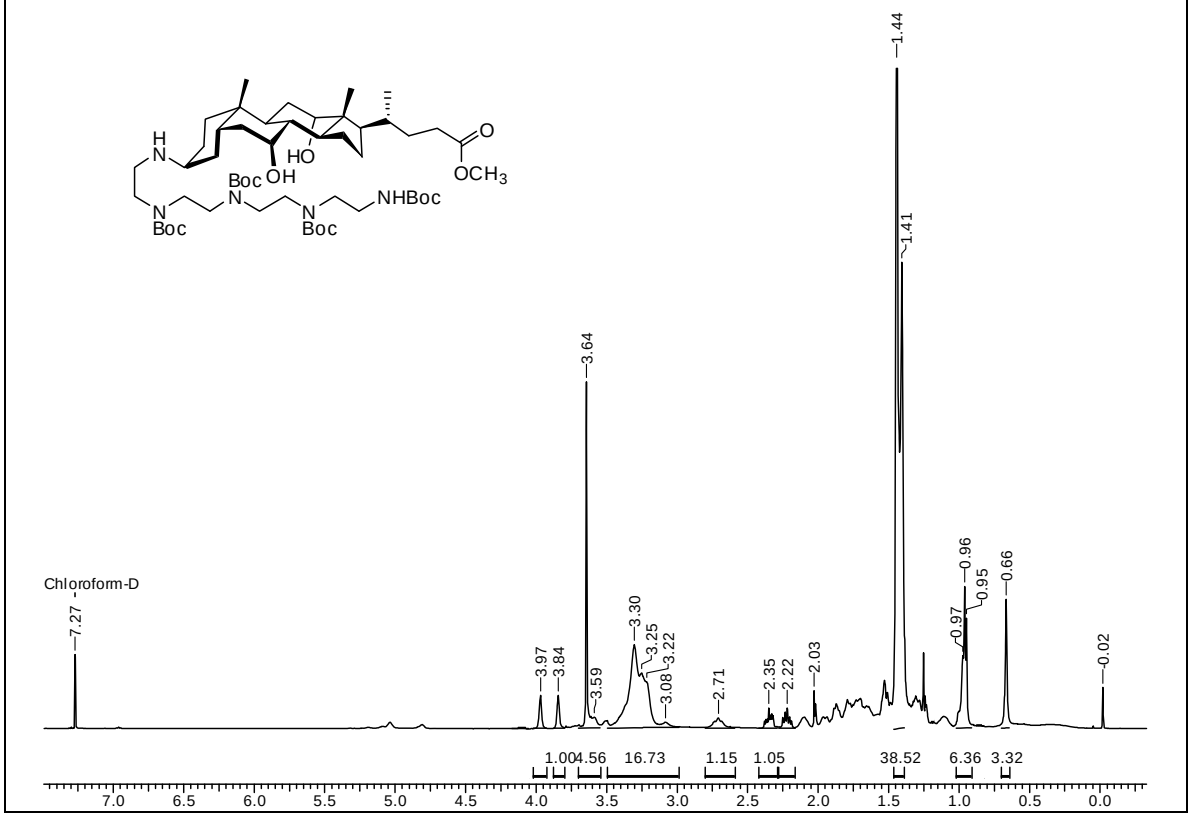
Compound 78: ^{1}H NMR, 400 MHz, $CDCl_3$



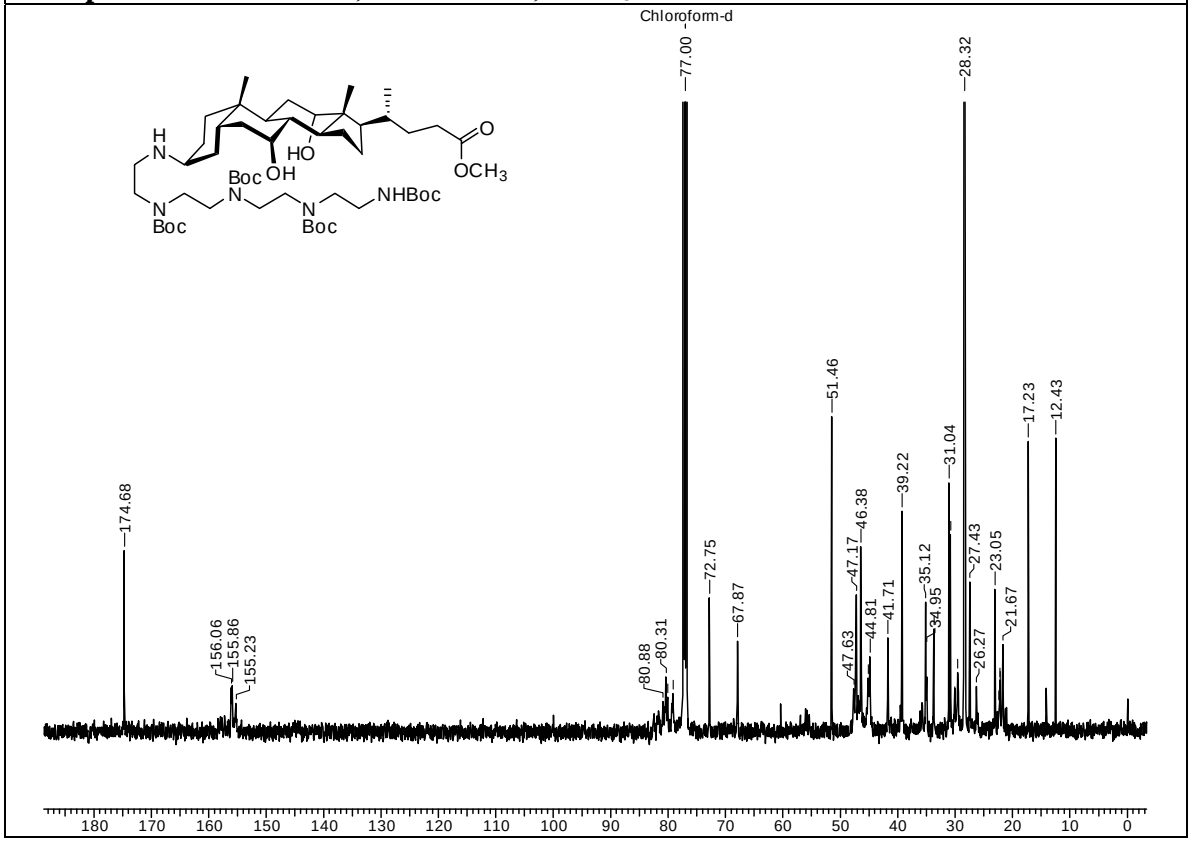
Compound 78: ^{13}C NMR, 125.76 MHz, $CDCl_3$



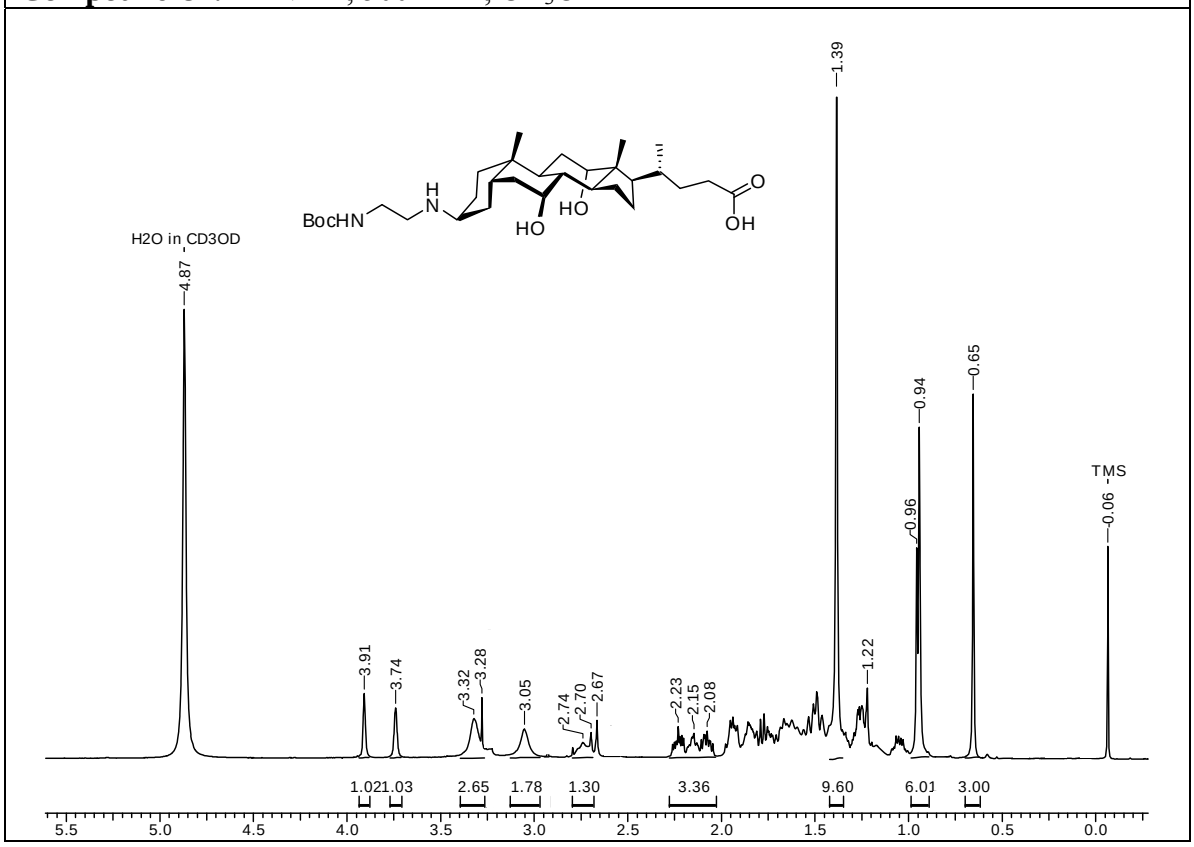
Compound 79: ^{1}H NMR, 400 MHz, $CDCl_3$



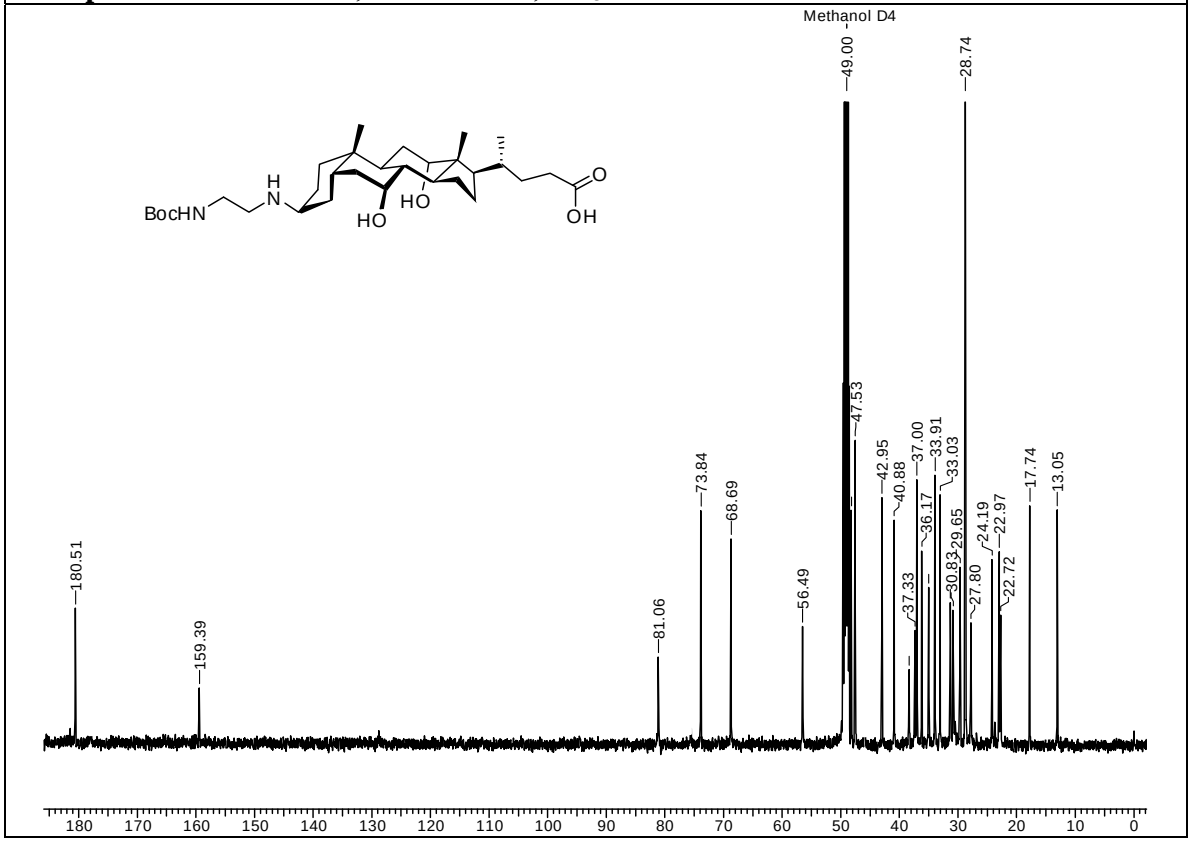
Compound 79: ^{13}C NMR, 125.76 MHz, $CDCl_3$



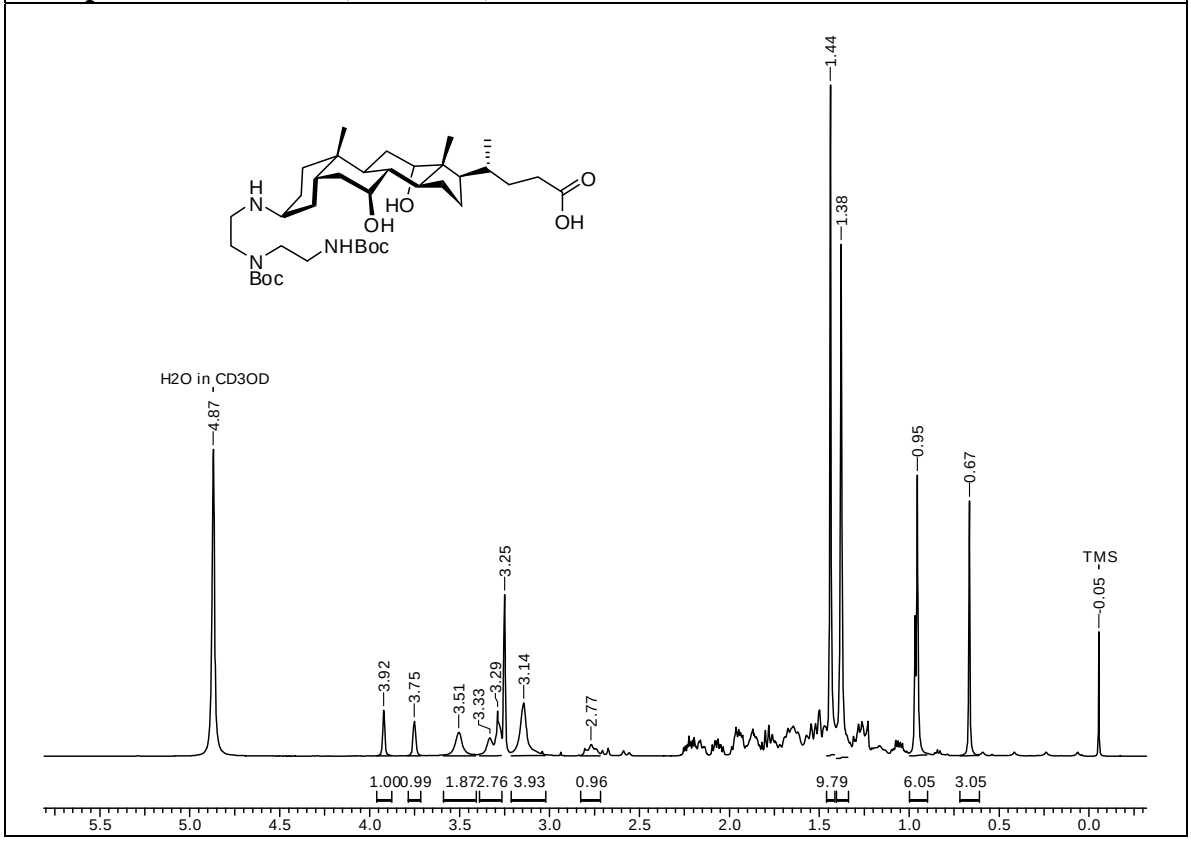
Compound 84: ^{1}H NMR, 500 MHz, CD_3OD



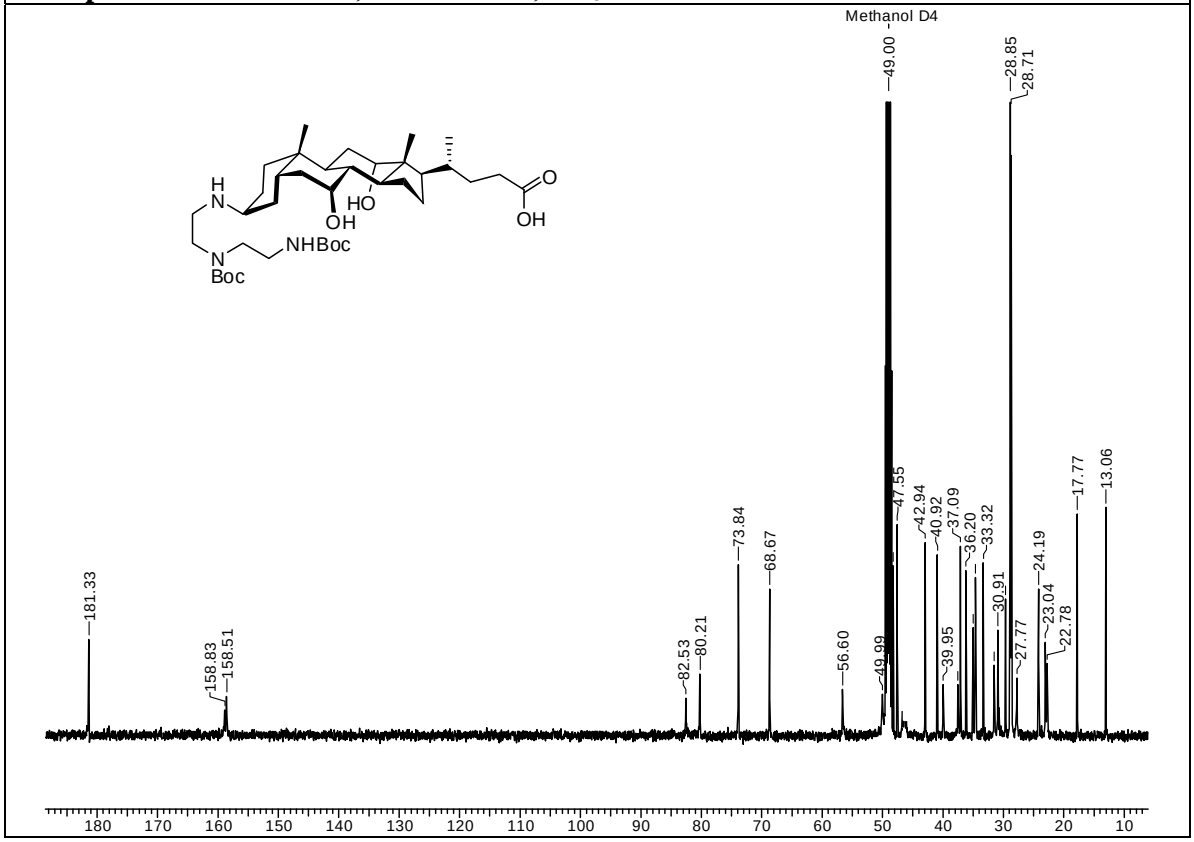
Compound 84: ^{13}C NMR, 125.76 MHz, CD_3OD



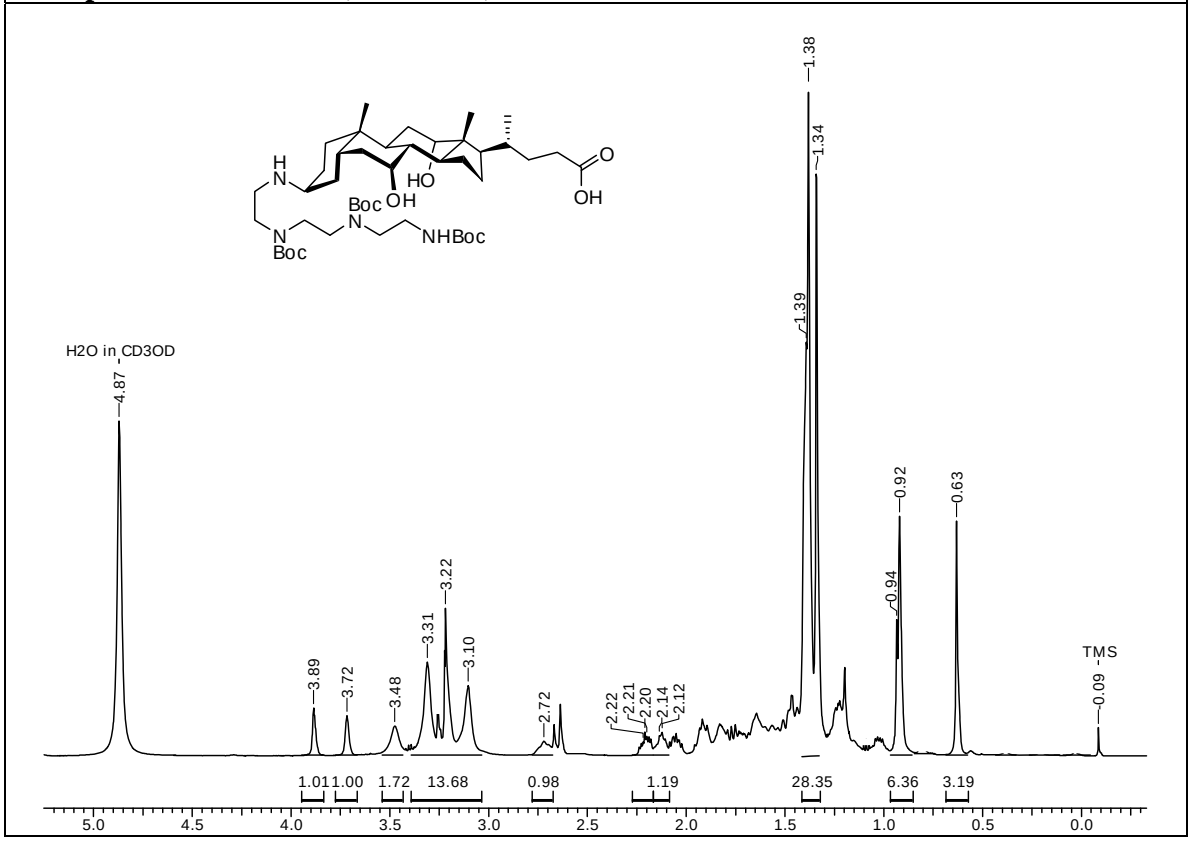
Compound 85: ^{1}H NMR, 500 MHz, CD_3OD



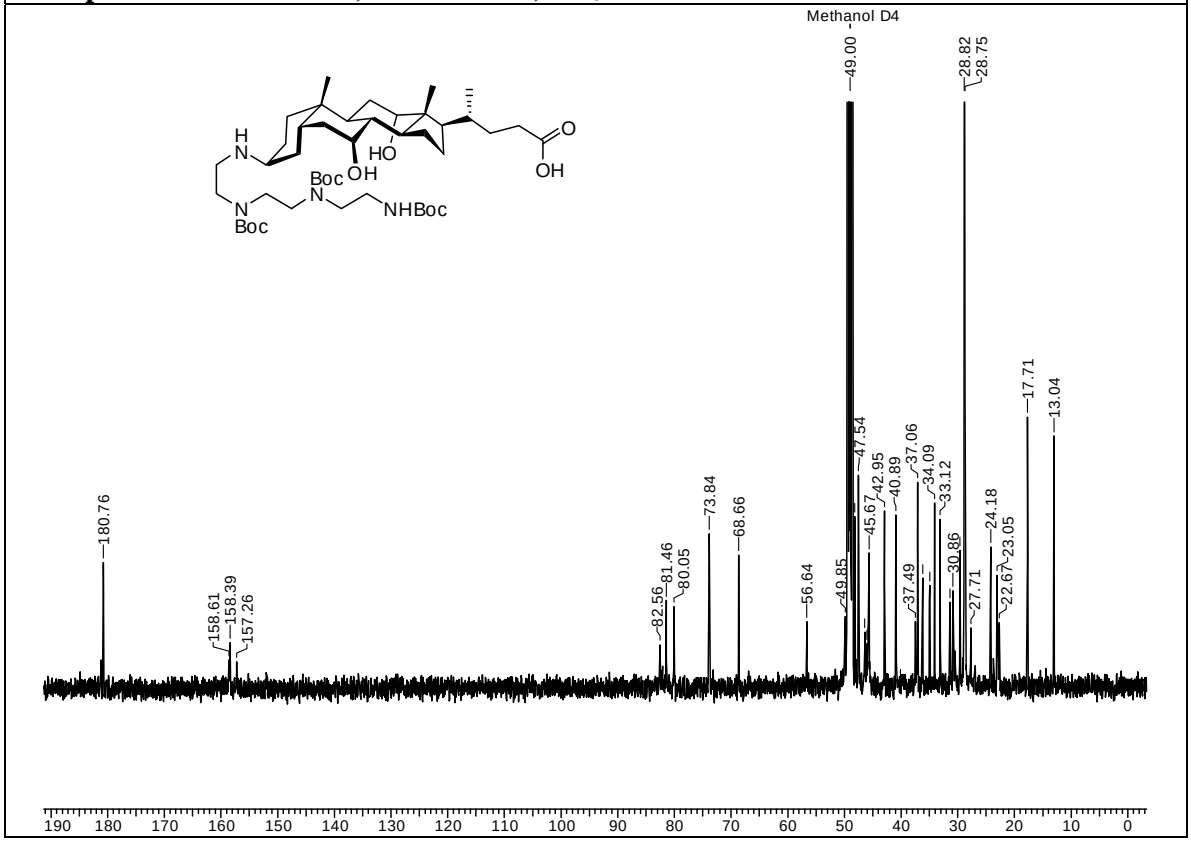
Compound 85: ^{13}C NMR, 125.76 MHz, CD_3OD



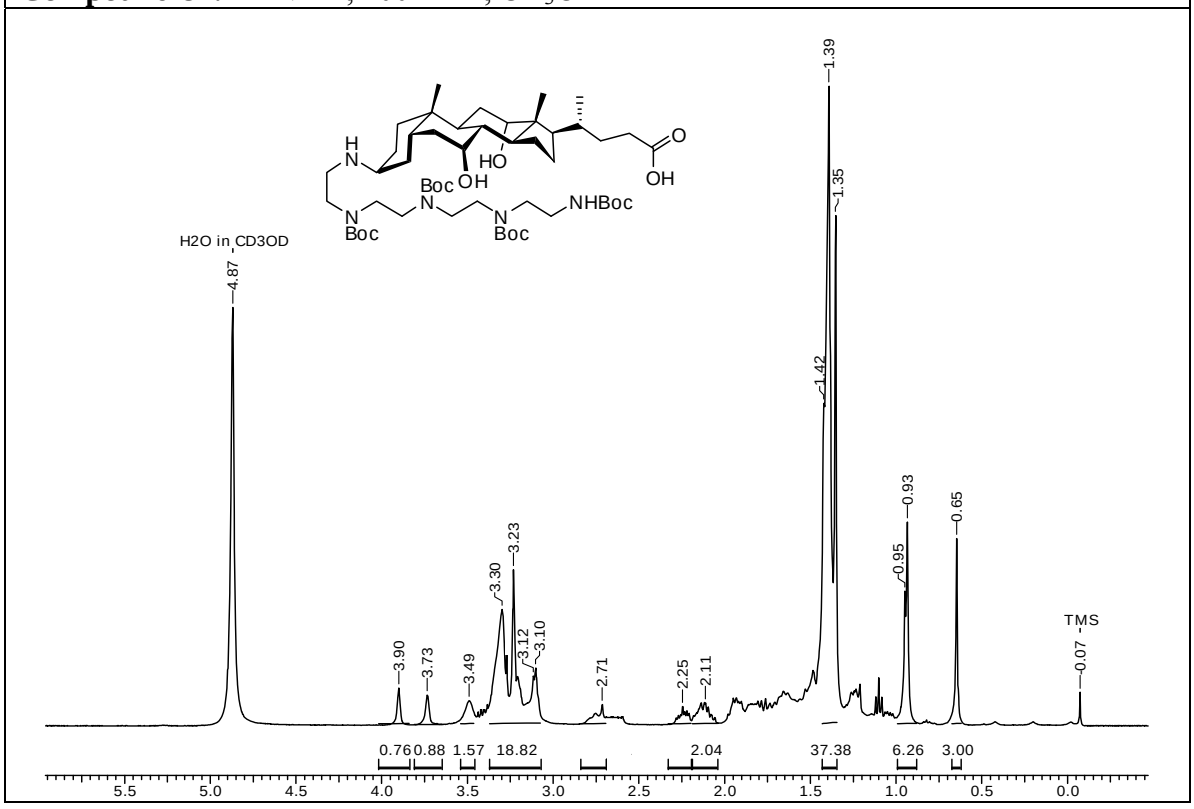
Compound 86: ^{1}H NMR, 500 MHz, CD_3OD



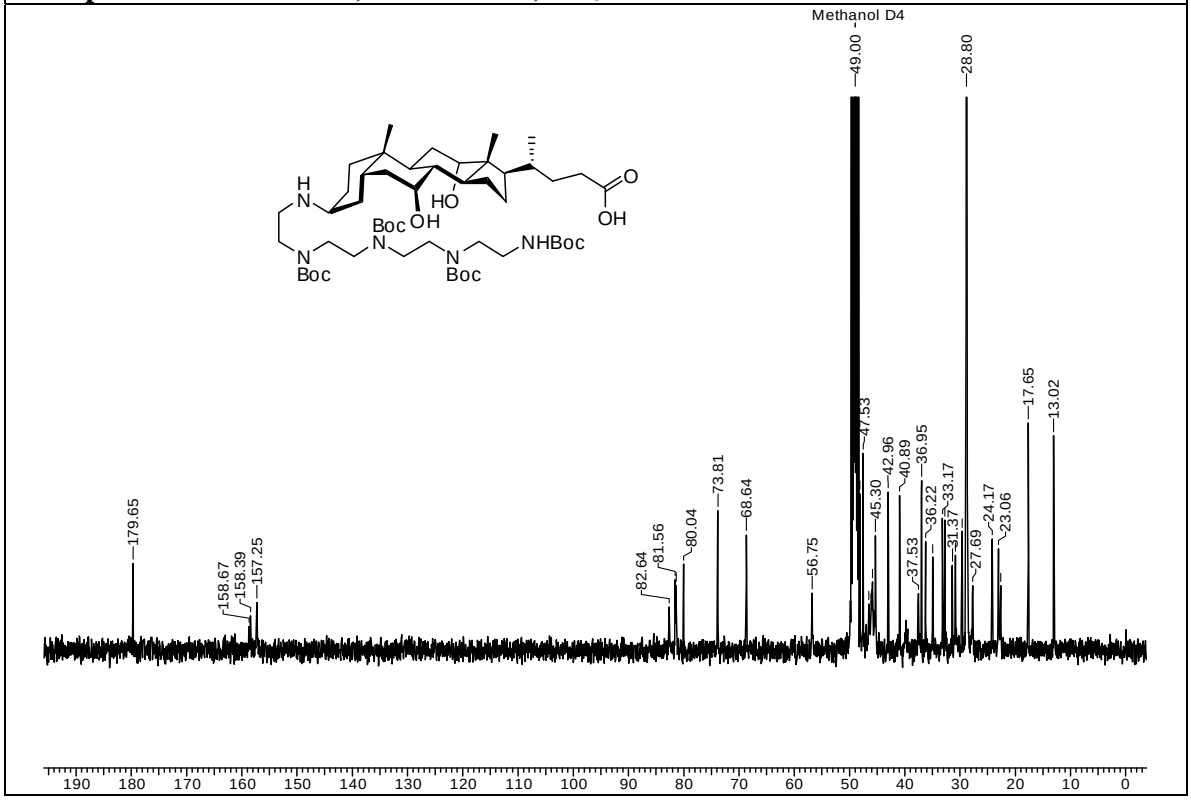
Compound 86: ^{13}C NMR, 125.76 MHz, CD_3OD



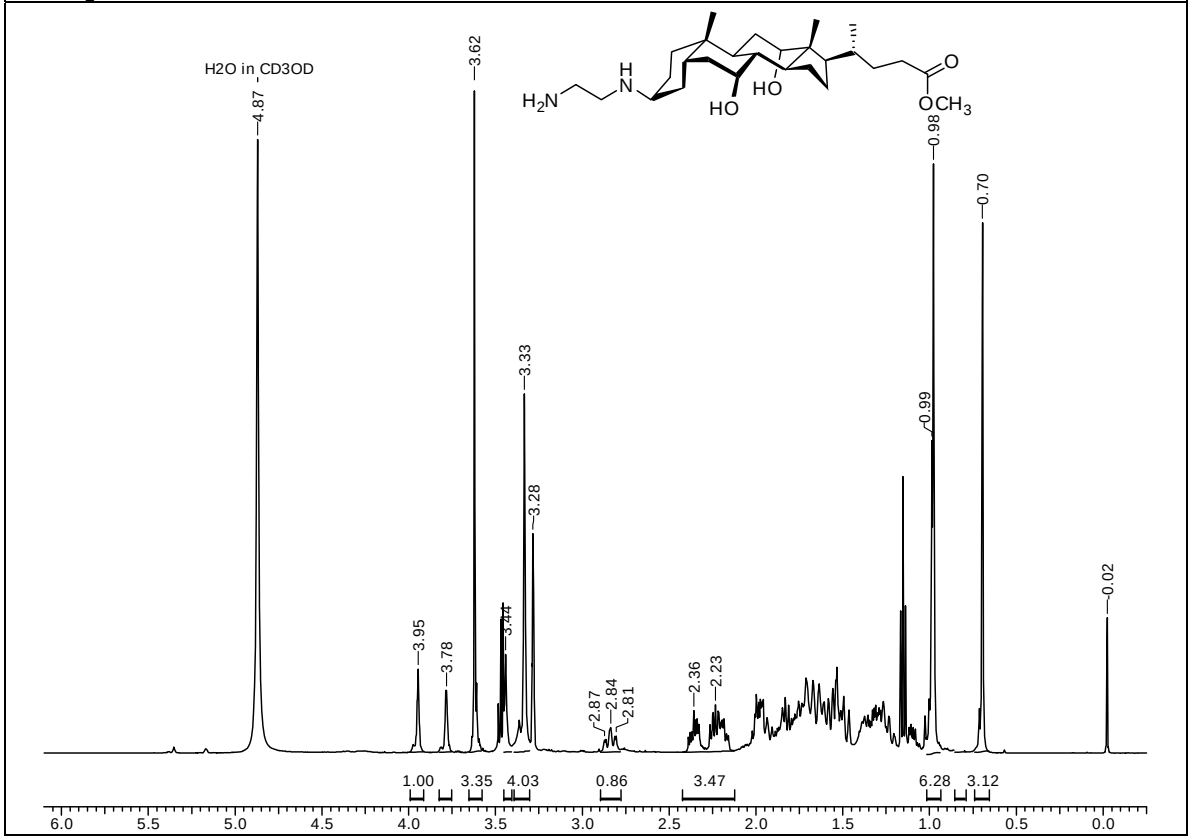
Compound 87: ^{1}H NMR, 400 MHz, CD_3OD



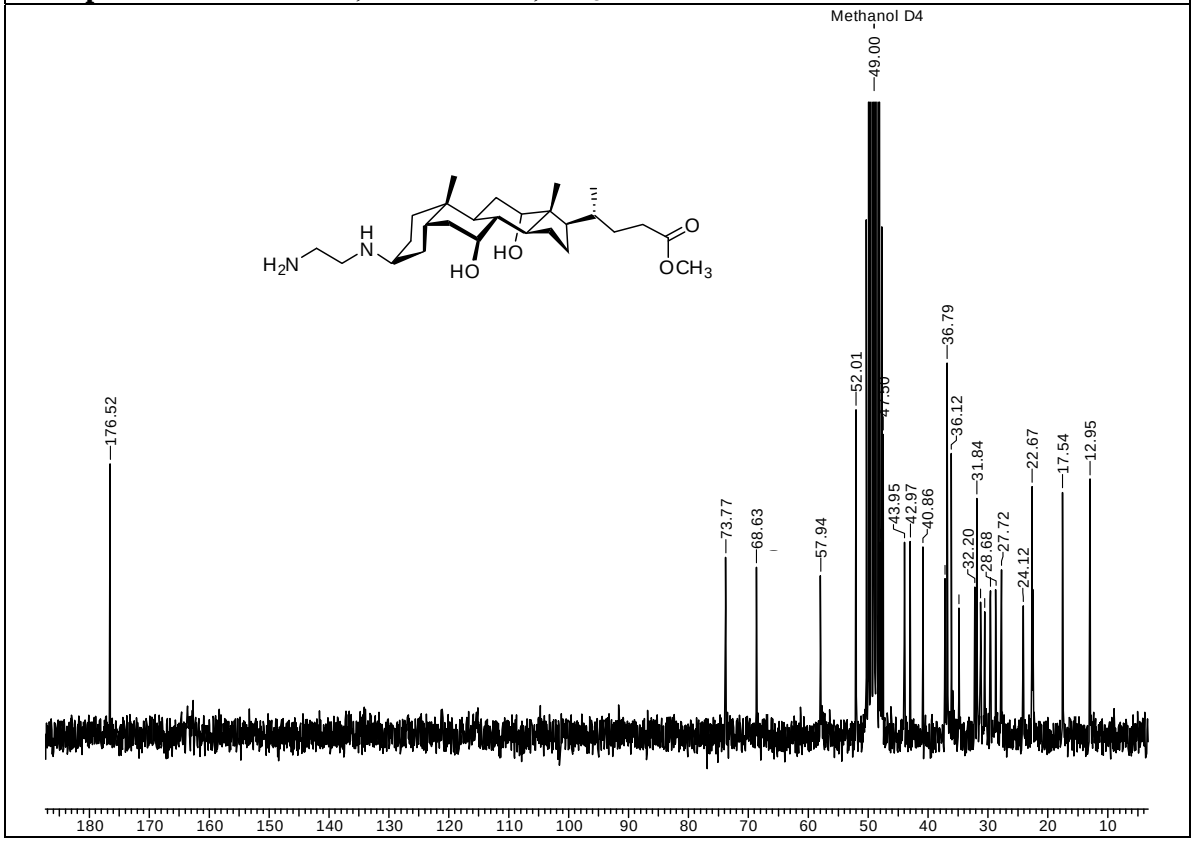
Compound 87: ^{13}C NMR, 100.61 MHz, CD_3OD



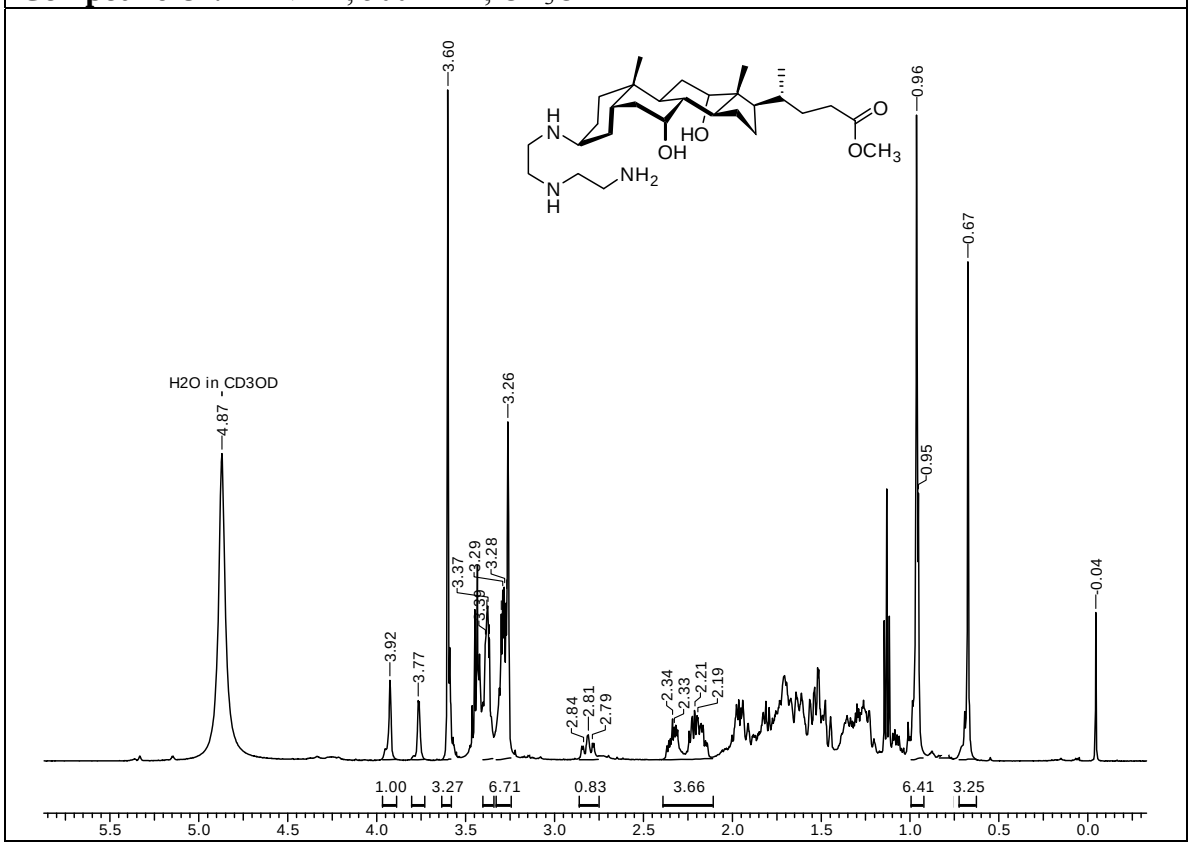
Compound 80: ^{1}H NMR, 500 MHz, CD_3OD



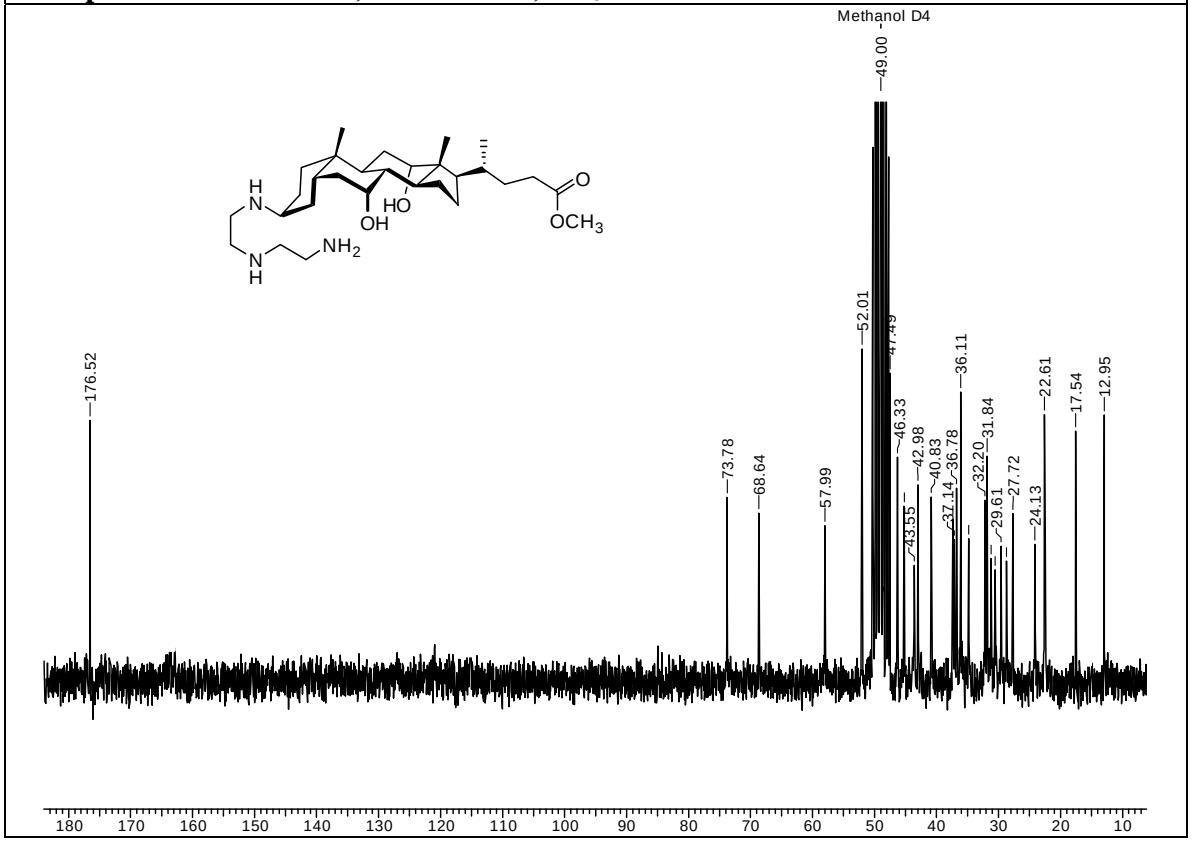
Compound 80: ^{13}C NMR, 125.76 MHz, CD_3OD



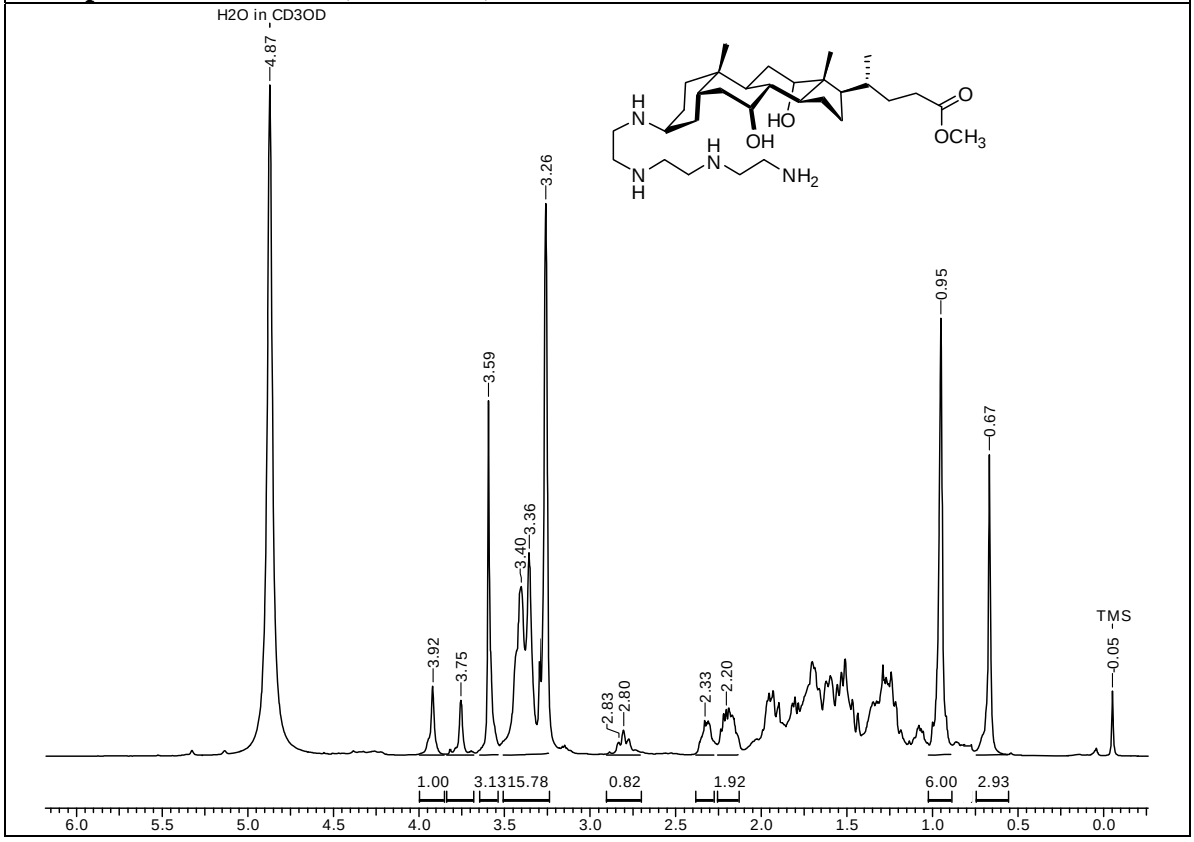
Compound 81: ^{1}H NMR, 500 MHz, CD_3OD



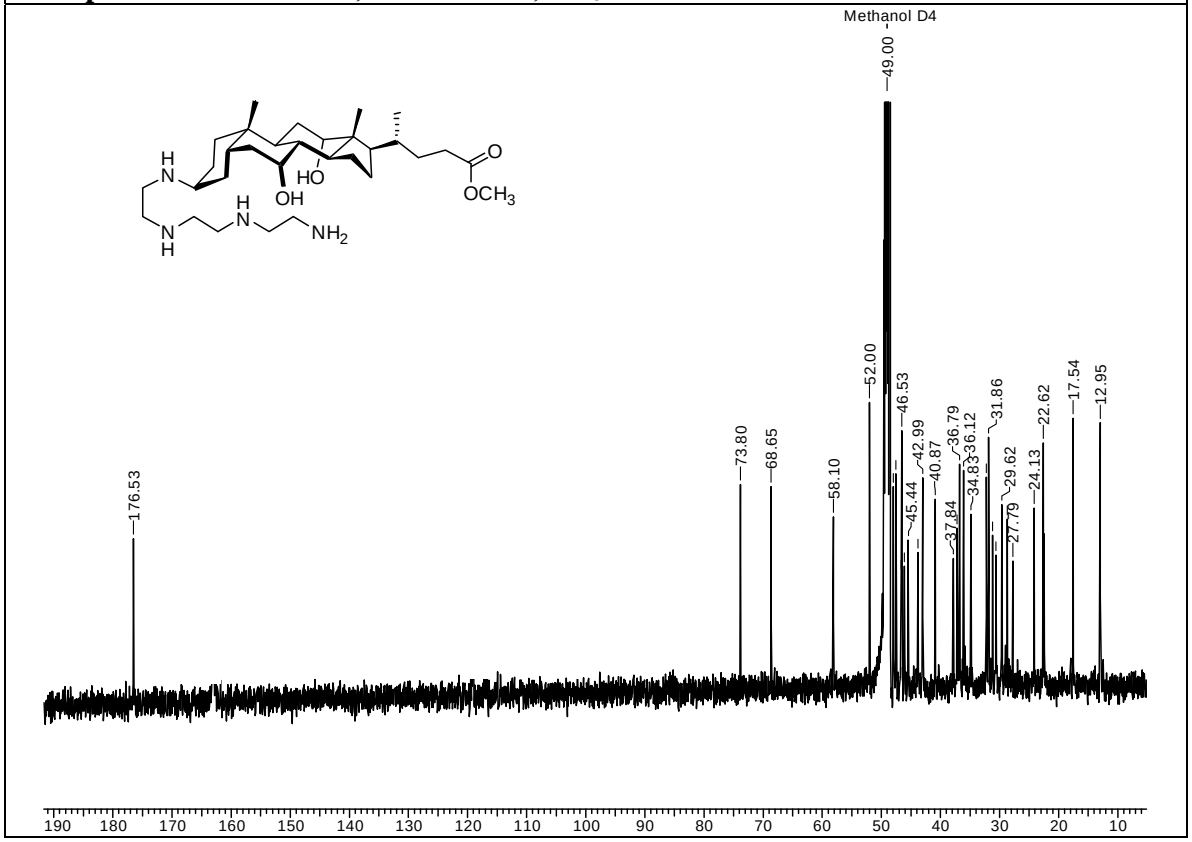
Compound 81: ^{13}C NMR, 125.76 MHz, CD_3OD



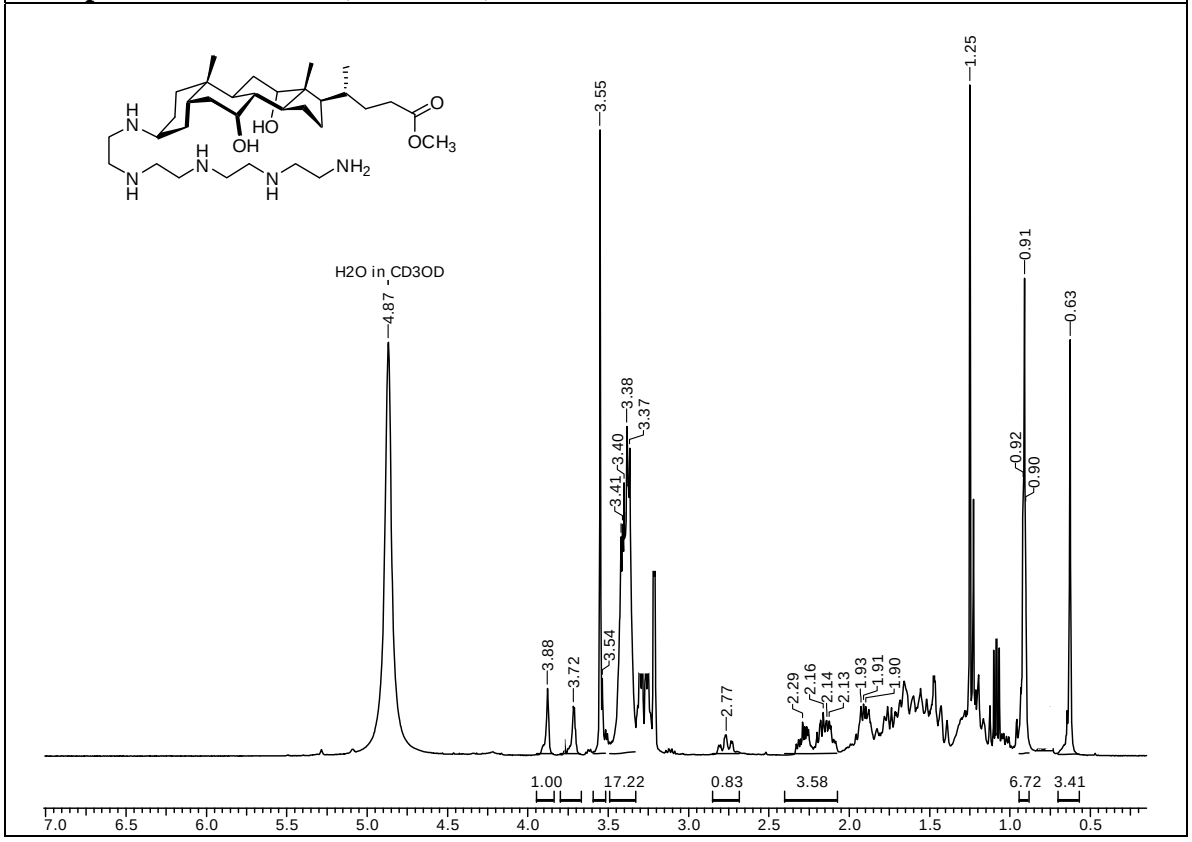
Compound 82: ^{1}H NMR, 500 MHz, CD_3OD



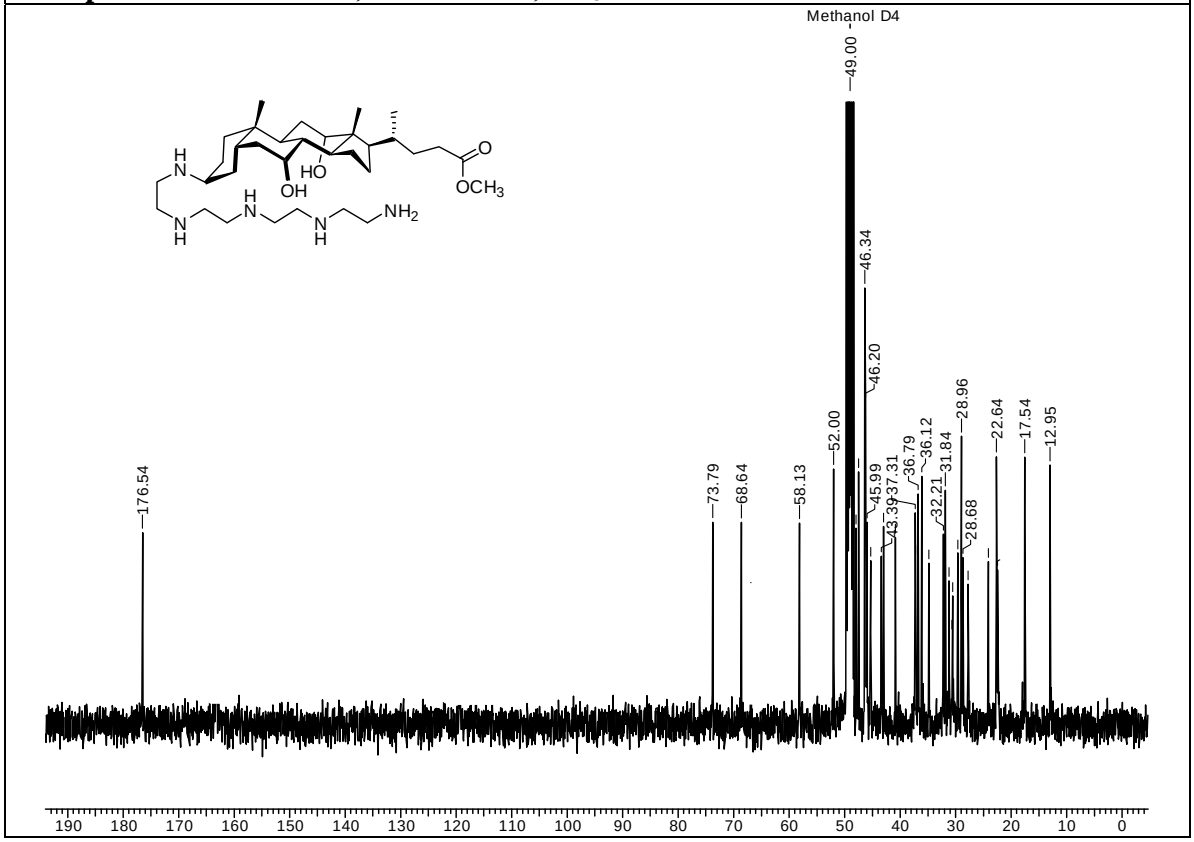
Compound 82: ^{13}C NMR, 125.76 MHz, CD_3OD



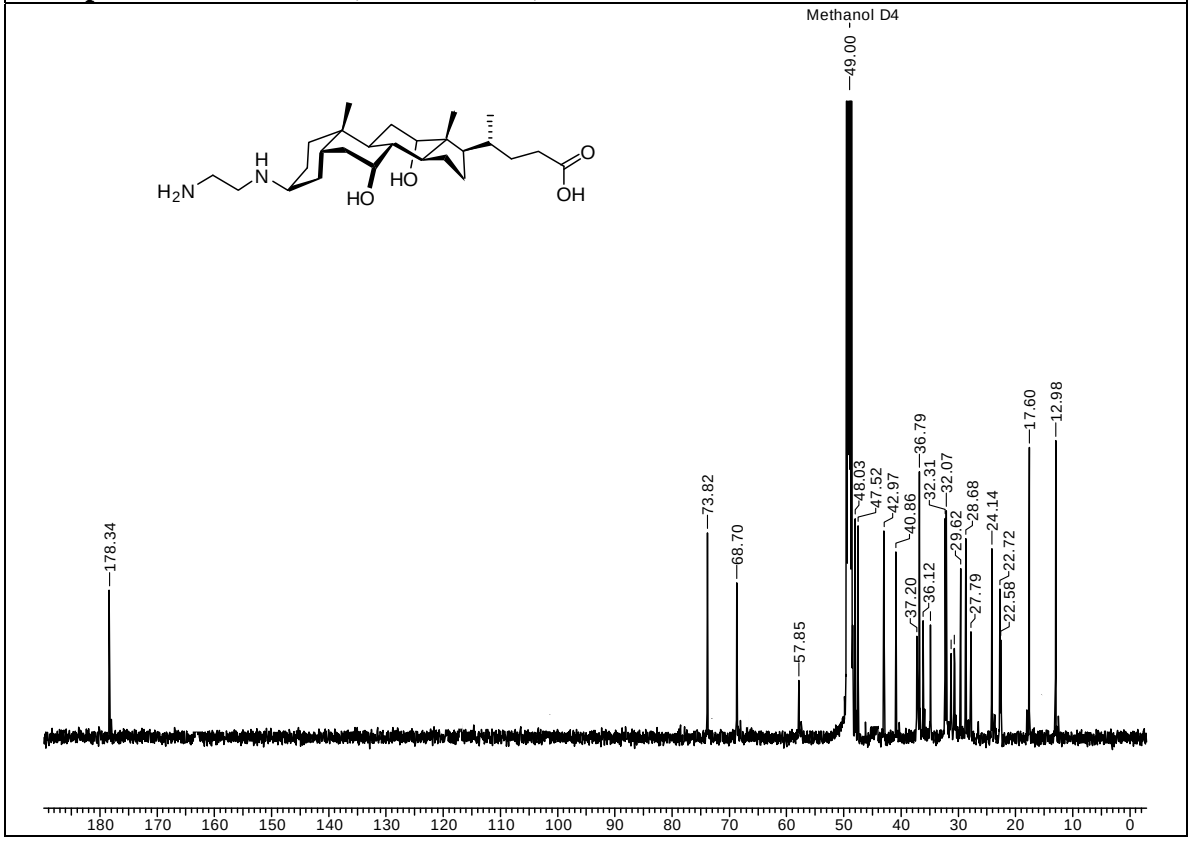
Compound 83: ^{1}H NMR, 400 MHz, CD_3OD



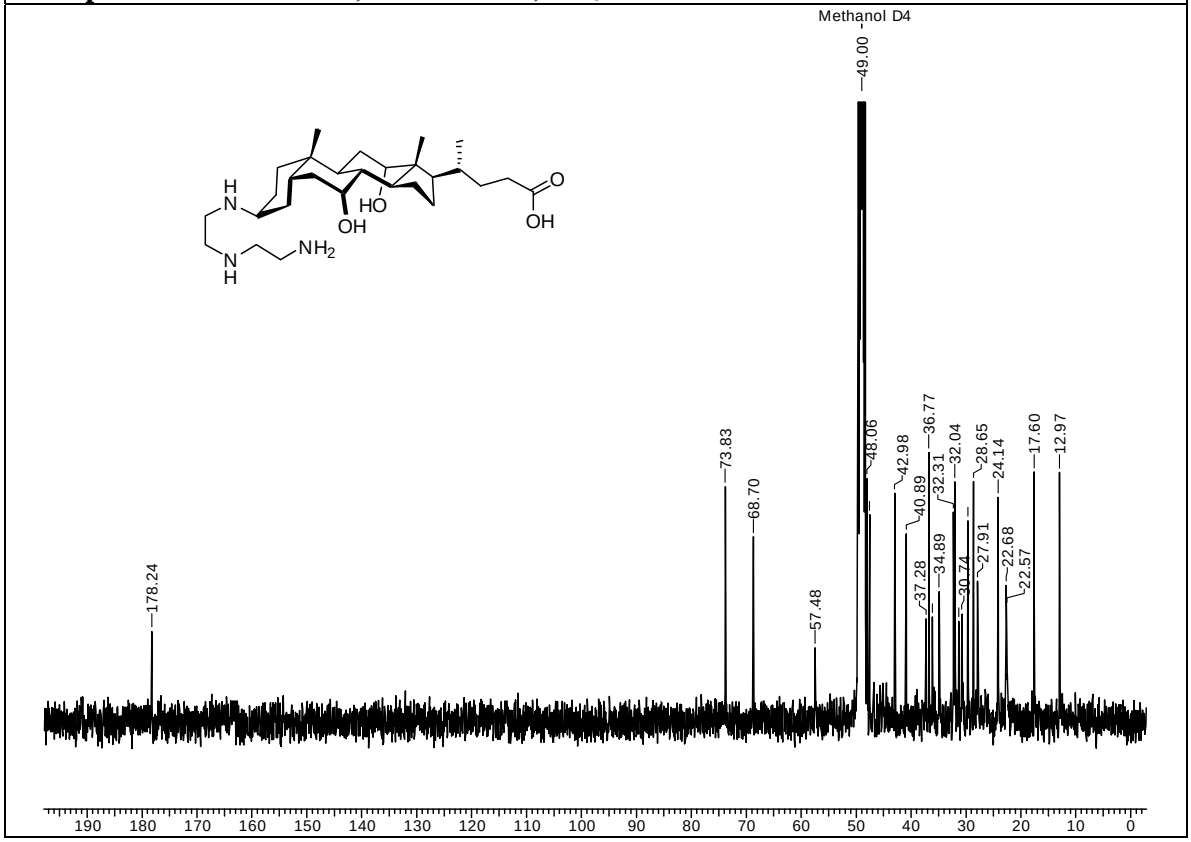
Compound 83: ^{13}C NMR, 100.61 MHz, CD_3OD



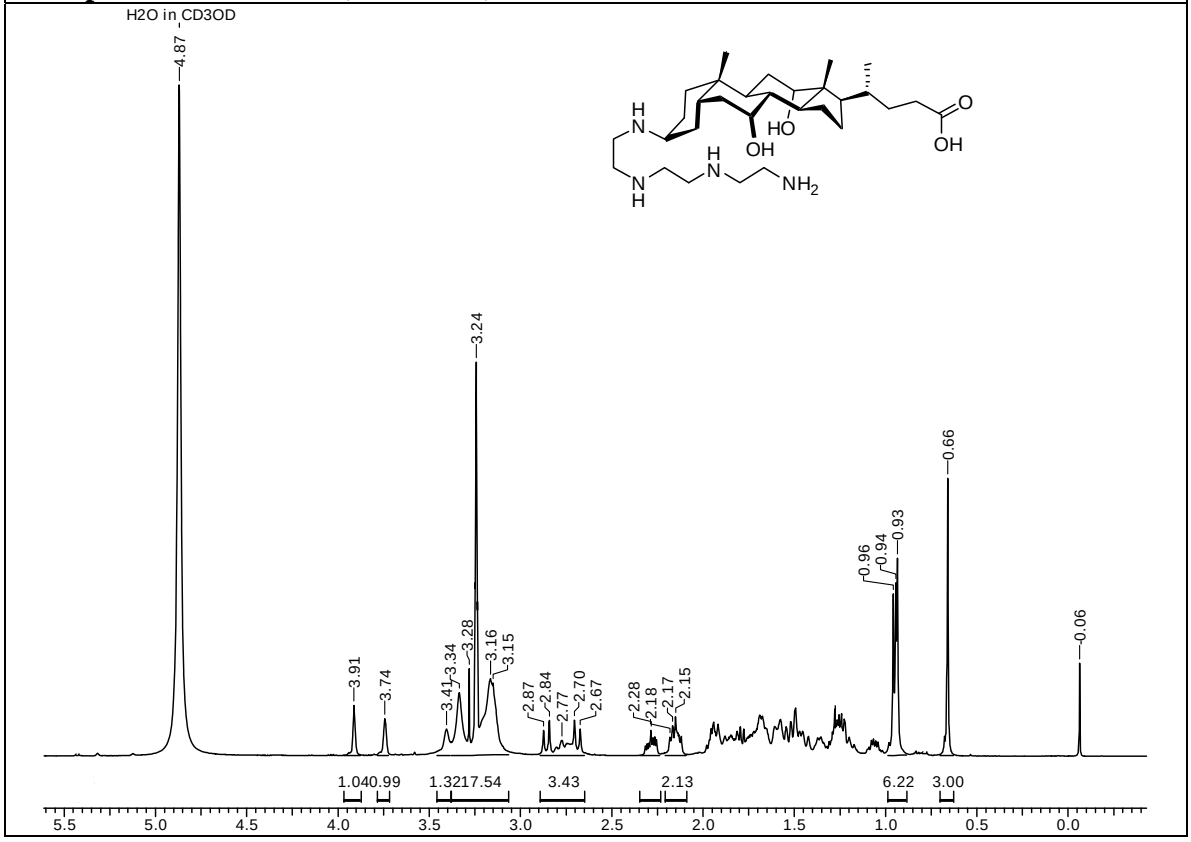
Compound 88: ^{13}C NMR, 125.76 MHz, CD_3OD



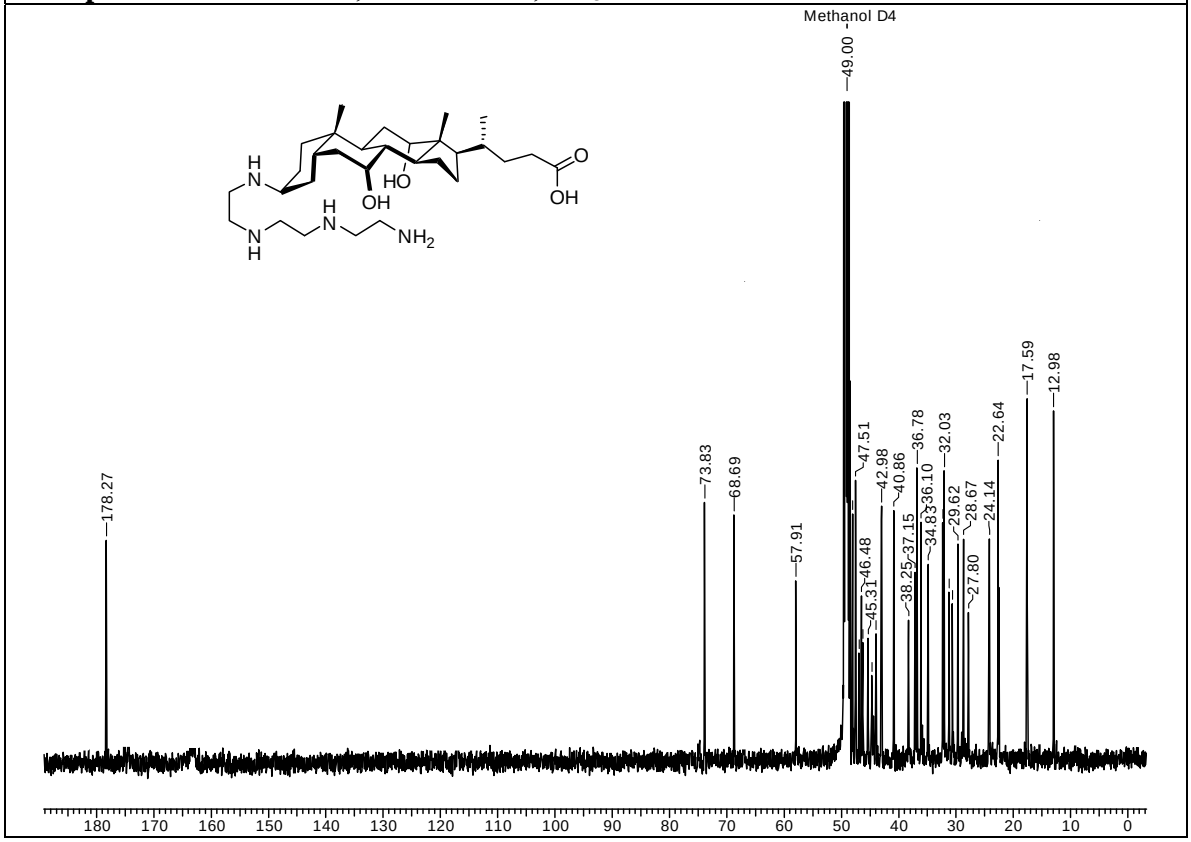
Compound 89: ^{13}C NMR, 125.76 MHz, CD_3OD



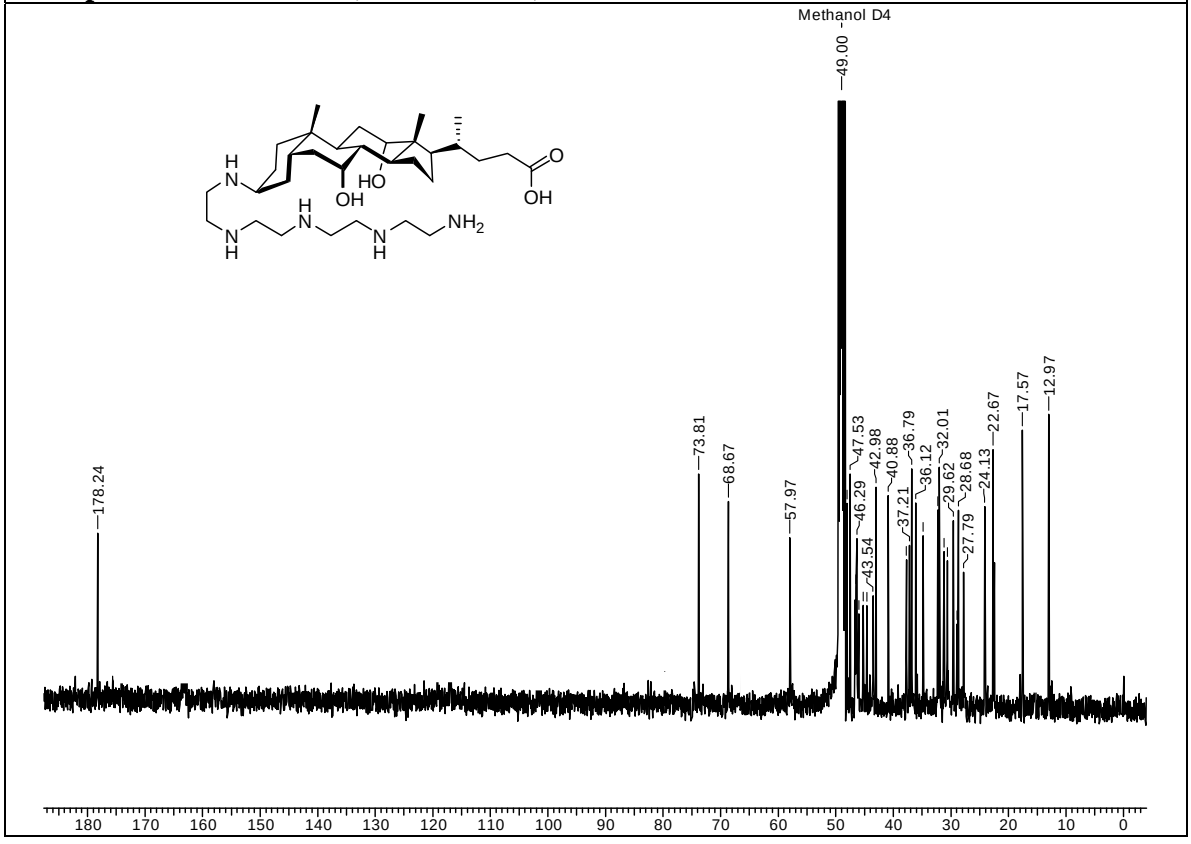
Compound 90: ^{1}H NMR, 500 MHz, CD_3OD



Compound 90: ^{13}C NMR, 125.76 MHz, CD_3OD



Compound 91: ^{13}C NMR, 125.76 MHz, CD_3OD



1.10. References:

1. (a) Zeelen, F.J. In *Advances in Drug Research*, Testa, B.; Meyer, U.A., Eds.; Academic Press: London, **1994**, *25*, 87-102. (b) Zeelen, F.J. *Medicinal Chemistry of Steroids*, Elsevier Science B. V: New York, **1990**. (c) Morgan, B.P; Moynihan, M.S. In *Kirk-Othmer Encyclopedia of Chemical Technology*, Kroschwitz, J.I.; Howe-Grant, M., Eds.; John Wiley & Sons: New York, **1997**, *22*, 851-921.
2. (a) Hancock, R. E. *Annu. Rev. Microbiol.* **1984**, *38*, 237; (b) Labischinski, G.; Bradaczek, H.; Naumann, D.; Rietschel, D. T.; Giesbrecht, P. *J. Bacteriol.* **1985**, *162*, 9; (c) Nikaido, H.; Vaara, M. *Microbiol. Rev.* **1985**, *49*, 1.
3. (a) Naka, K.; Sadownik, A.; Regen, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2278; b) Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961; (b) Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961.
4. (a) Cohen, M. L. *Science* **1992**, *257*, 1050. (b) Neu, H. C. *Science* **1992**, *257*, 1064.
5. (a) Barnett, J.; Ryman, B. E.; Smith, F. *J. Chem. Soc.* **1946**, 528; (b) Loncle, C.; Brunel, J. M.; Vidal, N.; Dherbomez, M. Letourneux, Y. *Eur. J. Med. Chem.* **2004**, *39*, 1067.
6. Sobotka, H. *Physiological Chemistry of the Bile*, Baltimore, Williams and Wilkins Co. 1937.
7. (a) Bellini, A.M.; Quaglio, M.P.; Guarneri, M.; *Eur. J. Med. Chem.* **1983**, *18*, 185. (b) Bellini, A.M.; Quaglio, M.P.; Guarneri, M.; *Eur. J. Med. Chem.* **1983**, *18*, 191.
8. Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N. Jr.; McCrimmon, D.; Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1354.
9. Stone, R. *Science* **1993**, *259*, 1125;

10. Brunel, J. M.; Salmi, C.; Loncle, C.; Vidal, N.; Letourneux, Y. *Curr. Cancer Drug Targets* **2005**, *5*, 267.

11. Rao, M. N.; Shinnar, A. E.; Noecker, L. A.; Chao, T. L.; Feibush, B.; Snyder, B.; Sharkansky, I.; Sarkhian, A.; Zhang, X.; Jones, S. R.; Kinney, W. A.; Zasloff, M. *J. Nat. Prod.* **2000**. *63*, 631-635.

12. (a) Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6138; (b) Merritt, M.; Lanier, M.; Deng, G.; Regen, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8494.

13. Deng, G.; Dewa, T.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 8975.

14. Pechulis, A. D.; Bellevue, F. H.; Cioffi, C. L.; Trapp, S. G.; Fojtik, J. P.; McKitty, A. A.; Kinney, W. A.; Frye, L. L. *J. Org. Chem.* **1995**, *60*, 5121-5126.

15. Brunel, J. M.; Letourneux, Y. *Eur. J. Org. Chem.* **2003**, 3897-3907.

16. Okumura, K.; Nakamura, Y.; Takeuchi, S.; Kato, I.; Fugimoto, Y.; Ikekawa, N. *Chem. Pharm. Bull.* **2003**, *51*, 1177-1182.

17. (a) Zhou, X. -D.; Cai, F.; Zhou, W. -S. *Tetrahedron*, **2002**, *58*, 10293-10299. (b) Zhang, D. -H.; Cai, F.; Zhou, X. -D.; Zhou, W. -S. *Org. Lett.* **2003**, *5*, 3257-3259. (c) Zhang, D. -H.; Cai, F.; Zhou, X. -D.; Zhou, W. -S. *Chinese J. Chem.* **2005**, *23*, 176-181.

18. Khabnadideh, S.; Tan, C.L.; Croft, S.L.; Kendrick, H.; Yardley, V.; Gilbert, I.H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1237.

19. Chitkul, B.; Atrash, B.; Bradley, M. *Tetrahedron Lett.* **2001**, *42*, 6211.

20. Kikuchi, K.; Bernard, E. M.; Sadownik, A.; Regen, S. L.; Armstrong, D. *Antimicrob. Agents Chemother.* **1997**, *41*, 1433.

21. Jones, S. R.; Kinney, W. A.; Zhang, X.; Jones, L. M.; Selinsky, B. S. *Steroids* **1996**, *61*, 565.

22. (a) Kim, H. -S.; Choi, B. -S.; Kwon, K. -C.; Lee, S. -O.; Kwak, H. J.; Lee, C. H. *Bioorg. Med. Chem.* **2000**, *8*, 2059; (b) Khan, S. N.; Bae, S. Y.; Kim, H. S. *Tetrahedron letters* **2005**, *46*, 7675; (c) Khan, S. N.; Kim, B. J.; Kim, H. S. *Bioorg. Med. Chem.* **2007**, *17*, 5139-5142.

23. Shu, Y.; Jones, S. R.; Kinney, W. A.; Selinsky, B. S. *Steroids* **2002**, *67*, 291.

24. (a) Choucair, B.; Dherbomez, M.; Roussakis, C.; Kihel, L. E. *Tetrahedron* **2004**, *60*, 11477; (b) Choucair, B.; Dherbomez, M.; Roussakis, C.; Kihel, L. E. *Bioorg. Med. Chem.* **2004**, *14*, 4213.

25. (a) Loncle, C.; Salmi, C.; Letourneux, Y.; Brunel, J. M. *Tetrahedron* **2007**, *63*, 12968; (b) Salmi, C.; Loncle, C.; Vidal, N.; Letourneux, Y.; Brunel, J. M. *Eur. J. Med. Chem.* **2008**, 4*3*, 540.

26. (a) Savage, P. B.; Li, C. *Exp. Opin. Invest. Drugs*, **2000**, *9*, 263-272 ; (b) Savage, P. B. *Eur. J. Org. Chem*. **2002**, 759-768; (c) Savage, P. B.; Li, C.; Taotafa, .; Ding, B.; Guan, Q. *FEMS Microbio. Lett.* **2002**, *217*, 1-7.

27. Vaara, M.; Vaara, T. *Nature* **1983**, *303*, 526-528.

28. Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961-2962.

29. Schmidt, E. J.; Boswell, S. R.; Walsh, J. P.; Schellenberg, M. M.; Winter, T. W.; Li, C.; Allman, G. W.; Savage, P. B. *J. Antimicrob. Chemother.* **2001**, *47*, 671-674.

30. Merritt, M.; Lanier, M.; Deng, G.; Regen, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8494.

31. Otto, S.; Osifchin, M.; Regen, S.L. *J. Am. Chem. Soc.* **1999**, *121*, 7276.

32. (a) Bandyopadhyay, P.; Janout, V.; Zhang, L.; Regen, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7691. (b) Bandyopadhyay, P.; Janout, V.; Zhang, L. H.; Sawko, J. A.; Regen, S.L. *J. Am. Chem. Soc.* **2000**, *122*, 12888. (c) Bandyopadhyay, P.; Regen, S. L. *J. Am. Chem. Soc.* **2002** *124*, 11254.

33. (a) Zhang, J.; Jing, B.; Regen, S.L. *J. Am. Chem. Soc.* **2003**, *125*, 13984. (b) Janout, V.; Jing, B.; Staina, I. V.; Regen, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 4436. (c) Chen, W.-H.; Regen, S.L. *J. Am. Chem. Soc.* **2005**, *127*, 6538.

34. Lasic, D. D. *Nature* **1992**, *355*, 279.

35. (a) Gale, E.F. In *Macrolide Antibiotics: Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: New York, **1984**, Chapter 11.

36. Stadler, E.; Dedek, P.; Yamashita, K.; Regen, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 6677.

37. (a) Vandenburg, Y. R.; Smith, B. D.; Perez-Payan, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2000**, *122*, 3252. (b) Davis, A. P.; Joos, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143.

38. a) Gokel, G. W.; Ferdiani, R.; Liu, J.; Pajewski, R.; Shabany, R.; Uetrecht, P. H. *Chem. Eur. J.* **2001**, *7*, 33. (b) Fyles, T. M.; Straaten-Nijenhuis, W. F. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D.N., Ed.; Elsevier Science: Oxford, **1996**; Vol. *10*, pp. 53-77.

39. Lee, E. R.; Marshall, J.; Siegel, C. S.; Jiang, C. W.; Yew, N. S.; Nicholas, M. R.; Nietupski, J. B.; Ziegler, R. J.; Lane, M. B.; Wang, K. X. *Hum. Gene Therapy* **1996**, *7*, 1701.

40. Ren, T.; Zhang, G. S.; Liu, F.; Liu, D. X. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 891.

41. Watanabe, M.; Hanashima, S.; Mizushina, Y.; Yoshida, H.; Oshige, M.; Sakaguchi, K.; Sugawara, F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 287.

42. Fujiwara, T.; Hirashima, N.; Hasegawa, S.; Nakanishi, M.; Ohwada, T. *Bioorg. Med. Chem.* **2001**, *9*, 1013.

43. Blagbrough, I. S.; Geall, A. J.; Neal, A. P. *Biochem. Soc. Trans.* **2003**, *31*, 397.

44. Walker, S.; Sofia, M. J.; Kakarla, R.; Kogan, N. A.; Wierichs, L.; Longley, C. B.; Bruker, K.; Axelrod, H. R.; Midha, S.; Babu, S.; Kahne, D. Proc. *Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1585.

45. (a) Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1589; (b) Bavikar, S. N.; Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J.; Shirazi, F.; Deshpande, M. V.; Kadreppa, S.; Chattopadhyay, S. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 5512.

46. Edmund, S.; Petr, D.; Keiji, Y.; Steven, L. R. *J. Am. Chem. Soc.* **1994,** *116*, 6677.

47. (a) Hancock, R.; Falla, T.; Brown, M. *Adv. Microb. Phys.* **1995**, *37*, 135-175; (b) Maloy, W. L.; Kari, U. P. *Biopolymers.* **1995**, *37*, 105; (c) Beamer, L. J.; Carrol, S. F.; Eisenberg, D. *Biochem. Pharm.* **1999**, *57*, 225.

48. Savage, P. B.; Chunhong Li. *Exp. Opin. Invest. Drugs.* **2000**, *9*, 263.

49. (a) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc*. **1994**, *116*, 7955; (b) Davis, A. P. *Chem. Soc. Rev.* **1993**, 243; (c) Davis, A. P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793.

50. (a) Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385; (b) Venkatasan, P.; Cheng, Y.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6955. (c) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7319.

51. Bowe, C. L.; Mokhtarzadeh, L.; Venkatesan, P.; Babu, S.; Axelrod, H. R.; Sofia, M. J.; Kakarla, R.; Chan, Y. Y.; Kim, J. S.; Lee, H. J.; Amidon, G. L.; Choe, S. Y.; Walker, S.; Kahne, D. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12218.

52. Enhsen, A.; Kramer, W.; Wess, G. *Drug Discovery Today* **1998**, *3*, 409.

53. (a) Behr, J. -P.; Demeneix, B.; Loeffler, J. -P.; Perez-Mutul, *J. Proc. Natl. Acad. Sci. U.S.A*. **1989**, *86*, 6982; (b) Donohue, R.; Mazzaglia, A.; Ravoo, B. J.; Darcy, R. *Chem. Commun*. **2002**, 2864; (c) Byk, G.; Dubertret, C.; Escriou, V.; Frederic, M.; Jaslin, G.; Rangara, R.; Pitard, B.; Crouzet, J.; Wils, P.; Schwartz, B.; Scherman, D. *J. Med. Chem*. **1998**, *41*, 224; (d) Ewert, K.; Ahmed, A.; Evans, H. M.; Schmidt, H. -W.; Safinya, C. R. *J. Med. Chem*. **2002**, *45*, 5023; (e) Nakaramura, E.; Isobe, H.; Tomita, N.; Sawamura, M.; Jinno, S.; Okayama, H. *Angew. Chem., Int. Ed*. **2000**, *39*, 4254; (f) McGeorge, C.; Perrin, C.; Monck, M.; Camilleri, P.; Kirby, A. J. *J. Am. Chem. Soc*. **2001**, *123*, 6215.

54. Geall, A. J.; Blagbrough, i. S. *Tetrahedron* **2000**, 56, 2249.

55. Choucair, B.; Dherbomez, M.; Roussakis, C.; Kihel, L. E. *Tetrahedron* **2004**, *60*, 11477.

56. (a) Zhang, D. H.; Cai, F.; Zhou, X-D.; Zhou, W-S. *Org. Lett.* **2003**, 5, 3257; (b) Tserng, K. *J. of Lipid Research* **1978**, *19*, 501-504.

57. (a) Hazra, B. G.; Pore, V. S.; Dey, S. K.; Datta, S.; Darokar, P. M.; Saikia, D.; Khanuja, S. P. S.; Thakur, A. P. *Bioorg. Med.Chem Lett.* **2004,** *14*, 773; (b) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Chavan, P. S.; Deashpande, M. V. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 2043; (c) Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Deashpande, M. V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R. *Org. Biomol Chem.* **2008**, *6*, 3823.

58. (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, 1997; (b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming

Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, 1998; (c) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, 5th ed.; NCCLS: Villanova, PA, 2000; M7–A5.

59. (a) Rapoport, H.; Reist, H. N. *J. Am. Chem. Soc.* **1955**, *77*, 480; (b) Reagents for Organic synthesis, Fieser & Fieser, Volume p-363.
60. Tserng, K. -Y. *Journal of lipid research* **1978**, *19,* 501-504.
61. Geldern, T. W.; Tu, N.; Kym, P. R.; Link, J. T.; Jae, H. -S.; Lai, C.; Apelqvist, T.; Rhonnstad, P.; Hagberg, L.; Koehler, K.; Grynfarb, M.; Goos-Nilsson, A.; Sandberg, J.; Österlund, M.; Barkhem, T.; Höglund, M.; Wang, J.; Fung, S.; Wilcox, D.; Nguyen, P.; Jakob, C.; Hutchins, C.; Färnegårdh, M.; Kauppi, B.; Öhman, L.; Jacobson, P. B. *J. Med. Chem.* **2004**, *47*, 4213-4230.
62. Ryu, E-H.; Ellern, A.; Zhao, Y. *Tetrahedron* **2006**, *62*, 6808.
63. Anelli, P. L.; Lattuada, L.; Uggeri, F. *Synth. Commun*. **1998**, *28*, 109-117.

CHAPTER - 2

Section A

Design, Synthesis and Bioevaluation of Novel Steroid Polypeptide Conjugates

2A.1. Abstract

A generic structure wherein fine-tuning of the molecular amphiphilicity is possible, has been designed based on squalamine and polymyxin B (PMB). Tetrapeptide derived from glycine and β-alanine was attached at the C-3β position of the modified cholic acid to realize novel linear tetrapeptide-linked cholic acid derivatives. All the synthesized compounds were tested against a wide variety of microorganisms (Gram negative bacteria, Gram positive bacteria and fungi) and their cytotoxicity was evaluated against human embryonic kidney (HEK293) and human mammary adenocarcinoma (MCF-7) cell lines. While relatively inactive by themselves, these compounds interact synergistically with antibiotics such as fluconazole and erythromycin to inhibit growth of fungi and bacteria respectively.

2A.2. Introduction

The dramatically rising prevalence of multidrug-resistant microbial infections over the past few decades has become a serious health problem. In order to circumvent this increasingly serious situation, there is an urgent need to develop new antimicrobial therapeutics having high efficacy and low toxicity in resistant strains of infectious organisms. The class of membrane-disrupting drugs is ideal as antimicrobial agents because microbes are unlikely to develop resistance to them.[1,2] As the outer membrane or cell wall of microbes provides a protective barrier against many types of antibiotics,[3] amphipathic molecules that can act synergistically with various hydrophobic antibiotics as outer membrane permeabilizers may represent a new class of antibiotic agents.

2A.3. Literature Survey on Steroid Polyamide Conjugates

Endogenous bioactive peptides and steroids play important roles in the normal physiology or disease process of mammalian system. Biologically active peptides are recognized to have significant therapeutic potential.[4] Introduction of the aminoacid or peptide to the steroid backbone offers a combination of a hydrophilic functional moiety as well as a hydrophobic carrier in a same molecule and therefore represents as an important class of molecules for drug design and development. Steroid-amino acid conjugates include such a class of compounds in which the amino acids are linked with steroids through amide or ester bonds. This class of conjugates covers a spectrum of important molecules in nature, such as bufetoxin **1**, a 3-arginyl derived steroid product isolated[5] from the chinese hoptoad (Figure 1). Cholyl glycine **2** and cholyl taurine **3**, which exist in the bile of animals and contains a glycyl or a taurinyl group attached to steroid. A starfish steroid, carolisterol C **4** in which a 24-carboxylic acid functionality is linked via an amide bond to D-cysteinolic acid was isolated from the polar extracts of the starfish *Styracaster caroli*

by Minale *et al*.[6] In view of the anti-HIV activity reported for polar sulphated sterols,[7] carolisterol C was tested in the NCI's primary anti-HIV screen and showed no protection against the cytopathic effects of HIV-1. Triseramide **5** is a new steroid conjugate from the starfish *Astropecten triseriatus*[8] and Myxodermoside A is a novel marine polyhydroxylated steroid from the starfish *Myxoderma platyacanthum* (Figure 1).[9]

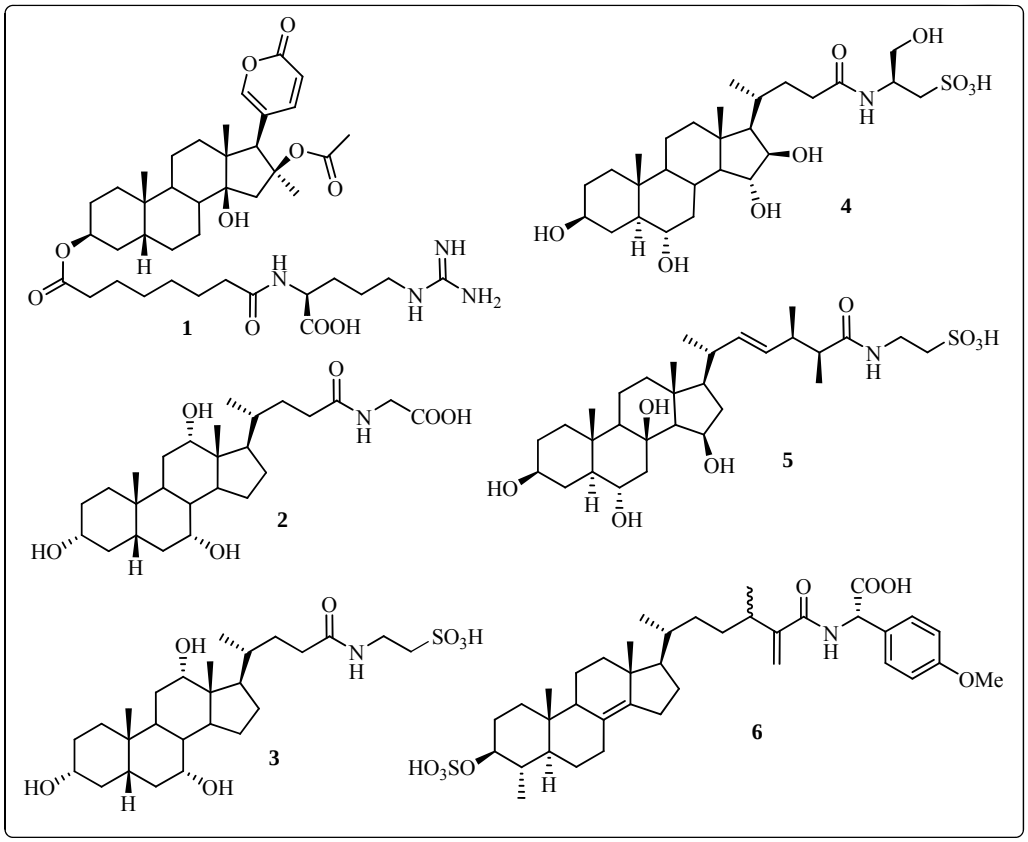


Figure 1

Myxodermoside A has been examined for the effects on the development of fertilized sea urchin eggs and showed a modest activity in comparison with other pentaglycosides.[10] Polymastiamide A **6**, is a tyrosine conjugated steroid analogue isolated from the Norwegion marine sponge *Polymastia boletiformis.*[11] It is an antimicrobial metabolite, and involves a linkage of steroid and a nonprotein aminoacid. It is the first example of a new type of marine natural product that is formed by combination of steroid and α-amino acid. It exhibited *in vitro* antimicrobial activity against various human and plant pathogens. These types of compounds are obtained in nature having various biological

properties and have been found to play a diversity of critical roles in a large number of organisms.

Virtually all-natural bile salts are conjugates of a bile acid with glycine or taurine. In certain instances small amounts of bile salts were found which were conjugates of ornithine, arginine and lysine.[12] Isolation of these conjugated acids from bile is troublesome and uncertain,[13] and pure conjugates required for experimentation are best prepared by synthetic conjugation of the components. Bondi and Muller[14] for the first time converted cholic acid through the ester and the acid hydrazide into the azide, which was then coupled with glycine or taurine in an alkaline medium. Bellini *et al* reported[15] number of amino acid conjugates of cholic acid other than with glycine or taurine. A number of them showed antimicrobial activity. To investigate the substrate specificity of bile acid transport across the liver *in vivo*, Ballatore *et al* have synthesized[16] and studied cholic acid conjugates of variety of amino acids. Considering both biological and chemical interest of steroidal polypeptides, Agarwal *et al* reported[17] steroids consisting of a repeating amino acid sequences. Based on the assumption that synthetic steroidal peptides might alter, or otherwise interfere with, established hormone production. Pettit *et al* synthesized[18] 3β-hydroxy-17β-(L-prolyl-L-prolyl) amino-5α-androstane and 3β-acetoxy-17β-(L-argenyl-L-argenyl-L-prolyl) amino-5α-androstane.

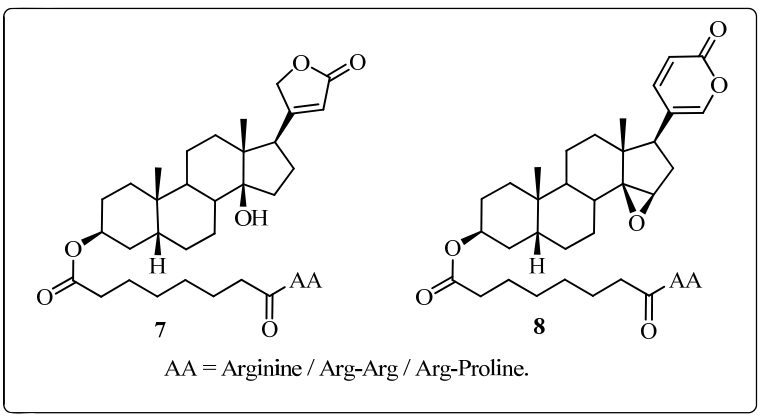


Figure 2

In order to examine the physiological activity, Shimada *et al* have synthesized the arginine linked cardiotonic steroids **7** and **8** as bufotoxin analogues from digitogenin and resibufogenin (Figure 2).[19] Novel bile acid-amino acid conjugates e.g. **9** (Figure 3) were synthesized in which the amide bond was reversed from its normal configuration.[20] These structural isomers of the β-alanyl conjugates of cholic acid and ursodeoxycholic acid were synthesized by reaction of succinic anhydride with the 24-nor-23-amine derivatives of cholic acid and ursodeoxycholic acid. The chemical and physical properties of these reverse amide conjugated bile acid analogues were compared with those of the normal glycine and β-alanine conjugates.

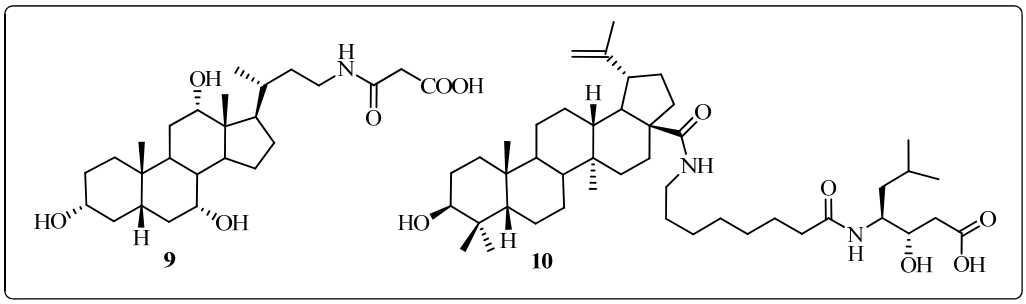


Figure 3

In recent years a wide variety of such steroid derivatives conjugated with amino acids or peptides have been prepared with various purposes. Mayaux *et al* synthesized,[21] N'-{N-[3β-hydroxyl-20(29)-ene-28-oyl]-8-aminooctanoyl}-L-statin **10** (Figure 3), by a five-step procedure starting from betulinic acid. This compound has been postulated to interfere specifically with virus-cell fusion and inhibited HIV replication at a concentration as low as 0.02 μg/mL.

The metabolism and intestinal absorption of bile salts was studied using cholic acid conjugates **11** (Figure 4) with small peptides of two to six amino acids.[22] To investigate the ability of the human intestinal bile acid transporter to transport cholic acid conjugates with potential HIV-1 protease inhibitory activity, cholic acid was conjugated at the 24

position of the sterol nucleus with various amino acids and amino acid analogues such as **12**.[23] In this study, one amino acid analogue-cholic acid conjugate showed HIV-1 protease inhibitory activity.

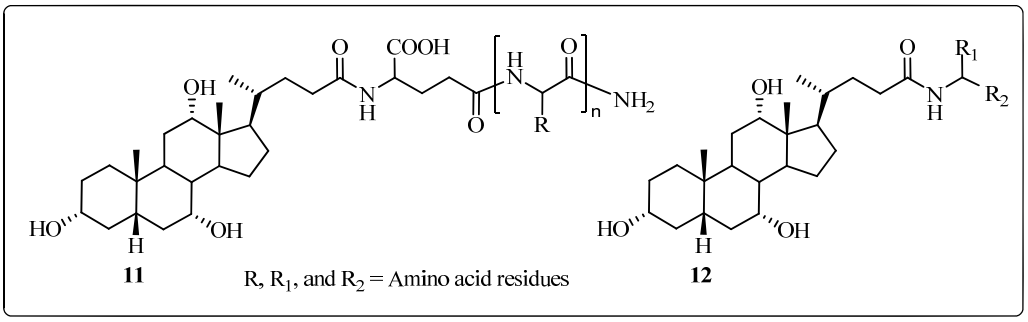


Figure 4

Kramer *et al* synthesized various peptide conjugates of modified bile acids,[24] (i) to investigate whether the hepatic and the intestinal bile acid transport system as well as the intestinal H^+/oligopeptide transporter can be used in drug therapy, (ii) to improve the membrane permeability and intestinal absorption of peptide drugs and (iii) to target a drug to the liver and the bilary system to obtain liver specific drugs, Amafalone **13** (Figure 5) (Am, 3α-amino-2β-hydroxy-5α-androstan-17-one) possessed interesting antiarrhythmic activity in animal models[25] and was developed to the stage of clinical testing, but did not become commercially available because it was deemed not to have sufficient oral bioavailability.[26] In China, Fang and co-workers,[27] in an attempt to develop aminosteroid derivatives with more desirable antiarrythmic properties, prepared a number of amide and N-alkyl derivatives of amafalone. These compounds exhibited lower toxicity and retained antiarrhythmic activity. Because of interest in peptides as potential therapeutic agents Mokotoff *et al* reported[28] the synthesis of peptidyl derivatives of the aminosteroid, amafalone. Six analogues were synthesized: the hydrochloride salts of Gly-Am **14**, Ala-gly-Am **15**, D-Ala-Gly-Am **16**, Pro-Am **17**, Pro-Pro-Am **18**, and D-Ala-Pro-Am **19**. Peptidyl aminosteroids **14**, **17**, **18** and **19** when administered to rat intravenously had protective antiarrythmic effects similar to those of amafalone. The oral route

observed less marked protection with **17**, in comparison to amafalone, while **18** and **19** were inactive.

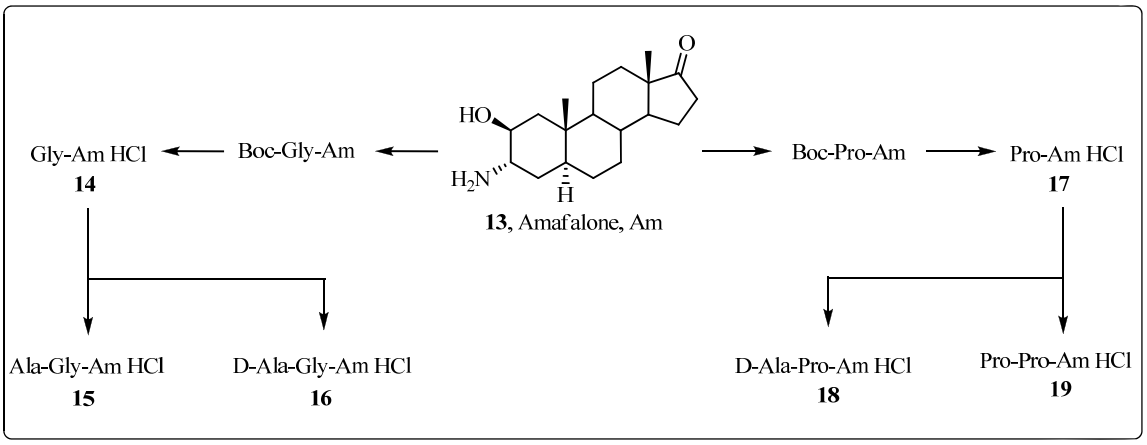


Figure 5

The phenomenon that the steroids enhanced the effects of the peptides through increasing their receptor numbers was named 'permissive action'.[29] Based on this concept, the urotoxins (Glu-Asp-Gly-OH, His-Gly-Glu-OH, His-Gly-Lys-OH, and His-Gly-Lys-$NHNH_2$) were introduced into the convenient sites of hydrocortisone and prednisolone via the amidation or condensation reactions to form the corresponding linkers.[30] The results suggested that the linkers of the steroids and peptides may simulate the 'permissive action' and this kind of conjugation of steroids and peptides may provide a special modification for steroids and oligopeptides. Similar such 'permissive action' was observed by the same group.[31]

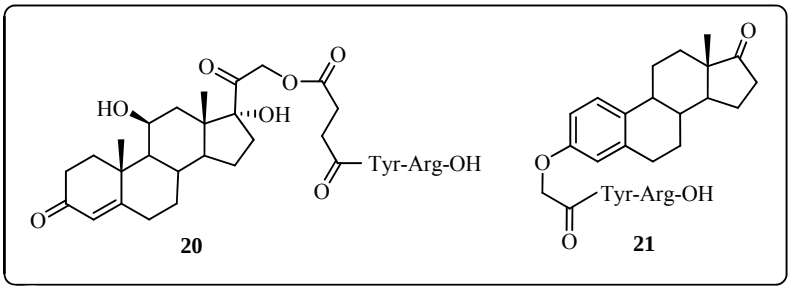


Figure 6

For this purpose kyotorphin (KTP, Tyr-ArgOH) was coupled with hydrocortisone and estrone to furnish the hybrid molecules **20** and **21** (Figure 6). The analgesic activities of

the corresponding hybrids were investigated using the tail flick test. In order to produce potential inhibitors of type 3, 17β-hydroxysteroid dehydrogenase (17β-HSD), a key steroidogenic enzyme, Poirier *et al* performed solid-phase synthesis of model libraries of 3β-peptido-3α-hydroxy-5α-androstan-17-ones. One of them, 3β-(N-heptanoyl)-L-phenylalanine-L-leucine-aminomethyl)-3α-hydroxy-5α-androstan-17-one inhibited the enzyme with an IC_{50} value of 117 nM, which is twice as potent as the natural substrate Δ^4-dione.[32]

A series of bile acid derivatives with variety of amino acids,[33] amino alcohols[34] and phenanthroline[35] coupled via an amide bond were reported. These conjugates form small micells in aqueous solutions and were found to behave as novel organogelators, forming stable, transperant and thermoreversible gels in aromatic solvents. Thiemann *et al* in their own studies on radiolabelled estradiol derivatives have become interested to find new connective approaches to radiolabelled estrane derivatives and to peptide estrane conjugates. For this they have developed a process for the synthesis of an estra-1,3,5(10),16-tetraenol [17,16-*e*] pyrimidine **23** from estrone **22** (Figure 7).

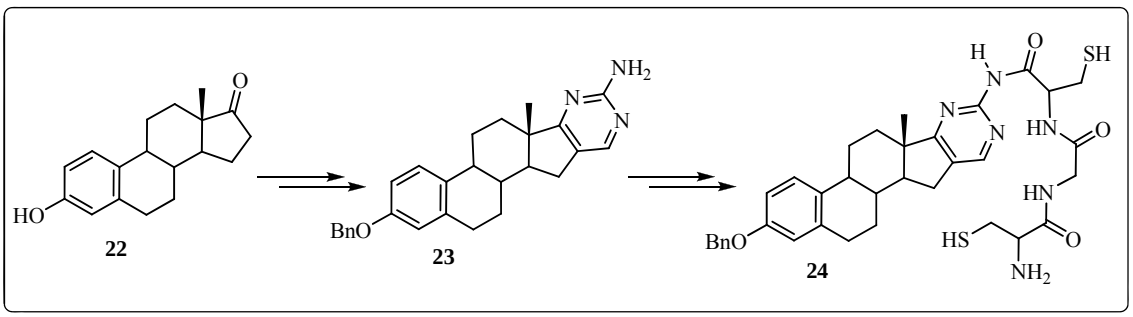


Figure 7

Compound **23** was then transformed to a steroid-heterocycle-tripeptide conjugate **24**.[36] Complexation of rhenium (Re) to compound **24** was observed in their first exploratory experiments.

A number of peptides have been identified that increases the permeability of the outer membranes of Gram-negative bacteria and sensitize these organisms to hydrophobic antibiotics that ineffectively transverse the outer membranes.[37] The best studied of these peptides are the polymyxin B derivatives. Based on these facts Savage *et al* modeled polymyxin B derivatives to determine potential active conformations and determined functionality conserved among antibiotics related to polymyxin B. This conserved functionality was incorporated on to a steroid scaffold yielding compounds **25** and **26** (Figure 8) that sensitize Gram-negative bacteria to hydrophobic antibiotics.[38]

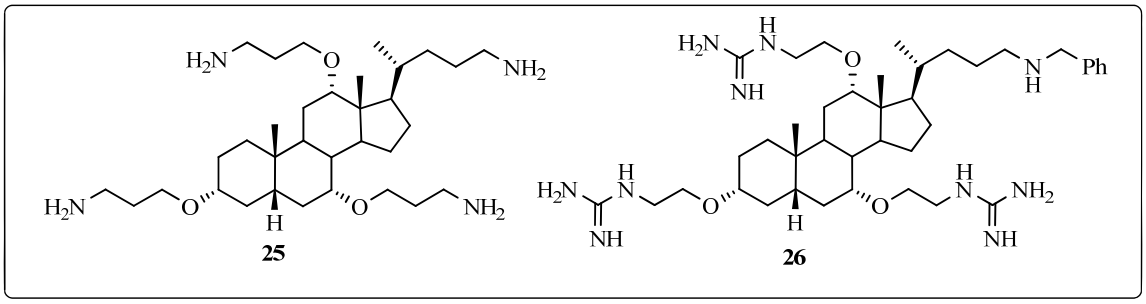


Figure 8

The same group reported[39] the preparation of cholic acid derivatives such as **27** in which amine bearing groups are attached to the steroid via ester or amide bonds (Figure 9). As a part of this effort, they have developed efficient means of appending cholic acid derivatives with three amino acids e.g. **28**. A number of the resulting compounds effectively sensitize gram-negative bacteria to erythromycin and novobiocin.

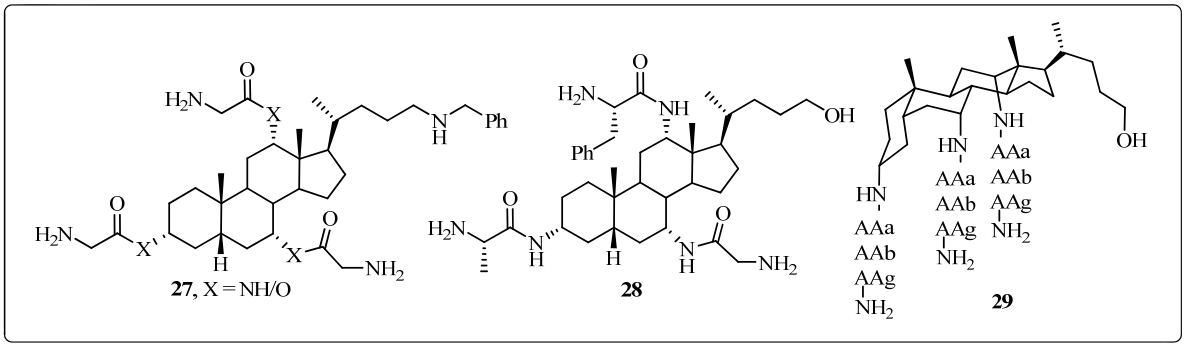


Figure 9

In continuation with this work new cationic steroid antibiotics (CSA) have been prepared[40] by Savage *et al* by conjugating tripeptides to a triamino analogue of cholic acid. These CSA-peptide conjugates such as **29** (Figure 9) were synthesized on a solid phase in an indexed library that was screened for antimicrobial activity against Gram-negative and Gram-positive bacteria. Similar such orthogonally protected triamino scaffold based on the bile acid framework was developed[41] by Davis *et al*. Researchers from Columbia University also described the preparation of a combinatorial library of synthetic receptors based on chenodeoxycholic acid and screening for members that bound a certain pentapeptide such as leuenkephalin.[42]

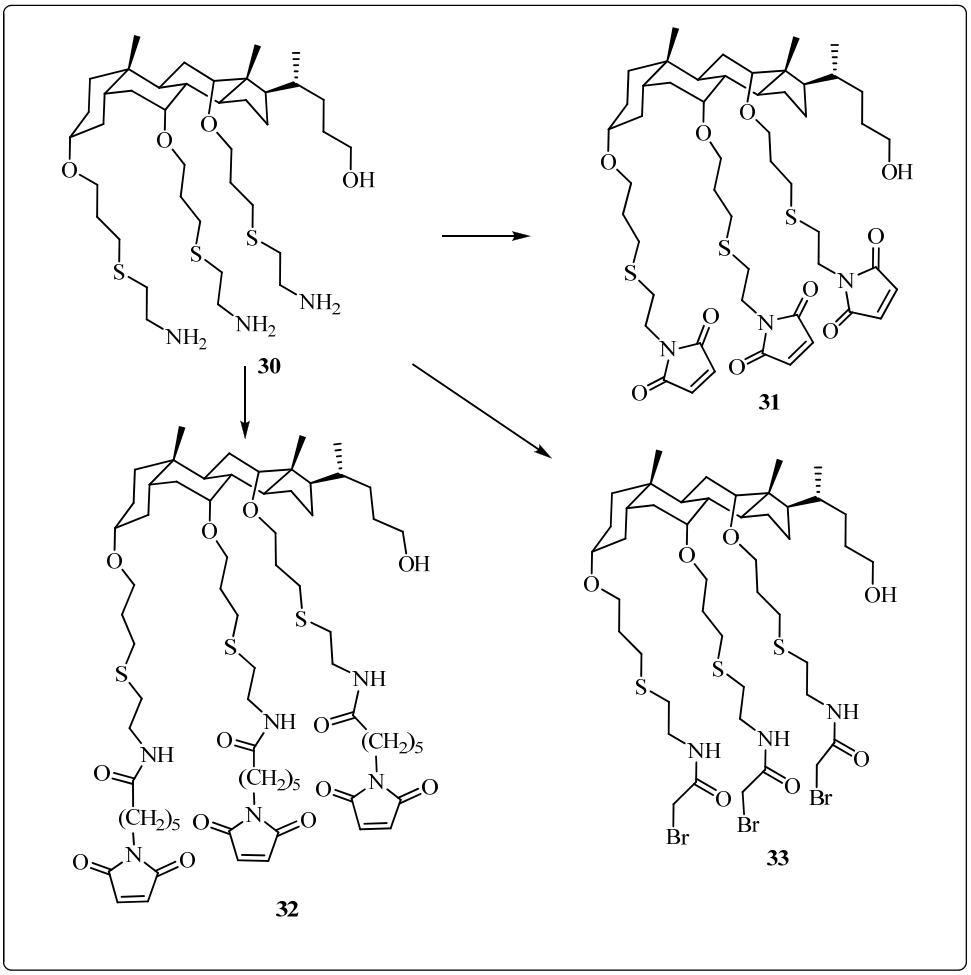


Figure 10

The use of cholic acid as a new template for multivalent peptide assembly has been reported recently[43] by Wang *et al*. The goal of their template-assembled peptide project

was to develop mimics of the trimeric gp41 fusion intermediates to serve as vaccines and inhibitors for blocking HIV-1 infection. Starting from cholic acid the intermediate tri-amine **30** was prepared in excellent yields. Using this tri-amine intermediate **30**, three different tempelates **31**, **32** and **33** for chemoselective peptide ligation were prepared (Figure 10).

To examine the chemoselective ligation, three antigenic peptides were chosen and tested. These include the potent HIV inhibitor DP178, a T-helper epitope from tetanus toxid and a minimum epitope sequence ELDKWA for HIV-neutralising antibody 2F5. For ligation a cysteine residue was introduced at either the C-or N-terminus of the peptides during solid phase synthesis to give the Cys-containing peptides: P37C, T-helper and P7C respectively.

The desired multivalent peptides were synthesized by a typical chemoselective ligation to afford trivalent peptides **34**, **35** and **36** (Figure 11). The resulting three-α-helix bundles of DP178 are believed to mimic the conformational epitopes of gp41 that are exposed during viral membrane fusion, which should be useful for HIV-1 vaccine development.

In order to synthesize orally active insulin analogues Byun *et al* modified the recombinant human insulin by covalently attaching deoxycholic acid derivatives. The recombinant insulin conjugates, [N^{B29}-deoxycholyl]insulin **37** and [N^{B29}-bisdeoxycholyl-L-lysil]insulin **38**, were studied for their chemical, structural and biological properties (Figure 12).[44]

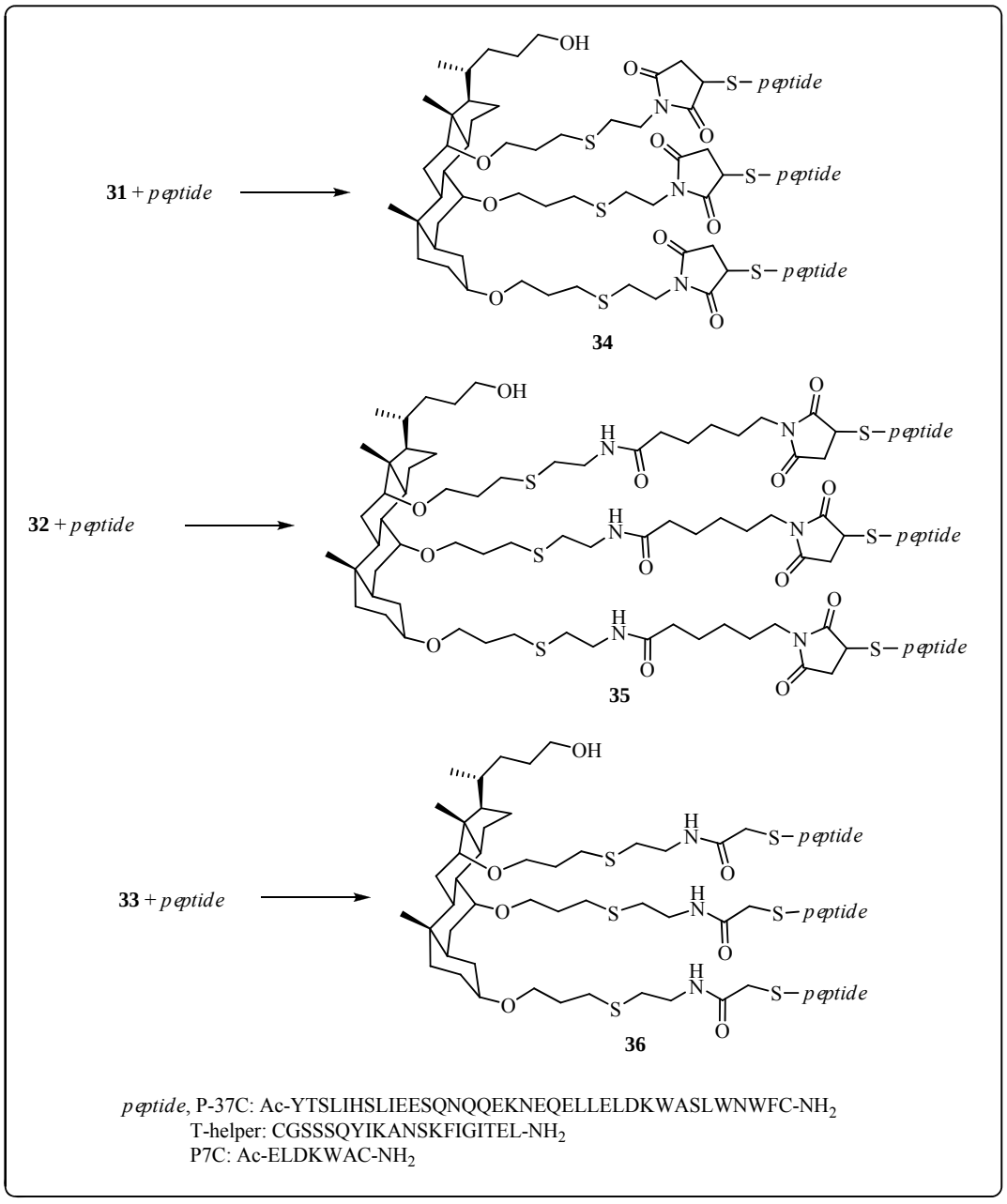


Figure 11

Competitive insulin binding assay with HepG2 cells revealed that monosubstituted insulin conjugates retains high binding affinity to the insulin receptor. When the insulin conjugates were intravenously administered (0.33 IU/Kg) to streptozotocin (STZ) induced diabetic rats, the conjugates showed sustained biological activity for a longer period with the similar lowest blood glucose level compared to native insulin. Ye *et al* synthesized[45] a series of N-protected amino acid-estradiol derivative conjugates such as **41** and **42** (Figure 13) by coupling of 17β-aminoestra-1,3,5(10)-trien-3-ol **39** or 17β-

hydrazonoestra-1,3,5(10)-trien-3-ol **40** with different amino acid via the catalysis of subtilisin Carlsberg in organic solvents.

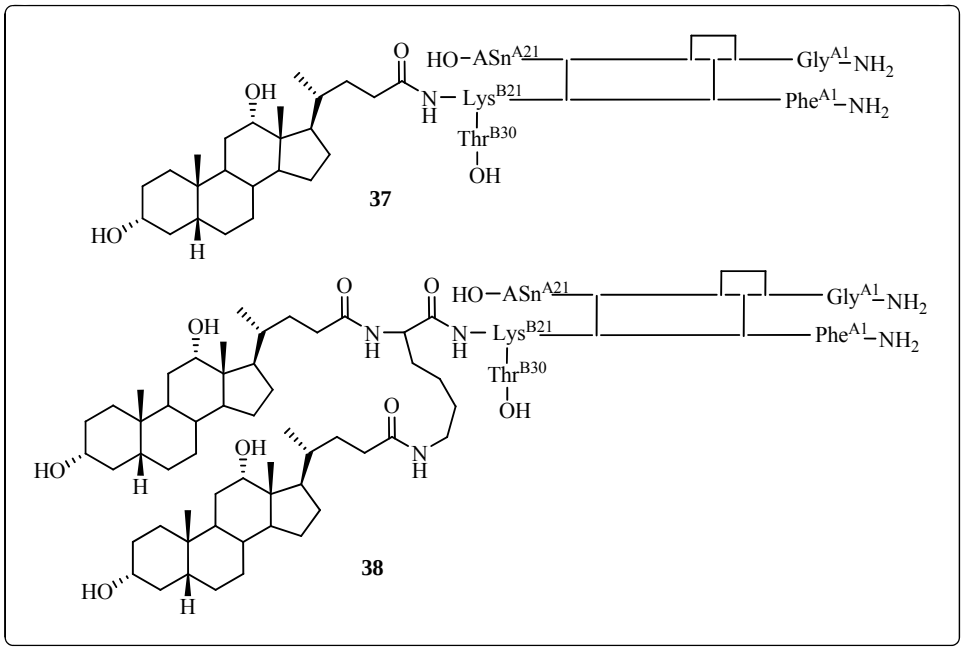


Figure 12

In vitro biological activity studies revealed that the binding interactions between estradiol derivative conjugates and estrogen receptors can be affected by the properties of the conjugated amino acid, but the effects of the change in binding properties did not result in changes in biological activities in both MCF-7 and HeLa cell lines.

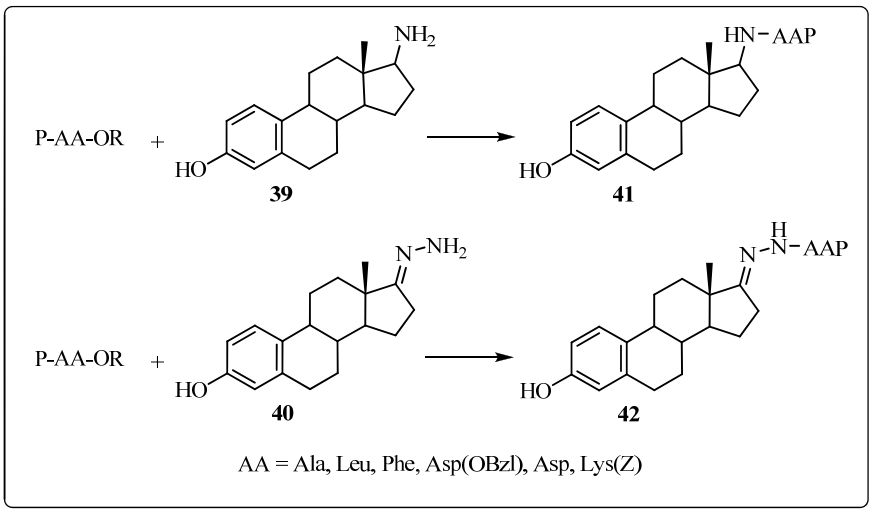


Figure 13

A few reports have been published in the literature on the synthesis of cyclic polypeptides involving steroid backbone.[46]

2A.4. Design of Novel Amphipathic Molecules

A literature survey of antimicrobial steroids reveals that several amino cholesterol derivatives exhibit profound antimicrobial activity.[47] The *in vitro* antibacterial properties of bile acids against certain Gram-positive microorganisms are well known.[48] Consequently, the preparation of various bile acid-based aminosterols was reported with a view to examine their activity as anti-microbial agents.[49] A recent approach to combat against pathogens is to introduce a polycationic chain onto a steroid scaffold. One such chimeric natural product namely squalamine[50] **43** has attracted considerable attention because of its potent antimicrobial activity against a broad spectrum of microorganisms[51,52] (Figure 14). Soon after the isolation of squalamine, Regen reported[53] rapid construction of squalamine mimic **44** which displays extraordinary antimicrobial properties. Naturally occurring steroid-amino acid conjugates such as bufotoxin **1** and polymastiamide A **6**, (Figure 1) exhibit *in vitro* antimicrobial activity.[54] Several peptides have been identified that increase the permeability of the outer membranes of Gram-negative bacteria and sensitize these organisms to hydrophobic antibiotics.[55] The best studied of these peptides are the polymyxin B (PMB) **45** derivatives. Savage and co-workers[56] designed a class of cationic steroid antibiotics (CSA) **46** as PMB mimics which display antibacterial activities comparable or superior to that of Squalamine **43**, bufotoxin **1** or PMB **45**.

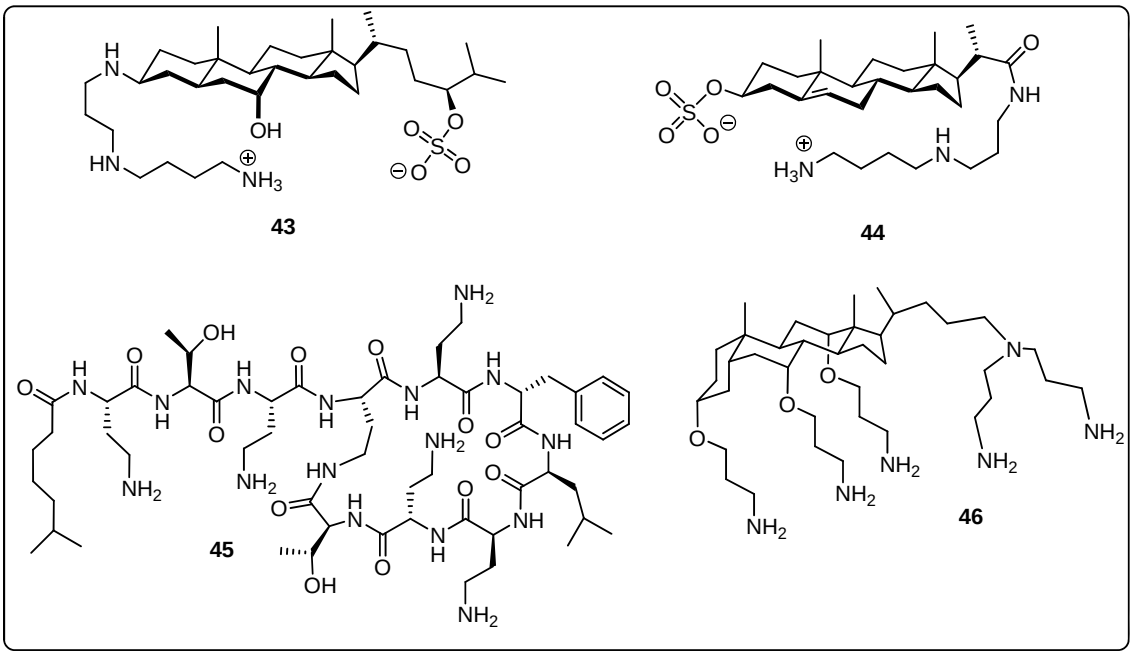


Figure 14

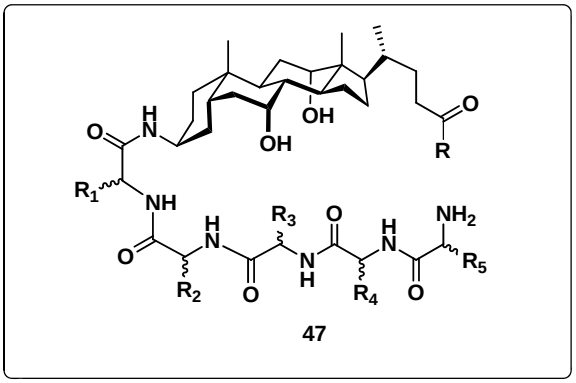
A number of intuitive assumptions can be drawn from this wide-ranging literature survey.

- A common feature of steroid derived antimicrobials is their potential to exhibit facially amphiphilic conformations containing polar and hydrophobic surfaces.[57]

- The overall amphiphilicity of the molecule plays a key role in determining the level of antimicrobial activity.

- A generic structure with fine-tuning of the molecular amphiphilicity may lead to novel molecules capable of selectively permeabilizing the microbial membranes.

With this in view, design of a generic structure with fine-tuning of the molecular amphiphilicity will lead to novel molecules. These molecules are expected to selectively permeabilize the outer membrane of the microbes. We have hypothesized that the cholic acid-polypeptide conjugate **47** could represent such a generic structure amenable to fine-tuning (Figure 15).



47

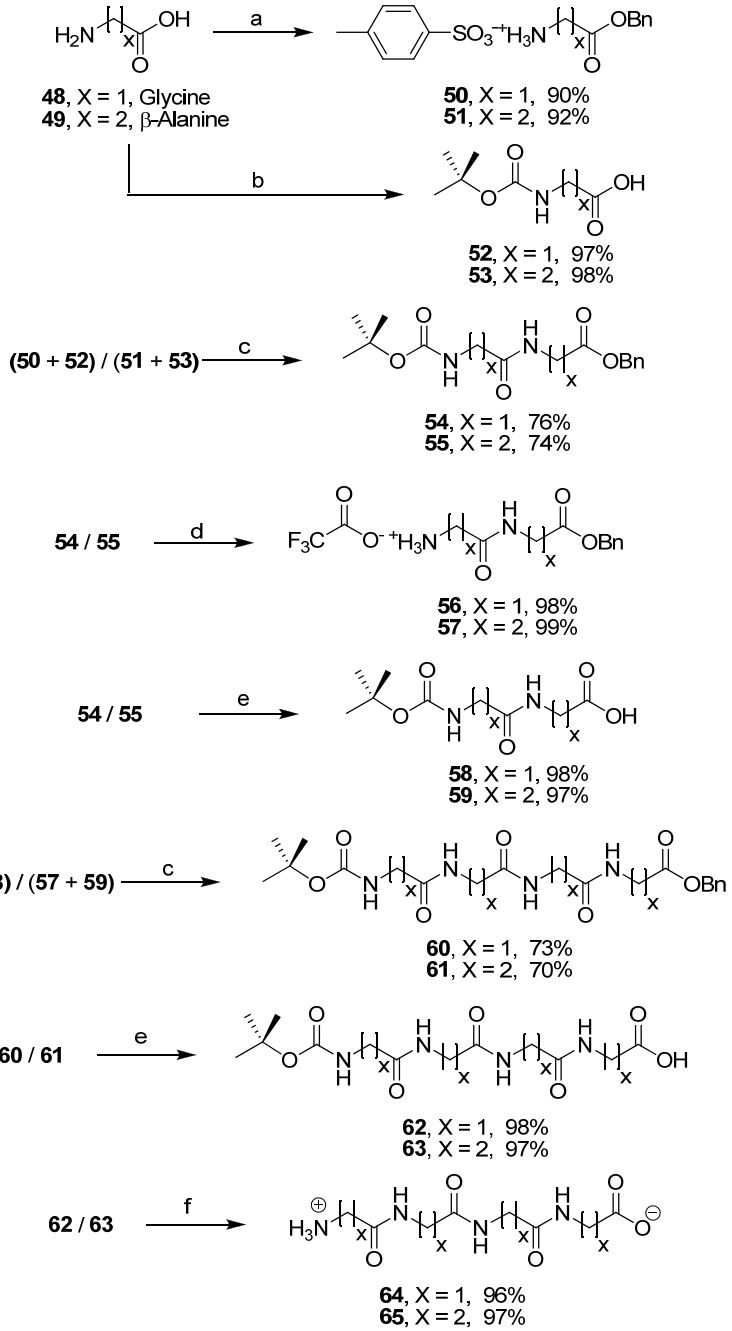
Figure 15

Introduction of an amino acid or peptide on cholic acid backbone offers a combination of a hydrophilic functional moiety as well as a hydrophobic carrier in the same molecule. Synthetic variations of the amino acid residues can produce library of compounds with variable amphiphilicity. There are very few reports of steroidal polypeptides consisting of a repeating amino acid sequence in which each amino acid unit is attached to a sterol molecule.[58] It was considered that such compounds would be of both biological as well as chemical interest.[59]

To support this hypothesis, novel cholic acid-tetrapeptide conjugates of glycine and β-alanine were synthesized and tested against a wide variety of microorganisms and were found to display remarkable synergistic activity with respect to fluconazole and erythromycin as documented herein for the first time. Cholic acid has been chosen because of its natural amphiphathic nature.[60] It differs from the conventional head-to-tail amphiphiles because the polar and non-polar domains are separated along the longitudinal axis of the molecule, which gives rise to distinct polar and non-polar faces.[61] A polypeptide segment has been introduced on to the cholic acid to have combination of a hydrophilic functional moiety as well as a hydrophobic carrier in the same molecule. Synthetic variations of the amino acid residues can produce a library of compounds with variable amphiphilicity.

2A.5. Chemistry

We undertook the synthesis and evaluation of a new family of cholic acid-polypeptide conjugates by exploiting a convergent approach, using classical solution phase synthesis.



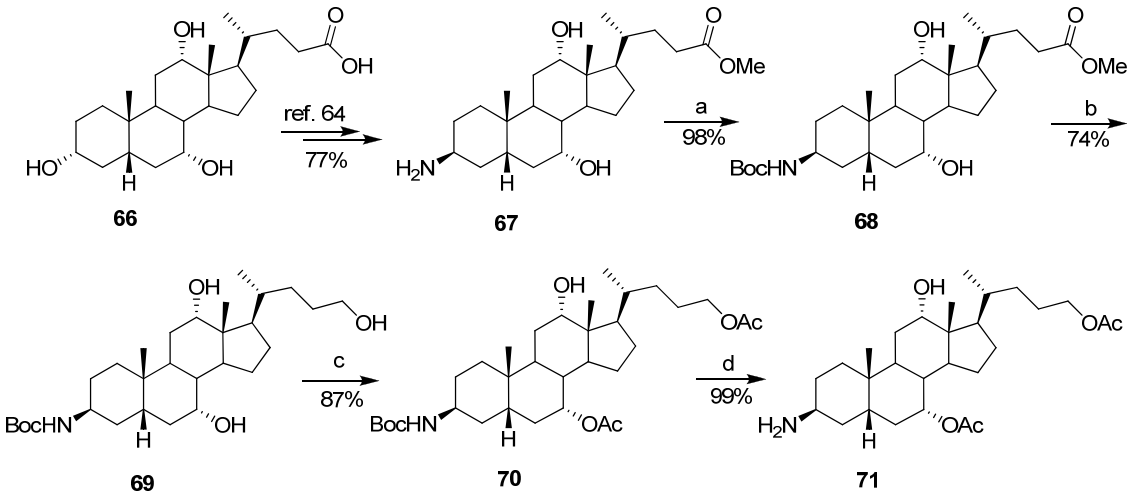
Scheme 1: Reagents and condition (a) Benzyl alcohol, *p*-TSA, toluene, reflux, 4h; (b) Boc anhydride, 1N NaOH, dioxane:water, 0-25 °C, 30 min.; (c) EDCI, HOBt, Et_3N, DCM, 0-25 °C, 6 h; (d) TFA, DCM, 0-25 °C, 2 h; (e) H_2, Pd-C, MeOH, 25 °C, 1 h; (f) 2M HCl:Et_2O, 0-25 °C, 1.5 h.

According to the literature procedures,[62] suitably protected monomers **50**, **52** and **51**, **53** were synthesized from glycine **48** and β-alanine **49**, respectively. The stepwise elongation was performed in dichloromethane (DCM) using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) activation to furnish Boc-$(Gly)_2$-OBn **54** in 76% yield and Boc-$(\beta\text{-Ala})_2$-OBn **55** in 74% yield (Scheme 1). Removal of the *tert*-butoxycarbonyl (Boc) group from peptides **54** and **55** was performed in trifluoroacetic acid (TFA)/DCM, to afford compounds **56** and **57** in quantitative yield. On the other hand, catalytic hydrogenolysis of the benzyl (Bn) group from peptides **54** and **55**, furnished compounds **58** and **59** in excellent yields. Compounds **60** and **61** were obtained by fragment condensation of **56** with **58** and **57** with **59** in DMF using EDCI activation in the presence of 1-hydroxybenzotriazole (HOBt) as catalyst. These tetrapeptides were purified by column chromatography to furnish compounds **60** and **61** as white solids in 73 and 70% yields, respectively.

The Benzyl groups of Boc-$(Gly)_4$-OBn **60** and Boc-$(\beta\text{-Ala})_4$-OBn **61** were removed by a similar catalytic hydrogenation reaction to furnish compounds **62** and **63** in 98 and 97% yield, respectively. Finally, the Boc groups of compounds **62** and **63** were removed using 2M HCl:Et_2O to afford the desired peptides **64** and **65** in 96 and 97% yield, respectively. Following the modified procedure (using EDCI and HOBt), we have drastically reduced the reaction time (7-8 h) compared to the earlier reported method, which required 3 days for the reaction to complete.[63]

Synthesis of the cholic acid intermediates, which are the building blocks for realization of the desired conjugates, is depicted in Scheme 2. 3β-Amino methyl cholate **67** was synthesized from cholic acid **66** in four steps in a straightforward way with an overall

yield of 77%.[64] The amine functionality in compound **67** was protected using Boc anhydride to afford compound **68** in excellent yield (Scheme 2).

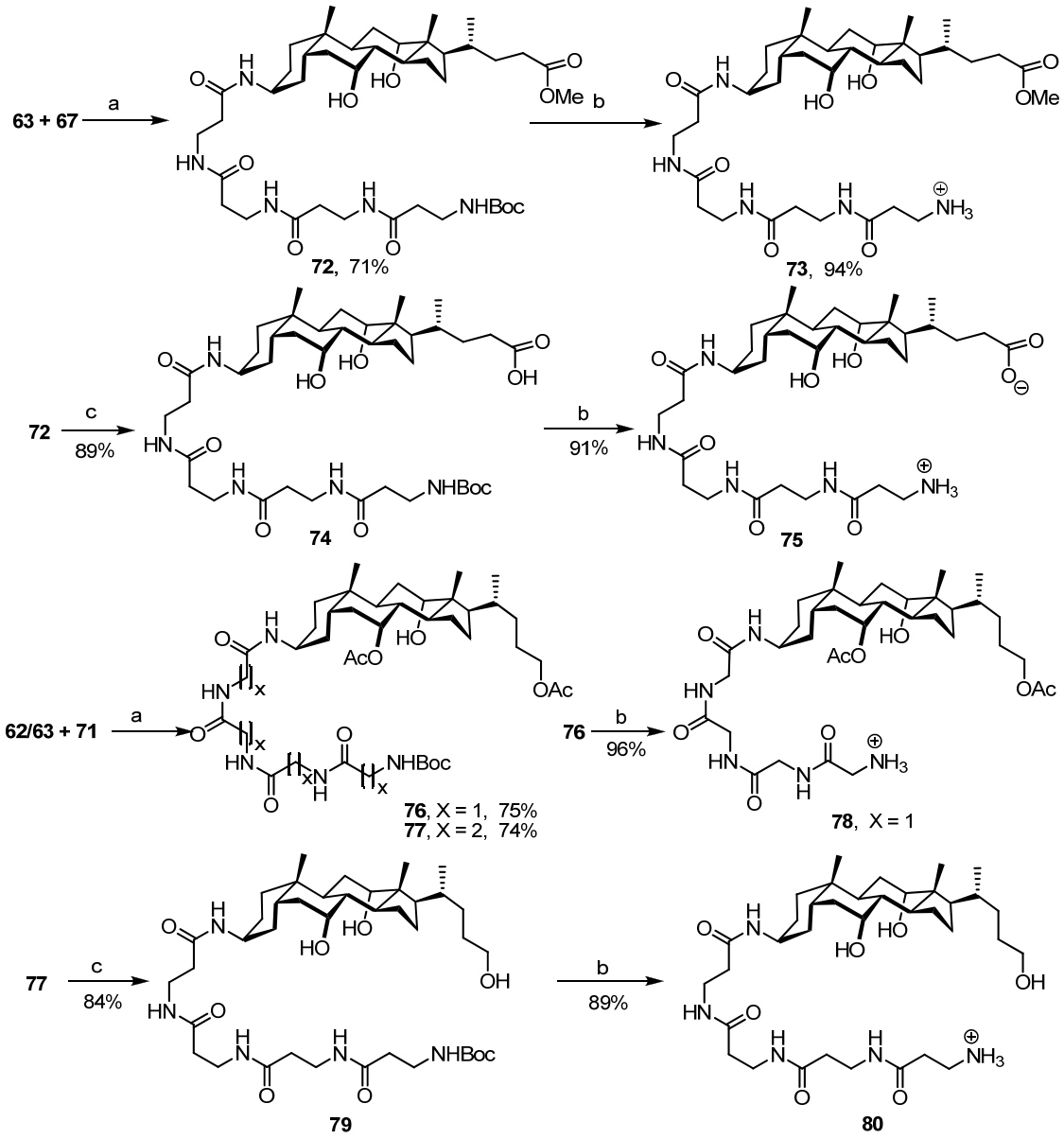


Scheme 2: Reagents and condition (a) Boc anhydride, Et_3N, dioxane:water, 25 °C 6 h; (b) LAH, THF, 0-25 °C, 1 h; (c) Ac_2O, DMAP, Et_3N, DCM, 25 °C, 8 h; (d) TFA, DCM, 0-25 °C, 2 h.

Reduction of the methyl ester of compound **68** was carried out using LAH to afford C-24 hydroxy compound **69** in 74% yield. Acylation of the C-7 and C-24 hydroxyl groups of compound **69** was carried out using acetic anhydride and a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) to furnish compound **70** in 87% yield. The protected C-3β amino functionality of compound **70** was unmasked using TFA to afford compound **71** in almost quantitative yield.

Coupling of 3β-amino cholic acid intermediates **67** with Boc-(β-Ala)$_4$-OH **63** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **72** in 71% yield (Scheme 3). Similarly coupling of 3β-amino cholic acid derivative **71** with Boc-(Gly)$_4$-OH **62** and Boc-(β-Ala)$_4$-OH **63** furnished compounds **76**, **77** in 75 and 74% yield, respectively. Subsequent hydrolysis of the methyl ester functionality in compound **72** and C-7 and C-24 acetates in compound **77** (LiOH 2M, MeOH) and aqueous work-up

provided the corresponding acid **74**, and hydroxy compound **79** in 89 and 84% yield, respectively.



Scheme 3: Reagents and condition (a) EDCI, HOBt, Et_3N, DMF, 0-25 °C, 6 h; (b) 2M HCl:Et_2O, 0-25 °C, 1.5 h; (c) 2M LiOH, MeOH, 25 °C, 12 h.

Cleavage of the Boc group in compounds **72**, **74**, **76** and **79** was accomplished with 2M HCl:Et_2O to afford free amino compounds **73**, **75**, **78**, and **80**, respectively in yields ranging from 89-96%. It is worth mentioning here that all the compounds **72-80** (Scheme 3) are new and characterized fully by IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

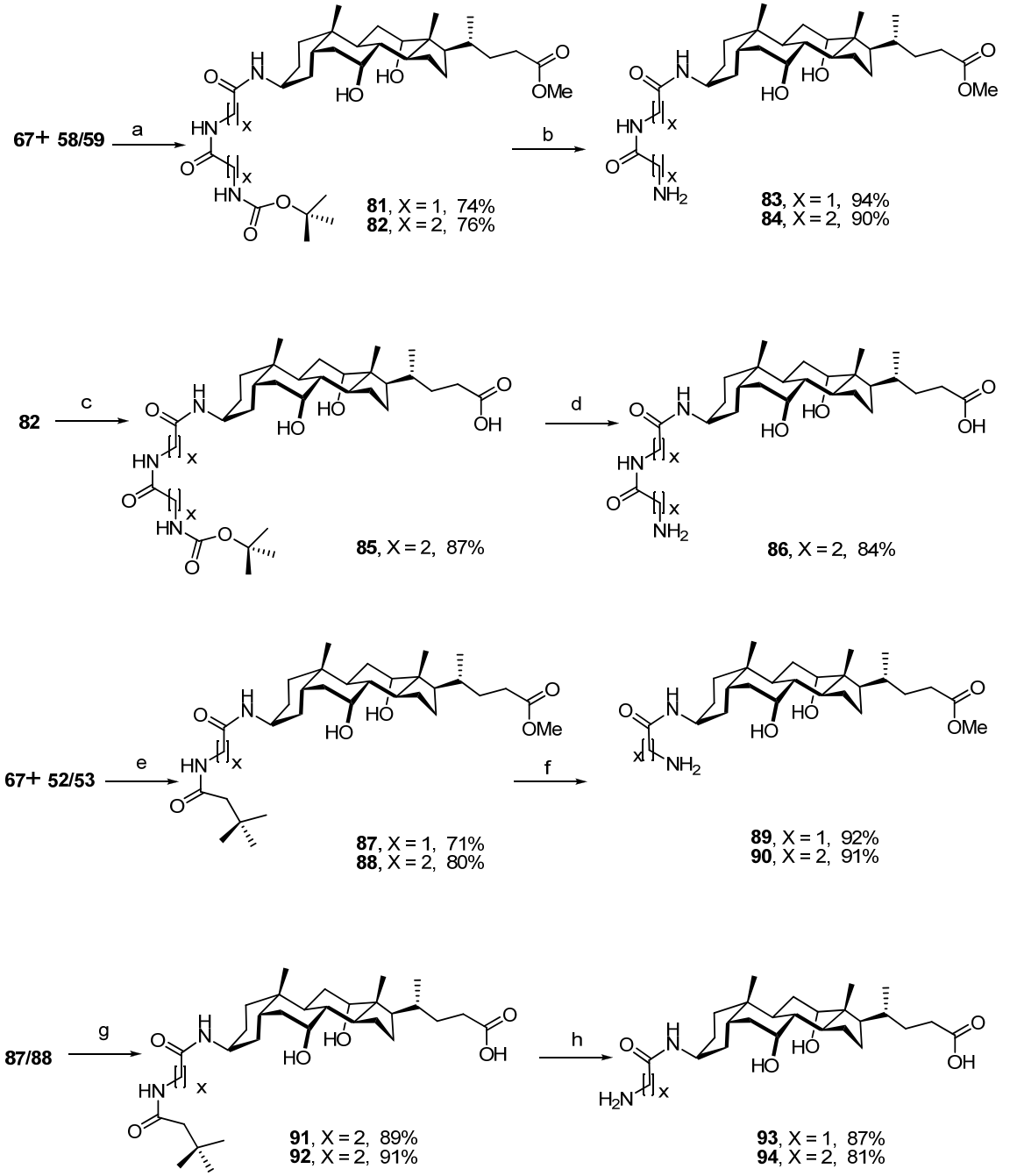
Tetrapeptides **60**-**65**, Cholic acid **66**, and cholic acid-tetrapeptide conjugates **72**-**80** were examined for *in vitro* antifungal, antibacterial activity as well as cytotoxic study. The bioassay results are shown in section 2A.4.

Bioassey result shown by cholic acid tetrapeptide conjugates **72**-**80** encouraged us to extend our objective. With this in view, we would like to synthesize novel cholic acid-dipeptide and monopeptide conjugates of glycine and β-alanine and their bioevolution study against a wide variety of microorganisms. This study has not been reported earlier. Cholic acid has been chosen because of its natural amphiphathic nature[60] and more about the choice of cholic acid has been described earlier. A dipeptide and monopeptide segment of glycine and β-alanine has been synthesized using classical solution phase synthesis (Scheme 1).

Coupling of 3β-amino cholic acid intermediates **67** with Boc-$(Gly)_2$-OH **58** and Boc-$(\beta\text{-Ala})_2$-OH **59** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **81** and **82** in 74% and 76% yield, respectively (Scheme 4). Subsequent hydrolysis of the C-24 methyl ester functionality in compound **82** (LiOH 2M, MeOH) provided the corresponding acid **85** in 87% yield. Cleavage of the Boc group in compounds **81**, **82** and **85** was accomplished with 2M HCl in Et_2O to afford free amino compounds **83**, **84** and **86** in 94%, 90% and 84% yields, respectively.

In the similar way coupling of 3β-amino cholic acid intermediates **67** with Boc-(Gly)-OH **52** and Boc-(β-Ala)-OH **53** under mild condition using EDCI/HOBt and Et_3N in DMF provided compounds **87** and **88** in 71% and 80% yield, respectively (Scheme 4). Subsequent hydrolysis of the C-24 methyl ester functionality in compound **87** and **88**

aq.LiOH 2M, MeOH provided the corresponding acid **91** and **92** in 89% and 91% yield, respectively. Cleavage of the Boc group in compounds **87**, **88**, **91** and **92** was



Scheme 4: Reagents and condition (a) EDCI, HOBt, Et_3N, DMF, 0-27 °C, 7-8 h,74% and 76%; (b) 2M HCl in Et_2O, 0-25 °C, 1 h, 90% and 94%; (c) 2M LiOH, MeOH, 28 °C, 10 h, 87%; (d) 2M HCl in Et_2O, 0-28 °C, 1 h, 84%; (e) EDCI, HOBt, Et_3N, DMF, 0-27 °C, 7 h,71% and 80%; (f) 2M HCl in Et_2O, 0-28 °C, 1 h, 92% and 91%; (g) 2M LiOH, MeOH, 27 °C, 9h, 89 and 91%; (h) 2M HCl in Et_2O, 0-28 °C, 1 h, 87% and 81%.

accomplished with 2M HCl in Et_2O to afford free amino compounds **89**, **90**, **93** and **94** respectively in yields ranging from 81-92%. It is worth mentioning here that all the compounds **81-94** (Scheme 4) are new and characterized fully by IR, ^{1}H NMR and ^{13}C NMR spectroscopy. Antimicrobial as well as cytotoxic study of Cholic acid-tetrapeptide conjugates **81-94** are in progress.

2A.6. Bioevaluation Study

2A.6.1. Antimicrobial activity

Tetrapeptides **60-65**, Cholic acid **66**, and cholic acid-tetrapeptide conjugates **72-80** were examined for *in vitro* antifungal as well as antibacterial activity. The antifungal activity was tested using NCL isolate fungal strains *Candida albicans* and *Cryptococcous neoformans* (human pathogens), *Benjaminiella poitrasii* and *Yarrowia lipolytica* (saprophytes), *Fusarium oxysporum* (plant pathogen). The antibacterial activity was evaluated against *Escherichia coli* (NCIM 2574), and *Staphylococcus aureus* (NCIM 2122). The MIC values were determined using standard broth microdilution technique as described by NCCLS.[65] 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 has been depicted in Table 2 as MIC values. *Candida albicans*, *Cryptococcous neoformans*, *Benjaminiella poitrasii*, *Yarrowia lipolytica*, *Fusarium oxysporum* strains and bacterial strains *Escherichia coli*, and *Staphylococcus aureus*, respectively to find out the minimum inhibitory concentration (MIC) values (Table 1). These compounds were also tested for their ability to permeabilize the outer membrane of Gram-negative bacteria such as *E. coli* causing sensitization to hydrophobic antibiotics that inefficiently cross the outer

membrane. We also demonstrated such permeabilization by cholic acid derivatives with *C. albicans*, a pathogenic fungus (Table 2).

In the preliminary bioevaluation, tetrapeptides **60-65** as well as cholic acid **66** did not show any appreciable antifungal or antibacterial effect. In comparison the tetrapeptide-linked cholic acid derivatives **72-80** showed good to moderate activity against *C. albicans*, *B. poitrasii* and *F. oxysporum* (columns A, C and E) whereas these compounds were less active towards bacteria (columns F and G) than fungi. The antifungal activity of most of the compounds was found to be similar to that of fluconazole (MIC, 32 μg/mL). The difference in the toxicity of the synthesized compounds against a wide variety of microorganisms can be attributed to the differences in their cell wall/cell membrane compositions which affect the passage of these compounds through cell wall/cell membrane.[66]

Table 1: Minimum inhibitory concentration (MIC) of modified steroids.

Entry	Compound Number	Antimicrobial Activity MIC (μg/mL)						
		Fungal Strains					Bacterial Strains	
		A	***B***	***C***	***D***	***E***	***F***	***G***
1	**66**	>128	>128	128	>128	>128	>128	>128
2	**72**	32	>128	16	96	64	>128	>128
3	**73**	128	>128	24	>128	32	96	64
4	**74**	32	>128	48	>128	64	>128	>128
5	**75**	32	>128	24	>128	64	32	>128
6	**76**	64	>128	32	>128	64	64	>128
7	**77**	32	>128	64	96	>128	>128	32
8	**78**	64	>128	16	>128	32	>128	64
9	**79**	128	>128	>128	>128	64	>128	>128
10	**80**	64	>128	64	>128	>128	32	>128
11	Amp. B	2	16	16	16	16	NT	NT
12	Fluconazole	32	32	32	64	8	NT	NT
13	Tetracycline	NT	NT	NT	NT	NT	8	16
14	Erythromycin	NT	NT	NT	NT	NT	64	32

A, *C. albicans*; **B**, *C. neoformans*; **C**, *B. poitrasii*; **D**, *Y. lipolytica*; **E**, *F. oxysporum*; **F**, *E. coli*; **G**, *S. aureus*; NT- Not Tested.

To characterize synergism of **60**-**66** and **72**-**80** with fluconazole (an antifungal agent) and erythromycin (a hydrophobic antibacterial agent), we determined the concentrations of these compounds necessary to lower the MIC values of the antibiotics to 1 μg/mL (a concentration at which many clinically useful antibiotics are active).[55b]

Table 2: MIC, Permeabilization and FIC Data for **60**-**66**, and **72**-**80** with *C. albicans* and *E. coli*.

Entry	Comp. Number	a (μg/ml)	a' (μg/ml)	a"	b (μg/ml)	b' (μg/ml)	b"
1	**60**	>128	128	1.03	>128	128	1.03
2	**61**	>128	128	1.03	>128	64	0.53
3	**62**	>128	64	0.53	>128	128	1.03
4	**63**	>128	64	0.53	>128	>128	1.03
5	**64**	128	>128	1.03	128	64	0.53
6	**65**	>128	128	1.03	>128	>128	1.03
7	**66**	>128	64	0.53	>128	64	0.52
8	**72**	32	6	0.22	>128	16	0.14
9	**73**	128	3	0.055	96	8	0.099
10	**74**	32	12	0.41	>128	16	0.14
11	**75**	32	1	0.062	32	4	0.14
12	**76**	64	12	0.22	64	24	0.39
13	**77**	32	8	0.28	>128	10	0.093
14	**78**	64	6	0.125	>128	8	0.078
15	**79**	128	12	0.125	>128	20	0.172
16	**80**	64	6	0.125	32	8	0.265

a: MIC of the synthesized compounds against *C. albicans*.
a': Concentration of the synthesized compounds required to lower the MIC of fluconazole from 32 μg/mL to 1 μg/mL (a concentration at which many clinically useful antibiotics are active).
a": FIC values with fluconazole.
b: MIC of the synthesized compounds against *E. coli*.
b': Concentration of the synthesized compounds required to lower the MIC of erythromycin from 64 μg/mL to 1 μg/mL.
b": FIC values with erythromycin.

This measurement entailed incubating a known population of yeast suspension (*C. albicans*) for 48 h in YPG and bacterial cells (*E. coli*) for 24 h in a nutrient broth with fluconazole and erythromycin, respectively, with incrementally varied concentrations of the synthesized compounds as described by Savage *et al*.[55b] Tetrapeptides **60**-**65** and cholic acid **66** did not showed any appreciable synergism, on the other hand, almost all

the cholic acid conjugates exhibited very good synergism. This suggests that the synergism effect shown by our novel compounds is due to their inherent amphiphilicity. To quantify the synergistic behaviour of our compounds with fluconazole and erythromycin, fractional inhibition concentration (FIC) values were also calculated.[67] Synergism between antibiotics is indicated by FIC values of less than 0.5. In the present case all the synthesised compounds **72**-**80** displayed FIC values of less than 0.5 with both fluconazole and erythromycin and many of the FICs shown in Table 2 are comparable to those reported for PMB derivatives.[55a]

2A.6.2. Antiproliferative Activity

Bile acids are known to promote proliferation and metastasis of cells of cancer origin and inhibit the proliferation of cells of non-cancer origin.[68] They are also known to be extremely toxic at high doses, presumably damaging cell membranes and mitochondrial membranes.[69] At low doses, bile acids stimulate the cell-signalling effects involving various pathways.[70] Hence we tested the cytotoxicity of the synthesized compounds in two different cell lines, one of cancer origin (human mammary carcinoma: MCF-7) and the other of non-cancer origin (human embryonic kidney: HEK293). Concentration of compounds needed to reduce the population growth of HEK293 and MCF-7 cells by 50% (IC_{50}) *in vitro* was evaluated using the MTT assay[71] (Table 3). Graphical representation of antiproliferative activities of all the compounds is included in the experimental section. Of all the cholic acid-tetrapeptide conjugates tested, none showed any substantial cytotoxicity up to 80 μM concentration. Incorporation of peptide residues on the cholic acid derivatives drastically reduced its cytotoxicity towards HEK293 cell line. Moreover, it was found that all the compounds except **79** enhanced the proliferation of MCF-7 cells and not HEK293 cells. This confirms the observation that cholic acid and its derivatives

promote the proliferation of cells from cancerous origin and not normal cells. This study also confirm that the synthesized cholic acid-peptide conjugates are not toxic to the cell lines tested at concentrations up to 80 μM.

Table 3: Cytotoxicity of modified steroids

Entry	Compound Number	IC_{50} (μM)	
		HEK293	MCF-7
1	**66**	50	>1000
2	**72**	100	>1000
3	**73**	600	700
4	**74**	>1000	>1000
5	**75**	200	>1000
6	**76**	500	200
7	**77**	150	80
8	**78**	150	800
9	**79**	>1000	800
10	**80**	>1000	800

While relatively inactive by themselves, these compounds interact synergistically with antibiotics such as fluconazole and erythromycin to inhibit growth of fungi and bacteria, respectively at 1-24 μg/mL.

2.7. Conclusion

Molecular amphiphilicity is one of the important factor which accounts for the easy transport of the molecules through the membranes, as a result a generic structure wherein fine-tuning of the molecular amphiphilicity is possible, have been designed utilizing amphiphilic nature of cholic acid. To realize a designed generic structure, tetra-peptide derived from glycine and β-alanine was linked at C3β-position of modified cholic acid. The synergism of the most active compounds with fluconazole and erythromycin greatly improves the activity of these antibiotics against *C. albicans* and *E. coli* respectively. Because the synthesized modified steroids act synergistically with unrelated hydrophobic antibiotics, these compounds most likely act as outer membrane permeabilizers. In

summary, we have demonstrated that the molecules having optimum amphiphilicity can produce potent sensitizers of Gram-negative bacteria and fungi.

2A.8. Experimental Section

Experimental Procedures:

Boc-Gly-Gly-OBn (54): H-Gly-OBn·*p*-TSA[62] **50** (3.37 g, 10 mmol), Boc-Gly-OH[62] **52** (1.75 g, 10 mmol) and 1-hydroxybenzotriazole (HOBt) (0.68 g, 5 mmol) were dissolved in dry DCM (80 mL) and the reaction mixture was cooled to 0 °C under argon atmosphere. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (2.1 g, 11 mmol) and Et_3N (2.02 g, 20 mmol) were added. The reaction mixture was allowed to warm to room temperature and was stirred for 6 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed successively with H_2O, 5% citric acid, H_2O, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from MeOH-Et_2O to give dipeptide **54** as colorless crystals (2.44 g, 76%). mp 78-79 ºC (lit.[72] colorless oil). The other spectroscopic data were consistent with that reported in literature.[72]

Boc-β-Ala-β-Ala-OBn[63] **(55):** Compound **55** was synthesized using H-β-Ala-OBn·*p*-TSA **51** and Boc-β-Ala-OH **53** as described for compound **54**. Boc-β-Ala-β-Ala-OBn **55** was recrystallized from ethyl acetate and heptane to afford a white crystalline solid, yield 74%. mp 77-79 ºC. ^{1}H NMR and other spectroscopic data was consistent with that reported in the literature.[63b]

Boc-$(Gly)_4$-OBn (60): Boc-Gly-Gly-OBn **54** (1.6 g, 5 mmol) was added to a mixture of TFA and DCM (1:1, 5 mL) at 0 °C and the reaction mixture was stirred for 1 h at 0 °C and additionally for 1 h at room temperature. The solvent was evaporated and the H-Gly-

Gly-OBn·TFA **56** obtained was used without further purification. Compound **54** (1.6 g, 5 mmol) and 10% Pd/C (0.18 g) were added to MeOH and hydrogenated for 1 h at 40 psi pressure. Pd/C was removed by filtration and the solvent was evaporated. This residue of compound **58** was dissolved in dry DMF (50 mL) under an argon atmosphere and the solution was cooled to -10 ºC. HOBt (0.337 g, 2.5 mmol) and EDCI (1.09 g, 5.5 mmol) were added and stirring was continued for 15 min. The H-Gly-Gly-OBn·TFA **56** obtained above and triethylamine (1.01 g, 10 mmol) were added and the reaction mixture was allowed to warm to room temperature and was stirred for 6h. The solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed successively with H_2O, 5% citric acid, H_2O, saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by flash chromatography using neutral alumina (DCM/ MeOH, 19:1) and further recrystallized using MeOH/*i*Pr_2O to afford Boc-$(Gly)_4$-OBn **60** (1.58 g, 73%) as a white powder. mp 173-174 ºC. (lit.[72] 174-175 ºC). The other spectroscopic data were consistent with that reported in literature.[72]

Boc-(β-Ala)$_4$-OBn (61): Compound **61** was synthesized using compound **57** and compound **58** as per the procedure described for compound **60**. Boc-$(\beta\text{-Ala})_4$-OBn **61** was purified by chromatography using neutral alumina (DCM/MeOH, 24:1) to afford a white solid (70%). IR, ^{1}H NMR spectroscopic data was consistent with that reported in the literature.[63b] mp 158-159 ºC; Anal. Calcd for $C_{24}H_{36}N_4O_7$: C, 58.5; H, 7.4; N, 11.4. Found: C, 58.6; H, 7.3; N, 11.3.; IR ν_{max} (Nujol)/(cm^{-1}) 1634, 1650, 1658, 1687, 1737, 3299; ^{13}C NMR (~60% $CDCl_3$ in CD_3OD, 50.32 MHz) δ 28.5x3, 34.3, 35.5, 36.0, 36.2, 36.3x2, 36.5, 37.2, 67.0, 79.8, 128.6x2, 128.7, 129.0x2, 136.1, 157.2, 172.6, 172.8, 172.9, 173.0; MS (LCMS) *m/z* 493.7 $[M+1]^+$, 515.7 $[M+Na]^+$.

Boc-(Gly)$_4$-OH (62): A solution of Boc-(Gly)$_4$-OBn (**60**) (0.872 g, 2 mmol) in anhydrous MeOH (40 mL) was treated with 10% Pd-C (0.087 g, 10% wt. equiv.) and hydrogenated for 1 h at 40 psi pressure at 25 °C. The reaction mixture was filtered through celite (MeOH wash), concentrated under vacuum, and dried thoroughly under vacuum to afford **62** (0.68 g, 98%) as a white solid, which was further recrystallized from 10% MeOH/*i*Pr$_2$O as a white powder. mp 126-127 ºC. (lit.[72] 126-128). The other spectroscopic data were consistent with that reported in literature.[72]

Boc-(β-Ala)$_4$-OH (63): Compound **63** was synthesized using Boc-(β-Ala)$_4$-OBn **(61)** as per the procedure mentioned for compound **62**. The linear tetrapeptide **63** was obtained as a white solid (97%). mp 182-183 ºC; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1658, 1681, 1722, 3301, 3382; ^{1}H NMR (CD$_3$OD, 500 MHz) δ 1.40 (s, 9H), 2.35-2.40 (m, 6H), 2.49 (t, 2H, J = 6.42 Hz), 3.28 (t, 2H, J = 6.88 Hz), 3.36-3.46 (m, 6H); ^{13}C NMR (CD$_3$OD, 125.76 MHz) δ 28.7x3, 34.7, 36.4, 36.5, 36.6, 37.0x2, 37.2, 37.9, 80.1, 158.1, 173.6, 173.7, 173.8, 175.4; MS (LCMS) *m/z* 403.5 [M+1]$^+$, 425.5 [M+Na]$^+$.

(Gly)$_4$-OH (64): A solution of compound **62** in 2M HCl:Et$_2$O (3 mL) was stirred at 0-25 ºC for 1.5 h. The volatiles were removed under vacuum, and the residue was dried thoroughly to afford compound **64** (96% yield). Anal. Calcd for $C_8H_{14}N_4O_5 \cdot HCl$: C, 34.0; H, 5.4; N, 19.8. Found: C, 33.9; H, 5.6; N, 20.1.; **IR** ν_{max} (Nujol)/(cm^{-1}) 1648, 1654, 1679, 1722, 2964, 3346, 3371; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 3.69-3.84 (m, 8H), 8.06 (bs, 3H); MS (LCMS) *m/z* 246.5 [M+1], 268.7 [M+Na].

(β-Ala)$_4$-OH (65): Compound **65** was synthesized using Boc-(β-Ala)$_4$-OH **(63)** as per the procedure mentioned for compound **64**. The linear tetrapeptide **65** was obtained as a gummy solid (97%). Anal. Calcd for $C_{12}H_{22}N_4O_5 \cdot HCl$: C, 42.5; H, 6.8; N, 16.5. Found: C,

42.7; H, 6.9; N, 16.4.; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1658, 1681, 1741, 3284, 3386; ^1H NMR (CD_3OD, 400 MHz) δ 2.37-2.41 (m, 4H), 2.48 (t, 2H, J = 6.42 Hz), 3.39-3.50 (m, 8H); MS (LCMS) *m/z* 302.8 [M+1], 324.4 [M+Na].

Compound 68: A solution of compound **67** (5.0 g, 11.9 mmol) in a mixture of dioxane (90 mL), water (10 mL) and triethylamine (1.8 g, 17.7 mmol) was stirred at 0 ºC in an ice bath. Di-*tert*-butyl dicarbonate (Boc anhydride) (5.0 g, 17.7 mmol) was added and stirring was continued at room temperature for 6 h. The standard work up gave a crude product, which on further column chromatographic purification using silica gel (DCM/MeOH, 49:1) afforded compound **68** (6.029 g, 98%) as a white solid. mp 142-144 ºC; $[\alpha]_D^{28}$ + 19.23 (*c* 1.156, $CHCl_3$); Anal. Calcd for $C_{30}H_{51}NO_6$: C, 69.1; H, 9.85; N, 2.7. Found: C, 68.85; H, 9.9; N, 2.9.; IR ν_{max} (Nujol)/(cm^{-1}) 1662, 1681, 1739, 3421; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.68 (s, 3H), 0.92 (s, 3H), 0.97 (d, 3H, J = 5.94 Hz), 1.44 (s, 9H), 3.66 (s, 3H), 3.84 (bs, 1H), 3.97 (bs, 1H), 4.78 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.2, 23.0, 23.2, 25.0, 25.8, 27.5, 28.4x3, 30.8, 30.9, 31.0x2, 33.9, 34.4, 35.1, 35.2, 37.1, 39.2, 41.7, 46.2, 46.4, 47.1, 51.4, 68.3, 73.0, 78.9, 155.3, 174.8; MS (LCMS) *m/z* 522.6 $[M+1]^+$, 544.5 $[M+Na]^+$.

Compound 69: To a 250 mL round bottom flask were added compound **68** (3.0 g, 5.75 mmol) in dry THF (200 mL) and $LiAlH_4$ (0.44 g, 11.5 mmol) at 0 ºC. After stirring the reaction mixture for 1 h at 0 to 25 ºC, saturated aqueous Na_2SO_4 was introduced slowly. The reaction mixture was stirred for further 30 min. The solvent was removed at reduced pressure and the residue was extracted with ethyl acetate. The usual work up afforded crude product. This was further purified by column chromatography on silica gel (DCM/MeOH, 97:3) to afford compound **69** (2.10 g, 74%) as a white solid. mp 117-119 ºC; $[\alpha]_D^{28}$ + 20.71 (*c* 1.255, $CHCl_3$); Anal. Calcd for $C_{29}H_{51}NO_5$: C, 70.55; H, 10.4; N,

2.8. Found: C, 70.3; H, 10.5; N, 2.7.; IR ν_{max} (Nujol)/(cm^{-1}) 1693, 3446; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.69 (s, 3H), 0.94 (s, 3H), 1.00 (d, 3H, J = 6.31 Hz), 1.44 (s, 9H), 3.62 (t, 3H, J = 6.07), 3.85 (bs, 2H), 3.99 (s, 1H), 4.78 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.7, 22.9, 23.3, 24.9, 25.6, 27.6, 28.3, 28.4x3, 29.5, 30.9x2, 31.9, 33.8, 34.6, 35.2, 35.6, 37.1, 39.3, 41.4, 46.4, 47.3, 63.3, 68.4, 73.1, 79.1, 155.4; MS (LCMS) *m/z* 516.3 $[M+Na]^+$.

Compound 70: A mixture of compound **69** (2.46 g, 5 mmol), Ac_2O (1.27 g, 12.5 mmol), DMAP (0.06 g, 0.5 mmol) and triethylamine (1.11 g, 11 mmol), in dry DCM was stirred at room temperature for 8 h. The solution was concentrated and the residue was extracted with ethyl acetate using the standard work up procedure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 99:1) to furnish compound **70** (2.50 g, 87%), as a white solid. mp 197-199 ºC; $[\alpha]_D^{28}$ + 13.71 (*c* 1.312, $CHCl_3$); Anal. Calcd for $C_{33}H_{55}NO_7$: C, 68.6; H, 9.6; N, 2.4. Found: C, 68.4; H, 9.4; N, 2.6.; IR ν_{max} (Nujol)/(cm^{-1}) 1690, 1705, 1726, 3454; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.69 (s, 3H), 0.95 (s, 3H), 0.99 (d, 3H, J = 6.32 Hz), 1.44 (s, 9H), 2.04 (s, 6H), 3.87 (m, 1H), 3.96-4.07(m, 3H), 4.78 (d, 1H, J = 6.19 Hz), 4.89 (d, 1H, J = 2.28 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.4, 20.8, 21.4, 22.8, 23.0, 25.0, 27.2, 27.4, 28.3x3, 28.7, 30.7, 30.9, 31.6, 33.1, 34.6, 34.9, 36.7, 37.9, 41.9, 46.0, 46.4, 47.1, 60.2, 64.8, 70.9, 72.5, 78.9, 155.2, 170.4, 171.1; MS (LCMS) *m/z* 600.2 $[M+Na]^+$.

Compound 71: Compound **70** (1.15 g, 2 mmol) was dissolved in DCM (10 mL) and a mixture of TFA/DCM (1:1, 5 mL) was added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and additionally for 1 h at room temperature. The solvent was evaporated and the residue was treated with saturated aqueous $NaHCO_3$ (20 mL). The resulting aqueous layer was extracted with ethyl acetate, washed with water (10 mL), brine (10

mL), dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. The crude product was purified by column chromatography using neutral alumina (DCM/MeOH, 19:1) to afford compound **71** as a white solid (0.994 g, 99%). mp 103-105 ºC; $[\alpha]_D^{28}$ + 13.67 (*c* 1.024, $CHCl_3$); Anal. Calcd for $C_{28}H_{47}NO_5$: C, 70.4; H, 9.9; N, 2.9. Found: C, 70.5; H, 10.1; N, 3.0.; IR ν_{max} (Nujol)/(cm^{-1}) 1714, 1737, 3444, 3492; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.69 (s, 3H), 0.95 (s, 3H), 0.99 (d, 3H, *J* = 6.44 Hz), 2.04 (s, 6H), 3.24 (bs,1H), 3.97-4.06 (m, 3H), 4.89 (d, 1H, *J* = 2.52 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4, 17.5, 20.9, 21.5, 22.9, 25.0, 27.1, 27.2, 27.4, 28.7, 29.5, 29.5, 31.0, 31.6, 34.8, 34.9, 35.2, 35.3, 37.9, 42.0, 46.1, 46.4, 47.1, 64.8, 71.1, 72.5, 170.5, 171.1; MS (LCMS) *m/z* 478.2 $[M+1]^+$.

Compound 72: Compound **67** (1.0 g, 2.37 mmol) and Boc-(β-Ala)$_4$-OH **63** (1.05 g, 2.61 mmol) were dissolved in dry DMF (25 mL) under an argon atmosphere and the solution was cooled to –10 ºC. HOBt (0.175 g, 1.3 mmol) and EDCI (0.550 g, 2.87 mmol) were added and stirring was continued for 30 min. The reaction mixture was allowed to warm to room temperature and was stirred for 6 h. The solvent was evaporated under reduced pressure and the residue was dissolved in DCM (300 mL). The organic phase was washed successively with H_2O, 5% citric acid, H_2O, sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was further purified by flash chromatography on silica gel (DCM/MeOH, 93:7) to afford compound **72** (1.35 g, 71%) as a white powder. mp 126-128 ºC; $[\alpha]_D^{28}$ + 16.02 (*c* 0.874, MeOH); Anal. Calcd for $C_{42}H_{71}N_5O_{10}$: C, 62.6; H, 8.9; N, 8.7. Found: C, 62.3; H, 9.1; N, 8.8.; IR ν_{max} (Nujol)/(cm^{-1}) 1647, 1654, 1701, 1714, 1737, 3292, 3334; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.67 (s, 3H), 0.90 (s, 3H), 0.97 (d, 3H, *J* = 5.33 Hz), 1.41 (s, 9H), 2.28-2.49 (m, 8H), 3.31-3.58 (m, 8H), 3.65 (s, 3H), 3.84 (bs, 1H), 3.97 (bs, 3H), 4.02 (bs, 1H), 5.59 (m, 1H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.5, 17.2, 22.9, 23.2, 24.6, 25.9, 27.5, 28.4x3, 29.6, 30.9, 31.1,

33.5, 34.3, 35.1, 35.3, 35.8, 36.0x2, 36.2x2, 36.3x2, 36.4, 37.1x2, 39.4, 41.8, 45.7, 46.5, 47.1, 51.5, 68.2, 72.9, 79.3, 156.3, 171.5, 172.0x2, 172.2, 174.8; MS (LCMS) *m/z*: 828.4 $[M+Na]^+$; HRESIMS *m/z* 828.5058 $[M+Na]^+$ ($C_{42}H_{71}N_5O_{10}Na$; calcd. 828.5069).

Compound 76: Compound **71** (0.477 g, 1 mmol) and Boc-$(Gly)_4$-OH **62** (0.416 g, 1.2 mmol) were coupled to furnish compound **76** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 93:7) to afford compound **76** (604 mg, 75%) as a white solid. mp 120-122 ºC; $[\alpha]_D^{29}$ + 15.47 (*c* 2.715, MeOH); Anal. Calcd for $C_{41}H_{67}N_5O_{11}$: C, 61.1; H, 8.4; N, 8.7. Found: C, 61.3; H, 8.25; N, 8.5.; IR ν_{max} (Nujol)/(cm^{-1}) 1648, 1654, 1701, 1716, 1733, 3294, 3454; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.66 (s, 3H), 0.93 (s, 3H), 0.96 (d, 3H, *J* = 6.0 Hz), 1.41 (s, 9H), 2.01 (s, 6H), 3.84 (bs, 2H), 3.98-4.03 (m, 5H), 4.10-4.15 (m, 5H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.5, 17.6, 21.0, 21.6, 23.0, 23.1, 24.6, 25.2x2, 27.3, 27.6, 28.4x3, 28.8, 30.7, 31.1, 31.8, 32.6, 34.7, 35.0, 36.5, 38.1, 42.1, 43.3x2, 43.5, 44.3, 45.8, 46.6, 47.3, 64.9, 71.1, 72.7, 80.2, 156.2, 168.3, 169.2, 169.4, 170.6, 170.7, 171.2; MS (LCMS) *m/z*: 828.4 $[M+Na]^+$; HRESIMS *m/z* 828.4752 $[M+Na]^+$ ($C_{41}H_{67}N_5O_{11}Na$; calcd. 828.4735).

Compound 77: Compound **71** (0.954 g, 2.0 mmol) and Boc-$(\beta$-Ala$)_4$-OH **63** (0.964 g, 2.4 mmol) were coupled to furnish compound **77** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 94:6) to afford compound **77** (1.27 g, 74%) as a white solid. mp 134-136 ºC; $[\alpha]^{28}_D$ + 16.54 (*c* 1.33, MeOH); Anal. Calcd for $C_{45}H_{75}N_5O_{11}$: C, 62.7; H, 8.8; N, 8.1. Found: C, 62.9; H, 8.6; N, 8.2.; IR ν_{max} (Nujol)/(cm^{-1}) 1647, 1716, 1733, 3292; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.66 (s, 3H), 0.91 (s, 3H), 0.97 (d, 3H, *J* = 6.52 Hz), 1.40 (s, 9H), 2.02 (s, 6H), 2.28-2.46 (m, 8H), 3.38 (m, 2H), 3.47 (m, 6H), 3.34-4.04 (m, 3H), 4.09

(bs, 1H), 4.84 (bs, 1H); ^{13}C NMR (~40% $CDCl_3$ in CD_3OD, 100 MHz) δ 12.4, 17.3, 20.9, 21.4, 22.8, 23.0, 24.4, 25.1, 27.5, 28.2x3, 28.7, 29.6, 30.7, 31.0, 31.8, 32.6, 34.7, 35.3, 35.7, 35.8, 35.9, 36.0, 36.1, 36.2, 36.5, 36.7, 36.9, 38.0, 42.0, 45.6, 46.5, 47.0, 65.3, 71.6, 72.4, 79.5, 156.8, 171.4, 171.8x2, 172.0, 172.5, 172.6; MS (LCMS) *m/z* 884.4 $[M+Na]^+$; HRESIMS *m/z* 884.5320 $[M+Na]^+$ ($C_{45}H_{75}N_5O_{11}Na$; calcd. 884.5361).

Compound 74: LiOH (2M in H_2O, 1 mL) was added to a solution of compound **72** (0.201 g, 0.25 mmol) in methanol (5 mL). The mixture was stirred at room temperature for 12 h and the solvent was removed in *vacuo*. The white residue was dissolved in cold water. Citric acid (5%) was added until pH = 7-8. The residue was filtered, washed with water and diethyl ether and dried thoroughly under vacuum to afford compounds **74** (176 mg, 89% yield) as a white solid. mp 114-117 ºC; $[\alpha]_D^{28}$ + 14.62 (*c* 1.095, MeOH); Anal. Calcd for $C_{41}H_{69}N_5O_{10}\cdot H_2O$: C, 60.8; H, 8.8; N, 8.65. Found: C, 61.0; H, 8.9; N, 8.4.; IR ν_{max} (Nujol)/(cm^{-1}) 1646, 1697, 1714, 3298; ^{1}H NMR (~10% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.96 (s, 3H), 1.01 (d, 3H, *J* = 6.02 Hz), 1.44 (s, 9H), 2.31-2.45 (m, 8H), 3.31-3.38 (m, 2H), 3.39-3.50 (m, 6H), 3.83 (bs, 1H), 3.97 (bs, 3H), 4.01 (bs, 1H); -^{13}C NMR (~40% $CDCl_3$ in CD_3OD, 100 MHz) δ 12.8, 17.5, 23.2, 23.6, 23.7, 24.8, 26.4, 28.0, 28.6x3, 28.8, 30.0, 31.3, 32.5, 33.8, 34.2, 34.6, 35.6, 36.1, 36.2, 36.4, 36.5, 36.6, 37.3, 37.4, 39.9, 42.1, 46.2, 46.9, 47.4, 53.9, 68.5, 73.3, 79.8, 157.2, 172.1, 172.8, 172.9, 179.1, 181.3; MS (LCMS) *m/z* 814.4 $[M+Na]^+$; HRESIMS *m/z* 814.4896 $[M+1]^+$ ($C_{41}H_{69}N_5O_{10}$; calcd. 814.4942).

Compound 79: Compound **79** was synthesized from compound **77** (0.215 g, 0.25 mmol) using the similar procedure as reported for compound **74**. Yield (0.163 g, 84%); mp 84-87 ºC; $[\alpha]_D^{28}$ + 17.72 (*c* 0.79, MeOH)); Anal. Calcd for $C_{41}H_{71}N_5O_9\cdot H_2O$: C, 61.9; H, 9.2; N, 8.8. Found: C, 62.1; H, 9.2; N, 8.9.; IR ν_{max} (Nujol)/(cm^{-1}) 1633, 1645, 1647, 1693, 3292;

^{1}H NMR (~20% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.70 (s, 3H), 0.95 (s, 3H), 1.00 (d, 3H, *J* = 6.28 Hz), 1.43 (s, 9H), 2.29-2.44 (m, 8H), 3.30-3.38 (m, 2H), 3.39-3.50 (m, 6H), 3.51-3.60 (m, 2H), 3.54 (bs, 2H), 3.83 (bs, 1H), 3.97 (s, 1H), 4.03 (bs, 1H); **^{13}C** NMR (~20% $CDCl_3$ in CD_3OD, 100 MHz) δ 12.7, 17.7, 23.1, 23.5, 24.7, 26.3, 27.9, 28.5x3, 28.7, 29.6, 31.1, 32.2, 33.7, 34.5, 35.5, 36.0, 36.2x2, 36.4, 36.5, 37.2, 37.3, 39.7, 42.0, 45.6, 46.1, 46.2, 46.3, 46.7, 47.6, 63.1, 68.5, 73.3, 79.8, 157.1, 172.0, 172.1, 172.7, 172.9, 181.5; MS (LCMS) *m/z*: 800.5 $[M+Na]^+$; HRESIMS *m/z* 800.5123 $[M+Na]^+$ ($C_{41}H_{71}N_5O_9Na$; calcd. 800.5149).

Compound 73: A solution of compound **72** (0.081 g, 0.1 mmol) in 2M HCl:Et_2O (5 mL) was stirred at 0 ºC for 30 min and additionally at 25 ºC for 1.5 h. The volatiles were removed under vacuum, and the residue was dried thoroughly to afford compound **73** (0.079 g, 94% yield); $[\alpha]_D^{28}$ +19.4 (*c* 0.615, MeOH); Anal. Calcd for $C_{37}H_{63}N_5O_8 \cdot HCl \cdot H_2O$: C, 58.4; H, 8.75; N, 9.2. Found: C, 58.4; H, 8.7; N, 9.35.; IR ν_{max} (Nujol)/(cm^{-1}) 1654, 1714, 1735, 3269, 3444; ^{1}H NMR (~5% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.72 (s, 3H), 0.98 (s, 3H), 1.01 (d, 3H, *J* = 6.77 Hz), 2.39-2.49 (m, 4H), 2.50-2.69 (m, 4H), 3.21 (m, 2H), 3.33 (bs, 2H), 3.40-3.55 (m, 6H), 3.66 (s, 3H), 3.82 (bs, 1H), 3.96 (bs, 1H), 4.05 (bs, 1H); ^{13}C NMR (~ 20% $CDCl_3$ in CD_3OD, 100 MHz) δ 13.0, 17.5, 23.3, 24.0, 25.2, 27.1, 28.4, 29.4, 30.5, 31.7, 31.8, 31.9, 32.8, 34.1, 35.1, 36.0, 36.1, 36.4, 36.5, 36.9, 37.1, 37.1, 37.9, 40.6, 42.7, 47.3, 47.6, 47.7, 49.9, 52.0, 68.9, 73.7, 171.9, 173.5, 173.7, 173.9, 176.4; MS (LCMS) *m/z* 706.2 $[M+1]^+$, 728.3 $[M+Na]^+$; HRESIMS *m/z* 706.4724 $[M+1]^+$ ($C_{37}H_{64}N_5O_8$; calcd. 706.4755).

Compound 75: Compound **75** was synthesized from compound **74** (0.079 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.066 g, 91% yield); $[\alpha]_D^{28}$ +23.16 (*c* 0.95, MeOH); Anal. Calcd for $C_{36}H_{61}N_5O_8 \cdot HCl \cdot 2H_2O$: C, 56.6; H, 8.7; N,

9.2. Found: C, 56.7; H, 8.9; N, 9.3.; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1711, 1716, 3265; ^1H NMR (CD_3OD, 400 MHz) δ 0.73 (s, 3H), 0.99 (s, 3H), 1.02 (d, 3H, J = 6.28 Hz), 2.35-2.53 (m, 8H), 3.22 (m, 2H), 3.41-3.50 (m, 6H), 3.82 (bs, 1H), 3.97 (bs, 3H), 4.03 (m, 1H); ^{13}C NMR (CD_3OD, 100.61 MHz) δ 13.0, 17.6, 23.4, 24.2, 25.5, 27.4, 28.6, 29.7, 31.9x2, 32.2, 33.0, 34.5, 35.4, 36.2, 36.5, 36.7x2, 37.1, 37.2x2, 37.3, 38.2, 40.9, 43.0, 47.3, 47.5, 48.0, 66.9, 69.1, 74.0, 172.2, 173.4, 173.8, 174.0, 176.6; MS (LCMS) *m/z*: 692.4 $[M+1]^+$, 714.4 $[M+Na]^+$; HRESIMS *m/z* 692.4584 $[M+1]^+$ ($C_{36}H_{62}N_5O_8$; calcd. 692.4594).

Compound 78: Compound **78** was synthesized from compound **76** (0.081 g, o.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.071 g, 96% yield); $[\alpha]_D^{26}$ +15.95 (*c* 1.63, MeOH); Anal. Calcd for $C_{36}H_{59}N_5O_9 \cdot HCl \cdot H_2O$: C, 56.9; H, 8.2; N, 9.2. Found: C, 56.7; H, 8.5; N, 9.5.; IR ν_{max} (Nujol)/(cm^{-1}) 1654, 1668, 1714, 1731, 1737, 3303, 3479; ^1H NMR (CD_3OD, 400 MHz) δ 0.72 (s, 3H), 1.00 (s, 3H), 1.02 (d, 3H, J = 6.0 Hz), 2.02 (s, 3H), 2.04 (s, 3H), 3.81 (bs, 2H), 3.90 (2H, d, J = 11.0 Hz) 3.95-4.07 (m, 6H), 4.87 (s, 1H), 5.82 (bs, 1H); ^{13}C NMR (CD_3OD, 100.61 MHz) δ 12.9, 17.9, 20.9, 21.5, 23.3, 24.1, 25.4, 26.3, 28.5, 28.8, 29.9, 31.7, 32.2, 33.1, 33.9, 35.8, 36.7, 37.7, 39.4, 41.7, 43.2, 43.4, 43.6, 43.8, 47.2, 47.6, 48.0, 66.2, 72.9, 73.5, 168.6, 170.7, 171.9, 172.0, 172.6, 173.1; MS (LCMS) *m/z* 706.2 $[M+1]^+$, 728.3 $[M+Na]^+$; HRESIMS *m/z* 706.440 $[M+1]^+$ ($C_{36}H_{60}N_5O_9$; calcd. 706.4391).

Compound 80: Compound **80** was synthesized from compound **79** (0.078 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.063 g, 89% yield); $[\alpha]_D^{28}$ +15.38 (*c* 0.91, MeOH); Anal. Calcd for $C_{36}H_{63}N_5O_7 \cdot HCl \cdot H_2O$: C, 59.0; H, 9.1; N, 9.6; Found: C, 59.3; H, 9.2; N, 9.5.; **IR** ν_{max} (Nujol)/(cm^{-1}) 1637, 1651, 1652, 3274; ^1H NMR (CD_3OD, 400 MHz) δ 0.72 (s, 3H), 0.97 (s, 3H), 1.02 (d, 3H, J = 6.84 Hz), 2.31-2.49 (m, 8H), 3.17 (m, 1H), 3.37 (m, 2H), 3.39-3.50 (m, 6H), 3.53 (m, 2H), 3.82 (bs,

1H), 3.98 (bs, 1H), 4.01 (bs, 1H); ^{13}C NMR (CD_3OD 100.61 MHz) δ 13.0, 18.0, 23.3, 24.0, 25.3, 26.9, 28.5, 29.4, 30.1, 30.4, 31.6, 32.8, 33.7, 34.2, 35.1, 36.0, 36.4, 36.5, 36.8, 36.9, 37.0, 37.3, 37.8, 40.4, 42.6, 46.7, 47.2, 48.0, 49.9, 63.4, 68.9, 73.8, 172.1, 172.8, 173.4, 173.5; MS (LCMS) *m/z* 679.3 $[M+2]^+$; HRESIMS *m/z* 678.4742 $[M+1]^+$ ($C_{36}H_{64}N_5O_7$; calcd. 678.4806).

Compound 81: Compound **67** (1.0 g, 2.37 mmol) and Boc-$(Gly)_2$-OH **58** (0.660 g, 2.84 mmol) were coupled to furnish compound **81** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 97:3) to afford compound **81** (1.113 g, 74%) as a white solid. mp 121-122 ºC; $[\alpha]_D^{25}$ + 27.02 (*c* 0.88, MeOH); Anal. Calcd for $C_{34}H_{57}N_3O_8$: C, 64.23; H, 9.04; N, 6.61 Found: C, C, 64.33; H, 8.81; N, 6.49; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1658, 1681, 1693, 1720, 1737, 3338; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.68 (s, 3H), 0.93 (s, 3H), 0.99 (d, 3H, *J* = 5.86 Hz), 1.44 (s, 9H), 3.66 (s, 3H), 3.76-4.01 (m, 6H), 4.05 (bs, 1H), 5.33 (bs, 1H), 6.76 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50.32 MHz) δ 12.4, 17.2, 22.8, 23.1, 24.4, 25.9, 27.4, 28.2x3, 30.8, 31.1, 33.2, 34.3, 35.1, 35.2, 36.9, 41.7, 43.3, 44.0, 45.9, 46.4, 47.1, 51.4, 68.2, 72.9, 80.1, 156.1, 168.4, 170.5, 174.8; MS (LCMS) *m/z*: 657.9663 $[M+Na]^+$.

Compound 82: Compound **67** (1.0 g, 2.37 mmol) and Boc-$(\beta$-Ala$)_2$-OH **59** (0.738 g, 2.84 mmol) were coupled to furnish compound **82** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 96:4) to afford compound **82** (1.195 g, 76%) as a white solid. mp 123-126 ºC; $[\alpha]_D^{25}$ + 23.64 (*c* 1.1, MeOH); Anal. Calcd for $C_{36}H_{61}N_4O_8$: C, 65.13; H, 9.26; N, 6.33 Found: C, 64.95; H, 9.57; N, 6.39; IR ν_{max} (Nujol)/(cm^{-1}) 1639, 1693, 1739, 3323; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.68 (s, 3H), 0.93 (s, 3H), 0.98 (d, 3H, *J* = 5.86 Hz), 1.42 (s, 9H), 2.38 (m, 4H), 3.26-3.64 (m, 4H), 3.66 (s, 3H), 3.85 (bs, 1H), 3.97 (bs, 1H), 4.10 (bs,

1H), 6.27 (bs, 1H), 7.10 (m, 1H); ^{13}C NMR ($CDCl_3$, 50.32 MHz) δ 12.4, 17.2, 22.7, 23.2, 24.5, 25.8, 27.4, 28.3x 3, 30.8, 31.0x2, 33.5, 34.2, 35.1, 35.2x2, 35.2, 35.6, 35.9, 36.3, 37.1, 39.3, 41.7, 45.4, 45.5, 46.4, 47.1, 51.4, 68.1, 72.9, 79.2, 156.0, 171.1, 172.0, 174.7; MS (MALDI-TOF) *m/z* 686.9378 $[M+Na]^+$.

Compound 87: Compound **67** (1.0 g, 2.37 mmol) and Boc-(Gly)-OH **52** (0.497 g, 2.84 mmol) were coupled to furnish compound **87** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 97:3) to afford compound **87** (0.973 g, 71%) as a white solid. mp 92-94 ºC; $[\alpha]_D^{26}$ + 25.38 (*c* 0.788, MeOH); Anal. Calcd for $C_{32}H_{54}N_2O_7$: C, 66.41; H, 9.40; N, 4.84 Found: C, 66.74; H, 9.56; N, 5.09; IR ν_{max} (Nujol)/(cm^{-1}) 1666, 1716, 1722, 1737, 3429; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, 3H, *J* = 6.0 Hz), 1.44 (s, 9H), 3.73 (d, 2H, *J* = 7 Hz), 3.83 (bs, 1H), 3.96 (s, 1H), 4.06 (bs, 1H), 5.52 (bs, 1H), 6.74 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50.32 MHz) δ 12.4, 17.2, 22.9, 24.5, 25.7, 27.4, 28.2x3, 28.3, 30.8, 31.0, 33.3, 35.1, 35.2, 37.2, 39.3, 42.6, 44.8, 45.4, 46.4, 47.0, 51.4, 68.2, 73.0, 80.1, 156.4, 169.3, 174.8; MS (MALDI-TOF) *m/z* 601.0773 $[M+Na]^+$.

Compound 88: Compound **67** (1.0 g, 2.37 mmol) and Boc-(β-Ala)-OH **58** (0.536 g, 2.84 mmol) were coupled to furnish compound **88** following the same procedure as described for compound **72**. The residue was purified by flash chromatography on silica gel (DCM/MeOH, 96:4) to afford compound **88** (1.124 g, 80%) as a white solid. mp 99-101 ºC; $[\alpha]_D^{25}$ + 26.98 (*c* 1.26, MeOH); Anal. Calcd for $C_{33}H_{56}N_2O_7$: C, 66.86; H, 9.52; N, 4.73. Found: C, 66.97; H, 9.35; N, 4.83; IR ν_{max} (Nujol)/(cm^{-1}) 1662, 1701, 1716, 1734, 3264, 3387; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.68 (s, 3H), 0.93 (s, 3H), 0.98 (d, 3H, *J* = 5.82 Hz), 1.42 (s, 9H), 2.39 (t, 2H, *J* = 6.04 Hz, *J* = 11.38 Hz), 3.84 (bs, 2H), 3.36 (m, 2H), 3.65 (s, 3H), 3.84 (bs, 1H), 3.96 (bs, 1H), 4.09 (bs, 1H), 5.36 (bs, 1H), 6.12 (bs, 1H);

^{13}C NMR ($CDCl_3$, 50.32 MHz) δ 12.4, 17.2, 22.9, 23.2, 24.5, 25.8, 27.4, 28.3x 3, 30.8, 31.0, 33.3, 34.2, 35.1, 35.2, 36.6, 37.2, 39.3, 41.6, 45.5, 46.4, 47.2, 51.4, 68.2, 72.9, 79.3, 156.3, 171.1, 174.8; MS (LCMS) *m/z*: 592.7398 $[M]^+$.

Compound 85: Compound **85** was synthesized from compound **82** (0.165 g, 0.25 mmol) using the similar procedure as reported for compound **74**. Yield (0.141 g, 87%); mp 139-141 ºC; $[\alpha]_D^{26}$ + 23.76 (*c* 1.01, MeOH)); Anal. Calcd for $C_{35}H_{59}N_3O_8$: C, 64.69; H, 9.15; N, 6.47 Found: C, 64.48; H, 9.19; N, 6.50; IR ν_{max} (Nujol)/(cm^{-1}) 1656, 1693, 1697, 1708, 3330; ^{1}H NMR (~10% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.72 (s, 3H), 0.98 (s, 3H), 1.02 (d, 3H, *J* = 6.37 Hz), 1.44 (s, 9H), 2.35 (t, 2H, *J* = 6.83 Hz, 13.37 Hz), 2.42 (t, 2H, *J* = 6.83 Hz, 13.35 Hz), 3.29-3.36 (m, 2H), 3.43 (t, 2H, *J* = 6.83 Hz, 13.37 Hz), 3.82 (bs, 1H), 3.97 (bs, 1H), 4.03 (bs, 1H); ^{13}C NMR (~10% $CDCl_3$ in CD_3OD, 100.61 MHz) δ 13, 17.6, 23.4, 24.0, 25.3, 26.9, 28.4, 28.7x3, 29.4, 31.6, 32.1, 32.2, 34.2, 35.1, 36.5, 37.0, 37.2, 37.8, 37.9, 40.5, 42.6, 46.8, 47.2, 47.8, 68.9, 73.8, 80.0, 157.9, 172.8, 173.7, 178.5; MS (MALDI-TOF) *m/z* 672.1835 $[M+Na]^+$, 688.1438 $[M+K]^+$.

Compound 91: Compound **91** was synthesized from compound **87** (0.144 g, 0.25 mmol) using the similar procedure as reported for compound **74**. Yield (0.125 g, 89%); mp 149-151 ºC; $[\alpha]_D^{26}$ + 27.63 (*c* 0.79, MeOH)); Anal. Calcd for $C_{31}H_{52}N_2O_7$: C, 65.93; H, 9.28; N, 4.96 Found: C, 66.14; H, 9.43; N, 4.81; IR ν_{max} (Nujol)/(cm^{-1}) 1650, 1697, 1705, 3351 (broad); ^{1}H NMR (~20% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.97 (s, 3H), 1.02 (d, 3H, *J* = 6.17 Hz), 1.47 (s, 9H), 3.69 (bs, 2H), 3.83 (bs, 1H), 3.96 (bs, 1H), 4.05 (bs, 1H); **^{13}C** NMR (~20% $CDCl_3$ in CD_3OD, 100 MHz) δ 12.9, 17.5, 23.4, 23.8, 25.0, 26.6, 28.2, 28.6x3, 29.1, 31.5, 31.7, 31.8, 34.0, 34.8, 35.8, 36.1, 37.9, 40.1, 42.3, 44.6, 46.3, 47.0, 47.5, 49.9, 68.6, 73.5, 80.6, 157.8, 170.8, 178; MS (MALDI-TOF) *m/z* 587.1513 $[M+Na]^+$, 603.1132 $[M+K]^+$.

Compound 92: Compound **92** was synthesized from compound **88** (0.148 g, 0.25 mmol) using the similar procedure as reported for compound **74**. Yield (0.131 g, 91%); mp 144-147 ºC; $[\alpha]_D^{25}$ + 27.1 (*c* 0.812, MeOH)); Anal. Calcd for $C_{32}H_{54}N_2O_7$ C, 66.41; H, 9.40; N, 4.84 Found: C, 66.38; H, 9.18; N, 4.99; IR ν_{max} (Nujol)/(cm^{-1}) 1647, 1693, 1712, 3342; ^{1}H NMR (~10% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.97 (s, 3H), 1.02 (d, 3H, *J* = 6.33 Hz), 1.47 (s, 9H), 2.16-2.30 (m, 2H), 3.69 (m, 2H), 3.83 (bs, 1H), 3.96 (bs, 1H), 4.05 (bs, 1H); ^{13}C NMR (~10% $CDCl_3$ in CD_3OD, 100.61 MHz) δ 12.9, 17.5, 23.3, 23.8, 25.1, 26.7, 28.2, 28.7x3, 29.2, 31.5, 31.8x2, 34.0, 35.0, 35.9, 36.0, 36.2, 36.9, 37.7, 37.8, 40.3, 42.4, 46.6, 47.1, 47.6, 68.8, 73.6, 80.0, 157.8, 172.7, 177.8; MS (MALDI-TOF) *m/z* 601.7855 $[M+Na]^+$, 617.0296 $[M+K]^+$.

Compound 83: Compound **83** was synthesized from compound **81** (0.063 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.050 g, 94% yield); $[\alpha]_D^{27}$ +28.28 (*c* 0.99, MeOH); IR ν_{max} (Nujol)/(cm^{-1}) 1649, 1658, 1685, 1716, 3240, 3363; MS (MALDI-TOF) *m/z* 558.2144 $[M+Na]^+$, 574.1624 $[M+K]^+$.

Compound 84: Compound **84** was synthesized from compound **82** (0.066 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.050 g, 90% yield); mp 206-208 ºC; $[\alpha]_D^{26}$ +20.9 (*c* 0.67, MeOH); Anal. Calcd for $C_{31}H_{53}N_3O_6$·HCl: C, 62.03; H, 9.07; N, 7.00 Found: C, 62.35; H, 9.23; N, 7.12; IR ν_{max} (Nujol)/(cm^{-1}) 1645, 1654, 1720, 1735, 3315, 3417; ^{1}H NMR (~10% $CDCl_3$ in CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.97 (s, 3H), 1.02 (d, 3H, *J* = 5.69 Hz), 2.35-2.50 (m, 4H), 3.06 (t, 2H, *J* = 6.03 Hz, *J* = 12.05 Hz), 3.44 (t, 2H, *J* = 7.03 Hz, *J* = 13.39 Hz), 3.68 (s, 3H), 3.84 (bs, 1H), 3.96 (bs, 1H), 4.01 (bs, 1H); ^{13}C NMR (~10% $CDCl_3$ in CD_3OD, 100.61 MHz) δ 12.8, 17.4, 23.1, 23.6, 24.8, 26.4, 27.9, 28.8, 31.2, 31.4, 31.5, 33.7, 34.6, 35.6, 35.8, 36.0, 36.6x2, 37.4, 37.4, 39.9,

42.1, 46.2, 46.8, 47.3, 51.9, 68.6, 73.3, 172.1, 172.4, 176.0; MS (MALDI-TOF) *m/z* 564.2188 $[M+1]^+$, 586.2240 $[M+Na]^+$, 602.1502 $[M+K]^+$.

Compound 86: Compound **86** was synthesized from compound **85** (0.065 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.046 g, 84% yield); mp 187-189 ºC; $[\alpha]_D^{26}$ +22.01 (*c* 1.09, MeOH); Anal. Calcd for $C_{30}H_{51}N_3O_6 \cdot HCl$: C, 61.47; H, 8.94; N, 7.17 Found: C, 61.39; H, 9.16; N, 7.16; IR ν_{max} (Nujol)/(cm^{-1}) 1641, 1713, 1735, 3359; ^1H NMR (CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.97 (s, 3H), 1.01 (d, 3H, *J* = 6.28 Hz), 2.46 (t, 2H, *J* = 6.73 Hz, *J* = 13.47 Hz), 2.49 (t, 2H, *J* = 6.74 Hz, *J* = 12.55 Hz), 3.18 (t, 2H, *J* = 5.81 Hz, *J* = 12.24 Hz), 3.44 (t, 2H, *J* = 6.43 Hz, *J* = 12.86 Hz), 3.80 (bs, 1H), 3.94 (bs, 1H), 4.00 (m, 1H); ^{13}C NMR (CD_3OD, 100.61 MHz) δ 13.0, 17.6, 23.4, 24.2, 25.5, 27.3, 28.6, 29.7, 31.8, 31.9, 32.2, 32.9, 34.5, 35.4, 36.3, 36.4, 36.7, 37.2, 37.3, 38.2, 40.9, 43.0, 47.2, 47.5, 48.0, 69.1, 74.0, 172.1, 173.2, 176.5; MS (MALDI-TOF) *m/z* 573.1524 $[M+Na]^+$, 589.1092 $[M+K]^+$.

Compound 89: Compound **89** was synthesized from compound **87** (0.058 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.044 g, 92% yield); mp 118-120 ºC; $[\alpha]_D^{25}$ +28.27 (*c* 1.91, MeOH); Anal. Calcd for $C_{27}H_{46}N_2O_5 \cdot HCl$: C, 62.95; H, 9.20; N, 5.44 Found: C, 63.19; H, 9.03; N, 5.27; IR ν_{max} (Nujol)/(cm^{-1}) 1680, 1731, 3417; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.68 (s, 3H), 0.93 (s, 3H), 0.97 (d, 3H, *J* = 5.29 Hz), 3.42 (m, 2H), 3.66 (s, 3H), 3.84 (s, 1H), 3.96 (s, 3H), 4.09 (bs, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 12.5, 17.2, 23.1, 23.2, 24.7, 26.0, 27.4, 28.5, 30.9, 31.1x2, 33.6, 34.3, 35.1, 35.2, 37.3, 39.4, 41.8, 45.0, 46.5, 47.2, 51.5, 68.2, 72.9, 172.8, 174.8; MS (MALDI-TOF) *m/z* 501.2392 $[M+Na]^+$, 517.1613 $[M+K]^+$.

Compound 90: Compound **90** was synthesized from compound **88** (0.059 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.046 g, 91% yield);

$[\alpha]_D^{25}$ +24.52 (*c* 1.06, MeOH); IR ν_{max} (Nujol)/(cm^{-1}) 1643, 1656, 1720, 1735, 3336, 3369; MS (MALDI-TOF) *m/z* 493.2378 $[M+1]^+$, 515.2359 $[M+Na]^+$.

Compound 93: Compound **93** was synthesized from compound **91** (0.056 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.040 g, 87% yield); mp 129-131 ºC; $[\alpha]_D^{27}$ +26.54 (*c* 1.13, MeOH); Anal. Calcd for $C_{26}H_{44}N_2O_5 \cdot HCl$: C, 62.32; H, 9.05; N, 5.59 Found: C, 62.41; H, 8.90; N, 5.50; IR ν_{max} (Nujol)/(cm^{-1}) 1670, 1711, 1714, 3245, 3357; ^1H NMR (CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.98 (s, 3H), 1.01 (d, 3H, *J* = 6.52 Hz), 3.54-3.70 (m, 2H), 3.80 (bs, 1H), 3.95 (bs, 3H), 4.07 (m, 1H); ^{13}C NMR (CD_3OD, 100.61 MHz) δ 13.0, 17.6, 23.4, 24.2, 25.6, 27.3, 28.6, 29.7, 31.8x2, 32.2, 34.5, 35.4, 36.3, 36.7, 38.3, 40.9, 41.6, 43.0, 47.5, 47.6 48.0, 69.0, 74.0, 166.5, 176.5; MS (MALDI-TOF) *m/z* 487.1214 $[M+Na]^+$, 504.1456 $[M+K]^+$.

Compound 94: Compound **94** was synthesized from compound **92** (0.058 g, 0.1 mmol) using the similar procedure as reported for compound **73**. Yield (0.039 g, 81% yield); mp 110-113 ºC; $[\alpha]_D^{26}$ +25.0 (*c* 1.04, MeOH); Anal. Calcd for $C_{27}H_{46}N_2O_5 \cdot HCl$: C, 62.95; H, 9.20; N, 5.44 Found: C, 63.10; H, 9.31; N, 5.68; IR ν_{max} (Nujol)/(cm^{-1}) 1643, 1650, 1713, 3269, 3368; ^1H NMR (CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.98 (s, 3H), 1.01 (d, 3H, *J* = 6.06 Hz), 2.66 (m, 2H), 3.17 (m, 2H), 3.80 (bs, 1H), 3.95 (bs, 3H), 4.04 (m, 1H); ^{13}C NMR (CD_3OD, 100.61 MHz) δ 13.0, 17.6, 23.4, 24.2, 25.6, 27.3, 28.7, 29.7, 31.86, 31.9, 32.2, 32.7, 34.5, 35.4, 36.3, 36.7, 37.3, 38.3, 40.9, 43.0, 47.2, 47.5, 48.0, 66.9, 69.1, 74.0, 171.6, 176.5; MS (MALDI-TOF) *m/z* 501.1702 $[M+Na]^+$, 518.2012 $[M+K]^+$.

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 ^{o}C. *E. coli* (NCIM 2574) and *S. aureus* (NCIM 2122) were maintained on NA (beef extract, 0.3%; peptone, 0.5%; sodium chloride, 0.5%) 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 ^{o}C where as *B. poitrasii* was incubated at 37 ^{o}C for 24 h. *F. oxysporum* was inoculated in potato dextrose and incubated at 28 ^{o}C for 48 h whereas bacterial strains *E. coli* and *S. aureus* in NA broth for 24 h. Compounds **60** to **66** and compounds **62** to **80** were solubilized in DMSO, and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, Fluconazole, Tetracycline and Erythromycin 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* (NCIM 2574), and *S. aureus* (NCIM 2122), respectively to find out MIC (Minimum Inhibitory Concentration) and IC_{50} (50%, Inhibition of Growth) values. Experiments were performed 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.[65] 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 for *B. poitrasii* (37 °C), 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.

Table 1: The half maximal inhibitory concentration (IC_{50}) of modified steroids.

Entry	Comp. No.	Antimicrobial Activity IC_{50} (μg/ml)						
		Fungal Strains					Bacterial Strains	
		A	**B**	**C**	**D**	**E**	**F**	**G**
1	**66**	128	>128	96	>128	64	64	64
2	**72**	16	>128	8	64	16	64	64
3	**73**	64	>128	12	>128	16	48	24
4	**74**	14	>128	24	>128	24	128	64
5	**75**	8	>128	16	>128	32	16	128
6	**76**	32	>128	16	>128	32	32	96
7	**77**	16	>128	16	32	96	96	16
8	**78**	28	>128	8	>128	16	64	32
9	**79**	50	>128	64	>128	32	64	64
10	**80**	16	>128	16	>128	48	16	128
11	**AmpB**	0.5	8	8	8	8	NT	NT
12	**Fluconazole**	4	16	16	32	4	NT	NT
13	**Tetracycline**	NT	NT	NT	NT	NT	1	4
14	**Erythromycin**	NT	NT	NT	NT	NT	16	16

A, *C. albicans*; **B**, *C. neoformans*; **C**, *B. poitrasii*; **D**, *Y. lipolytica*; **E**, *F. oxysporum*; **F**, *E. coli*; **G**, *S. aureus*; NT- Not Tested.

Antiproliferative 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. 3-(4,5-Dimethylthiazol-2-yl)-2,5-

diphenyltetra- zolium 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 triplicate.

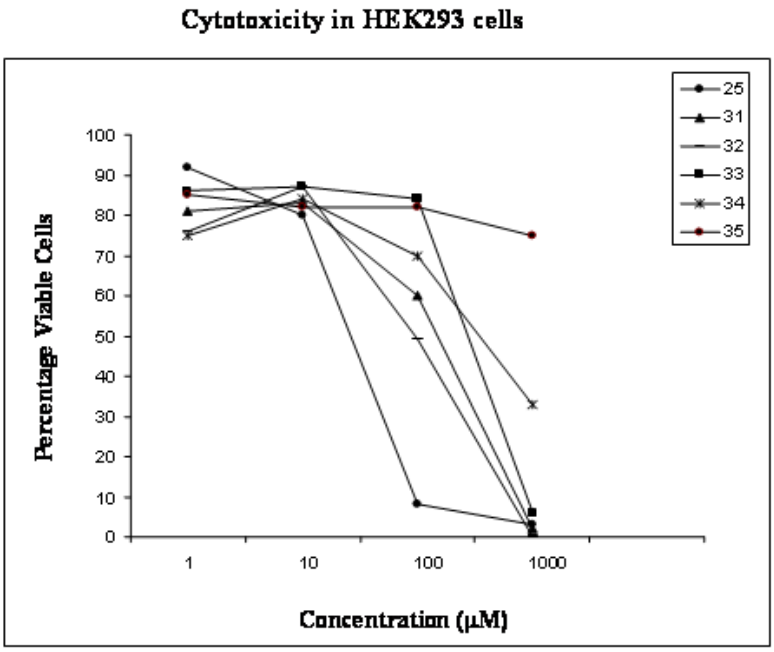


Figure 16. Cytotoxicity of **66** and **72-74** in HEK293 cells.

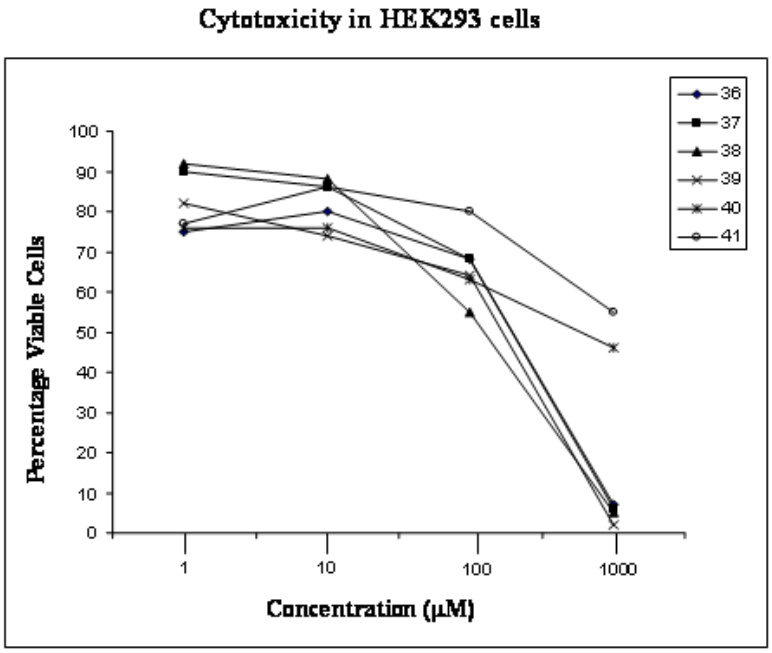


Figure 17. Cytotoxicity of **75-80** in HEK293 cells.

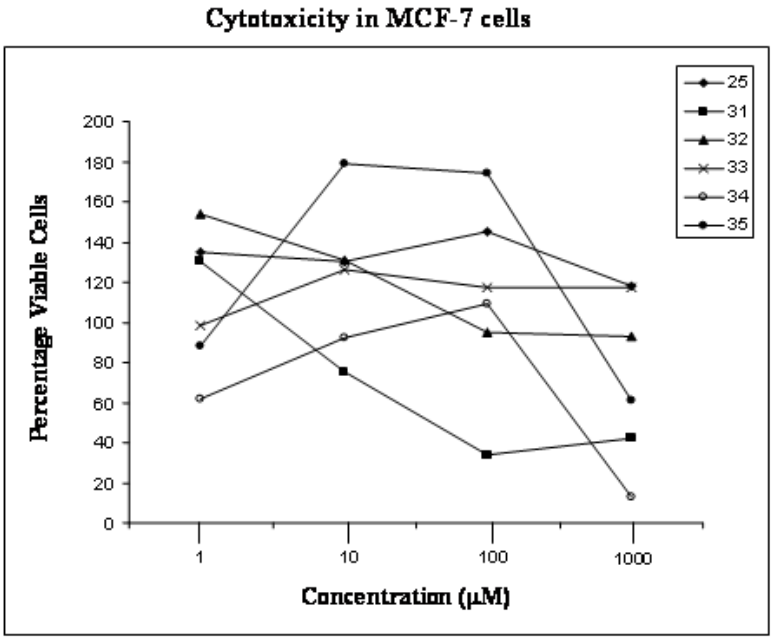


Figure 18. Cytotoxicity of **66** and **72-74** in MCF-7 cells.

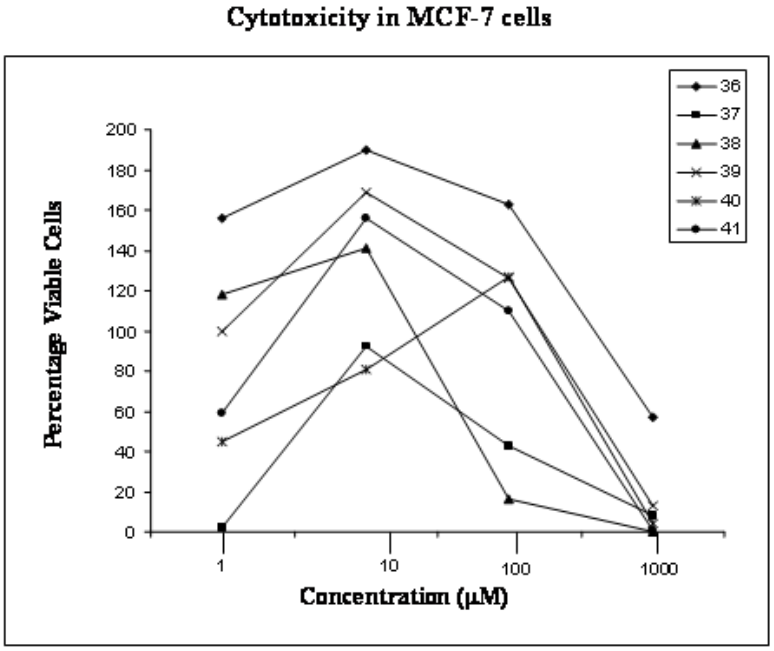
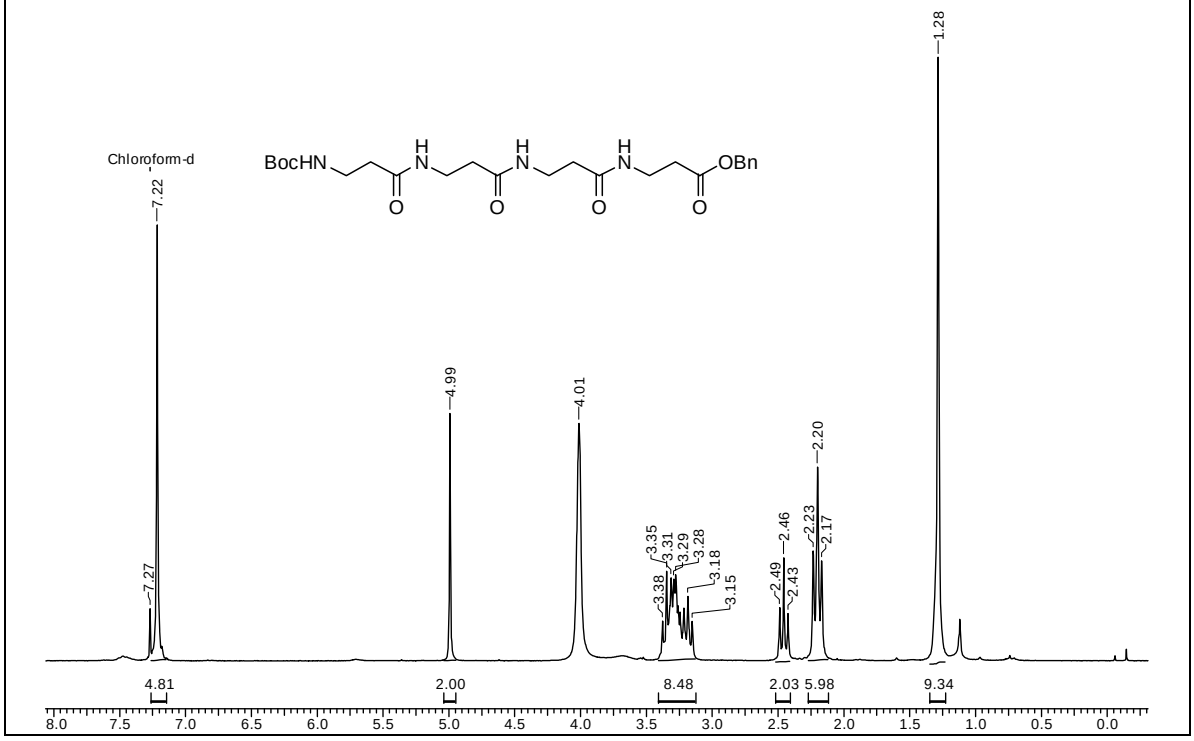


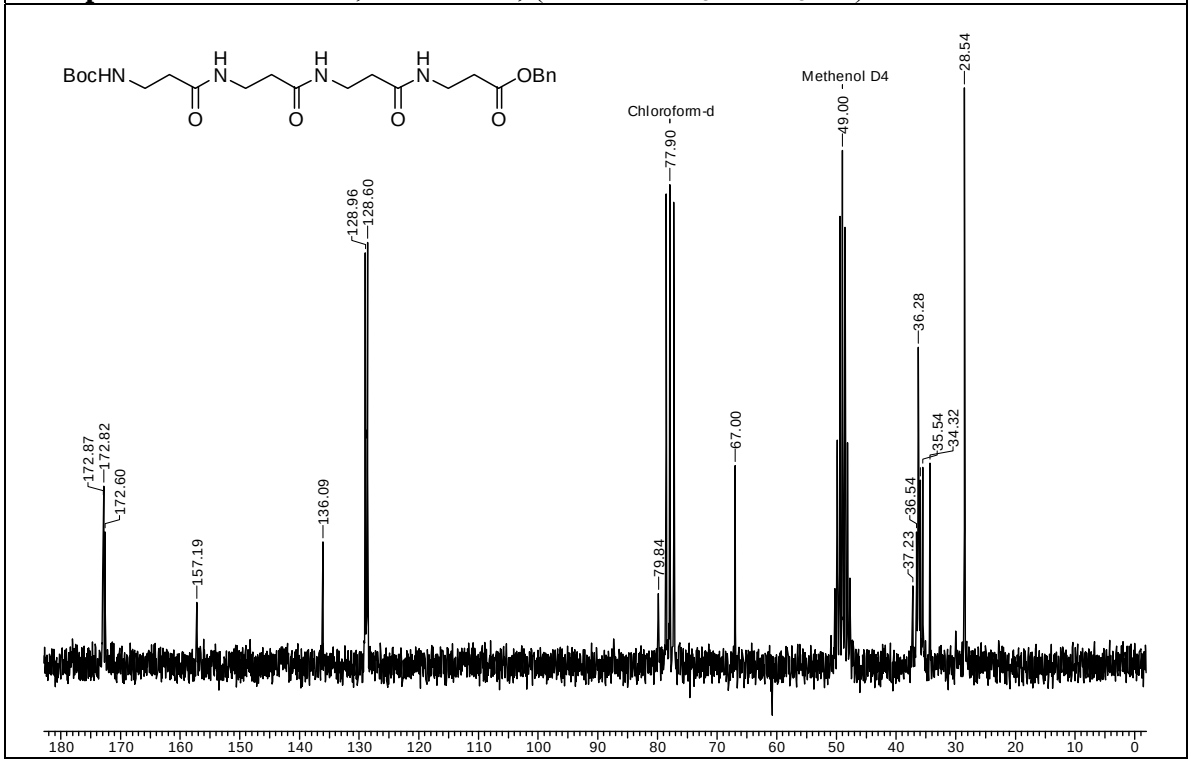
Figure 19. Cytotoxicity of **75-80** in MCF-7 cells.

2A.9. Selected Spectra

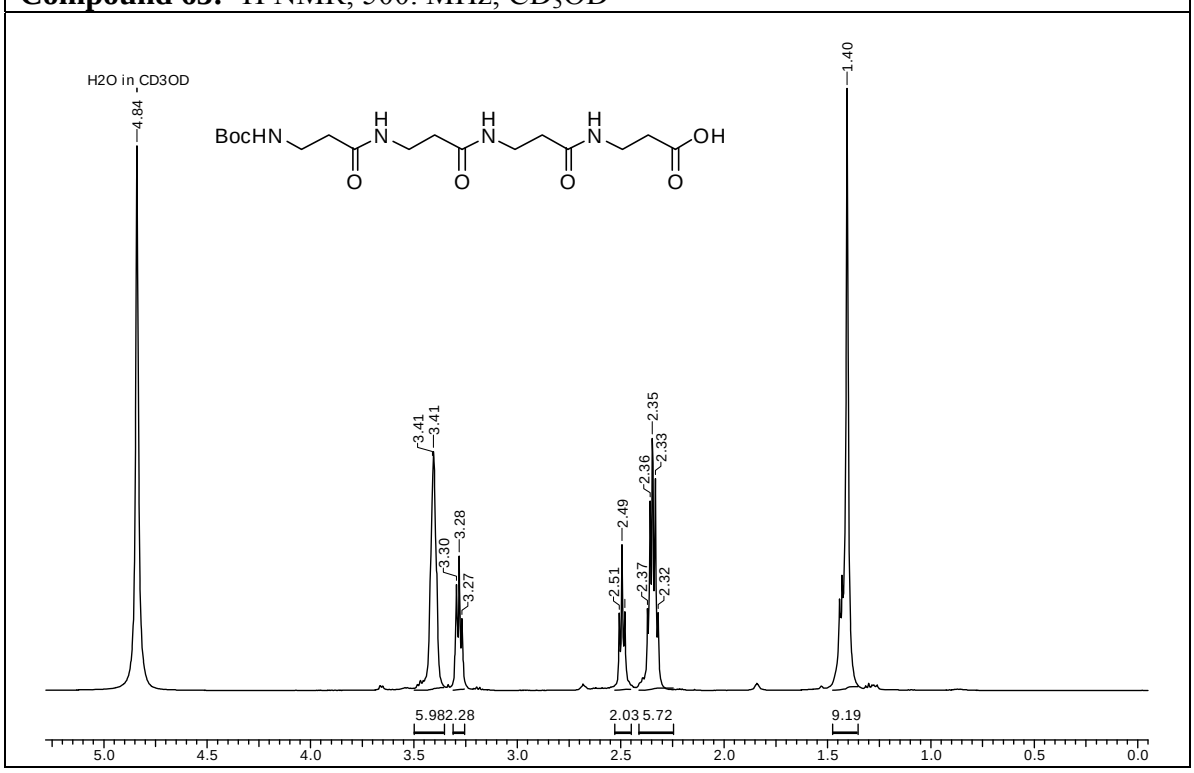
Compound 61: ^{1}H NMR, 200 MHz, (~30% CD_3OD in $CDCl_3$)



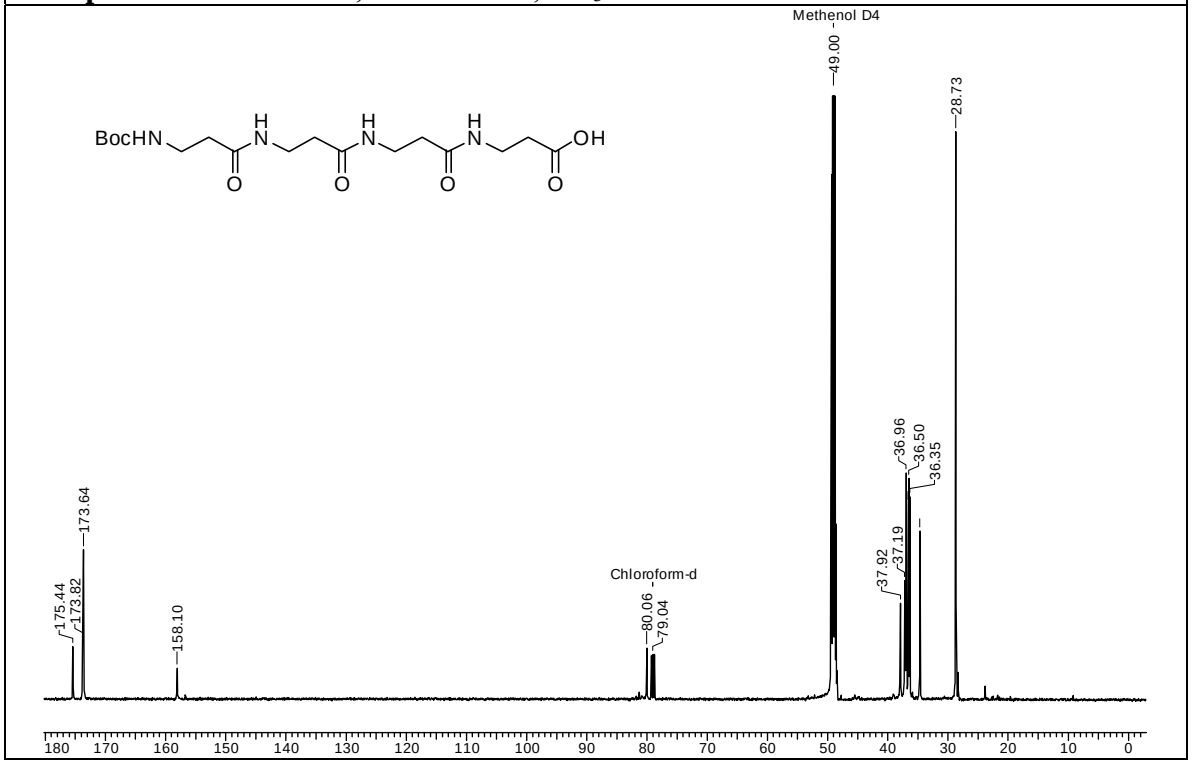
Compound 61: ^{13}C NMR, 50.32 MHz, (~60% $CDCl_3$ in CD_3OD)



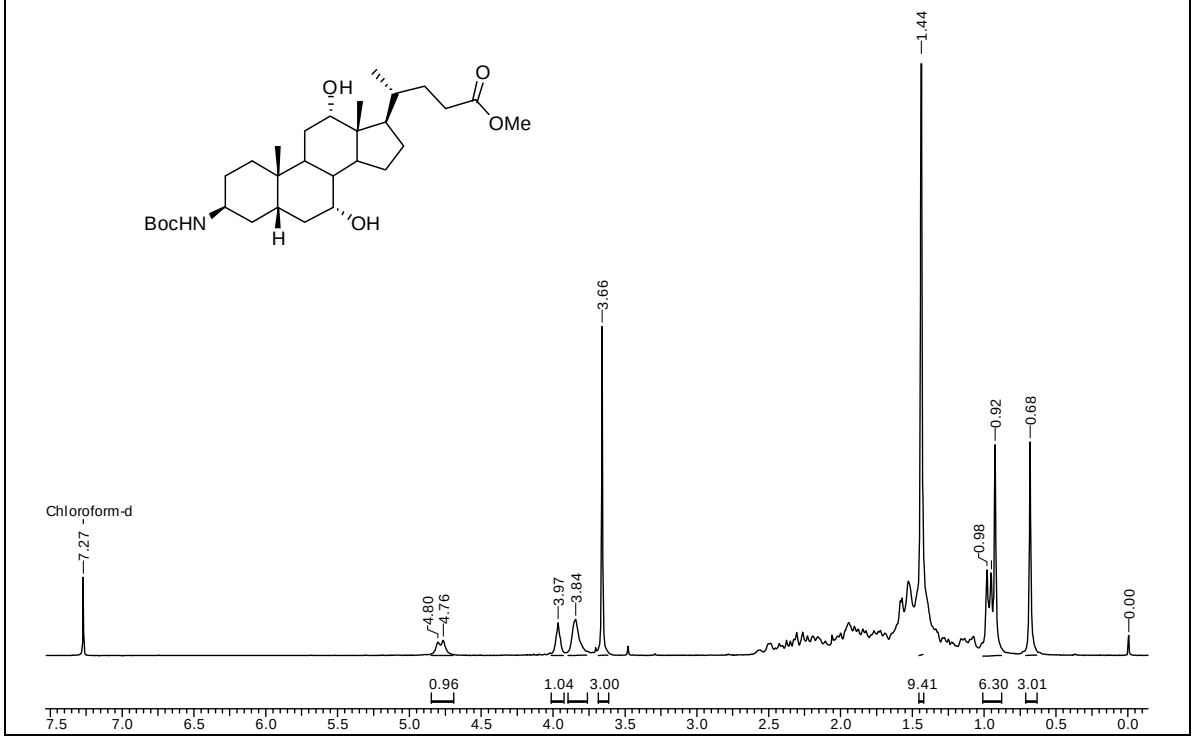
Compound 63: ^{1}H NMR, 500. MHz, CD_3OD



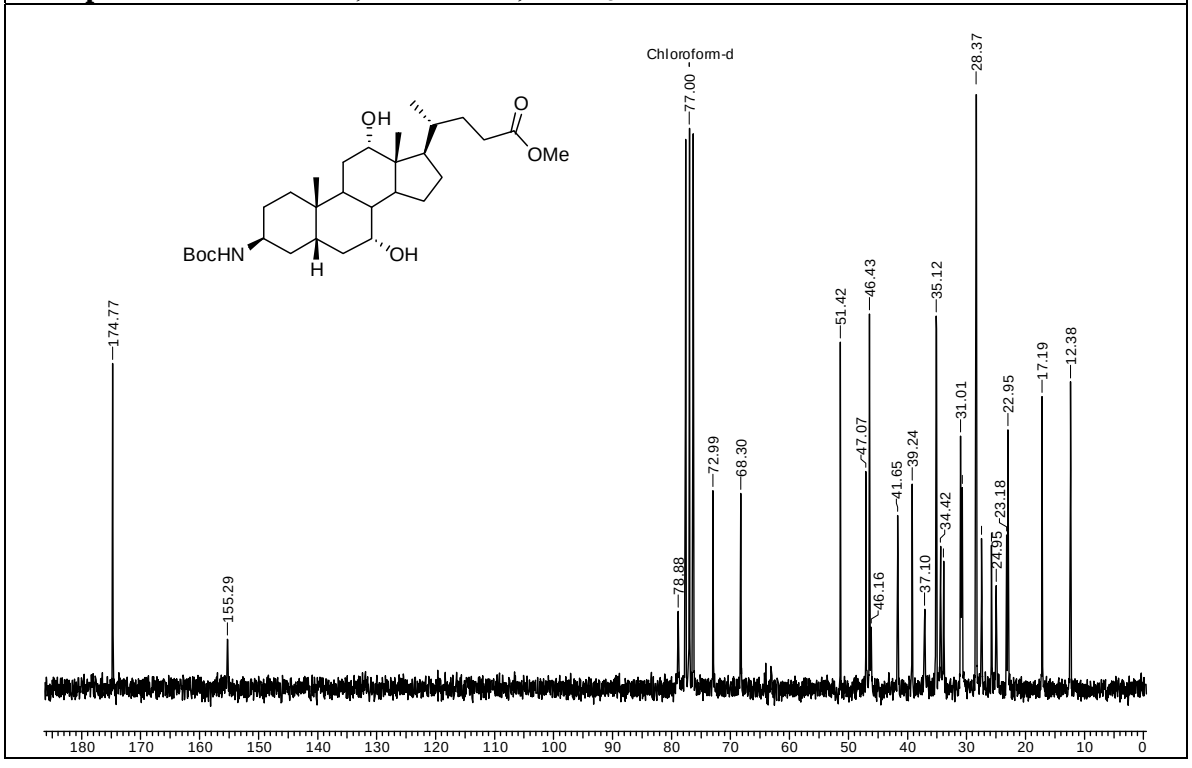
Compound 63: ^{13}C NMR, 125.76 MHz, CD_3OD



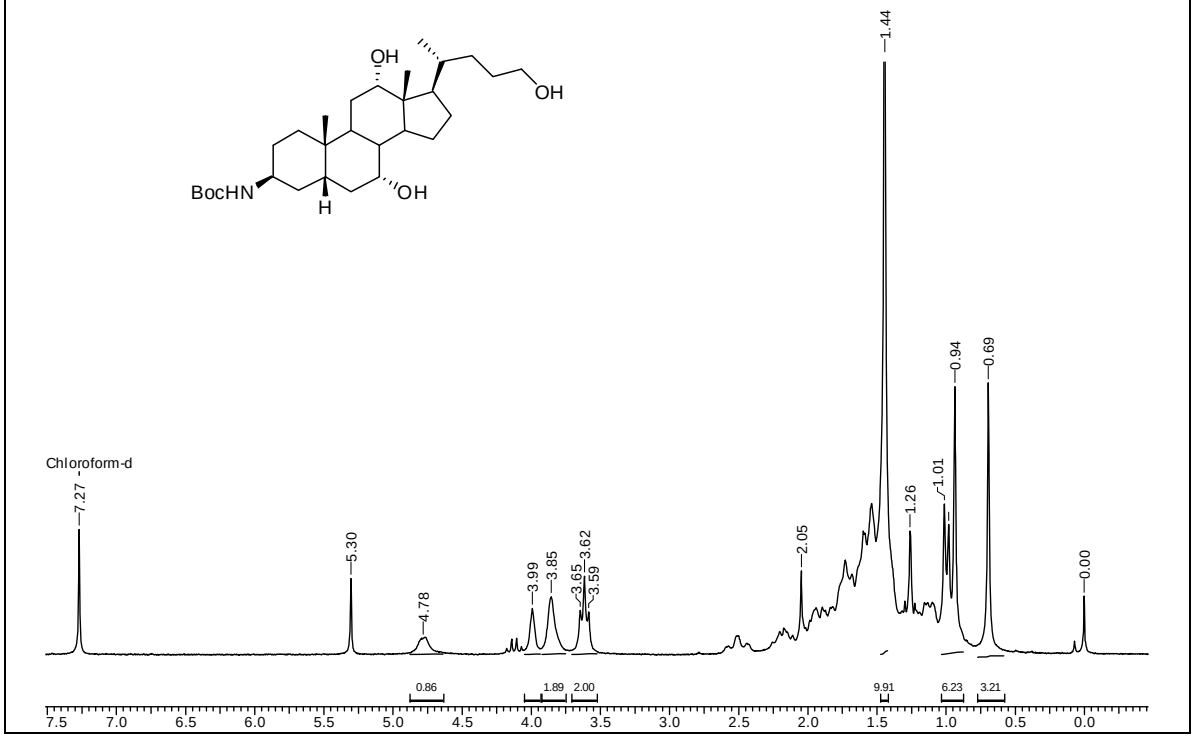
Compound 68: ^{1}H NMR, 200 MHz, $CDCl_3$



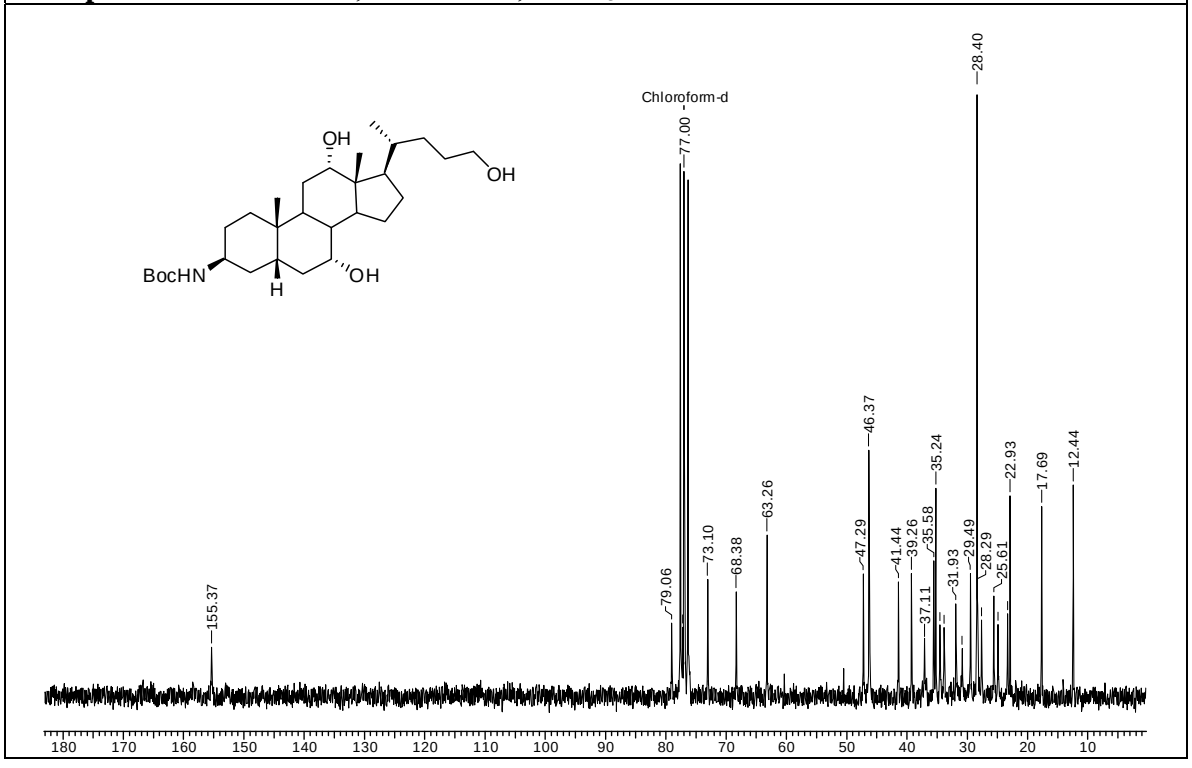
Compound 68: ^{13}C NMR, 50.32 MHz, $CDCl_3$



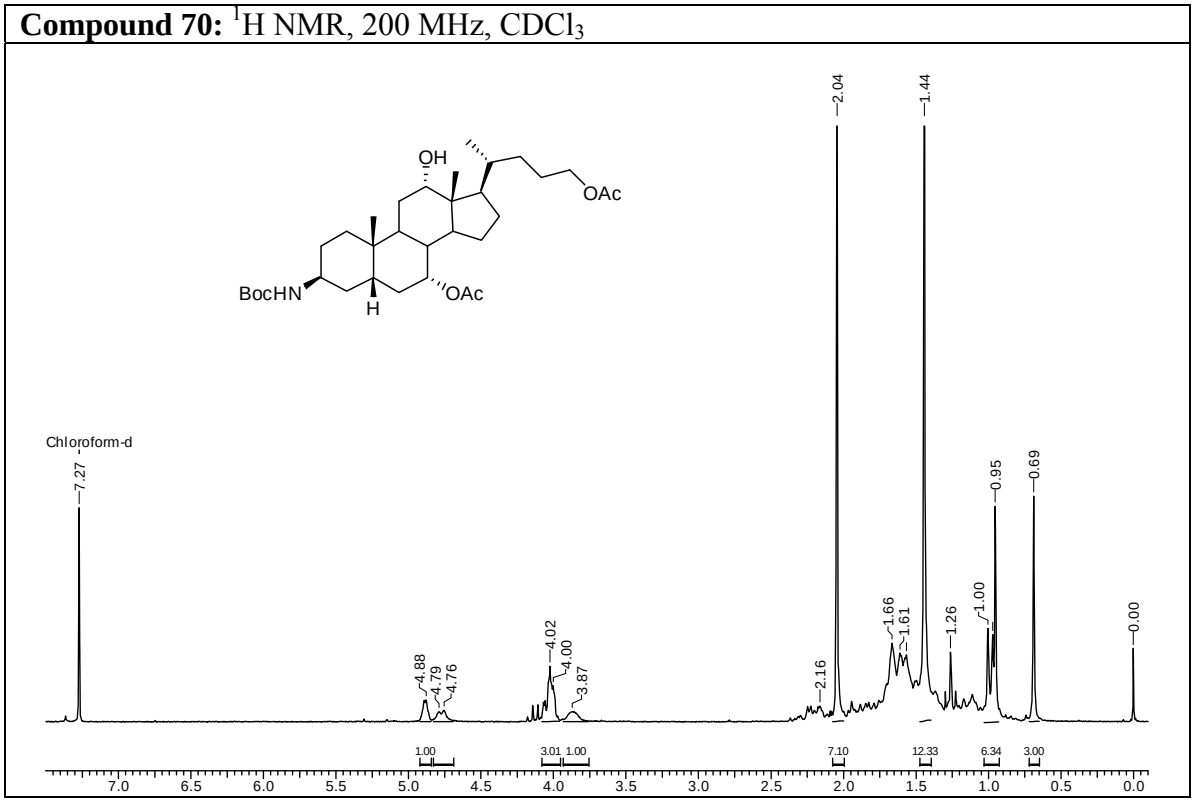
Compound 69: ^{1}H NMR, 200 MHz, $CDCl_3$



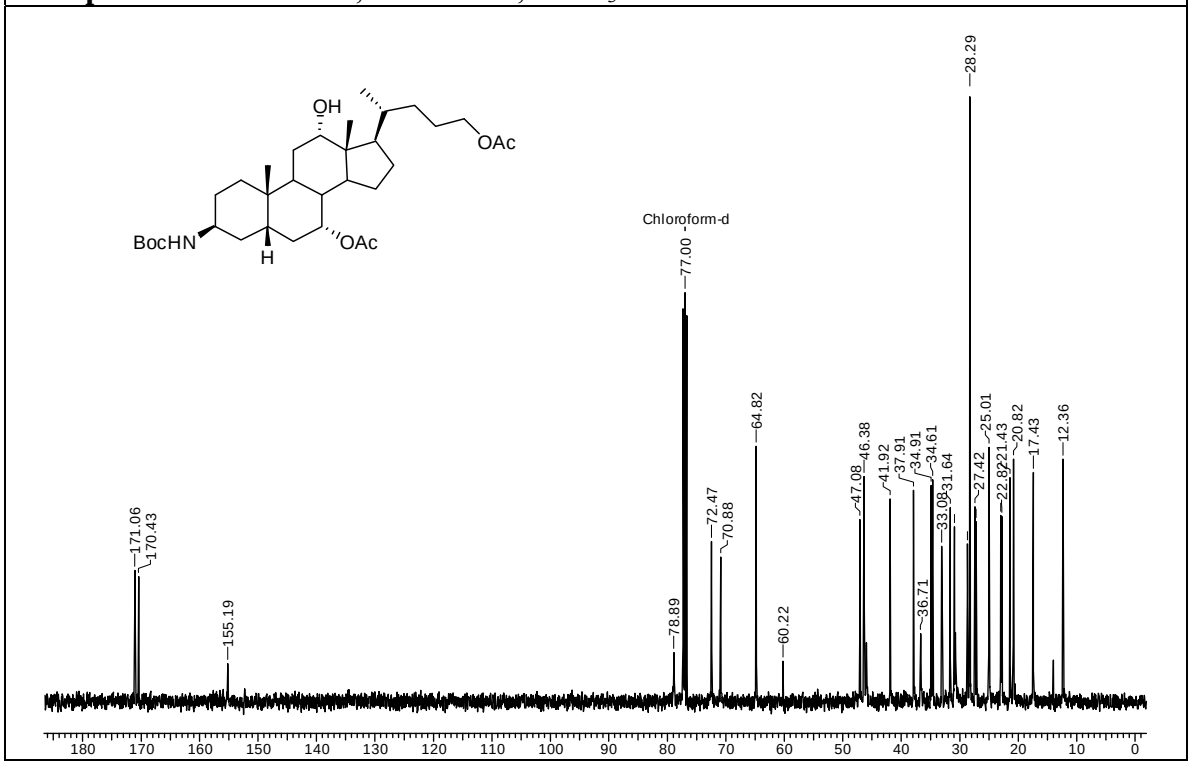
Compound 69: ^{13}C NMR, 50.32 MHz, $CDCl_3$



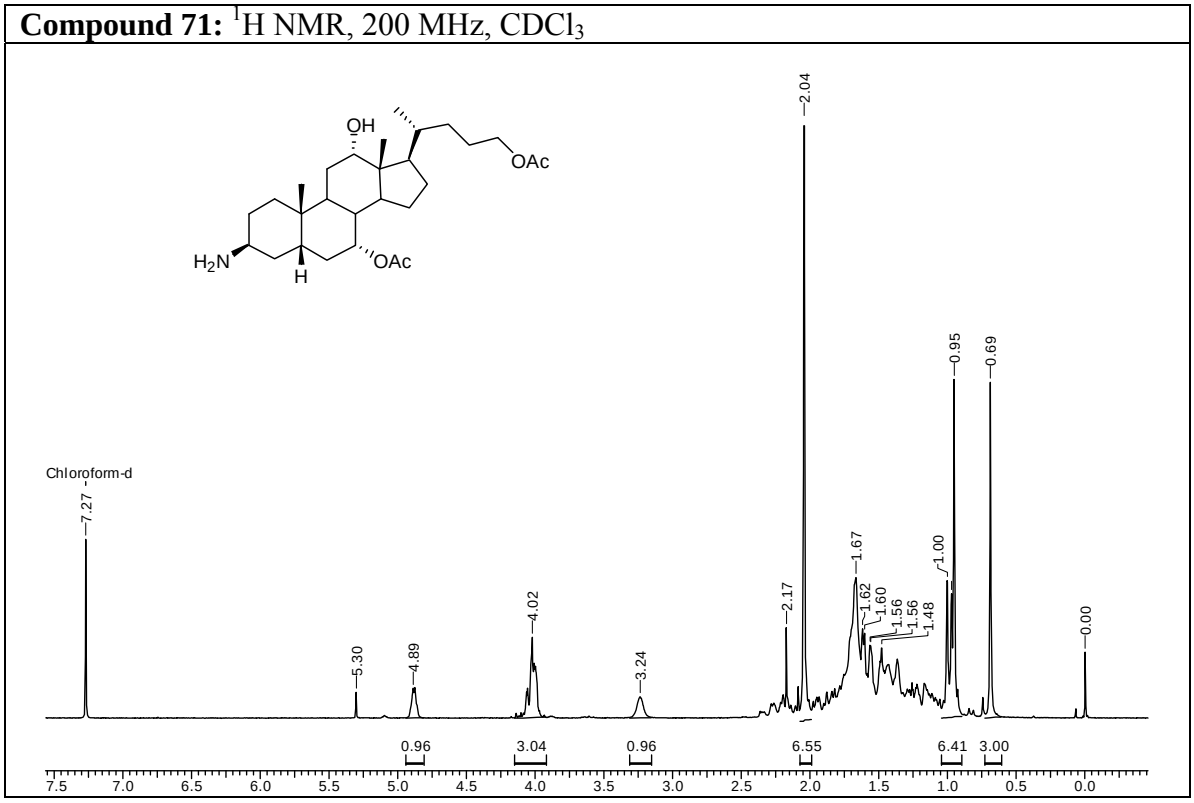
Compound 70: ^{1}H NMR, 200 MHz, $CDCl_3$



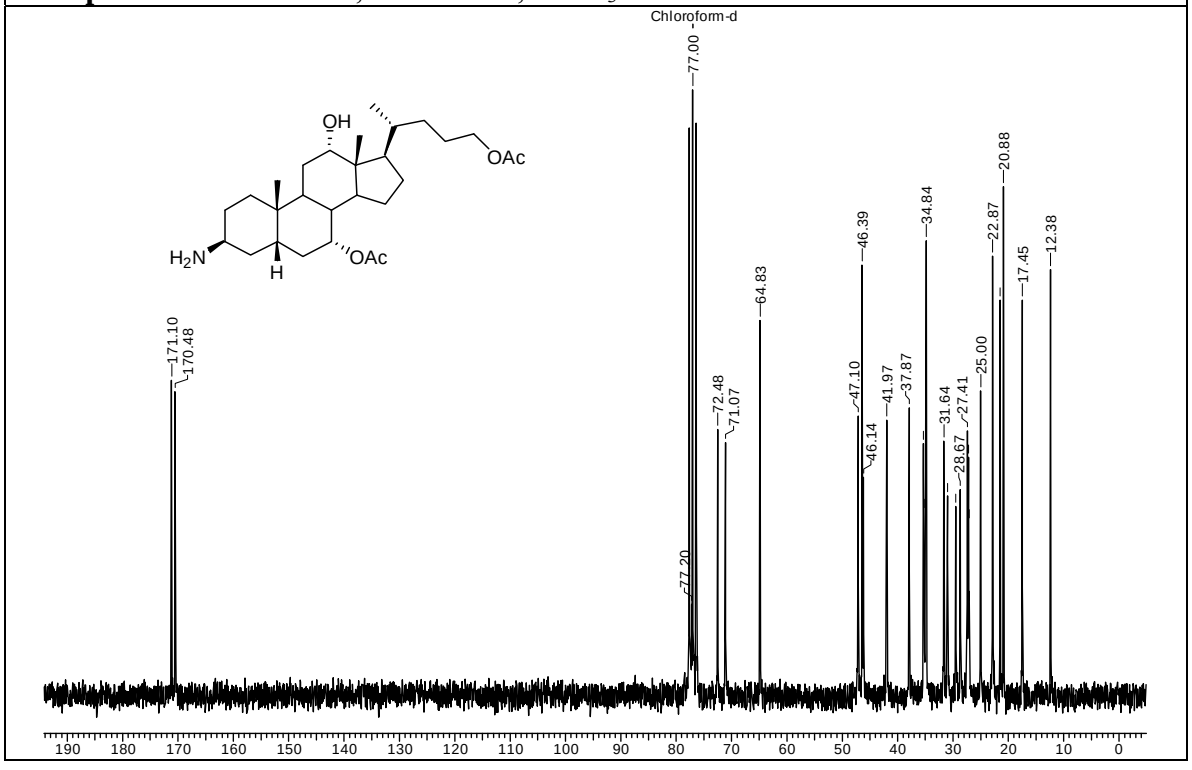
Compound 70: ^{13}C NMR, 50.32 MHz, $CDCl_3$



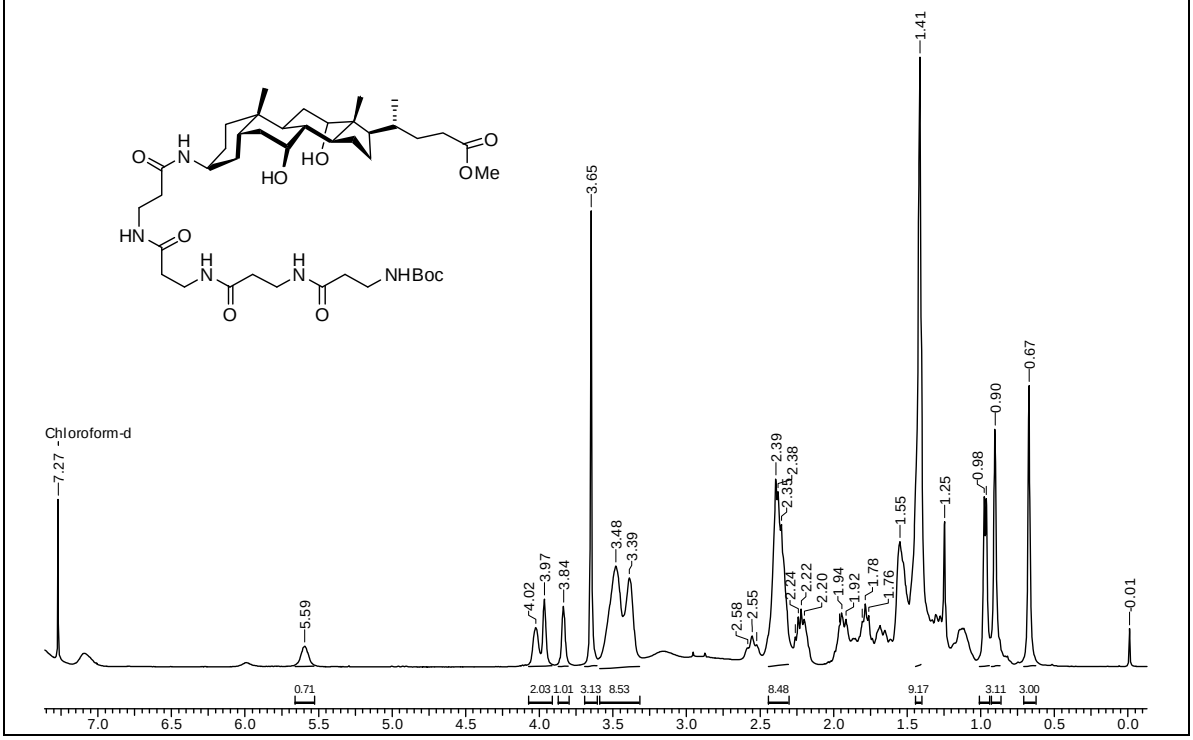
Compound 71: ^{1}H NMR, 200 MHz, $CDCl_3$



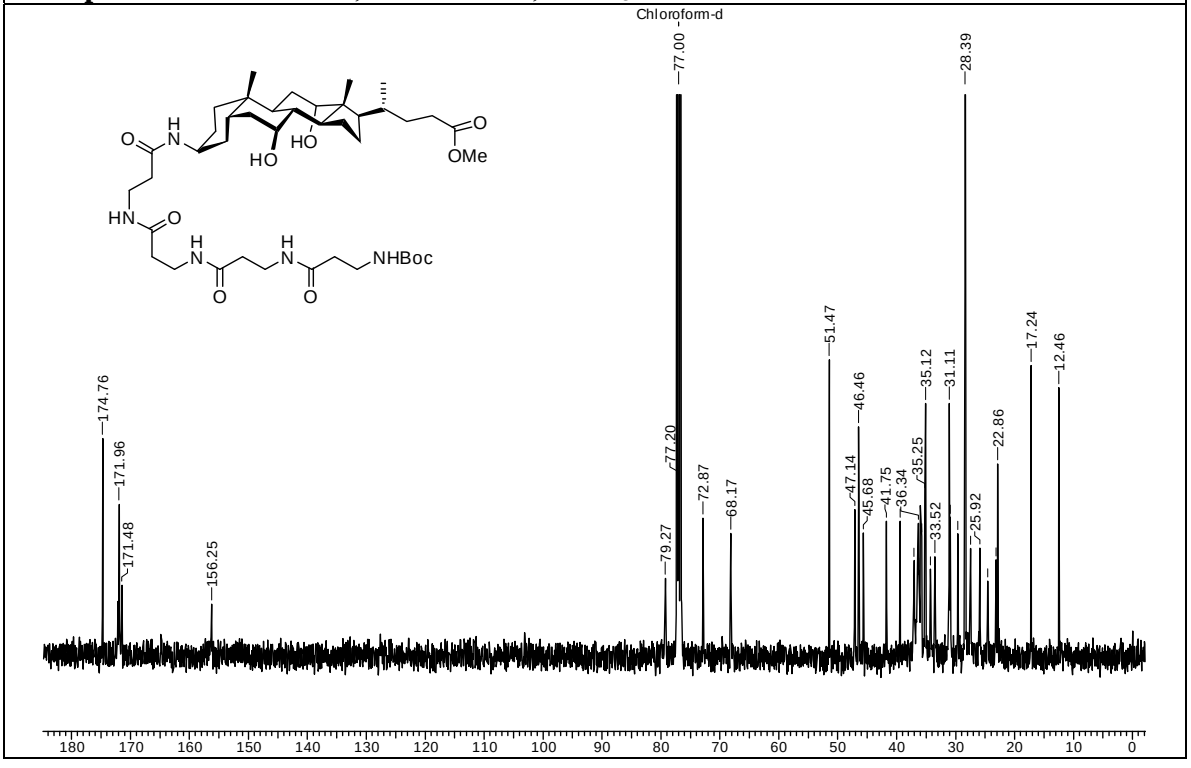
Compound 71: ^{13}C NMR, 50.32 MHz, $CDCl_3$



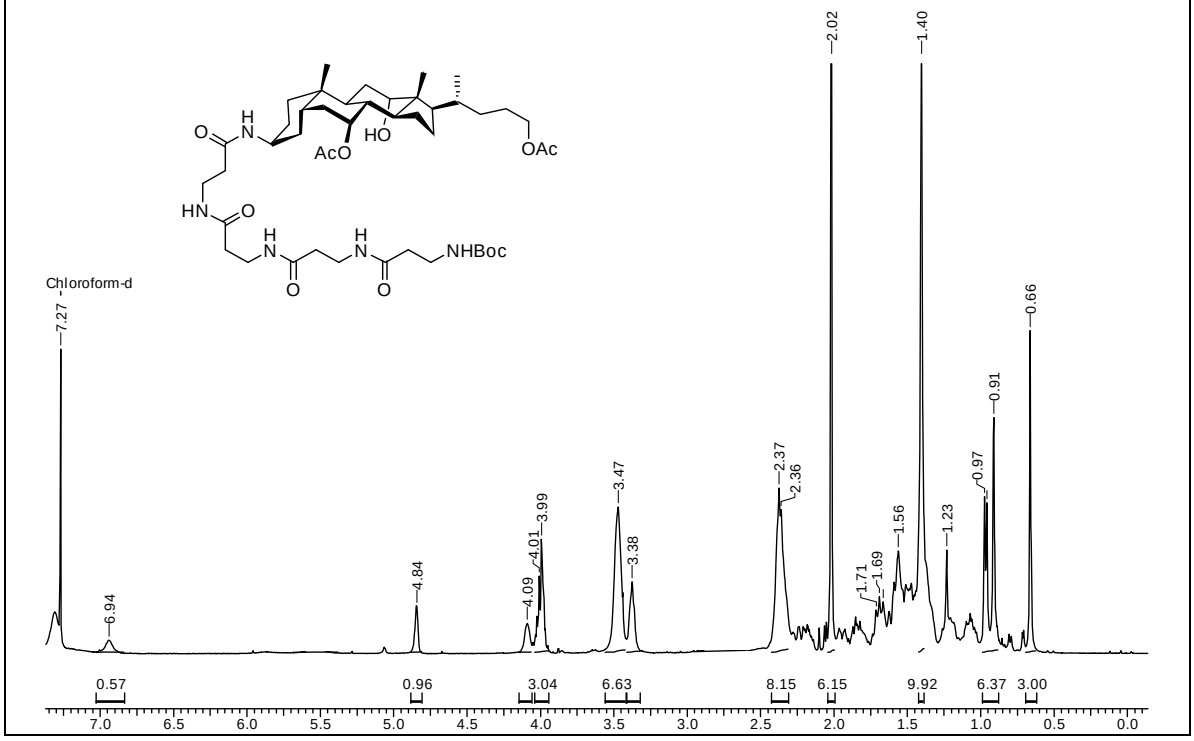
Compound 72: ^{1}H NMR, 400 MHz, $CDCl_3$



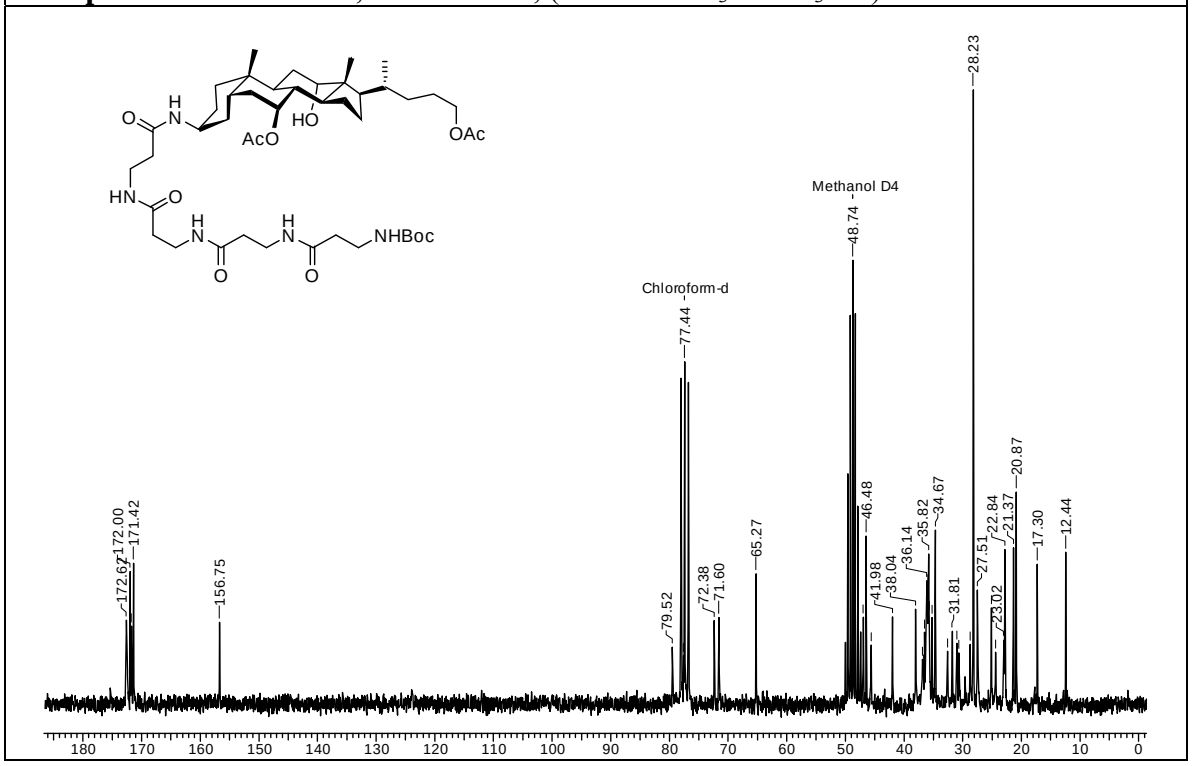
Compound 72: ^{13}C NMR, 100.61 MHz, $CDCl_3$



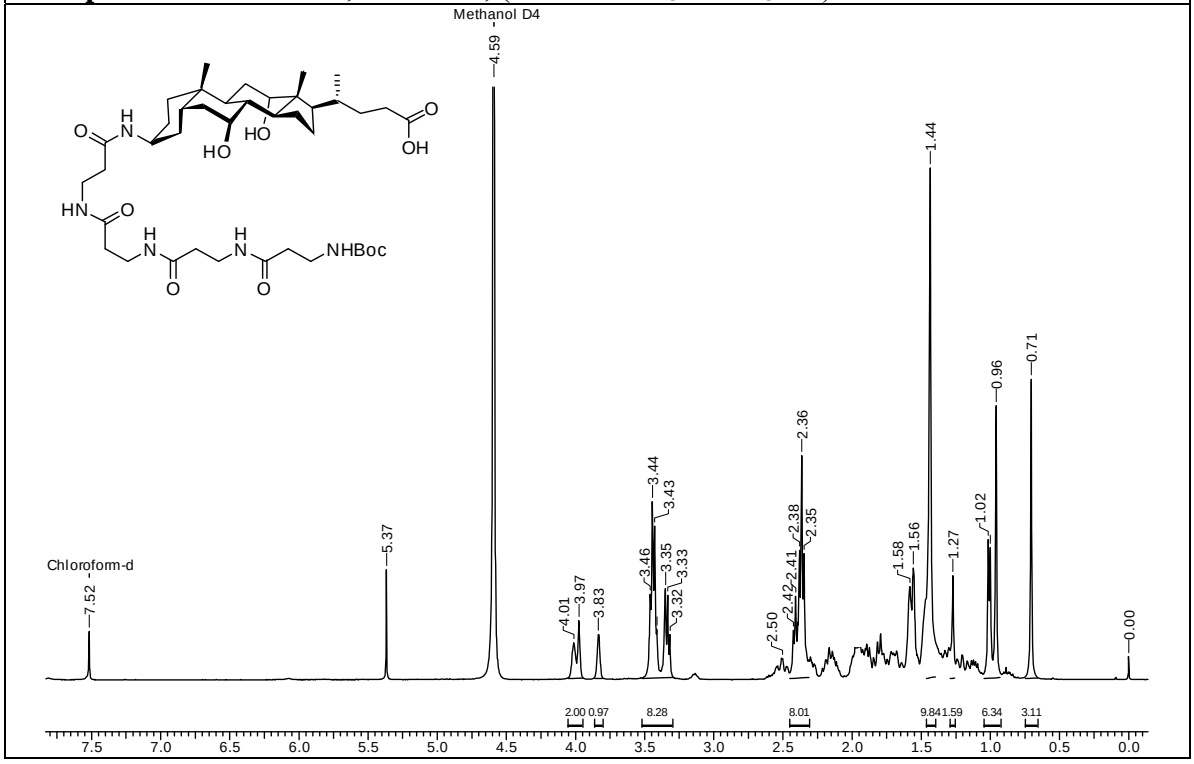
Compound 77: ^{1}H NMR, 400 MHz, $CDCl_3$



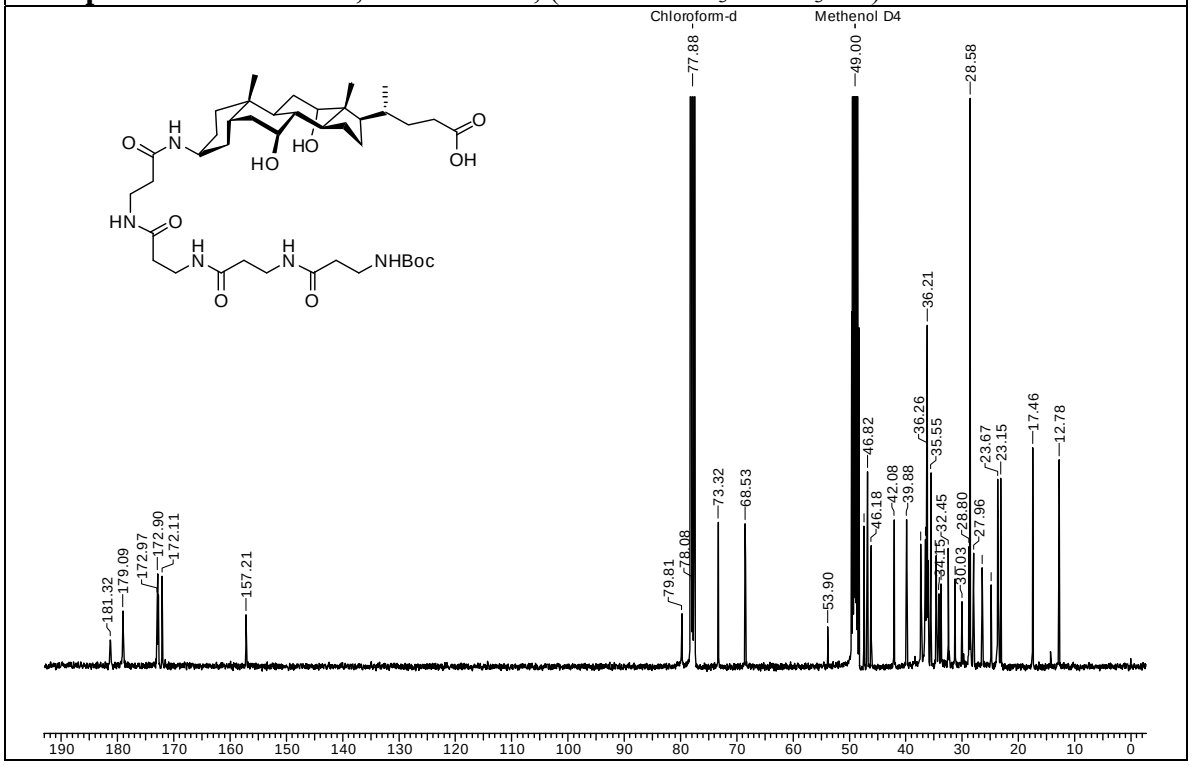
Compound 77: ^{13}C NMR, 100.61 MHz, (~40% $CDCl_3$ in CD_3OD)



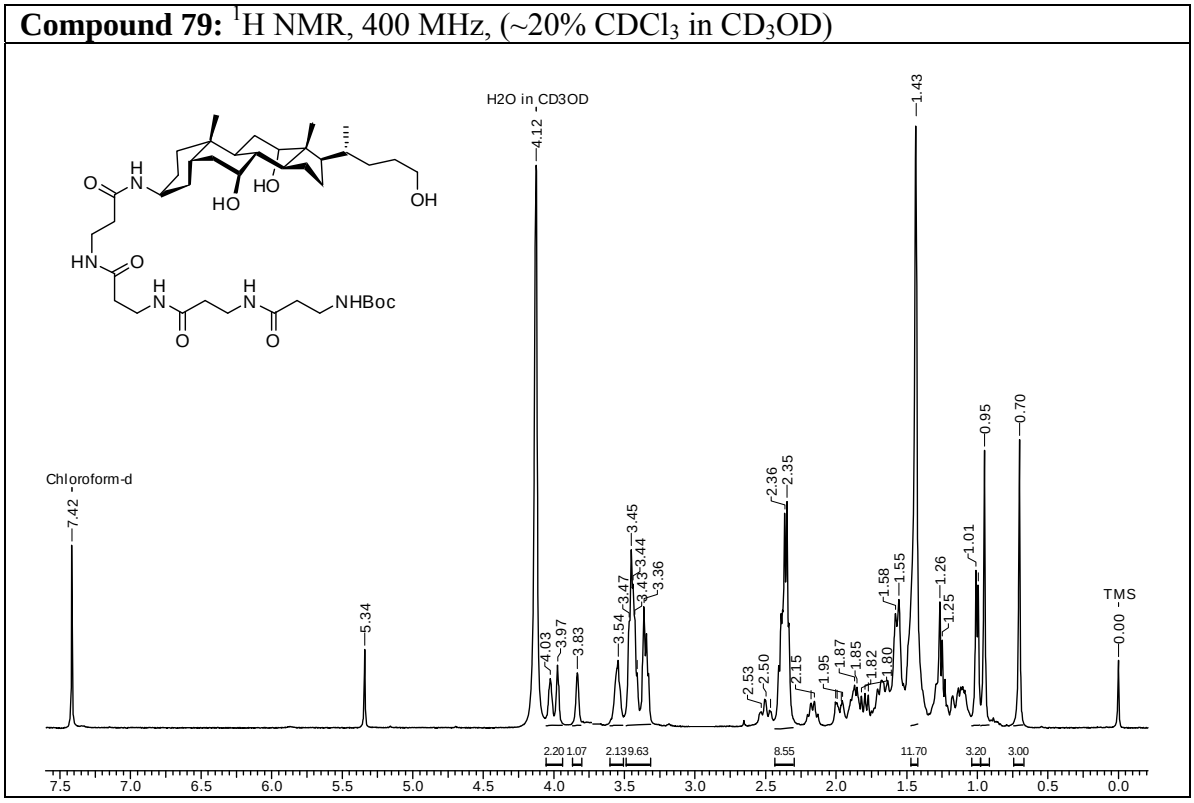
Compound 74: ^{1}H NMR, 400 MHz, (~10% $CDCl_3$ in CD_3OD)



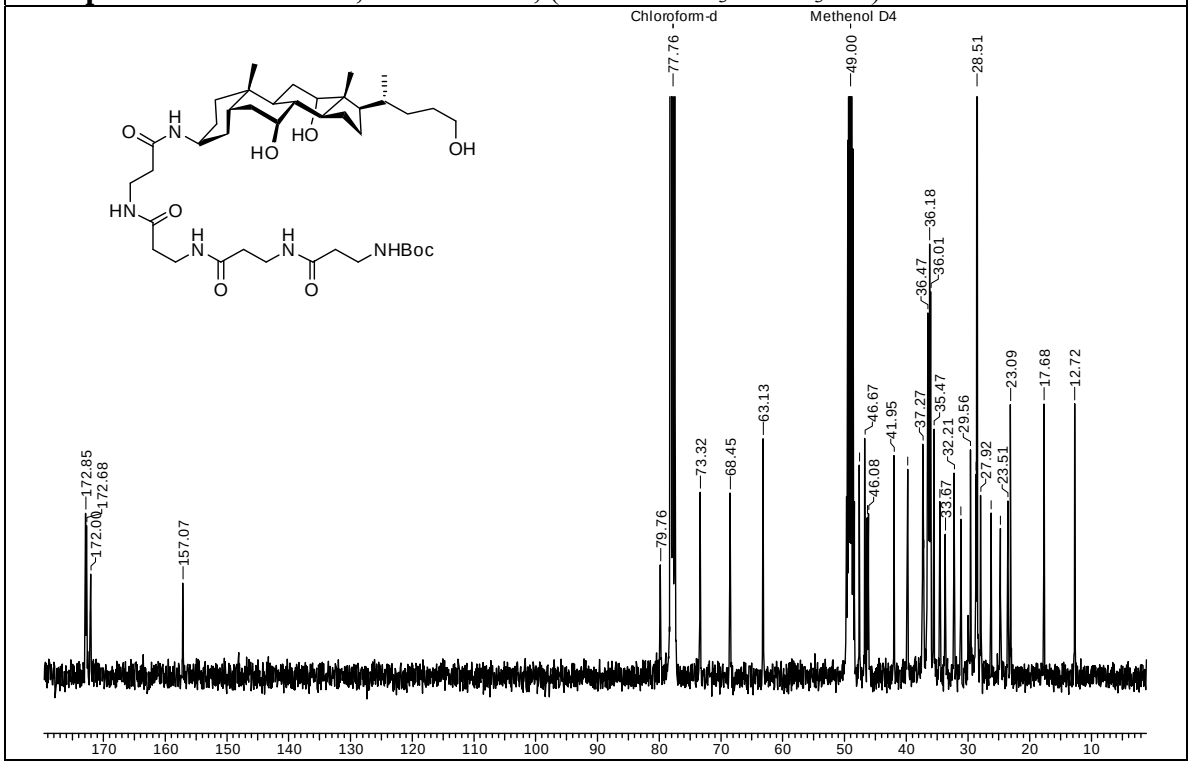
Compound 74: ^{13}C NMR, 100.61 MHz, (~40% $CDCl_3$ in CD_3OD)



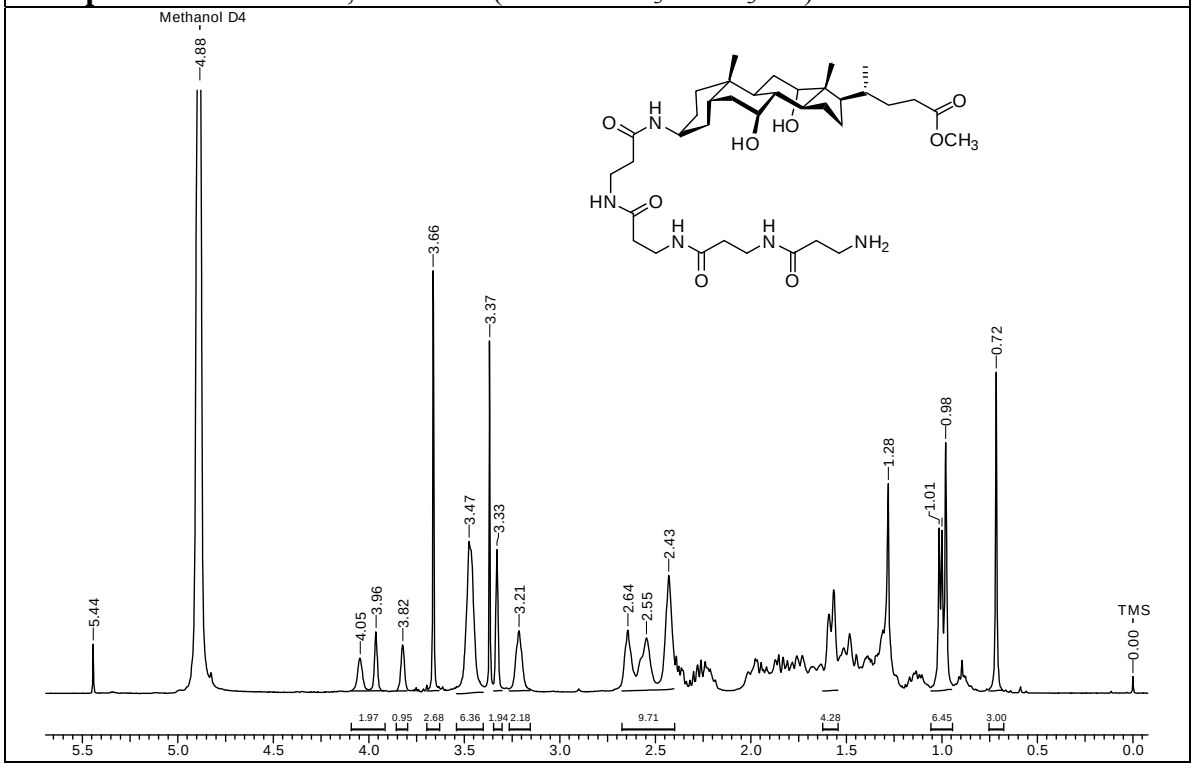
Compound 79: ^{1}H NMR, 400 MHz, (~20% $CDCl_3$ in CD_3OD)



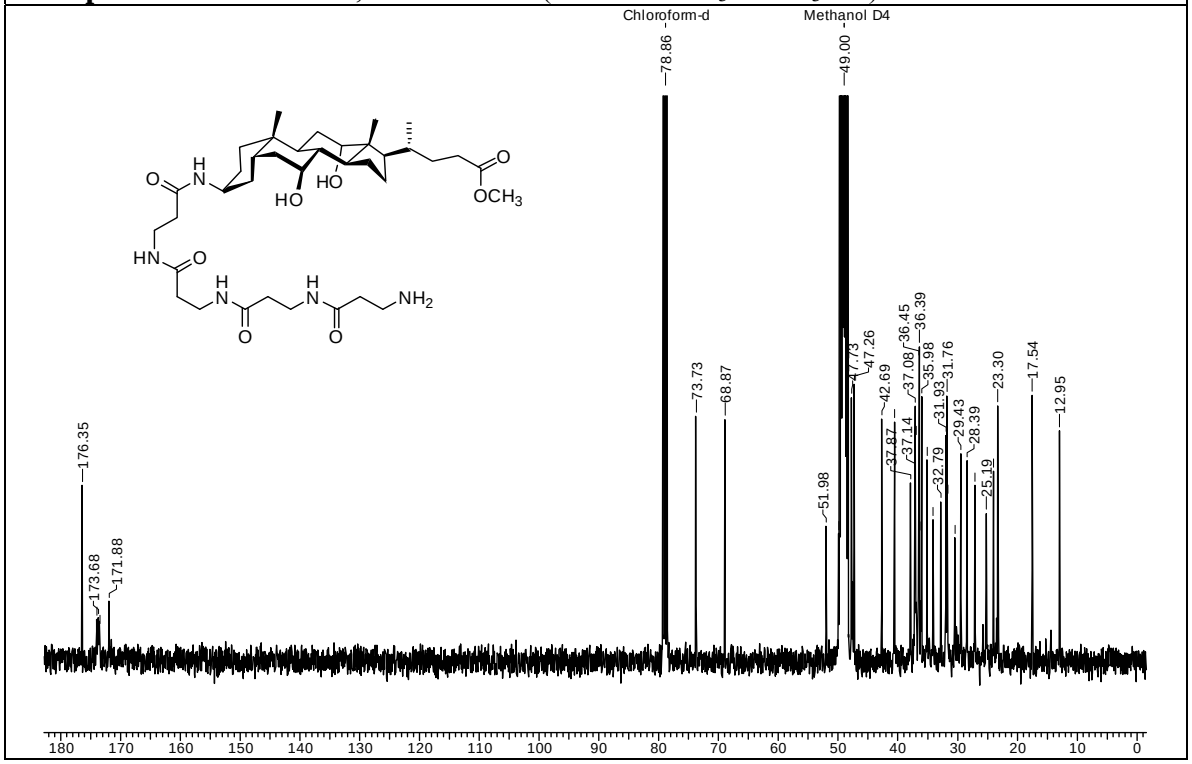
Compound 79: ^{13}C NMR, 100.61 MHz, (~20% $CDCl_3$ in CD_3OD)



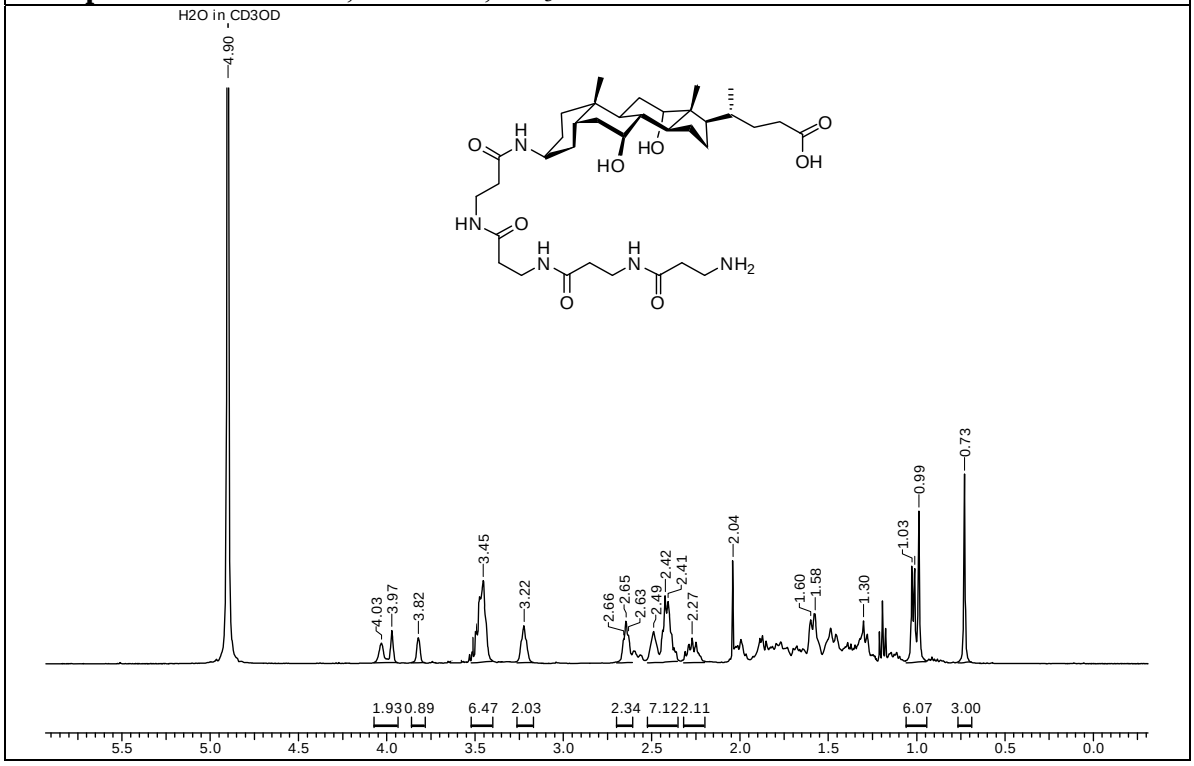
Compound 73: ^{1}H NMR, 400 MHz (~ 5% $CDCl_3$ in CD_3OD)



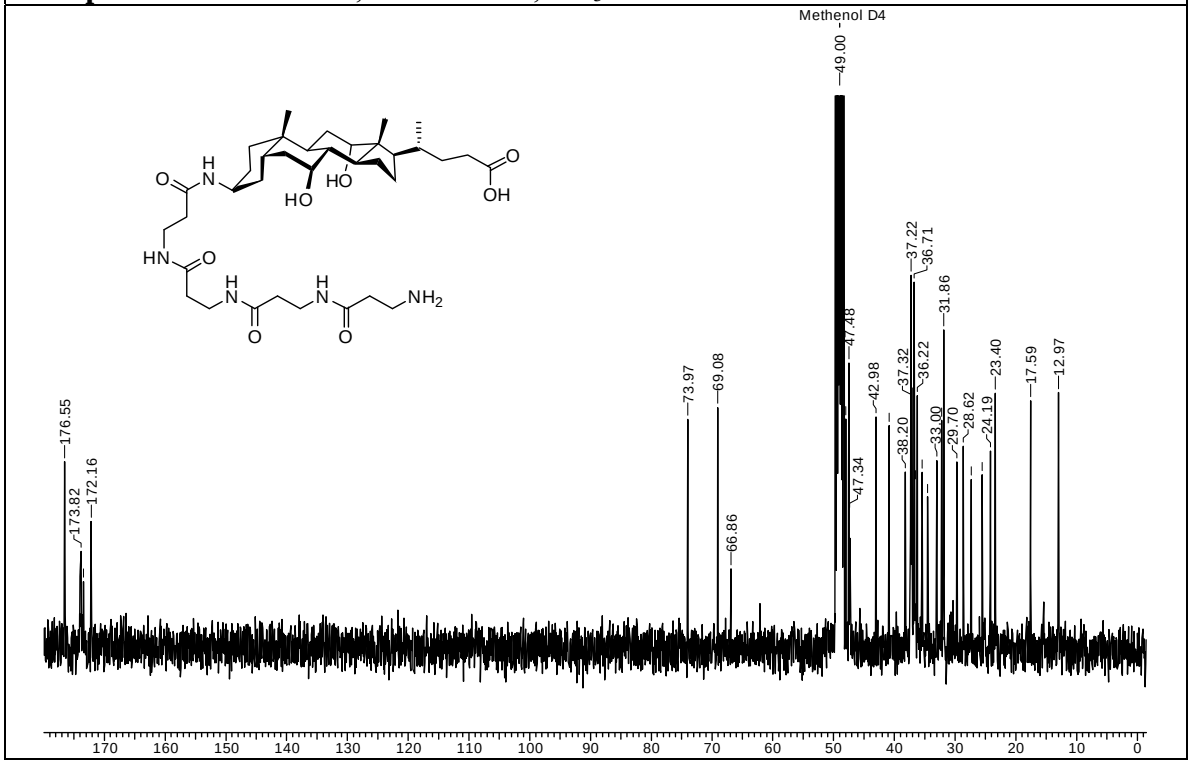
Compound 73: ^{13}C NMR, 100.61 MHz (~ 20% $CDCl_3$ in CD_3OD)



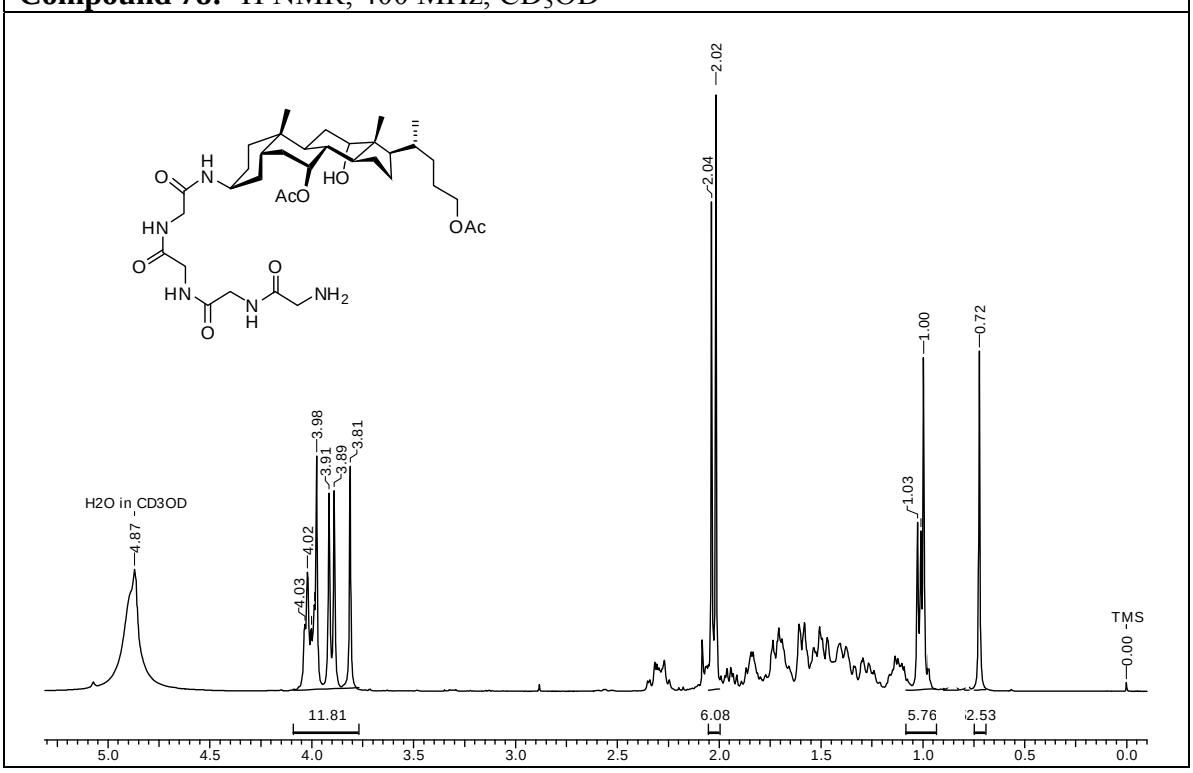
Compound 75: ^{1}H NMR, 400 MHz, CD_3OD



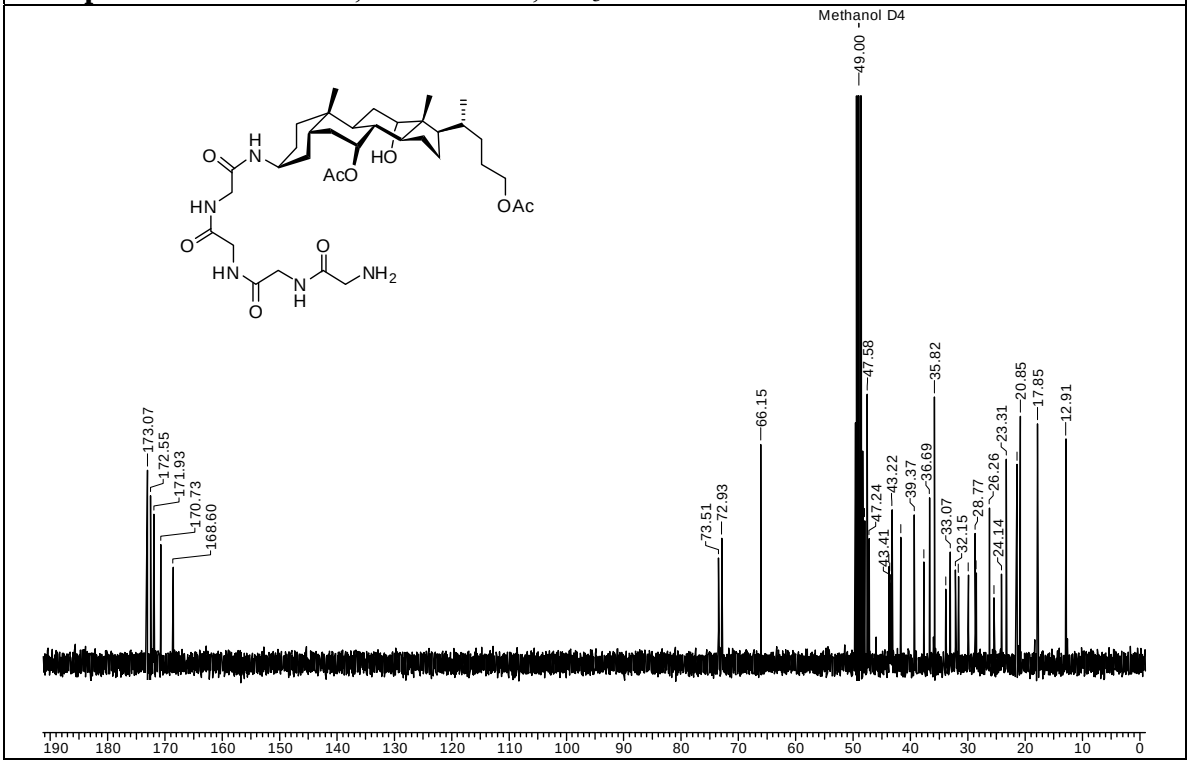
Compound 75: ^{13}C NMR, 100.61 MHz, CD_3OD



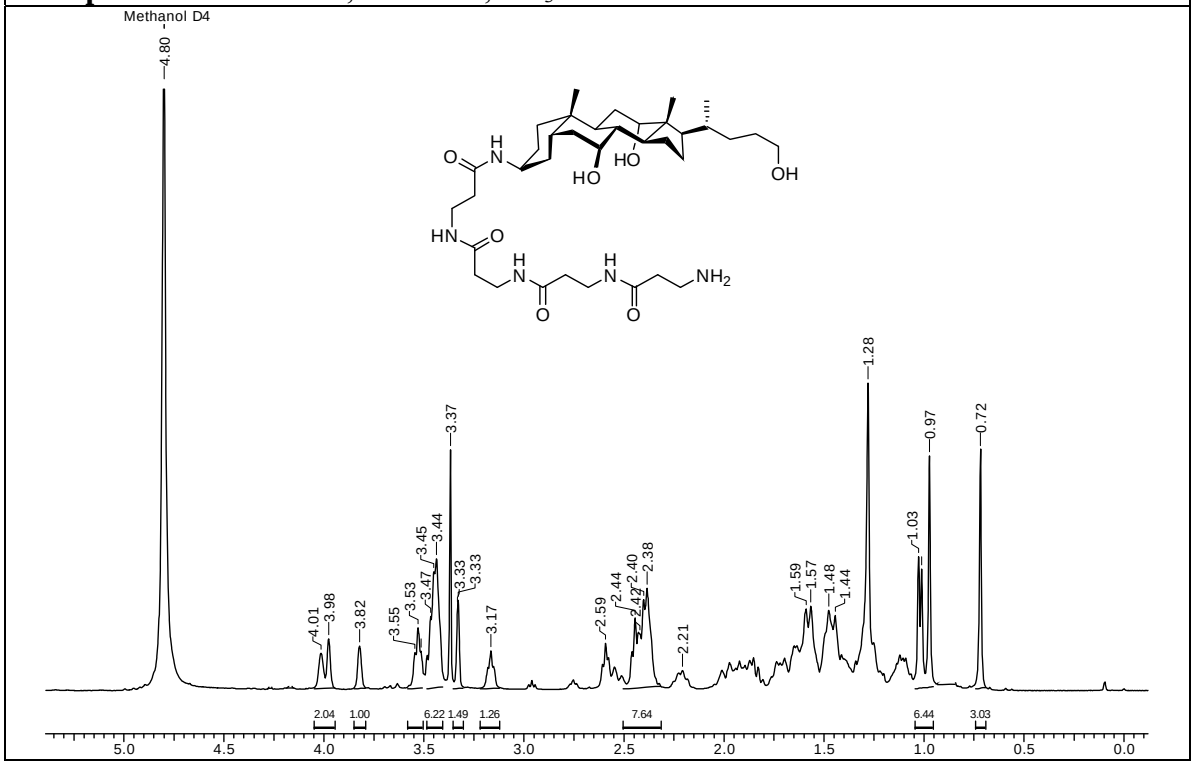
Compound 78: ^{1}H NMR, 400 MHz, CD_3OD



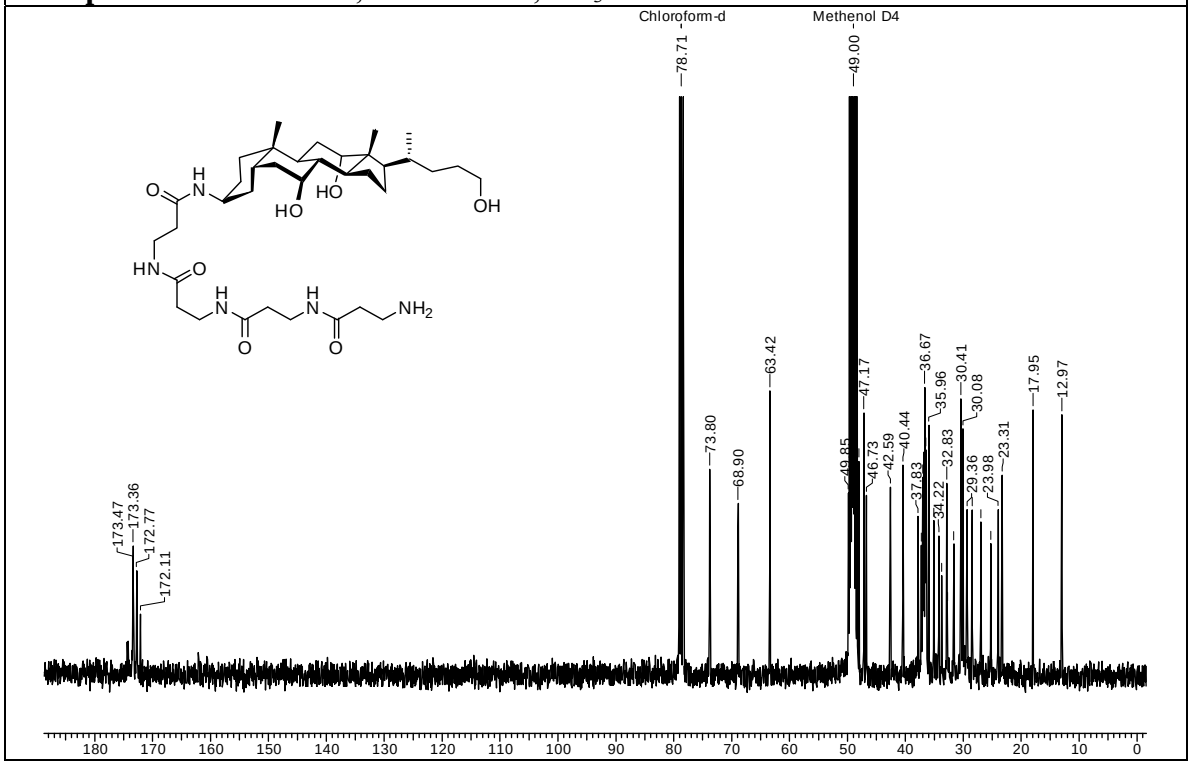
Compound 78: ^{13}C NMR, 100.61 MHz, CD_3OD



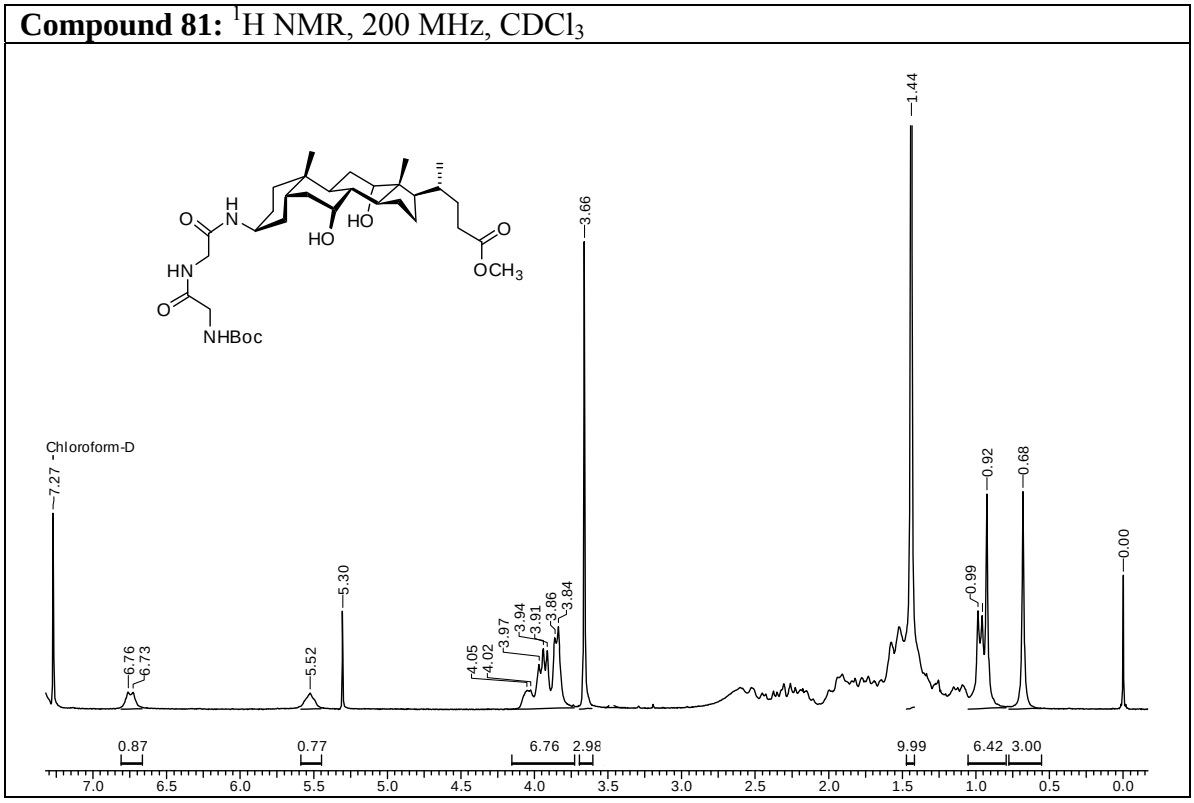
Compound 80: ^{1}H NMR, 400 MHz, CD_3OD



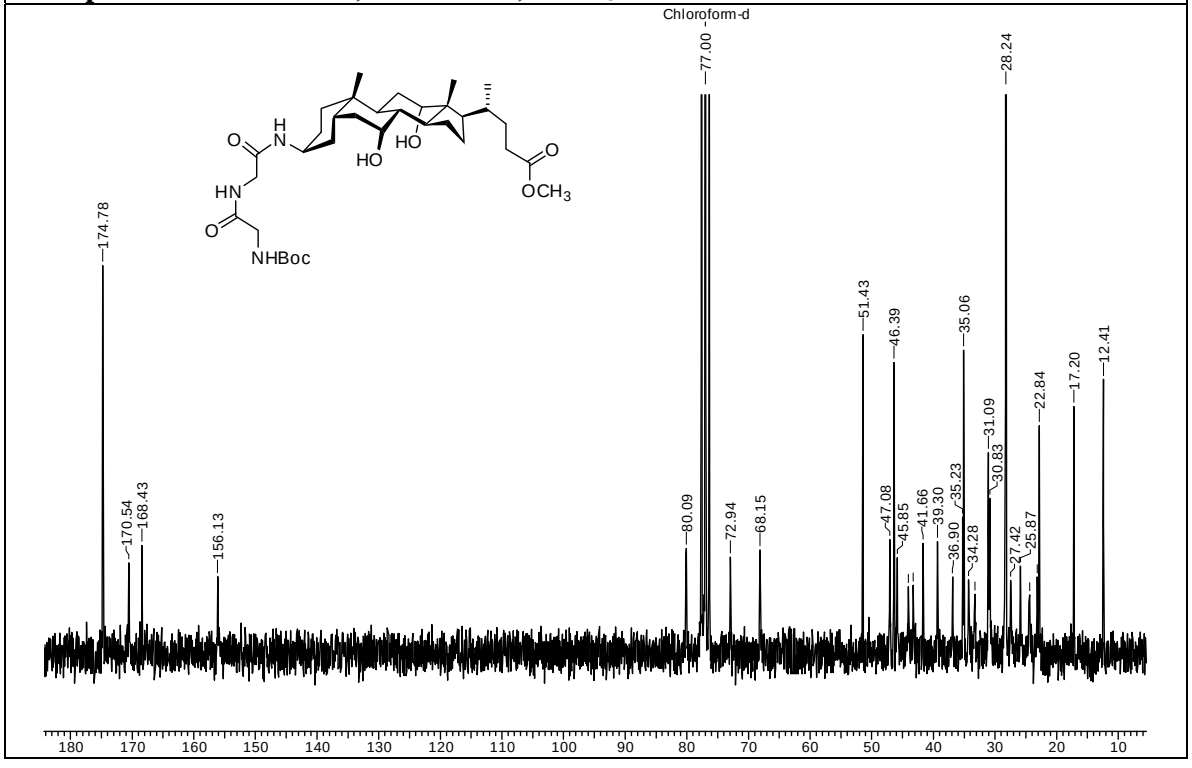
Compound 80: ^{13}C NMR, 100.61 MHz, CD_3OD



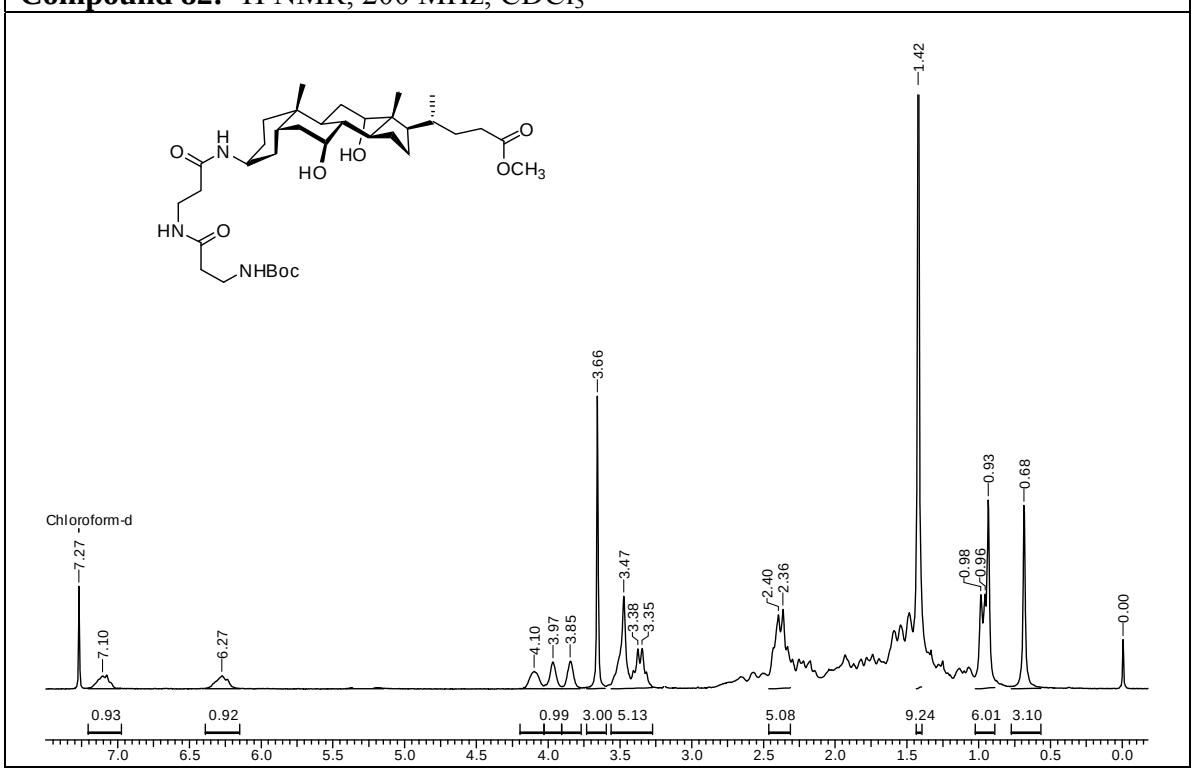
Compound 81: ^{1}H NMR, 200 MHz, $CDCl_3$



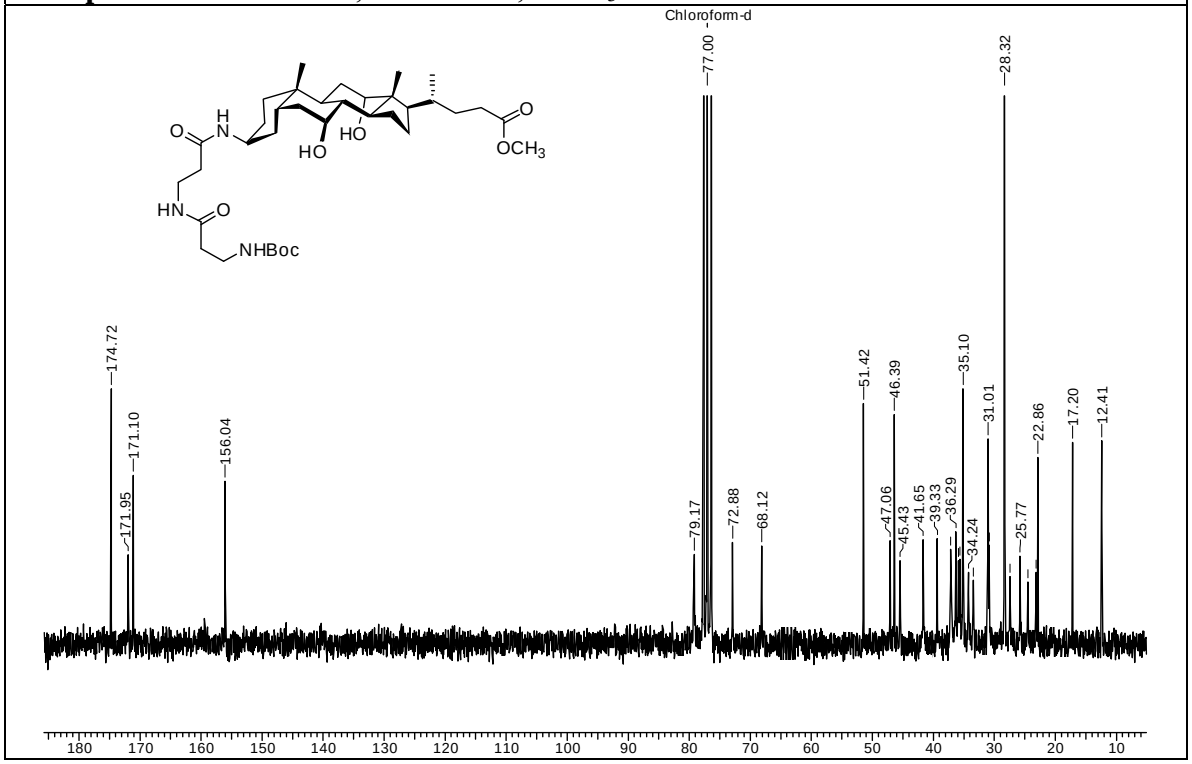
Compound 81: ^{13}C NMR, 50.32 MHz, $CDCl_3$



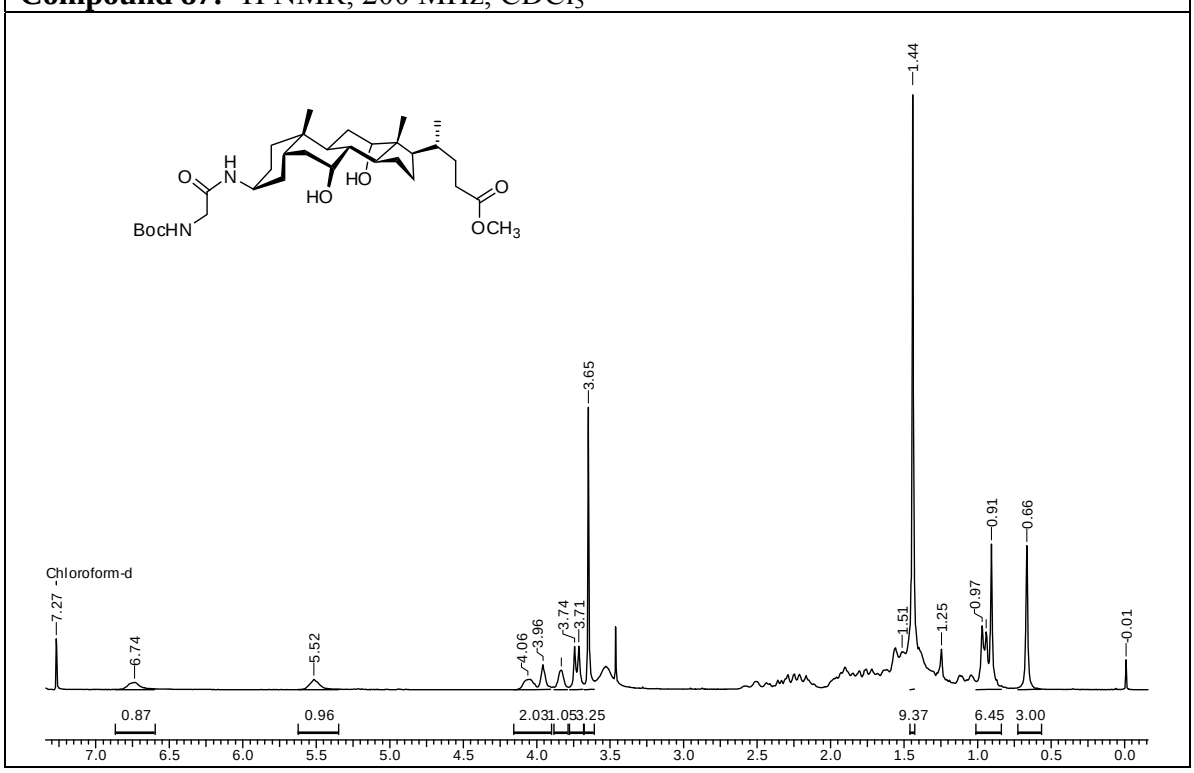
Compound 82: ^{1}H NMR, 200 MHz, $CDCl_3$



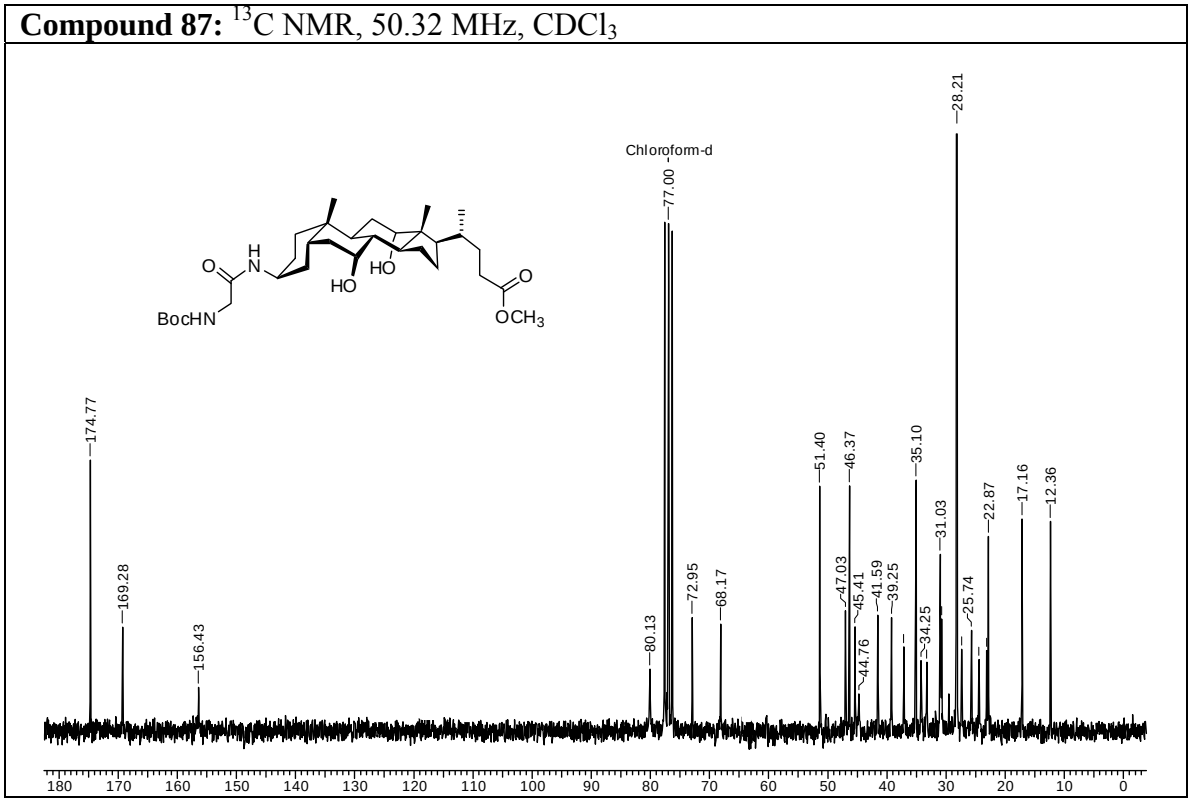
Compound 82: ^{13}C NMR, 50.32 MHz, $CDCl_3$



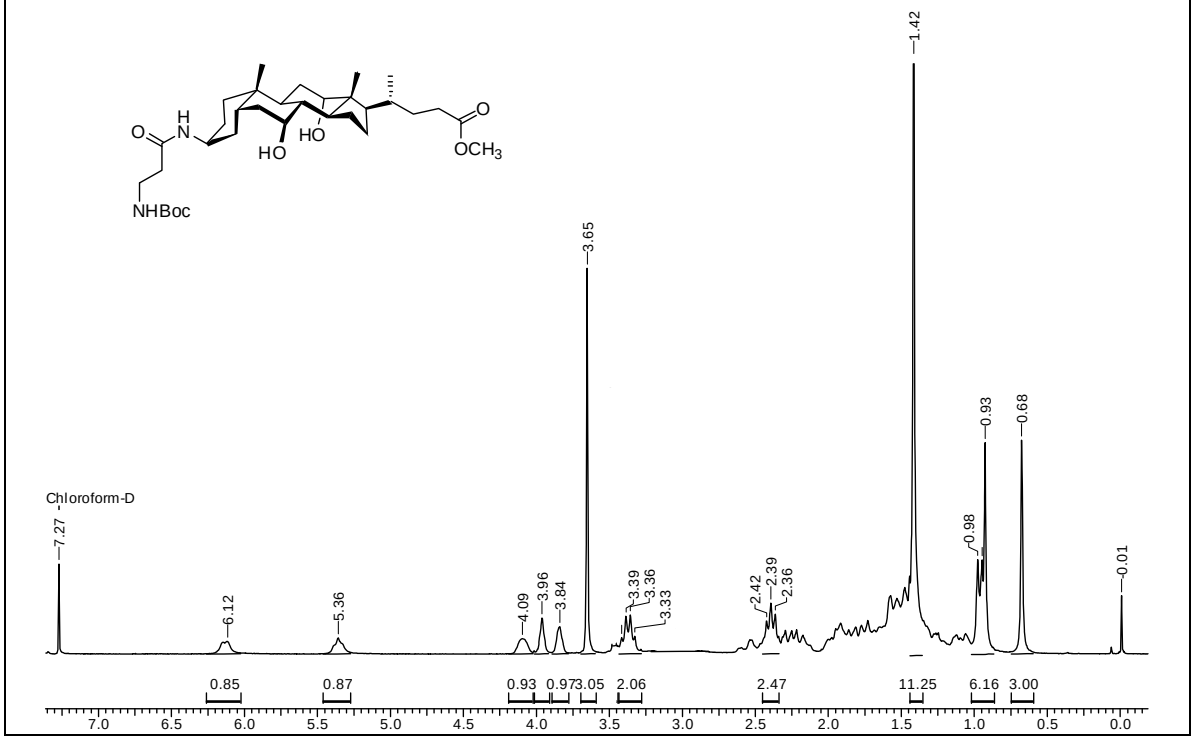
Compound 87: ^{1}H NMR, 200 MHz, $CDCl_3$



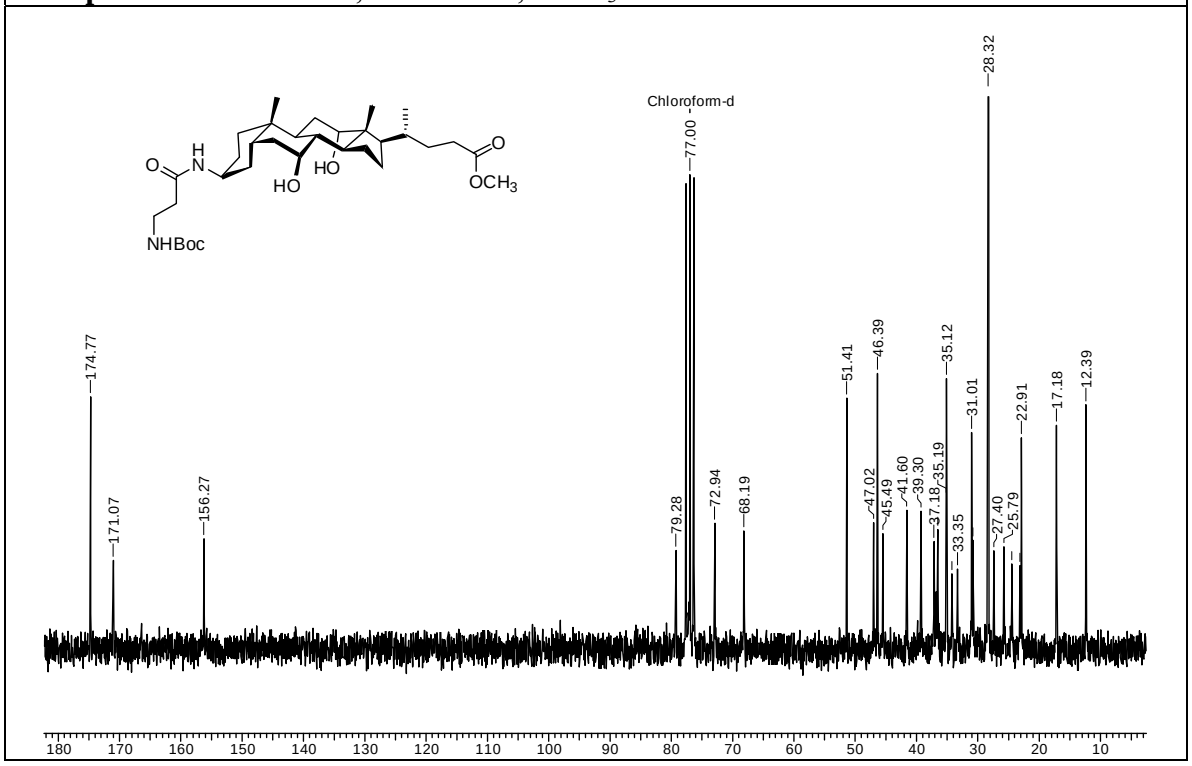
Compound 87: ^{13}C NMR, 50.32 MHz, $CDCl_3$



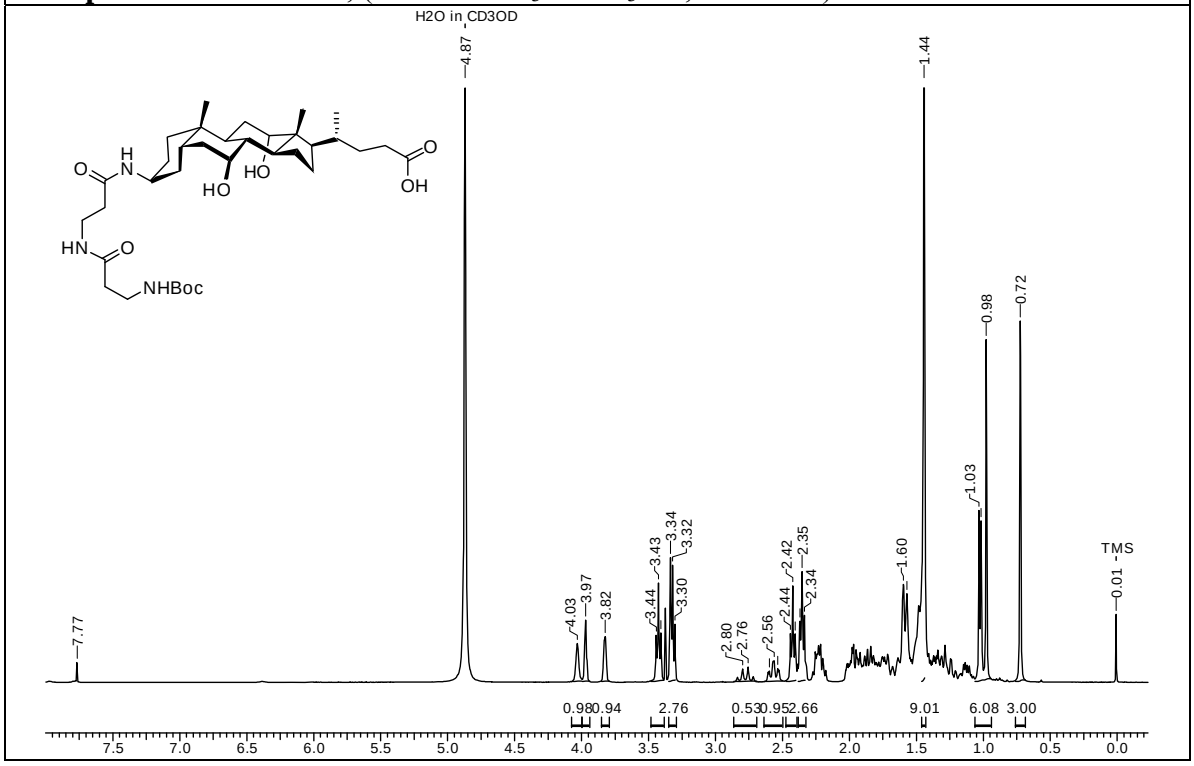
Compound 88: ^{1}H NMR, 200 MHz, $CDCl_3$



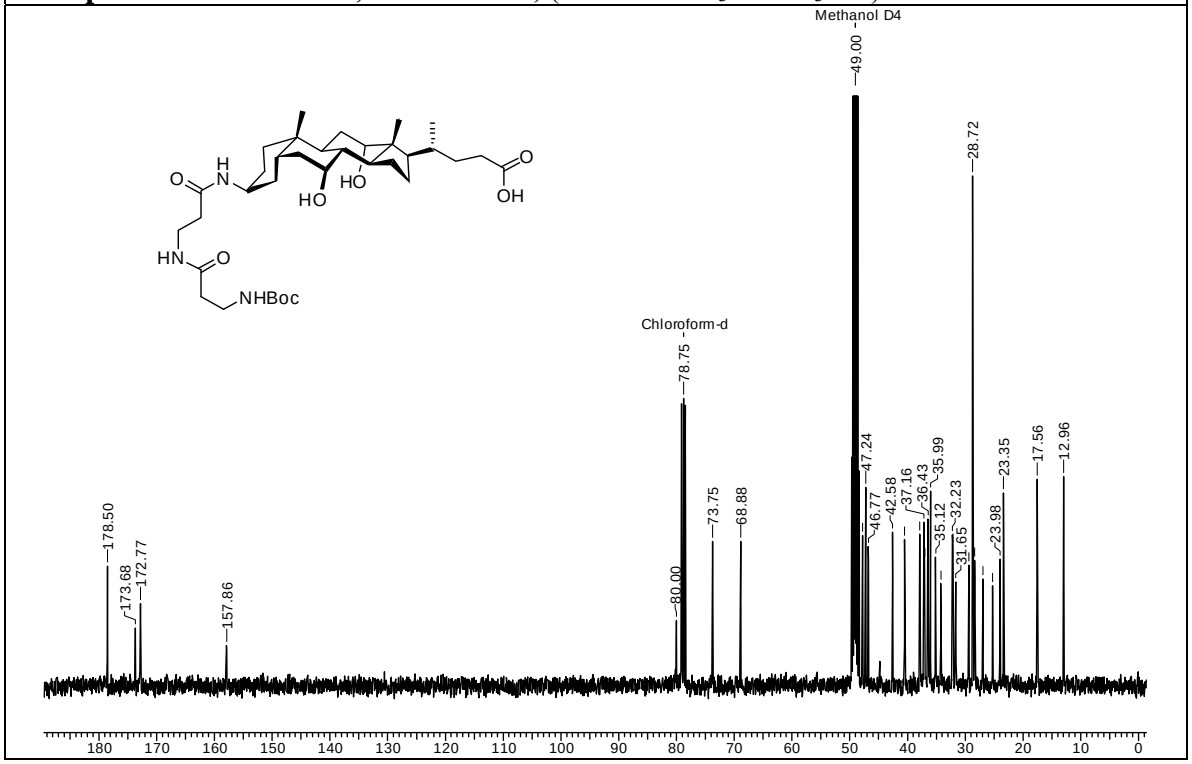
Compound 88: ^{13}C NMR, 50.32 MHz, $CDCl_3$



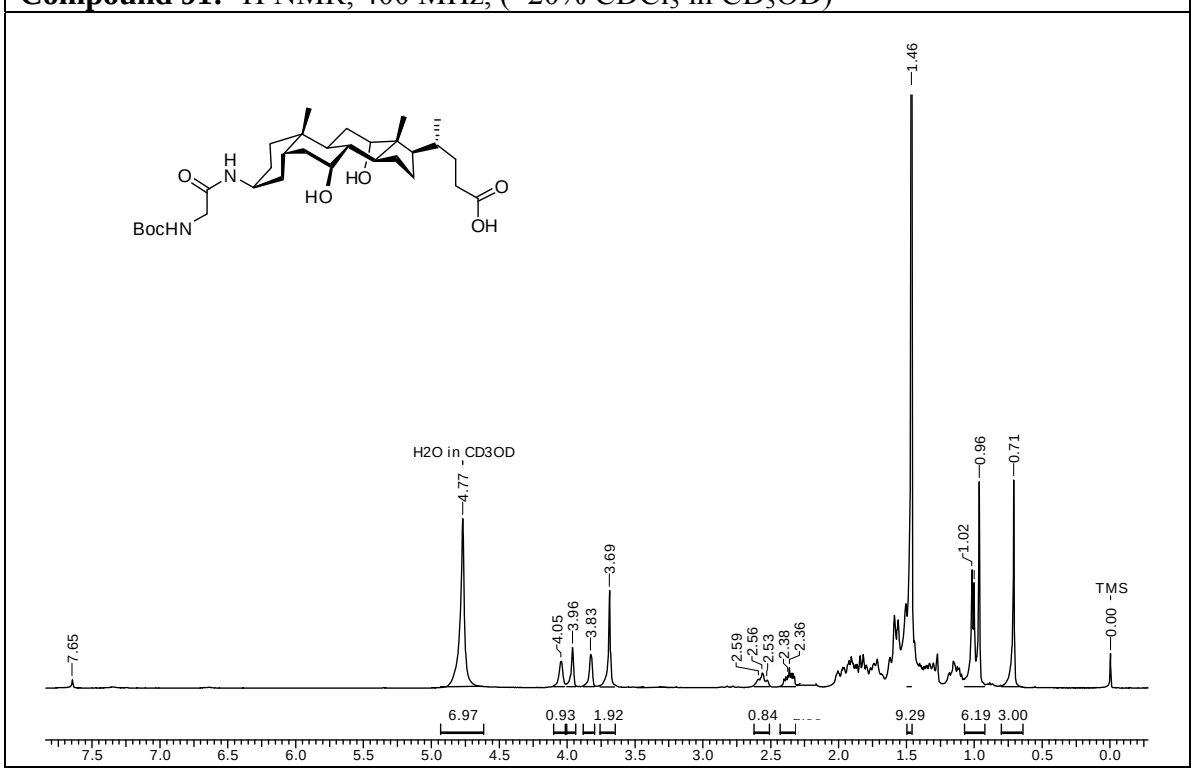
Compound 85: ^{1}H NMR, (~10% $CDCl_3$ in CD_3OD, 400 MHz)



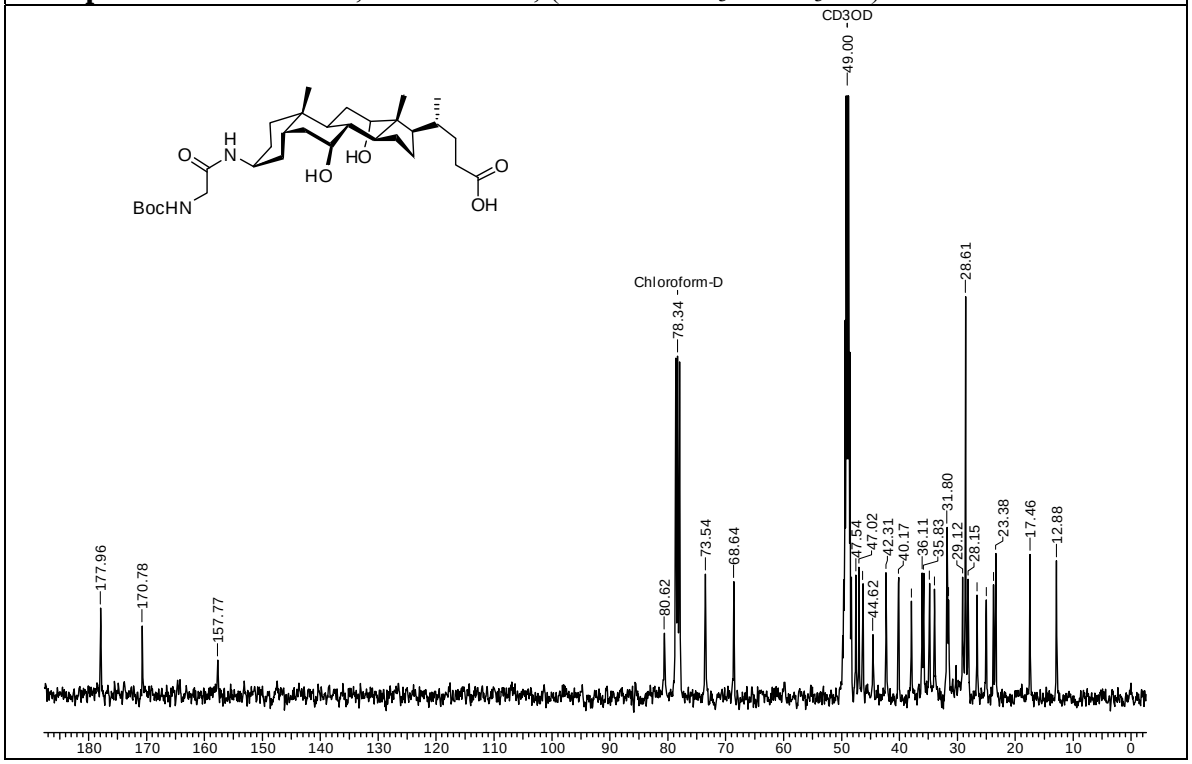
Compound 85: ^{13}C NMR, 100.61 MHz, (~10% $CDCl_3$ in CD_3OD)



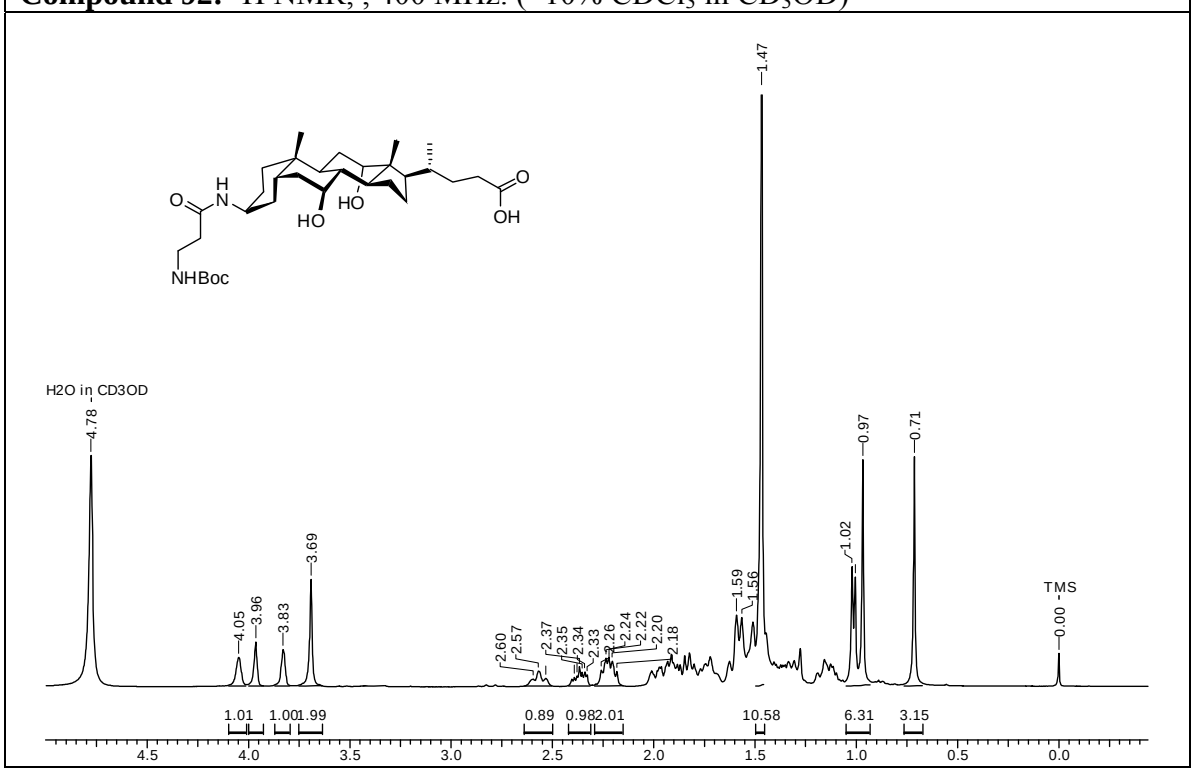
Compound 91: ^{1}H NMR, 400 MHz, (~20% $CDCl_3$ in CD_3OD)



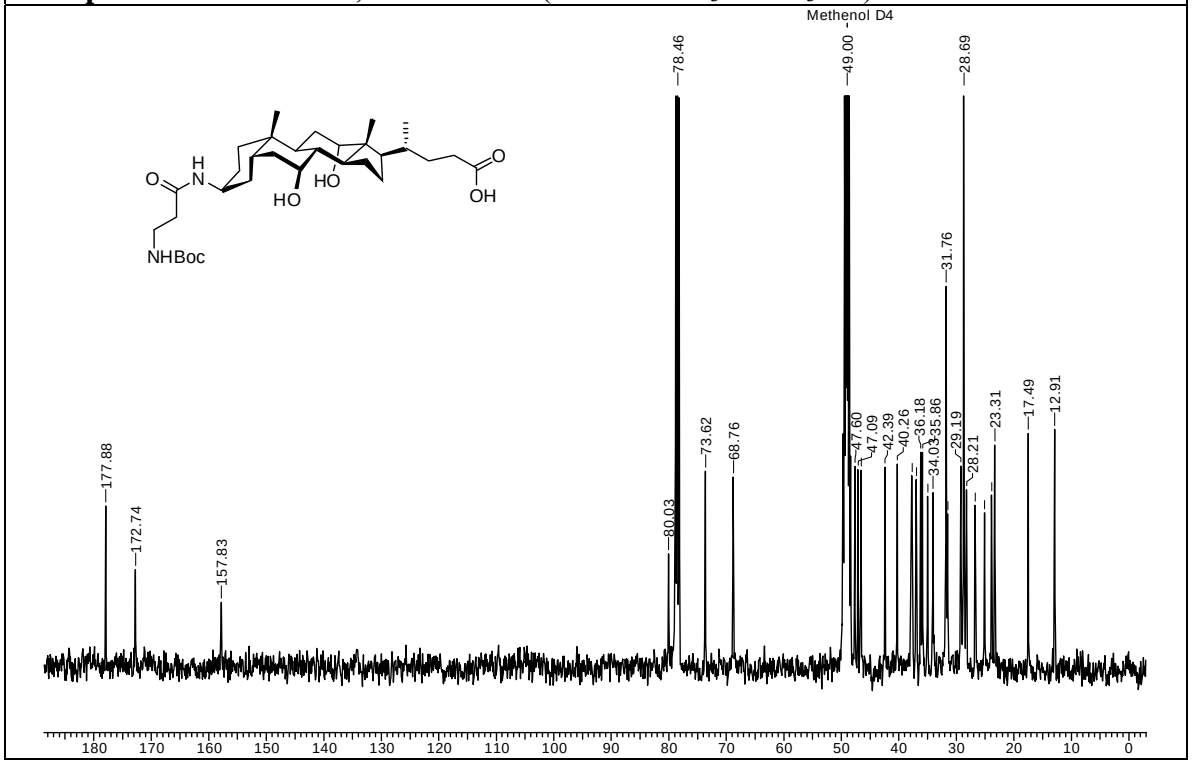
Compound 91: ^{13}C NMR, 100.61 MHz, (~20% $CDCl_3$ in CD_3OD)



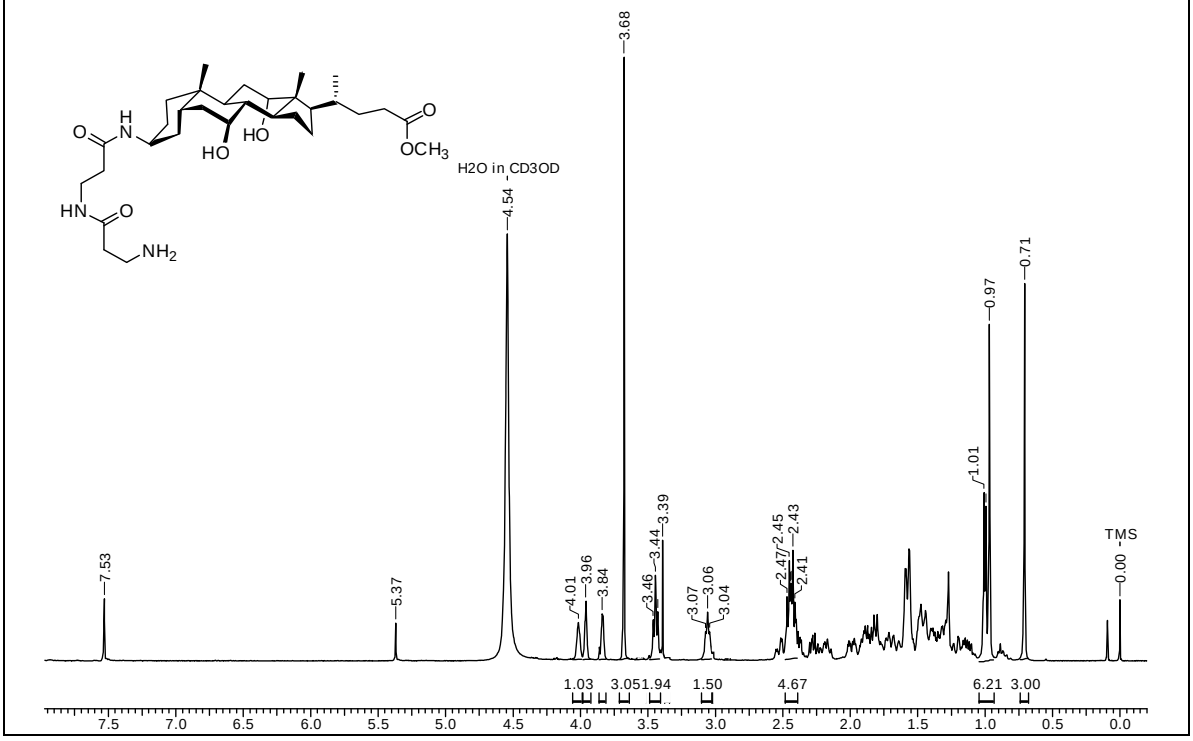
Compound 92: ^{1}H NMR, , 400 MHz. (~10% $CDCl_3$ in CD_3OD)



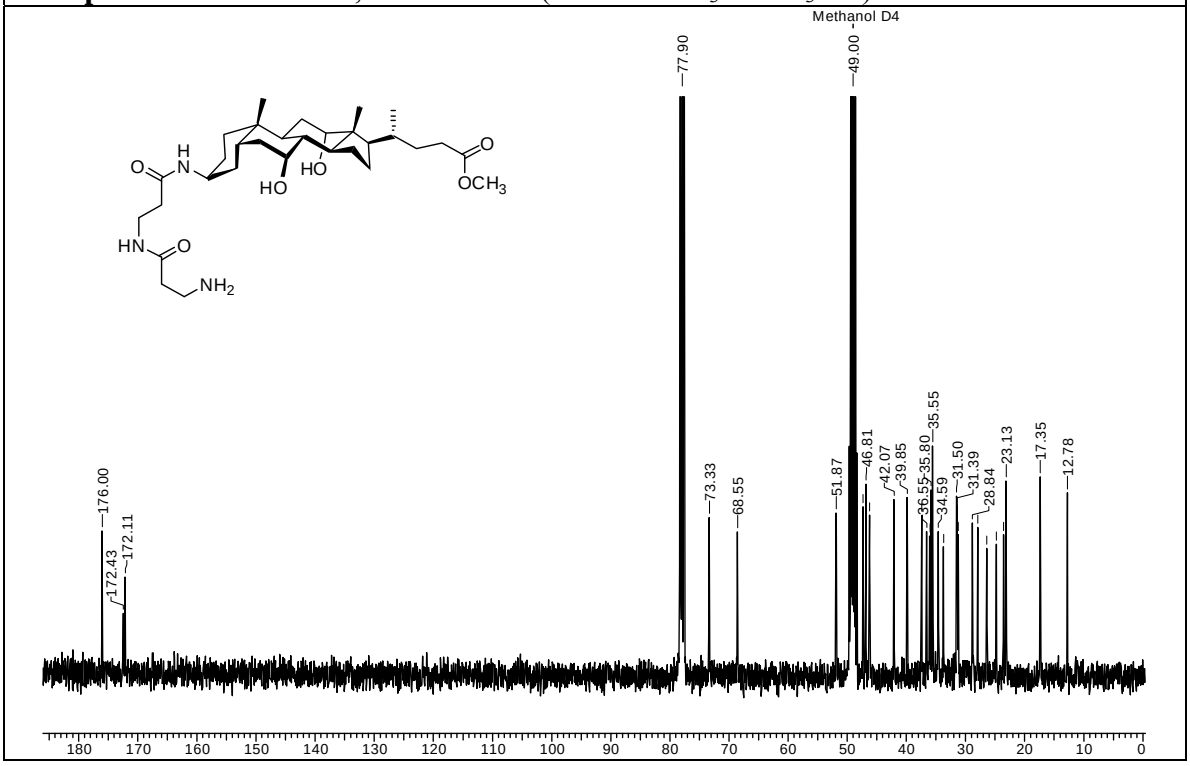
Compound 92: ^{13}C NMR, 100.61 MHz (~10% $CDCl_3$ in CD_3OD)



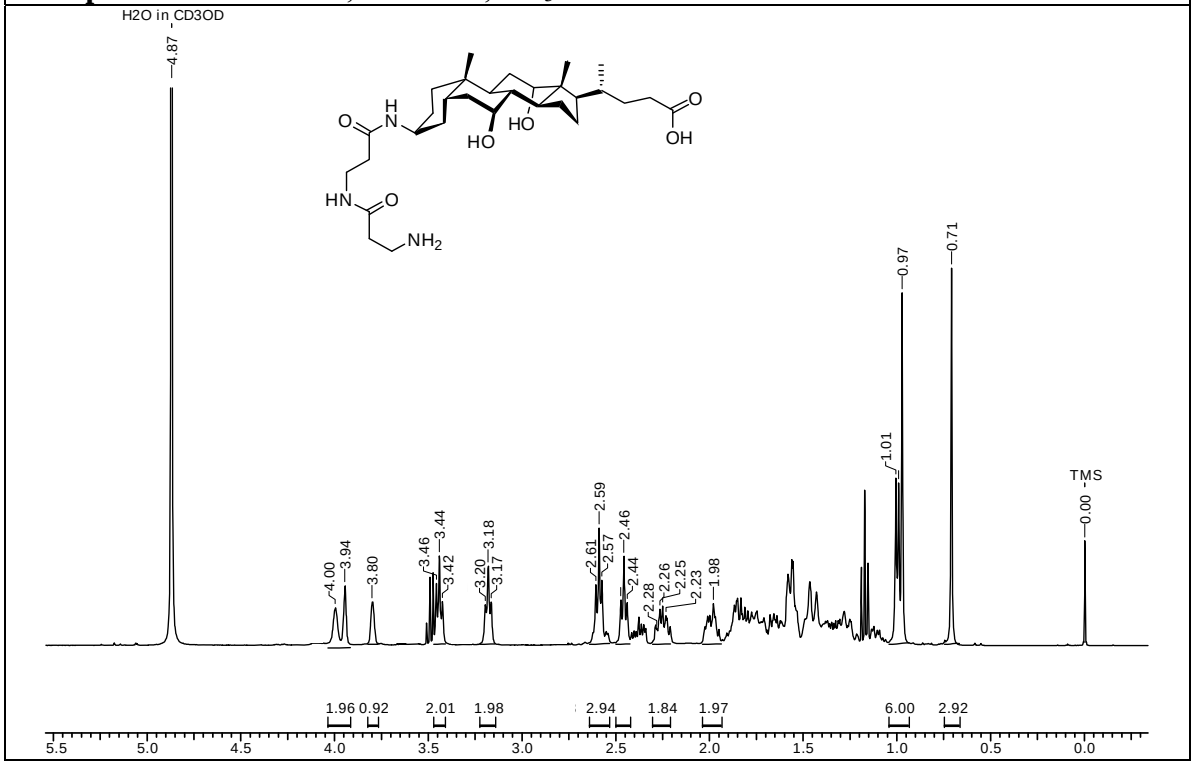
Compound 84: ^{1}H NMR, 400 MHz (~10% $CDCl_3$ in CD_3OD)



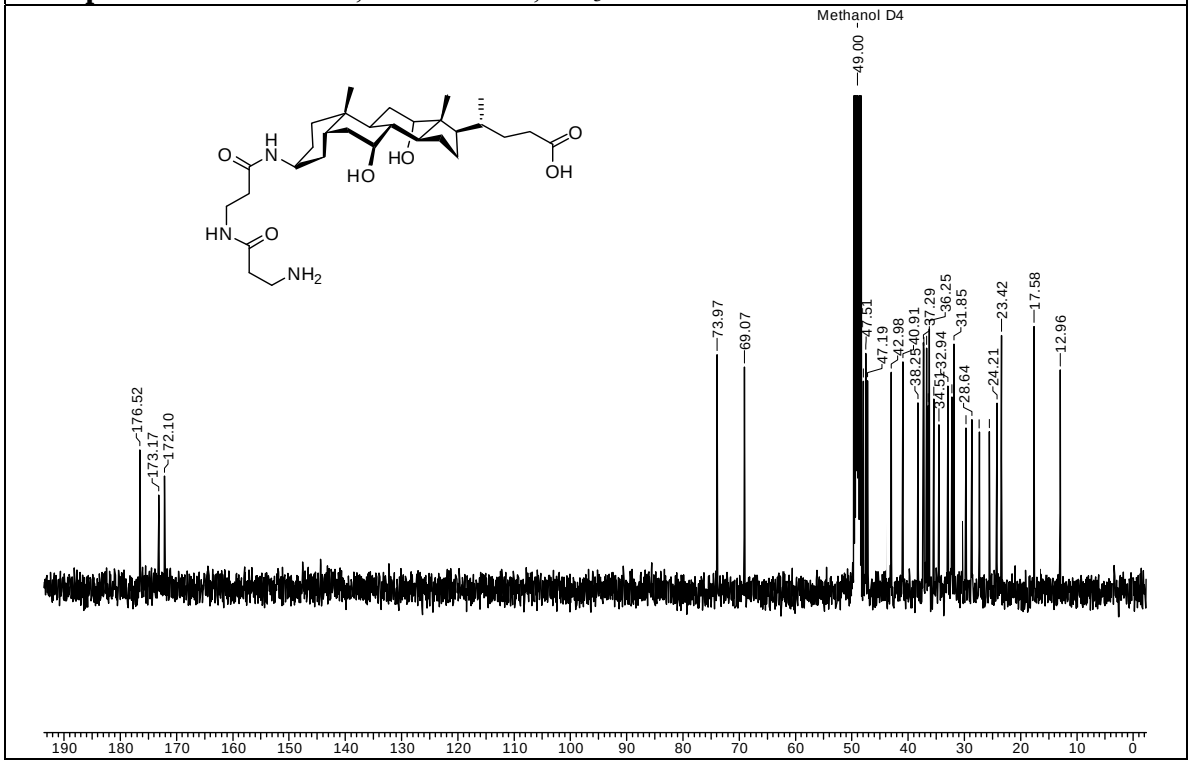
Compound 84: ^{13}C NMR, 100.61 MHz (~10% $CDCl_3$ in CD_3OD)



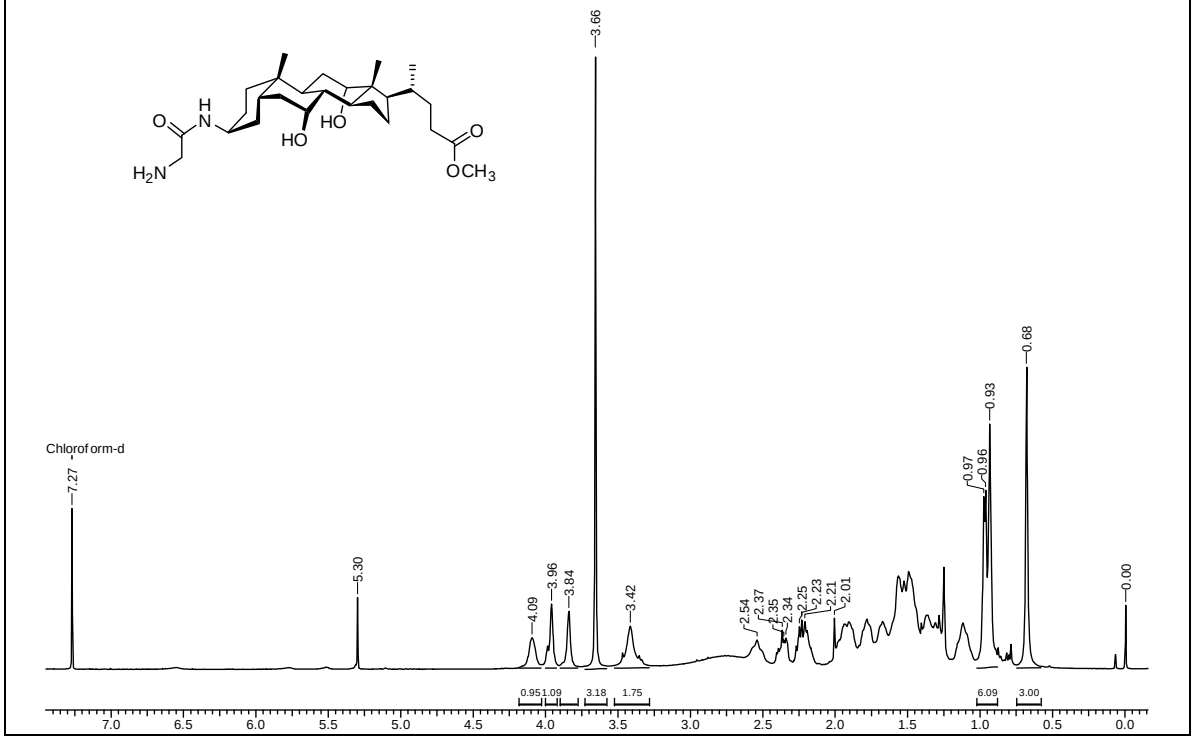
Compound 86: ^{1}H NMR, 400 MHz, CD_3OD



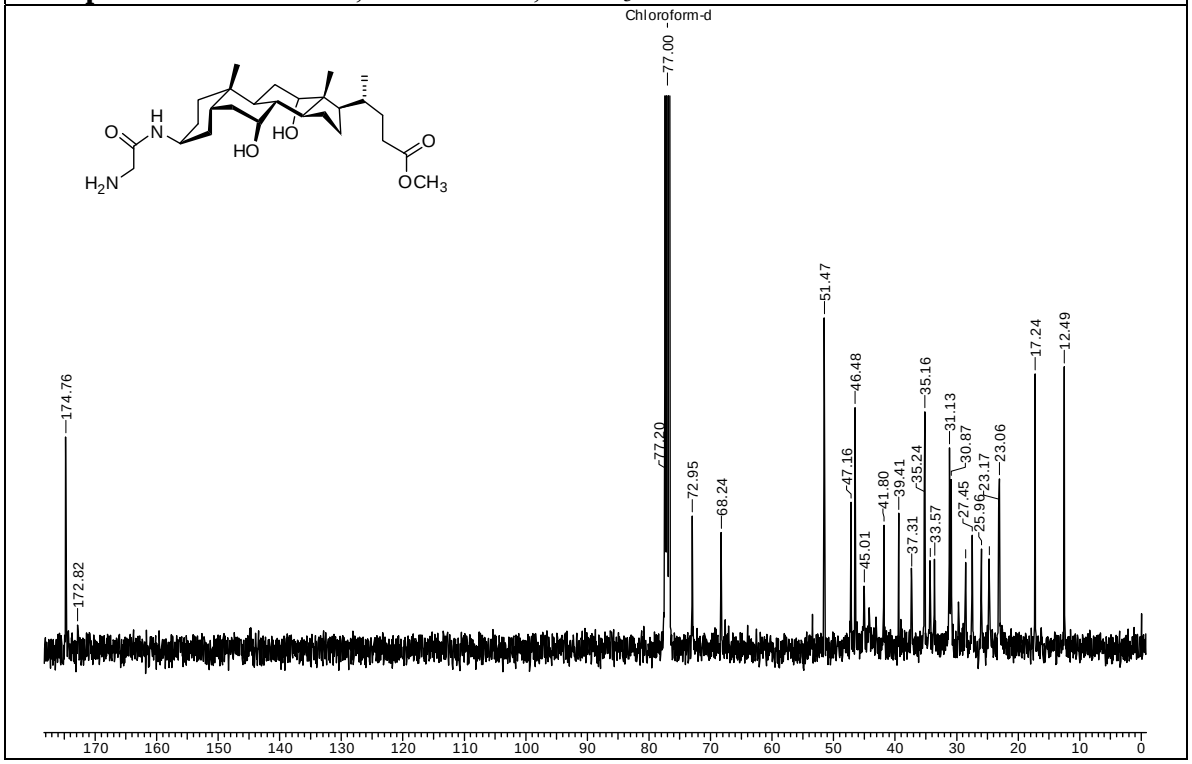
Compound 86: ^{13}C NMR, 100.61 MHz, CD_3OD



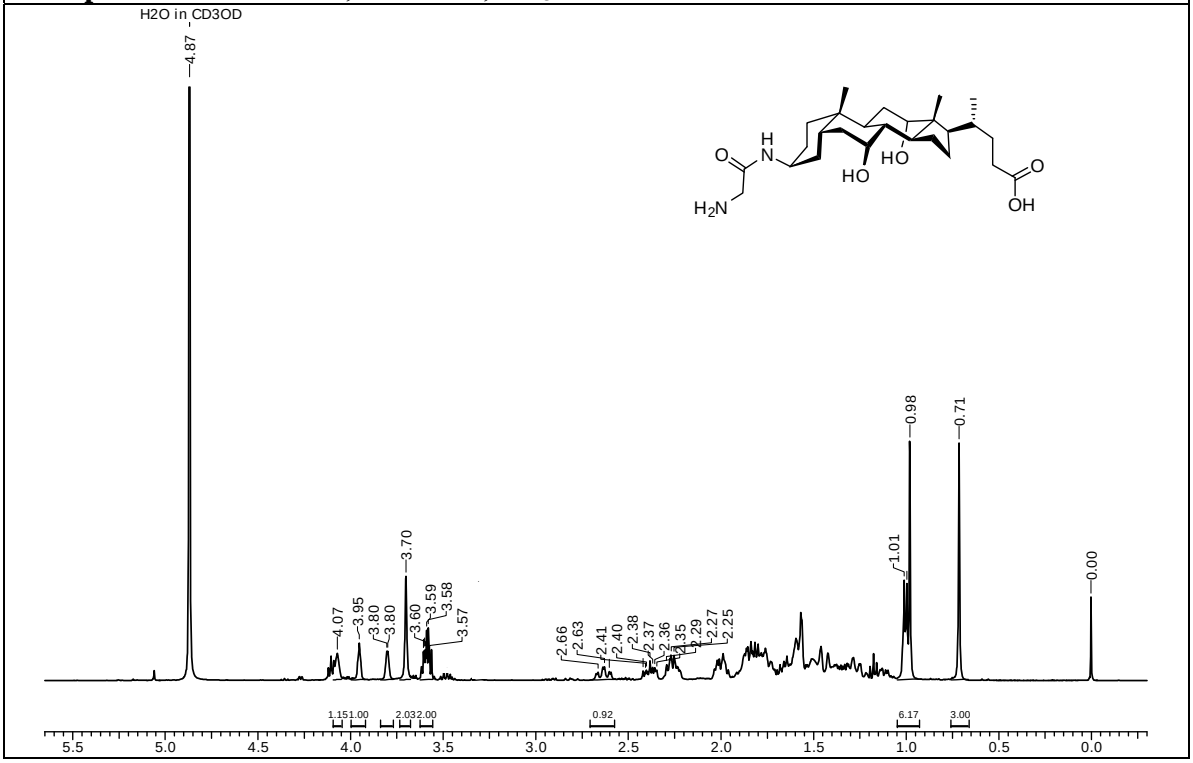
Compound 89: ^{1}H NMR, 400 MHz, $CDCl_3$



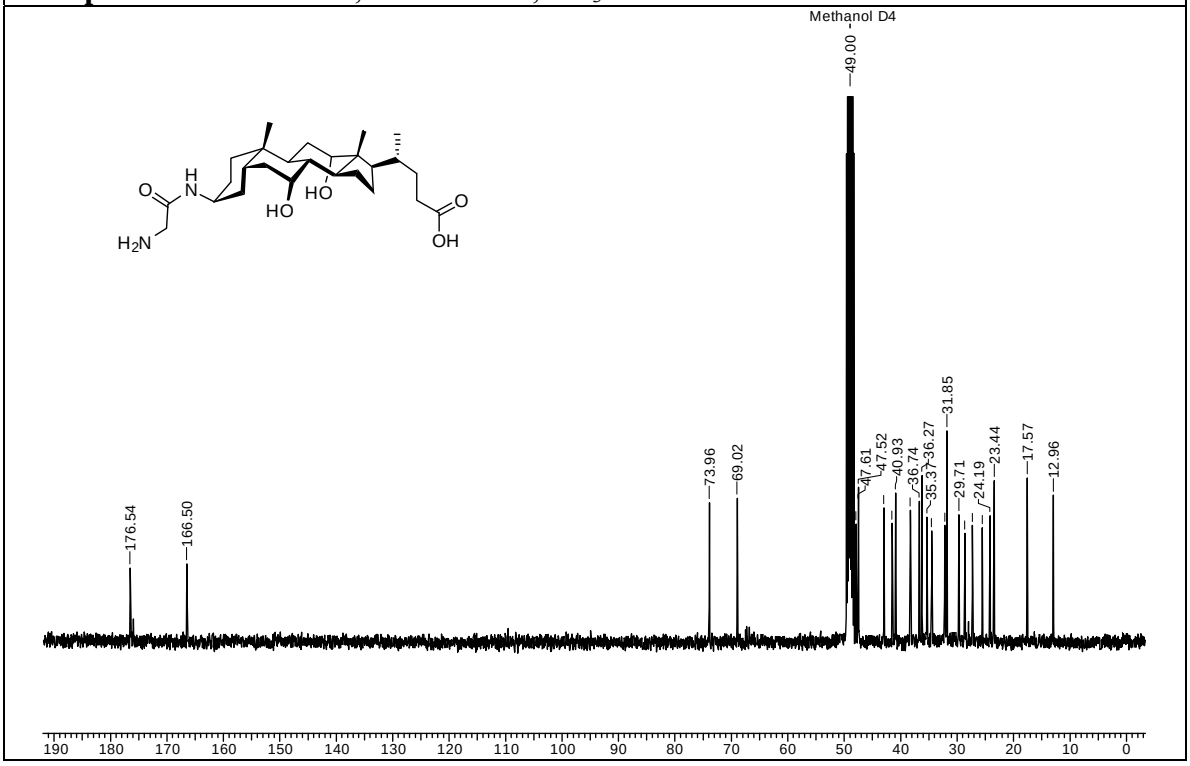
Compound 89: ^{13}C NMR, 100.61 MHz, $CDCl_3$



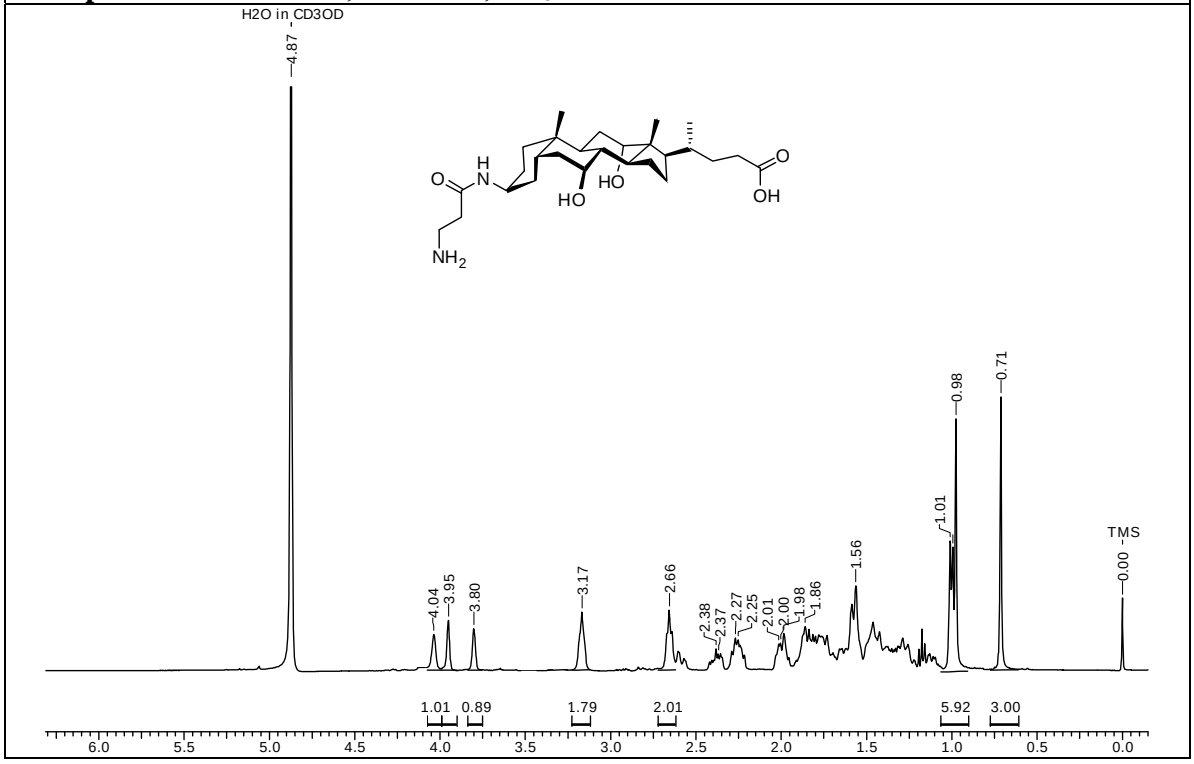
Compound 93: ^{1}H NMR, 400 MHz, CD_3OD



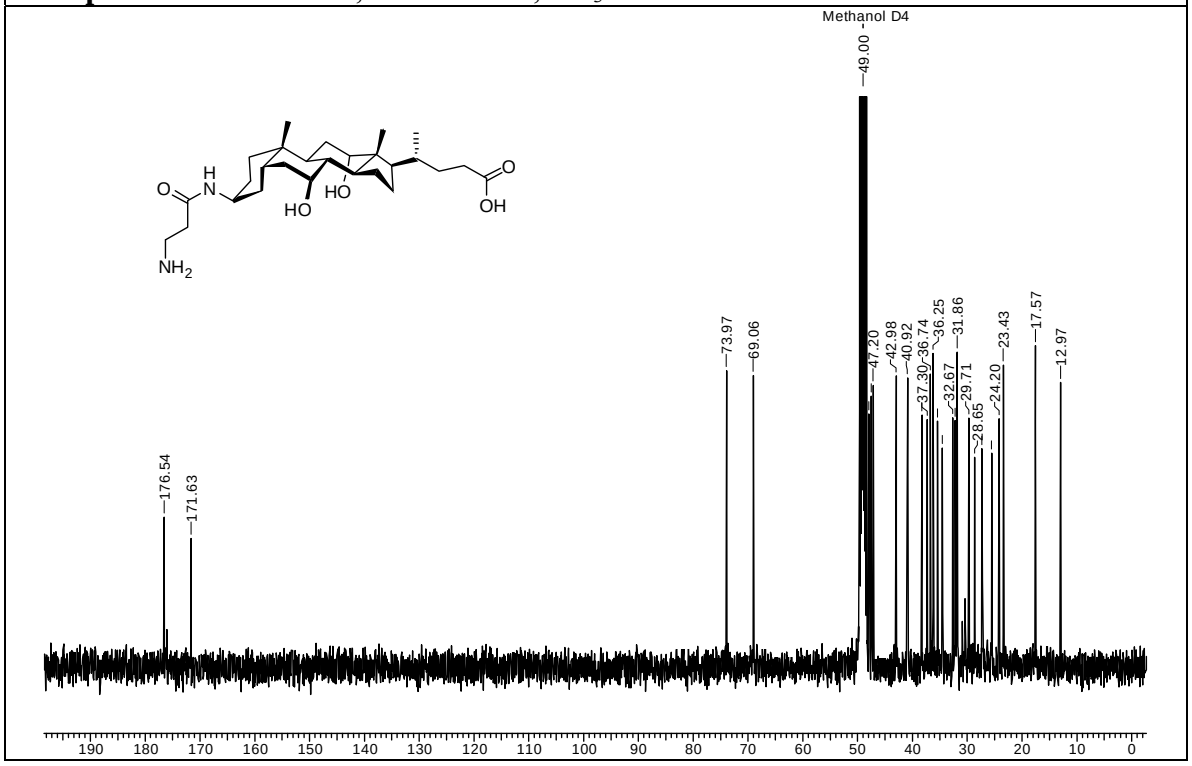
Compound 93: ^{13}C NMR, 100.61 MHz, CD_3OD



Compound 94: ^{1}H NMR, 400 MHz, CD_3OD



Compound 94: ^{13}C NMR, 100.61 MHz, CD_3OD



2A.10. References

1. Naka, K.; Sadownik, A.; Regen, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 2278.
2. Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961.
3. (a) Hancock, R. E. *Annu. Rev. Microbiol.* **1984**, *38*, 237; (b) Labischinski, G.; Bradaczek, H.; Naumann, D.; Rietschel, D. T.; Giesbrecht, P. *J. Bacteriol.* **1985**, *162*, 9; (c) Nikaido, H.; Vaara, M. *Microbiol. Rev.* **1985**, *49*, 1.
4. Freidinger, R. M. *J. Med. Chem.* **2003**, *46*, 5553.
5. (a) Wieland, H.; Alles, R. *Chem. Ber.* **1922**, *55*, 1789; (b) Wieland, H.; Hesse, G.; Huttel, R. *Annales de Chimie (Paris)* **1936**, *524*, 203; (c) Wieland, H.; Behringer, H. *Annales de Chimie (Paris)* **1941**, *549*, 209.
6. De Riccardis, F.; Minale, L.; Riccio, R. *Tetrahedron Lett.* **1993**, *34*, 4381.
7. (a) Sun, H. H.; Cross, S. S.; Gunasekera, M.; Kohen, F. E. *Tetrahedron* **1991**, *48*, 5467; (b) McKee, T. C.; Cardellina II, J. H.; Tischler, M.; Snader, K. M.; Boyd, M. R. *Tetrahedron Lett.* **1993**, *34*, 389.
8. Levina, E. V.; Kalinovskii, A. I.; Dmitrenok, P. S.; Prokofeva, N. G.; Andriyashchenko, P. V.; Stonik, V. A. *Doklady Biochem. Biophys.* **2004**, *396*, 171.
9. Finamore, E.; Minale, L.; Riccio, R.; Rinaldo, G.; Zollo, F. *J. Org. Chem.* **1991**, *56*, 1146.
10. Fusetani, N.; Kato, Y.; Hashimoto, K.; Komori, T.; Itakura, Y.; Kawasaki, T. *J. Nat. Prod.* **1984**, *47*, 997.
11. Kong, F.; Anderson, R.J. *J. Org. Chem.* **1993**, *58*, 6924.
12. (a) Turjman, N.; Nair, P. P. *Cancer Res.* 1981, 41, 3761; (b) Myhler, J. J.; Marai, L.; Kuksis, A.; Yousef, I. M.; Fisher, M. M. *Can. J. Biochem.* **1975**, *53*, 583.

13. Fieser, L. F.; Fieser, M. *Steroids*, Reinhold Publishing Corp.: New York, **1959**.

14. Bondi, S.; Muller, E. *Z. Physiol. Chem.* **1906**, *47*, 499.

15. (a) Crippa, G. B.; Bellini, A. M.; Crippa, A. *Ann. Chim. (Rome)* **1963**, *53*, 1496; (b) Bellini, A. M.; Vertuani, G.; Quaglio, M. P. *II Farmaco-Ed. Sci.* **1979**, *34*, 967; (c) Bellini, A. M.; Quaglio, M. P.; Guarneri, M.; Cavazinni, G. *Eur. J. Med. Chem.-Chim. Ther.* **1983**, *8*, 185.

16. Ballatore, A. M.; Beckner, C. F.; Caprioli, R. M.; Hoffman, N. E.; Liehr, J. G. *Steroids* **1983**, *41*, 197.

17. (a) Dhar, M. M; Agarwal, K. L. *Steroids* **1963**, *3*, 139; (b) Agarwal, K. L.; Dhar, M. M; *Steroids* **1964**, *4*, 495; (c) Agarwal, K. L.; Dhar, M. M; *Steroids* **1965**, *6*, 105.

18. (a) Pettit, G. R.; Gupta, A. K. D.; Smith, R. L. *Can. J. Chem.* **1966**, *44*, 2023; (b) Pettit, G. R.; Smith, R. L.; Klinger, H. *J. Org. Chem.* **1967**, *10*, 145.

19. Shimada, K.; Fujii, Y.; Nambara, T. *Chem. Pharm. Bull.* **1973**, *21*, 2183.

20. Coleman, J. P.; Kirby, L. C.; Klein, R. A. *J. Lipid Res.* **1995**, *36*, 901.

21. Mayaux, J.-F.; Bousseau, A.; Pauwels, R.; Huet, T.; Henin, Y.; Dereu, N.; Evers, M.; Soler, F.; Poujade, C.; De Clercq, E.; Le Pecq, J.-B. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3564.

22. Swaan, P. W.; Hillgren, K. M.; Szoka, F. C. Jr.; Oie, S. *Bioconj. Chem.* **1997**, *8*, 520.

23. Kagedahl, M.; Swaan, P. W.; Redemann, C. T.; Tang, M.; Craik, C. S.; Szoka, F. C. Jr.; Oie, S. *Pharm. Res.* **1997**, *14*, 176.

24. (a) Kramer, W.; Wess, G.; Schubert, G.; Bickel, M.; Girbig, F.; Gutjahr, U.; Kowalewski, S.; Baringhaus, K.-H.; Enhsen, A.; Glombik, H.; Mullner, S.; Neckermann, G.; Schulz, S.; Petzinger, E. *J. Biol. Chem.* **1992**, *267*, 18598; (b) Wess, G.; Kramer, W.; Schubert, G.; Enhsen, A.; Baringhaus, K.-H.; Glombik, H.; Mullner, S.; Bock, K.; Kleine, H.; John, M.; Neckermann, G.; Hoffmann, A. *Tetrahedron Lett.*

1993, *34*, 819; (c) Kramer, W.; Wess, G.; Enhsen, A.; Falk, E.; Hoffmann, A.; Neckermann, G.; Schubert, G.; Urmann, M. *J. Cont. Release* **1997**, *46*, 17.

25. Campbell, M. M.; Craig, R. C.; Boyd, A. C.; Gilbert, I. M.; Logan, R. T.; Redpath, J.; Roy, R. G.; Savage, D. S.; Sleigh, T. *J. Chem. Soc. Perkin Trans I* **1979**, 2235.
26. Marshall, R. J.; Winslow, E. *Gen. Pharmacol.* **1981**, *12*, 315.
27. Fang, C.; Liao, Q. -J, Xu, F. *J. Nanjing. Coll. Pharmacol.* **1984**, *15*, 45.
28. Mokotoff, M.; Zhao, M.; Marshal, R. J.; Winslow, E.; Wong, L. K.; Liao, Q. -J. *Steroids* **1990**, *55*, 399.
29. Ingle, D. J. *J. Endocrinol.* **1952**, *8*, 29.
30. (a) Wang, C.; Peng, S. Q.; Zhang, X. P.; Qiu, X. C. *Acta. Pharma. Sinica* **1998**, *33*, 111; (b) Wang, C.; Zhao, M.; Qiu, X.; Peng, S. *Bioorg. Med. Chem.* **2004**, *12*, 4403.
31. Wang, C.; Zhao, M.; Yang, J.; Peng, S. *Steroids* **2001**, *66*, 811.
32. Maltais, R.; Luu-The, V.; Poirier, D. *Bioorg. Med. Chem.* **2001**, *9*, 3101.
33. (a) Willemaen, H. M.; Vermonden, T.; Koudijs, A.; Marcelis, A. T. M.; Sudholter, E. J. R. *Colloids and Surfaces A: Physicochem. Eng. Aspects* **2003**, *218*, 59; (b) Willemaen, H. M.; Vermonden, T.; Marcelis, A. T. M.; Sudholter, E. J. R. *Eur. J. Org. Chem.* **2001**, 2329.
34. Valkonen, A.; Lahtinen, M.; Virtanen, E.; Kaikkonen, S.; Kolehmainen, E. *Biosens. Bioelectron.* **2004**, *20*, 1233.
35. Dukh, M.; Šaman, D.; Kroulik, J.; Černý, I.; Pouzar, V.; Král, V. Drašar, P. *Tetrahedron* **2003**, *59*, 4069.
36. Matsumoto, T.; Watanabe, M.; Mataka, S.; Thiemann, T. *Steroids* **2003**, *68*, 751.
37. (a) Vaara, M. *Microbiol. Rev.* **1992**, *56*, 395. (b) Vaara, M.; Porro, M. *Antimicrob. Agents Chemother.* **1996**, *40*, 1801.

38. Li, C.; Budge, L. P.; Driscoll, C. D.; Willardson, B. M.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1999,** *121,* 931.

39. (a) Rehman, A.; Li, C.; Budge, L. P.; Street, S. E.; Savage, P. B. *Tetrahedron Lett.* **1999**, *40*, 1865; (b) Zhou, X. -T.; Rehman, A.; Li, C.; Savage, P. B. *Org. Lett.* **2000**, *2*, 3015.

40. Ding, B.; Taotofa, U.; Orsak, T.; Chadwell, M.; Savage, P. B. *Org. Lett.* **2004**, *6*, 3433.

41. Amo, V.; Siracusa, L.; Markidis, T.; Baragana, B.; Bhattarai, K. M.; Galobardes, M.; Naredo, G.; Perez-Payan, M. N.; Davis, A. P. *Org. Biomol. Chem.* **2004**, *2*, 3320.

42. Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1814.

43. Li, H.; Wang, L. *Org. Biomol. Chem.* **2003**, *1*, 3507.

44. Lee, S.; Kim, K.; Kumar, T. S.; Lee, J.; Kim, S. K.; Lee, D. Y.; Lee, Y.; Byun, Y. *Bioconjugate Chem.* **2005**, *16*, 615.

45. (a) Yan, A. -X.; Chan, R. Y. K.; Lau, W. -S.; Lee, K. -S.; Wong, M. -S.; Xing, G. -W.; Tian, G. -L.; Ye, Y. -H. *Tetrahedron* **2005**, *61*, 5933; (b) Ye, Y. -H.; Huang, Y. -S.; Wang, Z. -Q.; Chen, S. -M.; Tiant, Y. *Steroids* **1993**, *58*, 35.

46. (a) Albert, D.; Feigel, M. *Tetrahedron Lett.* **1994**, *35*, 565; (b) Wess, G.; Bock, K.; Kleine, H.; Kurz, M.; Guba, W.; Hemmerle, H.; Lopez-Calle, E.; Baringhaus, K. -H.; Glombik, H.; Enhsen, A.; Kramer, W. *Angew. Chem. Int. Ed.* **1996**, *35*, 2222; (c) Albert, D.; Feigel, M.; Benet-Buchholz, J.; Boese, R. *Angew. Chem. Int. Ed.* **1998**, *37*, 2727; (d) Wessjohann, L. A.; Voigt, B.; Rivera, D. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 4785.

47. (a) Barnett, J.; Ryman, B. E.; Smith, F. *J. Chem. Soc.* **1946**, 528; (b) Loncle, C.; Brunel, J. M.; Vidal, N.; Dherbomez, M. Letourneux, Y. *Eur. J. Med. Chem.* **2004**, *39*, 1067.

48. Sobotka, H. *Physiological Chemistry of the Bile*, Baltimore, Williams and Wilkins Co. 1937.

49. (a) James, S. P.; Smith, F.; Stacy, M.; Webb, M. *J. Chem. Soc.* **1946**, 665; (b) Jones, A. S.; Webb, M.; Smith F. *J. Chem. Soc.* **1949**, 2164; (c) Hilton, M. L.; Jones, A. S.; Westwood, J. R. B. *J. Chem. Soc.* **1955**, 3449; (d) Fini, A.; Fazio, G.; Roda, A.; Bellini, A. M.; Mencini, E.; Guarneri, M. *J. Pharm. Sci.* **1992**, *81*, 726.

50. Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, J. N. Jr.; McCrimmon, D.; Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1354.

51. Stone, R. *Science* **1993**, *259*, 1125.

52. Brunel, J. M.; Salmi, C.; Loncle, C.; Vidal, N.; Letourneux, Y. *Curr. Cancer Drug Targets* **2005**, *5*, 267.

53. Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6138.

54. (a) Shimada, K.; Fujii, Y.; Mitsuishi, E.; Nambara, T. *Tetrahedron Lett.* **1974**, *15*, 467; (b) Kong, F.; Anderson, R. J. *J. Org. Chem.* **1993**, *58*, 6924.

55. (a) Vaara, M. *Microbiol. Rev.* **1992**, *56*, 395; (b) Rehman, A.; Li, C.; Budge, L. P.; Street, S. E.; Savage, P. B. *Tetrahedron Lett.* **1999**, *40*, 1865.

56. Savage, P. B.; Li, C.; Taotafa, U.; Ding, B.; Guan, Q. *FEMS Microbiol. Lett.* **2002**, *217*, 1.

57. Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1589.

58. (a) Agarwal, K. L.; Dhar, M. M. *Steroids* **1964**, *3*, 139; (b) Ding, B.; Taotofa, U.; Orsak, T.; Chadwell, M.; Savage, P. B. *Org. Lett.* **2004**, *6*, 3433; (c) Sievanen, E. *Molecules* **2007**, *12*, 1859.

59. (a) Kong, F.; Anderson, R. J. *J. Org. Chem.* **1993**, *58*, 6924; (b) Cunha Filho, G. A.; Schwartz, C. A.; Resack, I. S.; Murta, M. M.; Lemos, S. S.; Castro, C. K.; Pires, O. R.; Leite, J. R. S.; Bloch, C.; Schwartz, E. F. *Toxicon* **2005**, *45*, 777.

60. Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385.

61. Bowe, C. L.; Mokhtarzadeh, L.; Venkatesan, P.; Babu, S.; Axelrod, H. R.; Sofia, M. J.; Kakarla, R.; Chan, Y. Y.; Kim, J. S.; Lee, H. J.; Amidon, G. L.; Choe, S. Y.; Walker, S.; Kahne, D. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12218.

62. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, 2nd Edition; Springer-Verlag, New York, 1994.

63. (a) Narita, M.; Doi, M.; Kudo, K.; Terauchi, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3553; (b) Buttner, F.; Norgren, A. S.; Zhang, S.; Prabpai, S.; Kongsaeree, P.; Arvidsson, P. I. *Chem. Eur. J.* **2005**, *11*, 6145.

64. (a) Anelli, P. L.; Lattuada, L.; Uggeri, F. *Synth. Commun.* **1989**, *28*, 109; (b) Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178.

65. (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, 1997; (b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, 1998; (c) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, 5th ed.; NCCLS: Villanova, PA, 2000; M7–A5.

66. (a) Bartnicki-Garcia, S. *Annu. Rev. Microbiol.* **1968**, *22*, 87; (b) Debono, M.; Gordee, R. S. *Annu. Rev. Microbiol.* **1994**, *48*, 471.

67. (a) Elion, G. B.; Singer, S.; Hitchings, G. H. *J. Biol. Chem.* **1954**, *208*, 477; (b) Li., C.; Budge, L. P.; Driscoll, C. D.; Willardson, B. M.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1999**, *121*, 931.

68. (a) Yoon, H. S.; Rho, J. H.; Yoo, K. W.; Park, W. C.; Rho, S. H.; Choi, Y. H.; Suh, H.; Kim, N. D.; Yoo, K. S.; Yoo, Y. H. *Curr. Eye Res.* **2001**, *22*, 367; (b) Lamireau, T; Zoltowska, M.; Levy, E.; Yousef, I.; Rosenbaum, J.; Tuchweber, B.; Desmoulière, A. *Life Sci.* **2003**, *72*, 1401; (c) Pai, R; Tarnawski, A. S.; Tran, T. *Mol. Biol. Cell.* **2004**, *5*, 2156.

69. Wachs, F. P.; Krieg, R. C.; Rodrigues, C. M.; Messmann, H.; Kullmann, F.; Knüchel-Clarke, R.; Schölmerich, J.; Rogler, G.; Schlottmann, K. *Int. J. Colorectal Dis.* **2005**, *20*, 103.

70. Milovic, V.; Teller, I. C.; Murphy, G. M.; Caspary, W. F.; Stein, J. *Eur. J. Gastroenterol Hepatol.* **2001**, *13*, 945.

71. Sladowski, D.; Steer, S. J.; Clothier, R. H.; Balls, M. *J. Immunol. Methods* **1993,** *157*, 203.

72. Boger, D. L.; Zhou, J.; Winter, B.; Kitos, P. A. *Bioorg. Med. Chem.* **1995**, *3*, 1579.

73. Ryu, E-H.; Ellern, A.; Zhao, Y. *Tetrahedron* **2006**, *62*, 6808.

CHAPTER - 2

Section B

Pd Catalyzed One-pot Chemoselective Protocol for the Preparation of Carboxamides Directly from Azides

2B.1. Abstract

Carboxamides are obtained efficiently in high yields from azides on reaction with corresponding preformed activated carboxylic acids in a single-step reductive transformation using hydrogen atmosphere (balloon) under $Pd/CaCO_3$ and/or $Pd/BaSO_4$ catalysis. The method is highly chemoselective and compatible with highly labile functional groups such as benzyl carbamates, benzyl ethers, benzyl esters and olefins.

2B.2. Introduction

The conversion of azides to amines is an important transformation in organic synthesis.[1] The amines are further converted to the corresponding carboxamides which is the most common functionality in many biological interesting molecules. Several methods have been developed for the direct amidation of carboxylic acids and amines.[2] To overcome the situation where free amines can not be used because of structural wavering, azides have been used to directly form amide bonds.

2B.3. Literature Survey

The classical method of conversion of azide to amides includes Staudinger-type ligation involving the acylation of an iminophosphorane[3-8] and the amidation of carboxylic acid with an azide in the presence of triallyl phosphine[9,10] (Figure 1). A one pot self regulated approach for the synthesis of amides based on two redox reactions has been described by Ghosh and co-workers.[11] Recently Hu *et al* documented selenocaboxylate/azide amidation,[12] as an alternate method to the conventional nucleophilic acylation when an amide bond needs to be formed without going through an amine intermediate. The other attractive method was, Williams thio acid/azide amidation,[13] which was also documented as an improved method for the synthesis of *N*-acyl sulfonamides.[14] A new chemical ligation method was also reported by Raines *et al* in which phosphinobenzenethiol was used to link a thioester and azide to form an amide bond.[15]

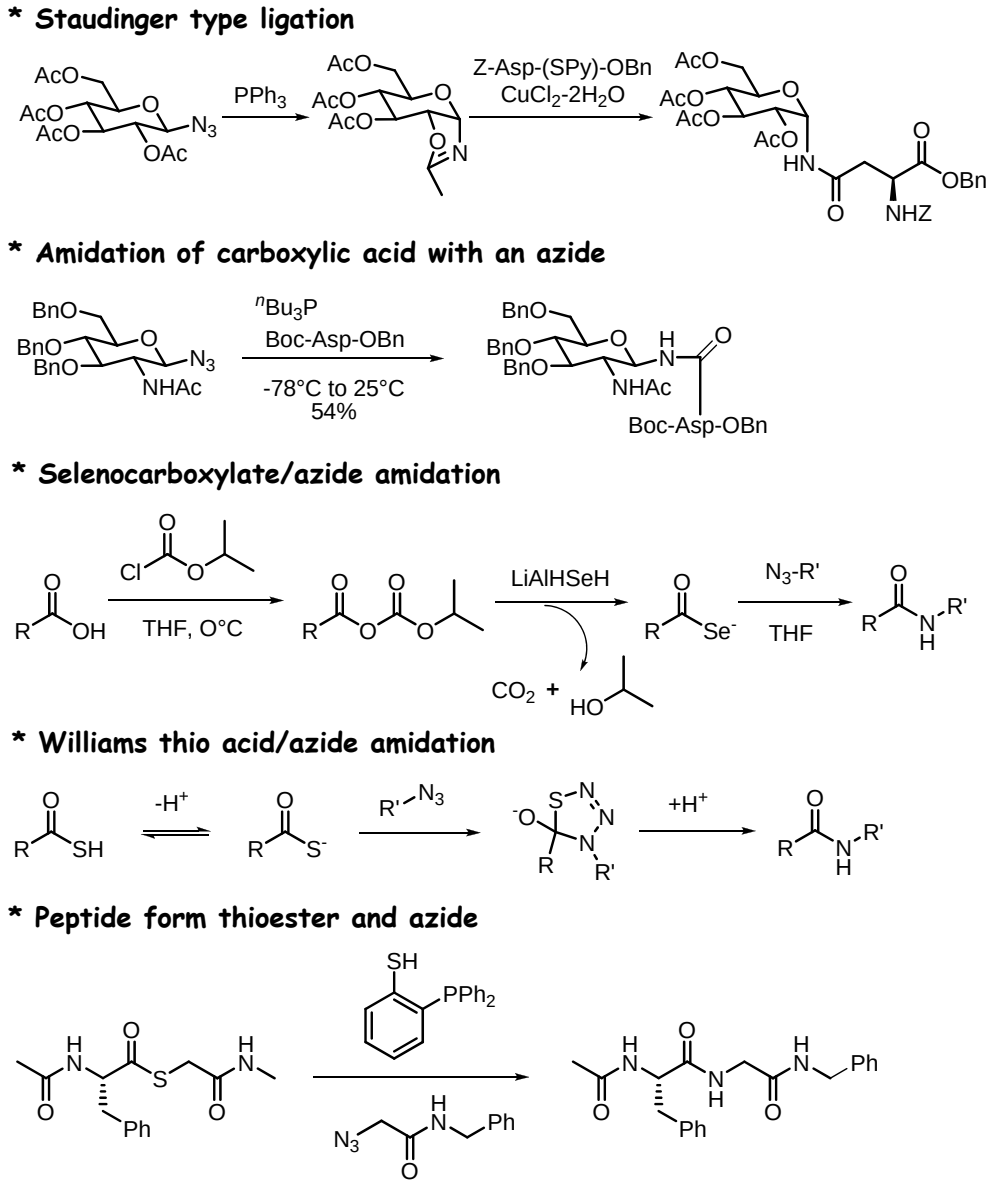


Figure 1: Literature reports on the conversion of azides to carboxamides

As a part of an ongoing program on synthesis of biologically interesting bile acid-aminoacid conjugates,[16] we required an efficient one pot method for the chemoselective transformation of azide to carboxamides. The major drawback of the Staudinger-type ligation involves with the overall yield and purification of products where as William thioacid/azide amidation and selenocarboxylate/azide amidation requires the conversion of acids to thio acids and selenocarboxylates respectively. Therefore there is an urgent need for the exceptionally chemoselective and operationally very simple protocol for the

synthesis of carboxamides directly from azides. What attracted our attention was the reductive transformation of azides to *N*-(*tert*- butoxycarbonyl)amines *via* catalytic hydrogenation by Saito *et al*[17] and Baskaran *et al*.[18] Various methods have been developed for such type of transformation using triethylsilane[19] or polymethylhydrosiloxane[20,21] as hydrogen source.

Based on the above-mentioned observations and in continuation of our recent work in our laborotary,[22] here we describe a new and efficient "one pot" chemoselective protocol for the preparation of carboxamides. In this practice carboxamides were obtained efficiently in high yields from azides on reaction with corresponding preformed activated carboxylic acids in a single-step reductive transformation using hydrogen atmosphere (balloon) under $Pd/CaCO_3$ and/or $Pd/BaSO_4$ catalysis.

2B.4. Result and Discussion

The first successful result for the amidation of benzyl azide **1a** with Boc-Gly-OSu **2a** was obtained using $Pd\text{-}BaSO_4$ in THF to furnish compound **3** in 86 % yield. Continuous 18 hrs of H_2 (balloon) atmosphere was required for the complete consumption of the starting materials at 25 °C (Table 1, Entry 1), whereas at 0 °C we have noticed no reaction for 20 h. Compound **4** was obtained in good yield when benzyl azide **1a** was treated with Boc-Glu-(O*t*Bu)-OSu **2b** (Entry 2). Similarly, compound **5** was obtained in 82 % yield when benzyl azide **1a** was treated with Boc-Thr-(Bzl)-OSu **2c** (Entry 3). This was our first evidence for chemoselective reductive transformation of azide to carboxamides in the presence of benzyl ethers. Unfortunately we could not achieve the required chemoselectivity for the reduction of azides over benzyl esters (Entry 4), as the benzyl ester functionality in compound Boc-Asp-(OSu)-OBzl **2d** was hydrogenolyzed under the present reaction conditions. The longer reaction time may be one of the reasons for the

observed hydrogenolysis of the benzyl ester functionality. To reduce the reaction time we planned to use polar-protic solvents such as ethanol instead of THF. For this the stability of the *N*-hydroxy succinimide (NHS) ester in compound **2a** was verified by simply stirring the activated ester in EtOH. The ester **2a** was found to be stable in EtOH for the period of about 12 hrs. We observed ethanolysis of compound **2a** at elevated temperatures as well as when the reaction time was more than 12 hrs. This suggests that EtOH can be utilized for the reaction at room temperature provided reaction completes within 10-12 hrs. The first trial experiment for the amidation of benzyl azide **1a** with Boc-Gly-OSu **2a** in EtOH at 25 °C using H_2 balloon and Pd-$BaSO_4$ catalysis produced the desired amide **3** within 0.5 hrs (Entry 1). Similar way we have drastically reduced the reaction time for carboxamide **4** and **5** in Ethanol (0.5 h) compared to the earlier reported method, which required 18-19 h for the reaction to complete in THF.

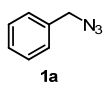
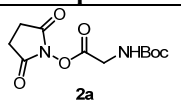
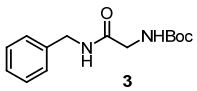
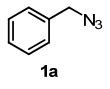
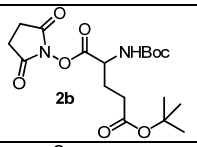
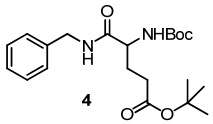
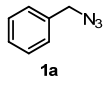
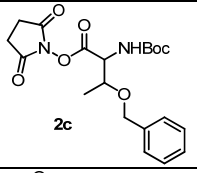
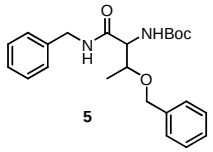
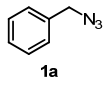
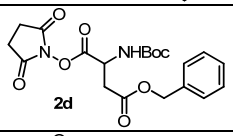
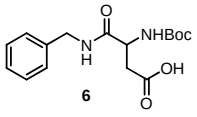
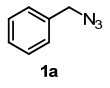
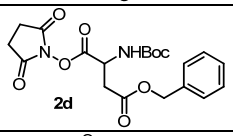
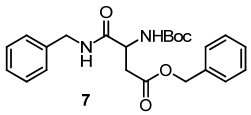
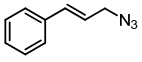
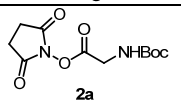
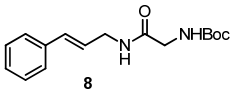
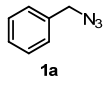
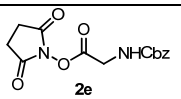
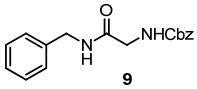
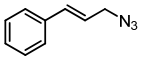
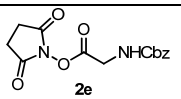
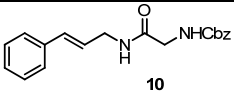
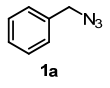
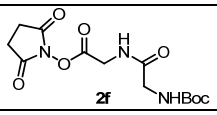
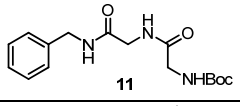
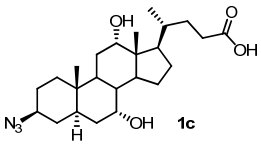
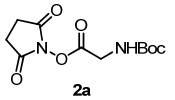
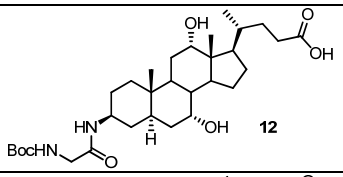
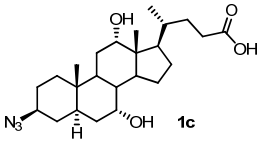
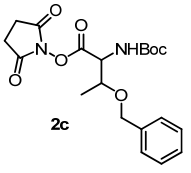
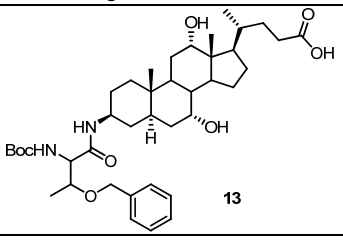
Yet again, we could not realize the synthesis of carboxamide **7**, instead; compound **6** was formed when benzyl azide **1a** was treated with Boc-Asp-(OSu)-OBzl **2d** under the catalytic hydrogenation condition using Pd-$BaSO_4$ in EtOH within 0.5 hrs (Entry 4). After surveying several reagent systems for this transformation the best result was achieved with the use of Lindlar catalyst (Pd-$CaCO_3$) in EtOH. In a typical experimental procedure, benzyl azide **1a** when treated with Boc-Asp-(OSu)-OBzl **2d** in EtOH at 25 °C using H_2 balloon and Pd-$CaCO_3$ catalysis produced the desired amide **7** in 81 % yield. within 0.5 hrs (Entry 1). Successful result obtained with substrate **2d** encouraged us to extend our objective. The simplicity, remarkable chemoselectivity and mildness of this catalytic transformation were exhaustively studied with compounds bearing different sensitive functional groups such as benzyl ethers (Entry 3 and 11), olefins (Entry 6 and 8) and benzyl carbamates (Entry 7 and 8). In particular, synthesis of compound **10** (Entry 8)

was realized when azide bearing double bond **1b** and NHS ester bearing *N*-Cbz protecting group **2e** were exposed to the present modified protocol. As expected, azide possessing olefin functional group was smoothly converted into the corresponding carboxamides **10** in excellent yield without affecting the carbon-carbon double bonds and *N*-Cbz protecting group. The scope and generality of this protocol was utilized in our current research for the synthesis of peptide **11** (Entry 9) and steroidal carboxamides **12** as well as **13** (Entry 10 and 11, respectively).

2B.5. Conclusion

In the present manuscript, we have demonstrated a new robustic, efficient "one pot" chemoselective protocol for the preparation of carboxamides. In this practice carboxamides were obtained efficiently in high yields from azides on reaction with corresponding preformed activated carboxylic acids in a single-step reductive transformation using hydrogen atmosphere (balloon) under $Pd/CaCO_3$ and/or $Pd/BaSO_4$ catalysis. Along with this, the simplicity, remarkable chemoselectivity and mildness of this catalytic transformation were exhaustively studied with compounds bearing different sensitive functional groups such as benzyl ethers, olefins and benzyl carbamates and benzyl esters. The scope and generality of this protocol was utilized in our current research for the synthesis of peptide and steroidal carboxamides .

Table 1.

Entry	Azide Component	NHS ester Component	Product	Catalyst[†]	Yield
1	 1a	 2a	 3	A B	89/86[‡] 91
2	 1a	 2b	 4	A B	85/83[‡] 87
3	 1a	 2c	 5	A B	82/82[‡] 81
4	 1a	 2d	 6	A	86/79[‡]
5	 1a	 2d	 7	B	81
6	 1b	 2a	 8	A B	85 84
7	 1a	 2e	 9	A B	88 87
8	 1b	 2e	 10	A B	77 80
9	 1a	 2f	 11	A B	87 83
10	 1c	 2a	 12	B	76
11	 1c	 2c	 13	B	70

[†] **A**- Palladium, 5 wt. % on barium sulphate; **B**- Palladium, 5 wt. % on calcium carbonate.

[‡]Yields for the reactions carried out in THF (Other all yields are for the reaction carried out in EtOH).

Entry 1A, 2A, 3A was earlier reported in our laboratory.[22]

2B.6. Experimental Section

General Methods:

Palladium, 5 wt. % on calcium carbonate, poisoned with lead (Lindlar Catalyst) and Palladium, 5 wt. % on barium sulphate, unreduced were purchased from Aldrich, Benzyl azide **1a** was obtained from Alfa Aesar, Boc-L-Glycine *N*-hydroxysuccinimide Ester (Boc-Gly-OSu) **2a**, *N*α-*t*-Boc-L-Glutamic acid γ-*t*-butyl ester *N*-hydroxysuccinimide ester (Boc-Glu-(O*t*Bu)-OSu) **2b**, Boc-O-benzyl-L-threonine *N*-hydroxysuccinimide ester (Boc-Thr(Bzl)-OSu) **2c**, Boc-L-aspartic acid β-*N*-hydroxysuccinimide ester α-benzyl ester (Boc-Asp(OSu)-OBzl) **2d,** *N*-(Benzyloxycarbonyl)glycine *N*-hydroxy-succinimidyl ester (Z-Gly-OSu) **2e** were purchased from Bachem. The non-commercial 1-scuunimidyl-*N*-tert-butyloxycarbonylglycylglycinate[23] (Boc-Gly-Gly-OSu **2f)** and azides (cinamyl azide[24] **1b** and steroidal azide[25]**1c**) and were synthesized according to the literature procedures.

Experimental Procedure:

Compound 3: (***N*-Benzyl-α-[(*tert*-butoxycarbonyl)amino)acetamide)** Palladium, 5 wt. % on calcium carbonate, poisoned with lead (30 mg, 15 % by wt.) was added successively to a stirred solution of Boc-Gly-OSu **2a** (200 mg, 0.73 mmol) and benzyl azide **1a** (108 μL, 0.87 mmol) in EtOH (5 mL). The reaction flask was evacuated and flushed with hydrogen gas. The resultant mixture was stirred under hydrogen atmosphere (balloon) at 25 ˚C for 30 min. After completion of the reaction, the catalyst was filtered through a pad of celite, the filter cake was washed with EtOH (20 mL) and the filtrate was concentrated under reduced pressure. This crude product was dissolved in EtOAc (100 mL) washed with 10 % citric acid (2x10 mL), 20 % $NaHCO_3$ (2x10 mL), cold water (2×10 mL), brine (10 mL) and was dried over Na_2SO_4. The residue was purified over

silica gel (100-200 mesh) using 2 % MeOH/CH_2Cl_2 to afford compound **3** as a white solid (176 mg, 91 % yield); mp 65-66 °C (lit.[26] mp 64-68 °C); Anal. Calcd for $C_{14}H_{20}N_2O_3$: C, 63.62; H, 7.63; N, 10.60. Found: C, 63.8; H, 7.5; N, 10.9; IR ν_{max} (Nujol) 3308, 1703, 1658, 1530 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.41 (s, 9H), 3.82 (d, 2H, J = 6Hz), 4.45 (d, 2H, J = 6Hz), 5.30 (bs, 1H), 6.67 (bs, 1H), 7.24-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.1, 43.1, 44.1, 80.0, 127.3, 127.5x2, 128.5x2, 137.9, 156.1, 169.5; MS (LCMS) *m/z* 264.2 2253 $[M+H]^+$, 287.2134 $[M+Na]^+$.

Compound 4: White solid, mp 71-72 °C; Anal. Calcd. for $C_{21}H_{32}N_2O_5$: C, 64.26; H, 8.22; N, 7.14; Found: C, 64.4; H, 8.3; N, 7.4.; IR ν_{max} (Nujol) 3347, 1739, 1652, 1537 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.38 (s, 9H), 1.86 (m, 1H), 2.01 (m, 1H), 2.24 (m, 1H), 2.31 (m, 1H), 4.09 (bs, 1H), 4.36 (bs, 2H), 5.33 (bs, 1H), 6.71 (bs, 1H), 7.18-7.25 (m, 5H); MS (LCMS) *m/z* 637.4189 $[M+H]^+$, 660.4564 $[M+Na]^+$.

Compound 5: White solid, mp 92 °C; Anal. Calcd for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.59; N,7.03. Found: C, 69.3; H, 7.7; N,7.103; IR ν_{max} (Nujol) 3320, 1706, 1667, 1547, 1223 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.10 (d, 3H, J = 6.0 Hz), 1.45 (s, 9H), 4.10-4.60 (m, 6H), 5.50 (m, 1H), 6.82 (m, 1H), 7.20-7.38 (m, 10H); ^{13}C NMR (CD_3OD, 75 MHz) δ 15.8, 28.1, 43.2, 47.8, 71.5, 74.8, 79.9, 127.5, 127.7, 127.8, 128.4, 128.7, 138.0, 156.1, 170.1; MS (LCMS) *m/z* 421.2420 $[M+Na]^+$.

Compound 6: Yellowish white semisolid; IR ν_{max} (Nujol) 3297, 1719, 1693, 1689 $cm^{-}1$; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.38 (s, 9H), 2.87 (m, 2H), , 4.27-4.5 (m, 3H), 5.95 (s, 1H), 7.24 -7.31 (m, 5H); Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.6; H, 6.7; N, 8.7; MS (LCMS) *m/z* 323.4621 $[M+H]^+$, 345.4657 $[M+Na]^+$.

Compound 7: White solid. mp 90-91 °C (lit.[25] mp 91 °C); Anal. Calcd for $C_{23}H_{28}N_2O_5$: C, 66.97; H, 6.84; N, 6.79. Found: C, 67; H, 6.8; N, 6.679; IR ν_{max} (Nujol) 3331, 1735, 1888, 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.43 (s, 9H), 2.77 (dd, 1H, *J* = 6 & 17 Hz), 3.09 (dd, 1H, *J* = 5.4 & 17 Hz), 4.25-4.5 (m, 1H), 4.56 (bs, 2H), 5.13 (s, 2H), 5.71 (s, 1H), 6.87 (s, 1H), 7.23 -7.39 (m, 10H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.1x3, 36.0, 43.3, 50.6, 66.7, 80.4, 127.3, 127.4x2, 128.1x2, 128.3, 128.48x2, 128.5x2, 135.3, 137.8, 155.5, 170.5, 171.5; MS (LCMS) *m/z* 413.5396 $[M+H]^+$,435.5512 $[M+Na]^+$.

Compound 8. Yellowish gummy material; Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.3; H, 7.5; N, 9.7; IR ν_{max} (Nujol) 3347, 1701, 1681 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 1.44 (s, 9H), 3.83 (d, 2H, *J* = 6Hz), 4.05 (m, 2H), 5.33 (bs, 1H), 6.09-6.23 (dt, *J* = 15.9 & 6Hz, 1H), 6.42 (d, 1H, *J* = 16Hz), 6.56 (bs, 1H), 7.23 -7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.2x3, 41.3, 42.2, 80.1, 125.1, 126.2x2, 127.6, 128.5x2, 131.9, 136.3, 156.1, 169.4; MS (LCMS) *m/z* 291.4285 $[M+H]^+$, 313.5347 $[M+Na]^+$.

Compound 9: White solid. mp 118-119 °C (lit.[27] mp 119-121 °C); Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.6; H, 6.2; N, 9.3; IR ν_{max} (Nujol) 3324, 1693, 1650 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.89 (d, 2H, *J* = 5.8 Hz), 4.44 (d, 2H, *J* = 6 Hz), 5.08 (s, 2H), 5.53 (bs, 1H), 6.50 (bs, 1H), 7.20 -7.41 (m, 10H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 43.3, 44.4, 67.1, 127.5, 127.6x2, 128x2, 128.2, 128.5 x2, 128.6x2, 136, 137.7, 156.6, 169; MS (LCMS) *m/z* 299.3563 $[M+H]^+$, 321.3293 $[M+Na]^+$.

Compound 10: White solid, mp 107-108 °C; Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64; Found: C, 70.4; H, 6.4; N, 8.6; IR ν_{max} (Nujol) 3334, 1699, 1647 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.90 (d, 2H, *J* = 5.3 Hz), 4.04-4.2 (m, 2H), 5.13 (s, 2H), 5.51 (bs, 1H), 6.17 (dt, 1H, *J* = 15.7 & 6 Hz), 6.28 (bs, 1H), 6.52 (d, 1H, *J* = 16 Hz), 7.22 -7.40

(m,10H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 41.5, 44.6, 67.3, 124.8, 126.4x2, 127.8, 128.1x2, 128.3, 128.55x2, 128.58x2, 132.5, 136, 136.3, 168.8; MS (LCMS) *m/z* 347.4477 $[M+Na]^+$.

Compound 11: White solid; Anal. Calcd for $C_{16}H_{23}N_3O_4$: C, 70.35; H, 6.21; N, 8.64; Found: C, 70.4; H, 6.4; N, 8.6; IR ν_{max} (Nujol) 1699, 1667, 1651 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.38 (s, 9H), 3.75 (d, 2H, *J* = 5 Hz), 3.95 (d, 2H, *J* = 5 Hz), 4.40 (d, *J* = 5 Hz, 2H), 5.23 (bs, 1H), 6.83 (bs, 1H), 6.94 (bs, 1H), 7.24-7.30 (m, 5H); MS (LCMS) *m/z* 322.17$[M+H]^+$, 344.15 $[M+Na]^+$; HRESIMS *m/z* 344.1586 $[M+Na]^+$ ($C_{16}H_{23}N_3NaO_4$; calcd. 344.1586).

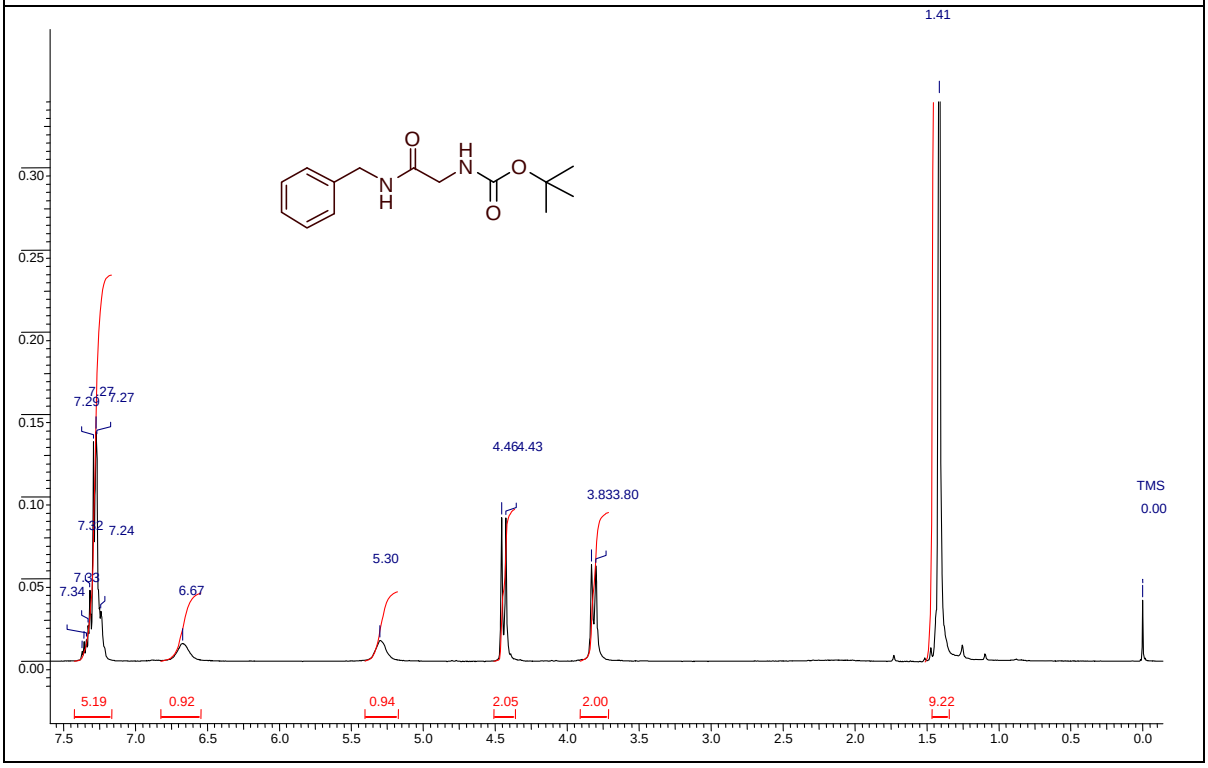
Compound 12: White solid, mp 149-151 °C; IR ν_{max} (Neat) 1650, 1697, 1705, 3351 (broad) cm^{-1}. ^{1}H NMR ($CDCl_3$+CD_3OD, 400 MHz) δ 0.71 (s, 3H), 0.97 (s, 3H), 1.02 (d, 3H, *J* = 6.0 Hz), 1.47 (s, 9H), 2.22 (m, 2H), 2.35 (m, 1H), 2.57 (m, 1H), 3.69 (bs, 2H), 3.83 (bs, 1H), 3.96 (bs, 1H), 4.05 (bs, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 100 MHz) δ 12.9, 17.5, 23.4, 23.8, 25.0, 26.6, 28.2, 28.6x3, 29.1, 31.5, 31.8, 34.0, 34.8, 35.8, 36.1, 37.9, 40.2, 42.3, 44.6, 46.3, 47.0, 47.5, 50.0, 68.6, 73.5, 80.6, 157.8, 170.8, 178.0; MS (LCMS) *m/z* 587.4 $[M+Na]^+$, HRESIMS *m/z* 587.3701 $[M+Na]^+$ ($C_{31}H_{52}N_2NaO_7$; calcd. 587.3672).

Compound 13: White solid, mp 115-116 °C; IR ν_{max} (Neat) 3380 (broad), 1712, 1660, 1546 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.66 (s, 3H), 0.69 (s, 3H), 0.97, (s, 3H), 1.15 (d, 3H, *J* = 6 Hz), 1.46 (s, 9H), 3.81 (bs, 1H), 3.95 (bs, 1H), 4.11 (bs, 2H), 4.31 (bs, 1H), 4.60 (s, 2H), 5.70 (bs, 1H), 6.90 (bs, 1H), 7.32 (m, 5H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.4, 14.9, 17.2, 22.7, 23.2, 24.6, 25.8, 27.5, 28.3x3, 29.6, 30.7, 30.9, 31.0, 33.5, 34.2, 35.0, 35.3, 37.2, 39.3, 41.7, 45.5, 46.5, 46.9, 56.9, 60.4, 68.3, 71.8, 73.0, 75.1, 79.9,

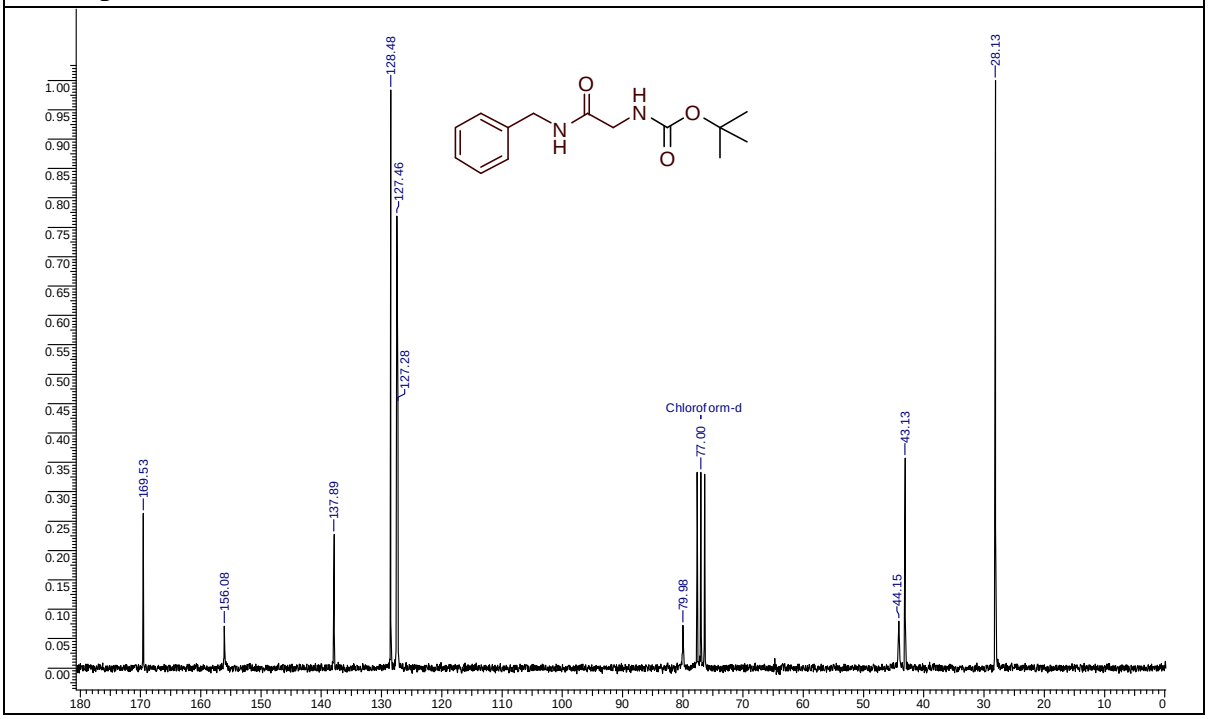
127.8x3, 128.4, 137.8, 155.8, 168.6, 178.3; MS (LCMS) *m/z* 699.4511 $[M+H]^+$, 721.4352 $[M+Na]^+$.

2B.7. Selected Spectra

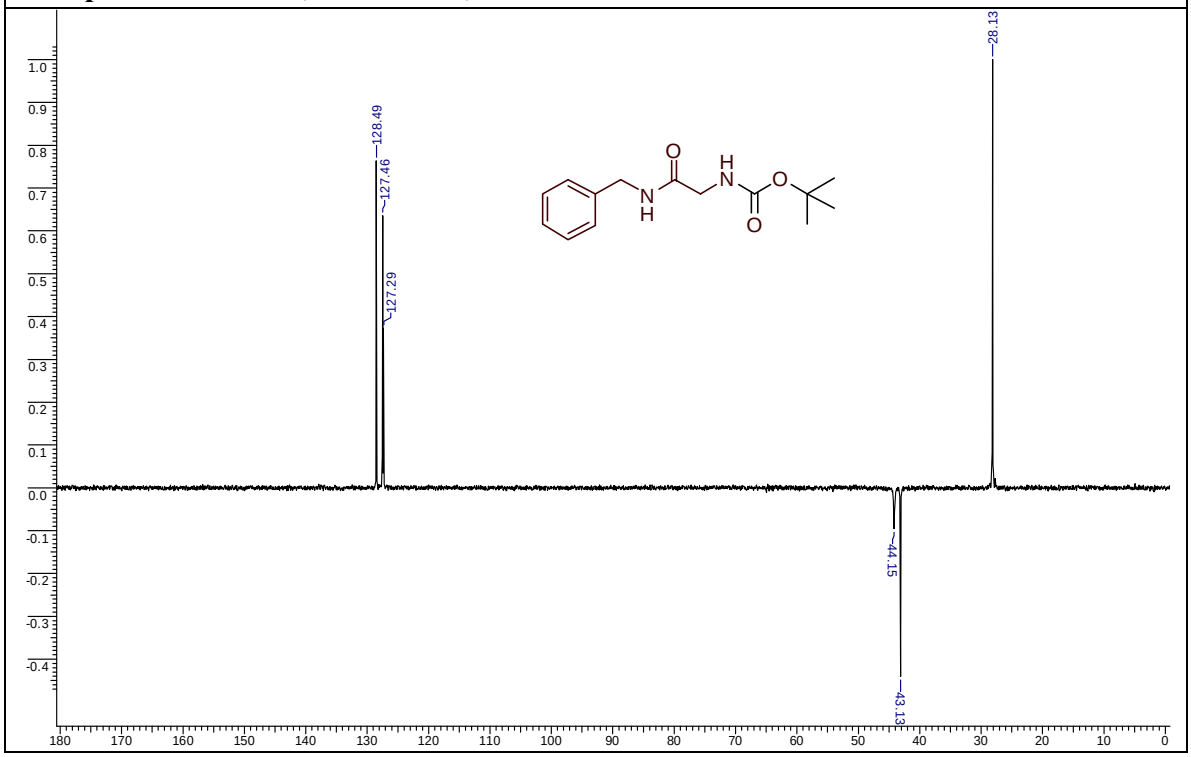
Compound 3: ^{1}H NMR, 200 MHz, $CDCl_3$



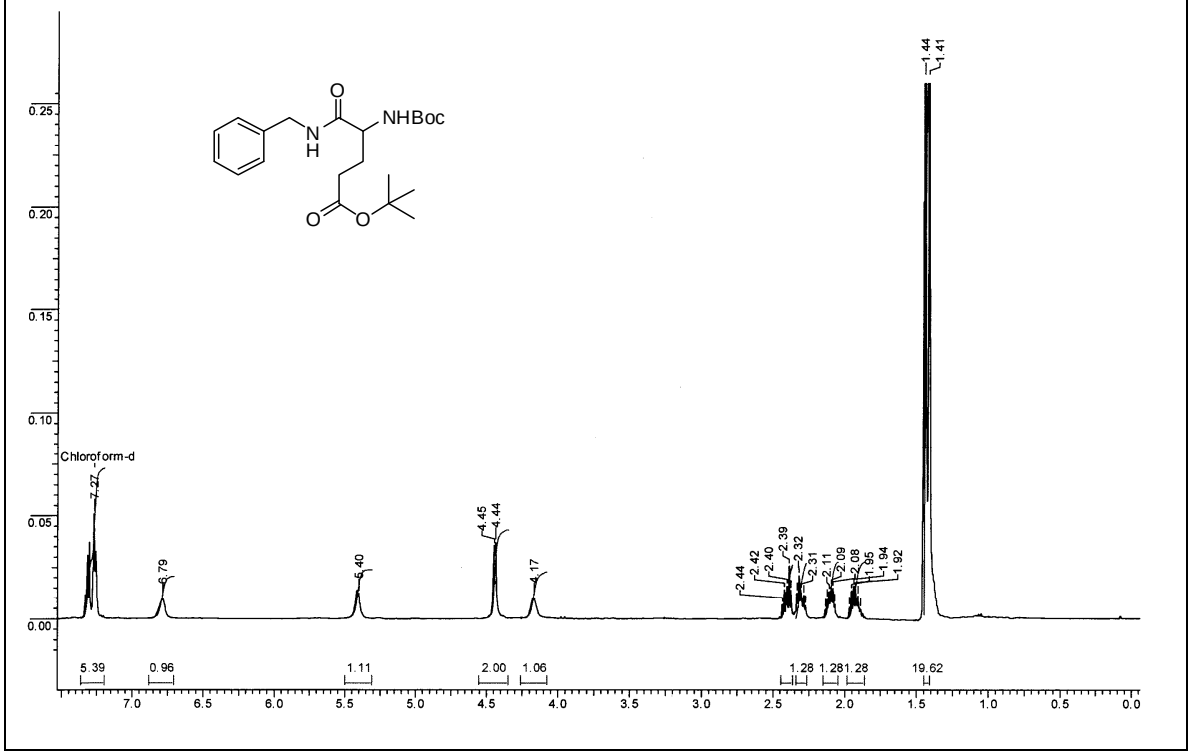
Compound 3: ^{13}C NMR, 50.32 MHz, $CDCl_3$



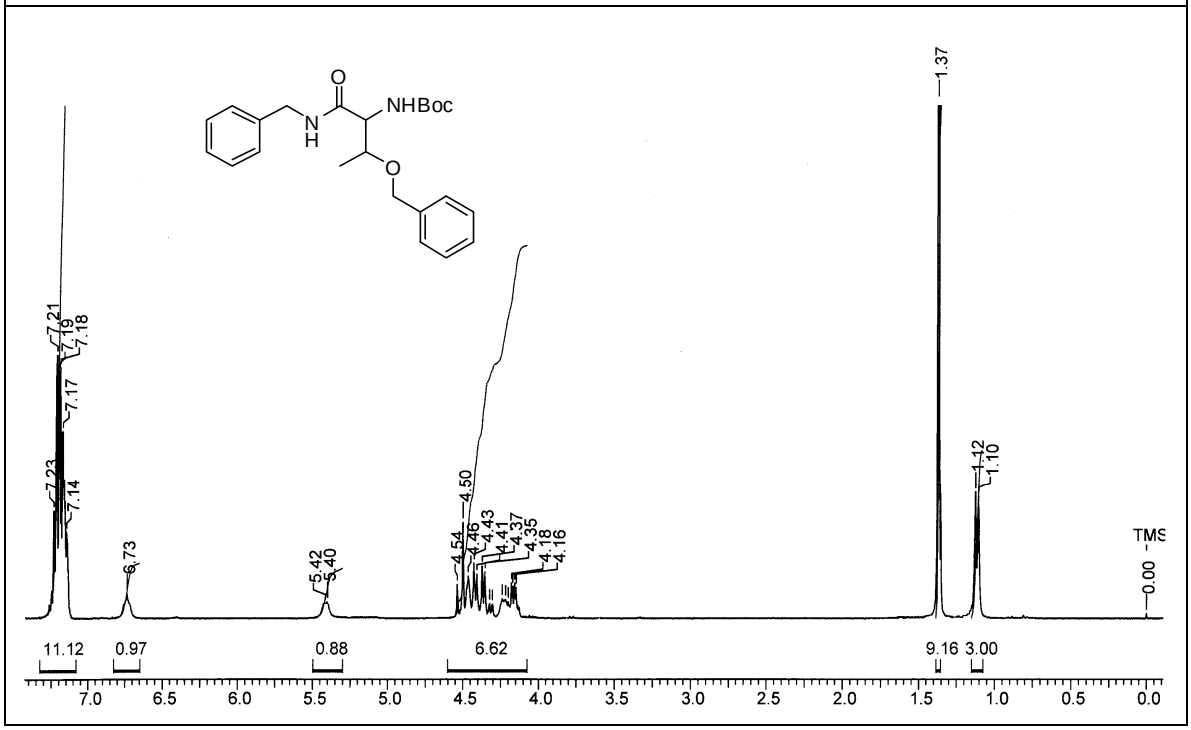
Compound 3: DEPT, 50.32 MHz, $CDCl_3$



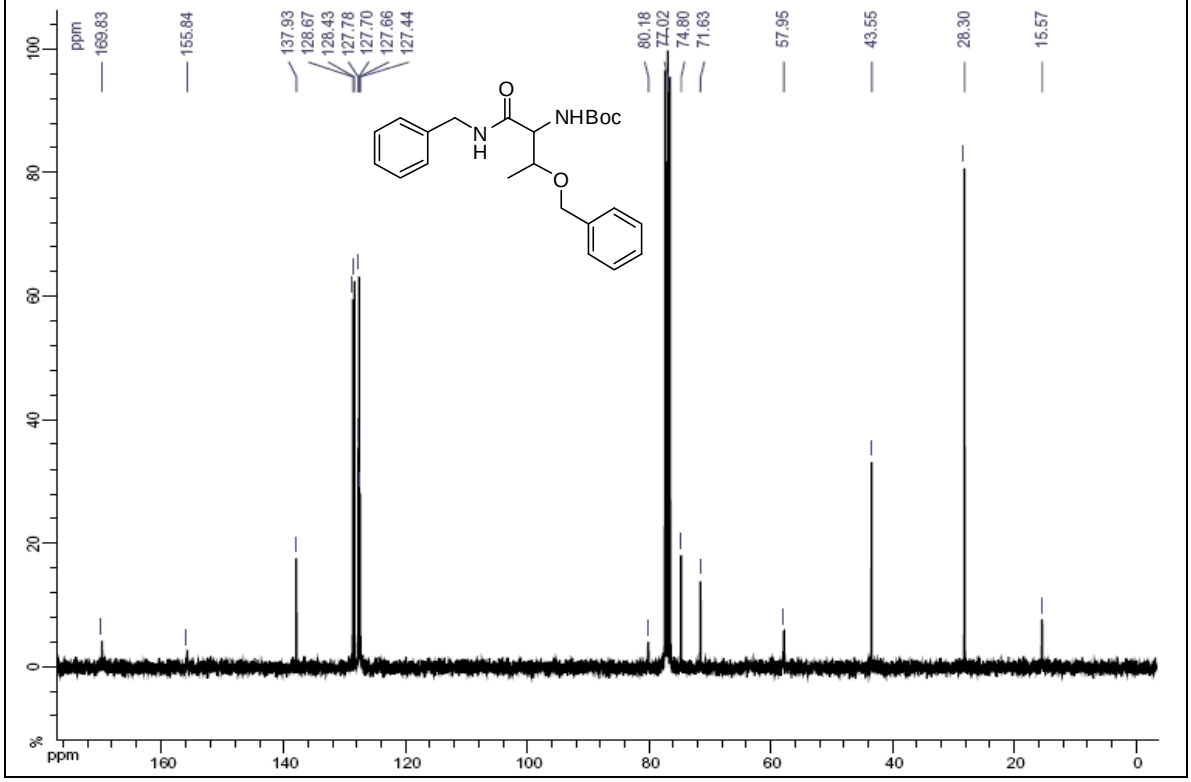
Compound 4: ^1H NMR, 300 MHz, $CDCl_3$



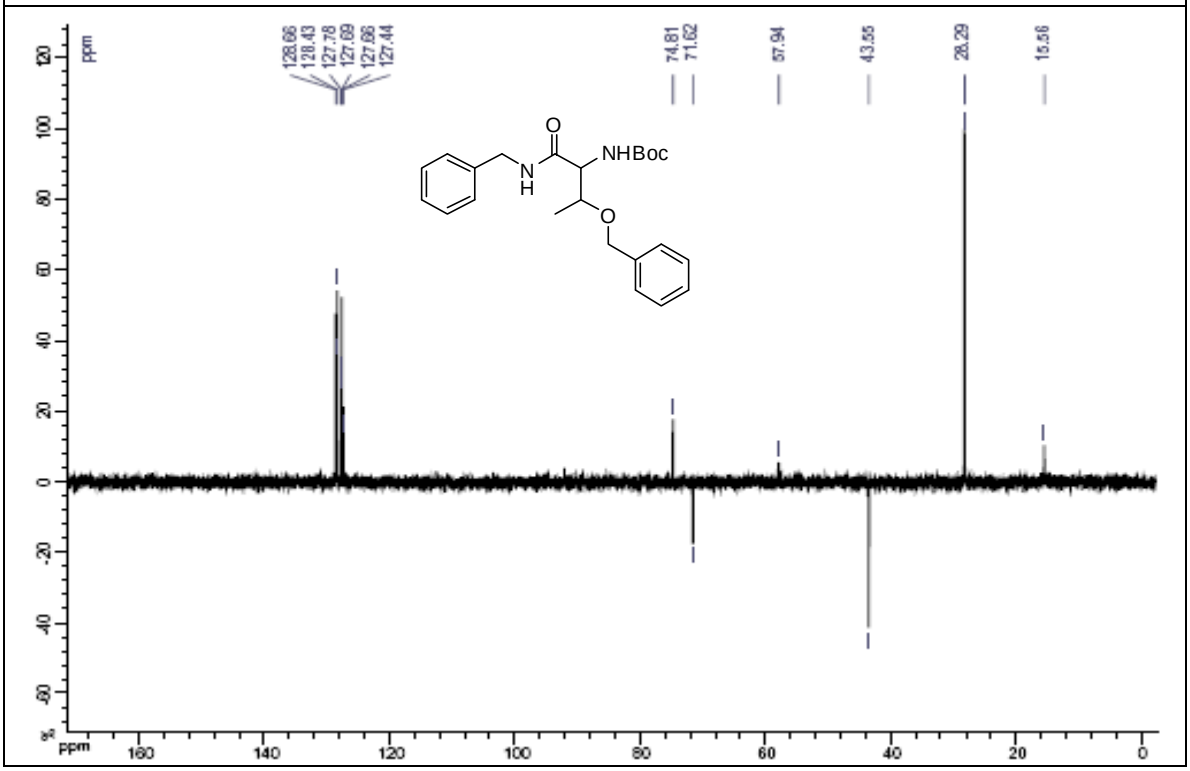
Compound 5: ^{1}H NMR, 300 MHz, $CDCl_3$



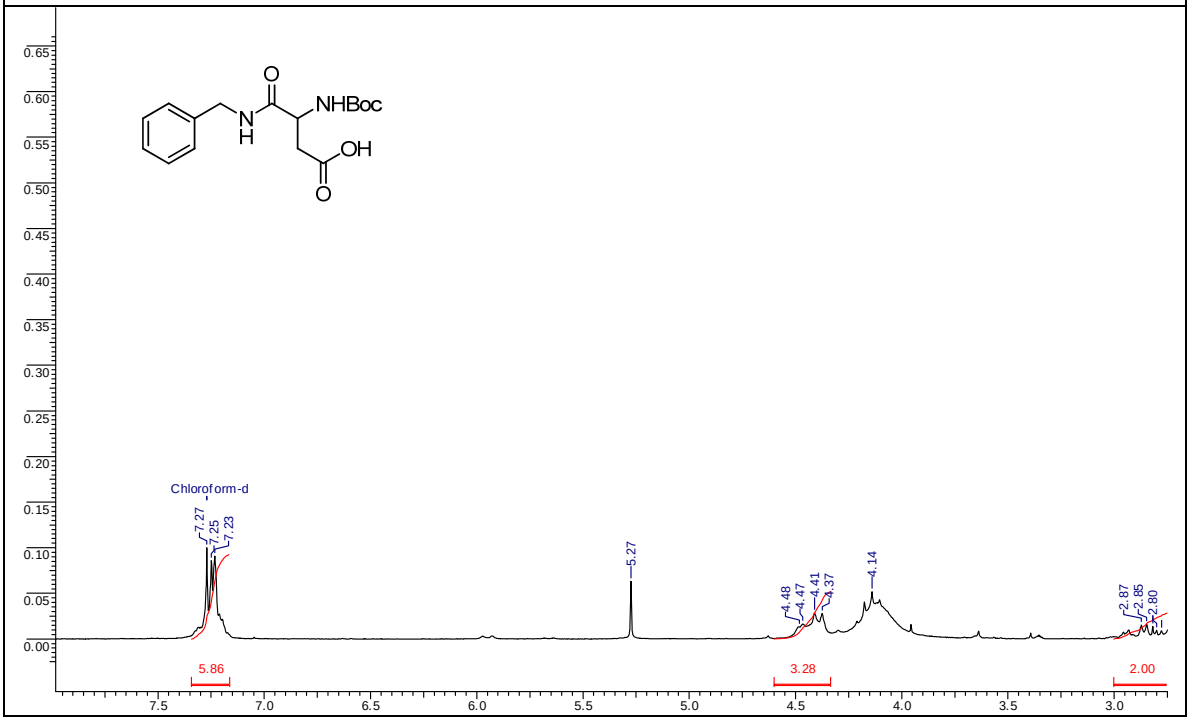
Compound 5: ^{13}C NMR, 75.51 MHz, $CDCl_3$



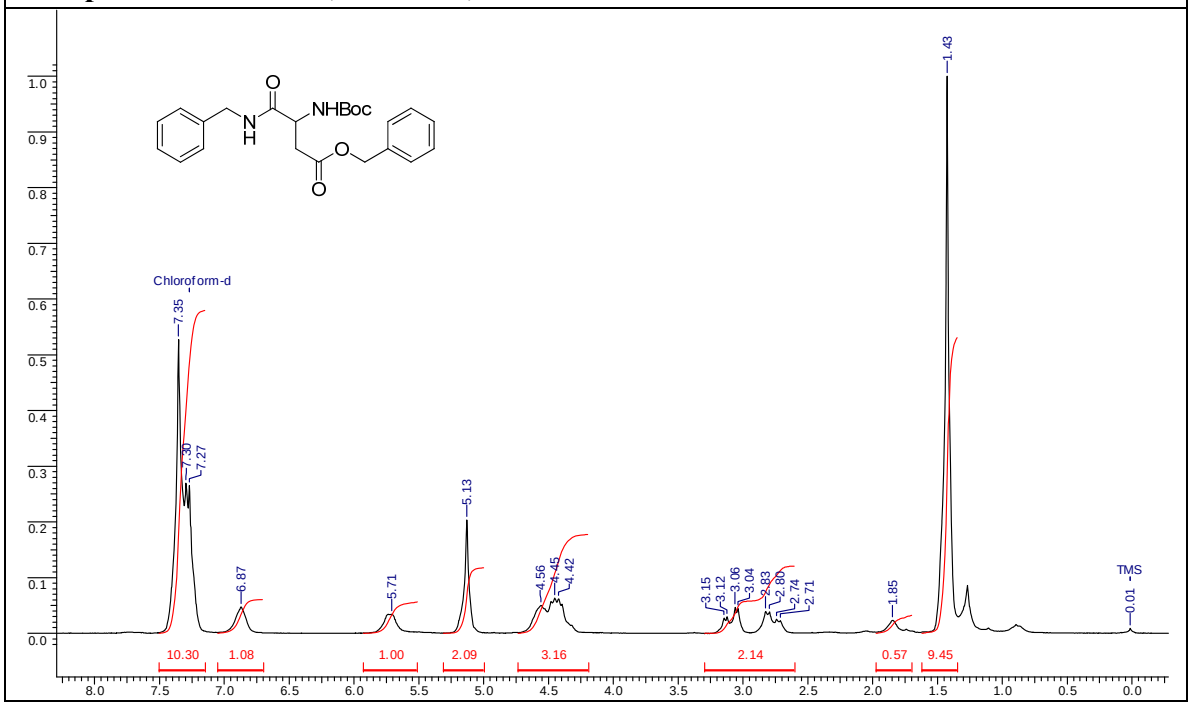
Compound 5: ^{13}C NMR, 75.51 MHz, $CDCl_3$



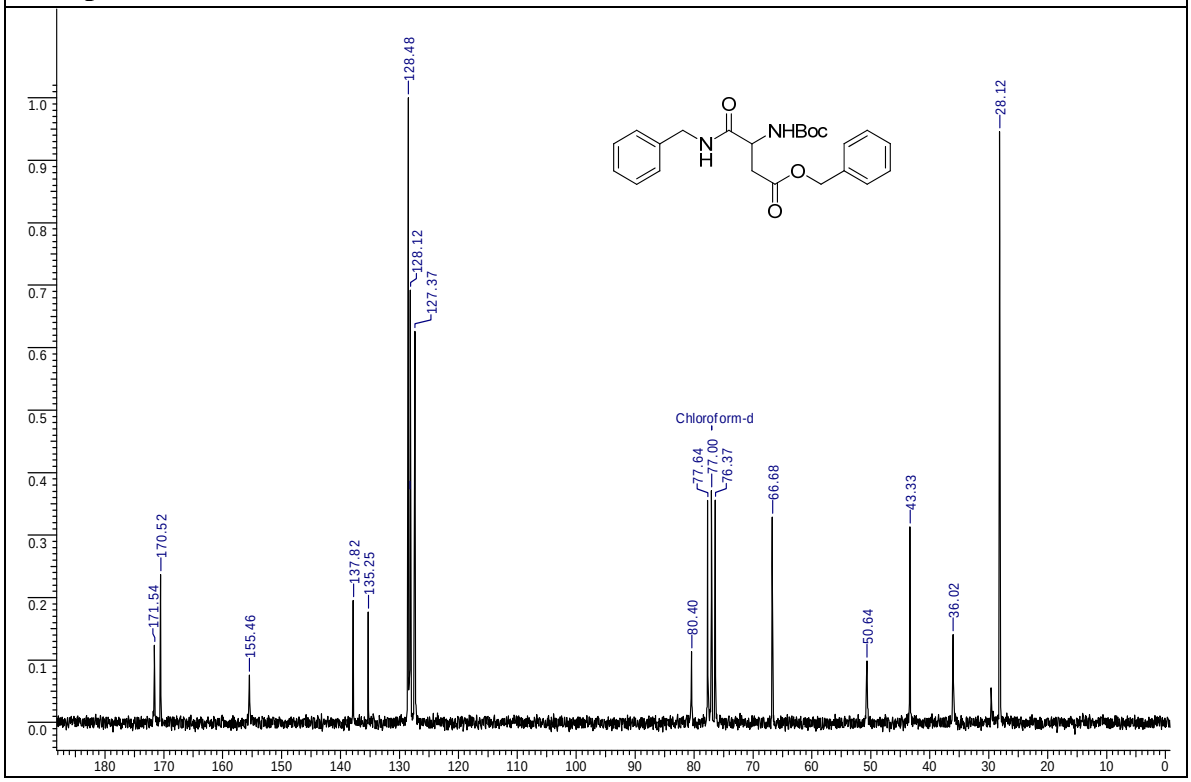
Compound 6: ^{1}H NMR, 200 MHz, $CDCl_3$



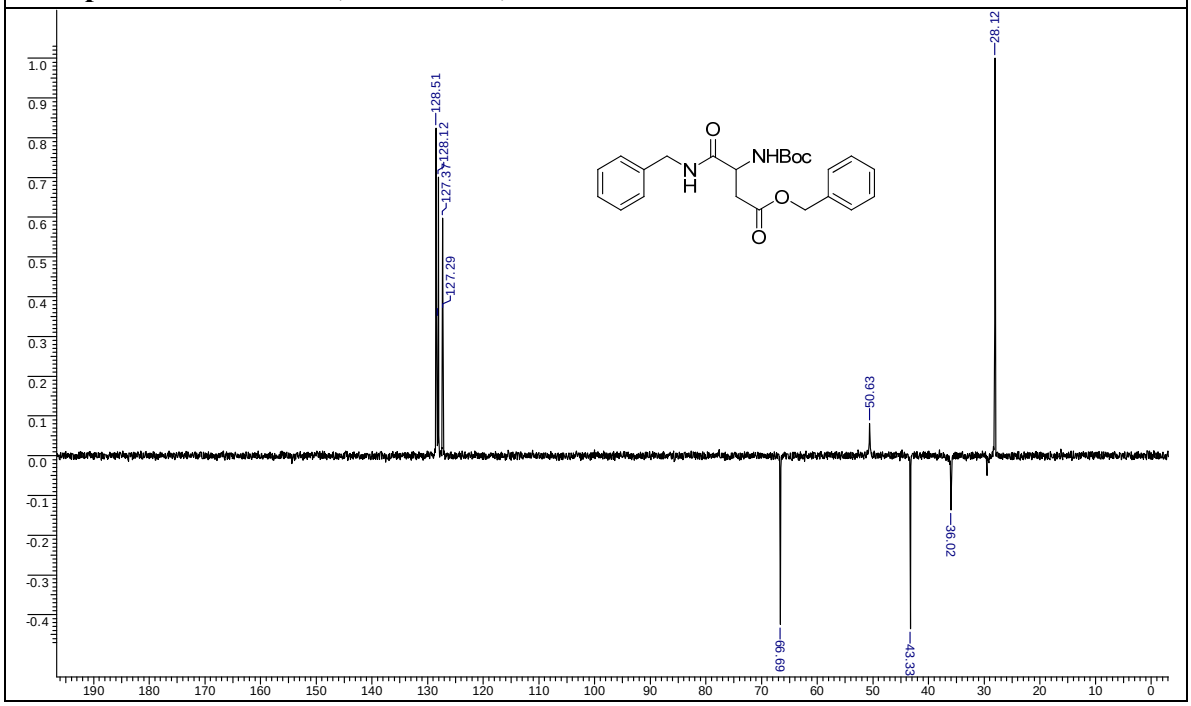
Compound 7: ^{1}H NMR, 200 MHz, $CDCl_3$



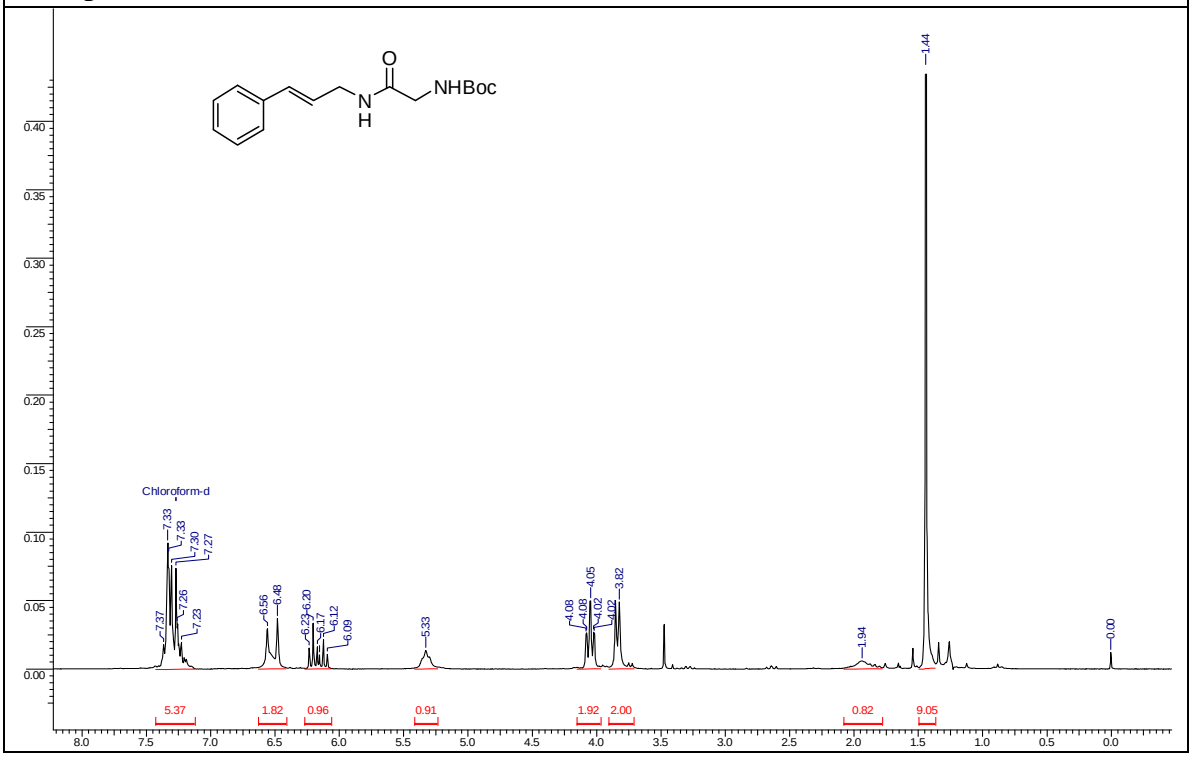
Compound 7: ^{13}C NMR, 50.32 MHz, $CDCl_3$



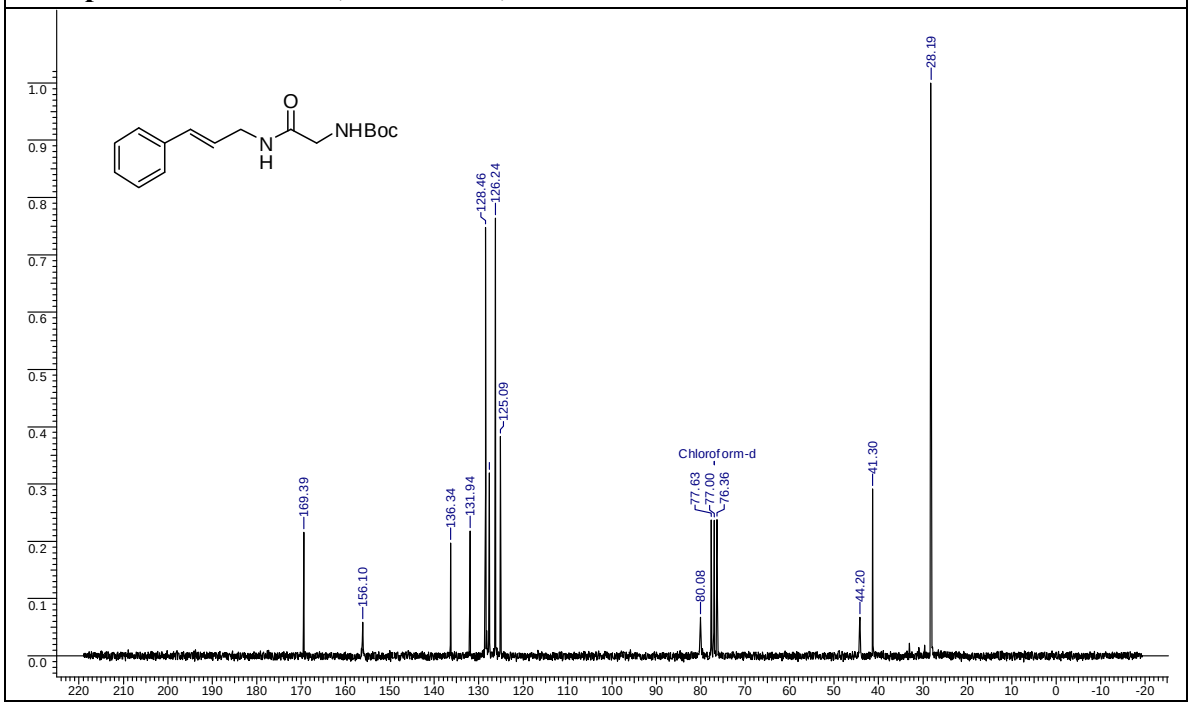
Compound 7: ^{13}C NMR, 50.32 MHz, $CDCl_3$



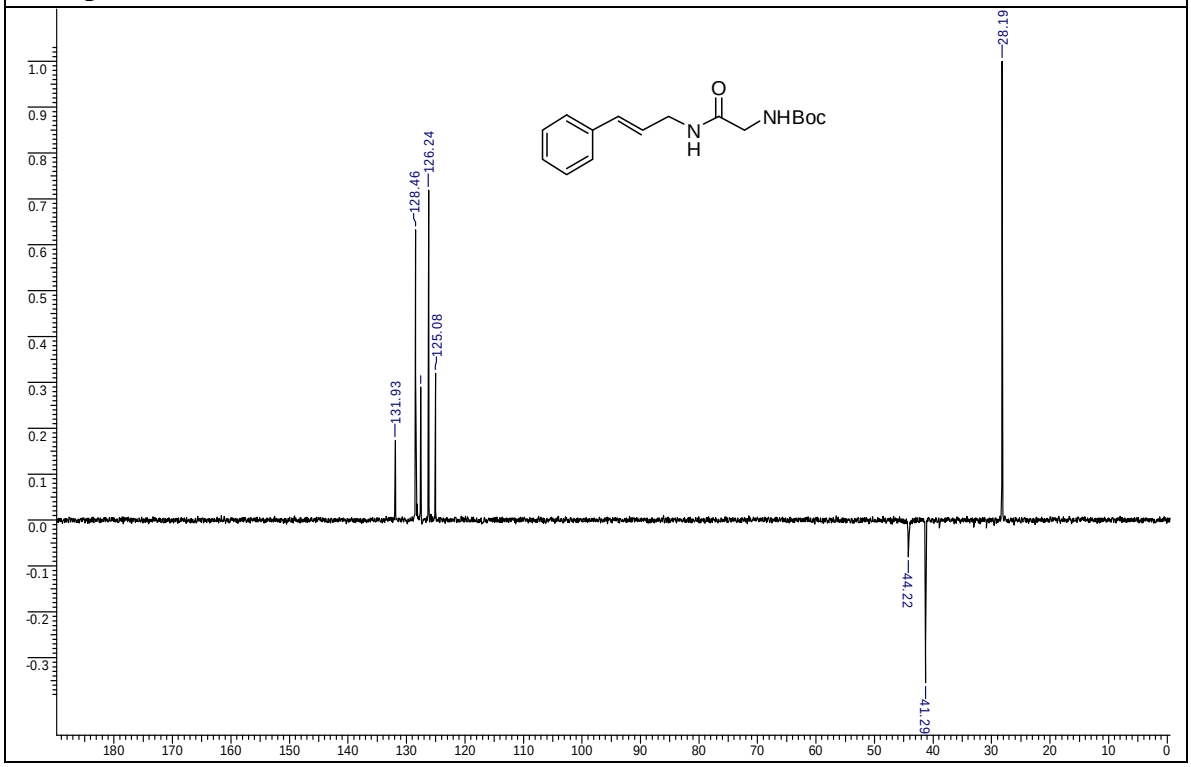
Compound 8: ^{1}H NMR, 200 MHz, $CDCl_3$



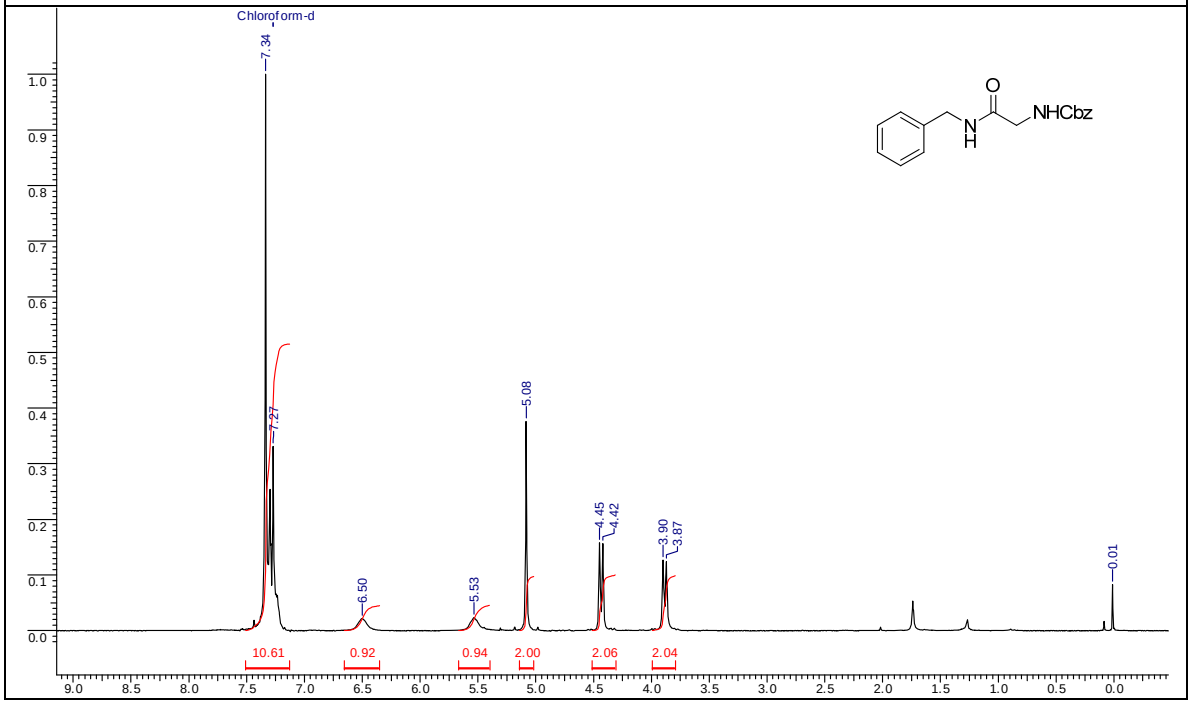
Compound 8: ^{13}C NMR, 50.32 MHz, $CDCl_3$



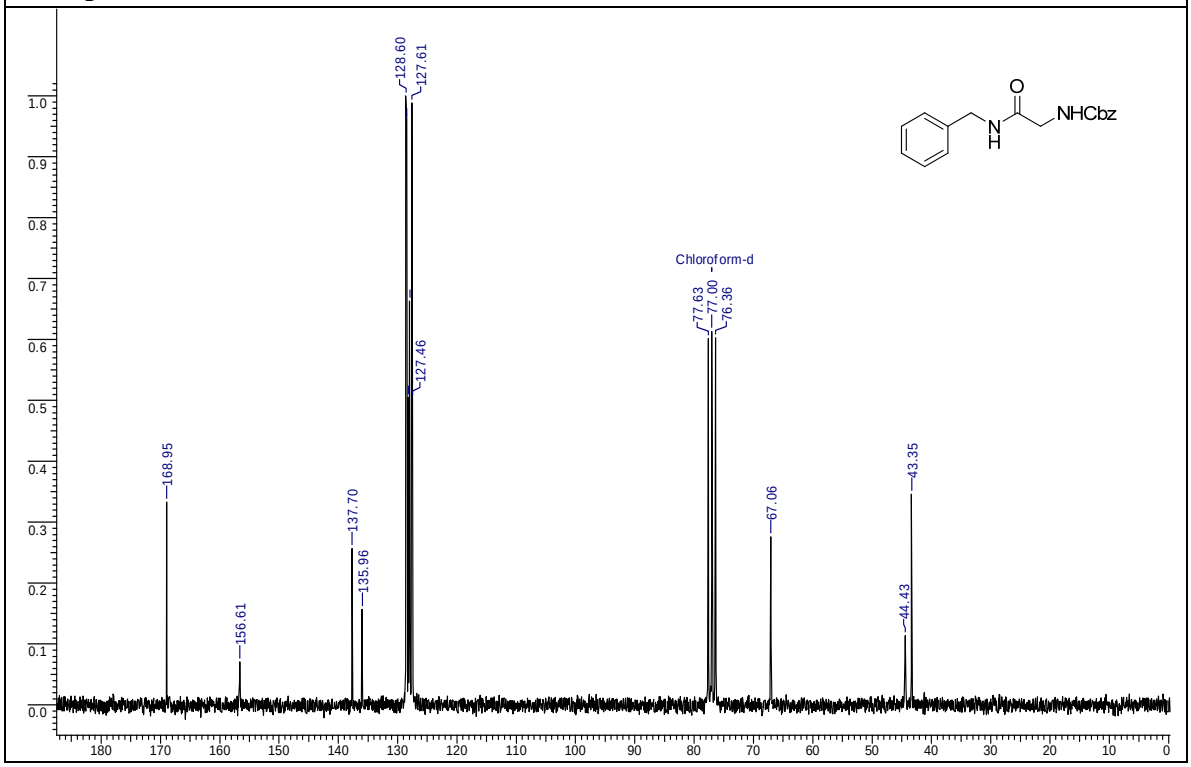
Compound 8: ^{13}C NMR, 50.32 MHz, $CDCl_3$



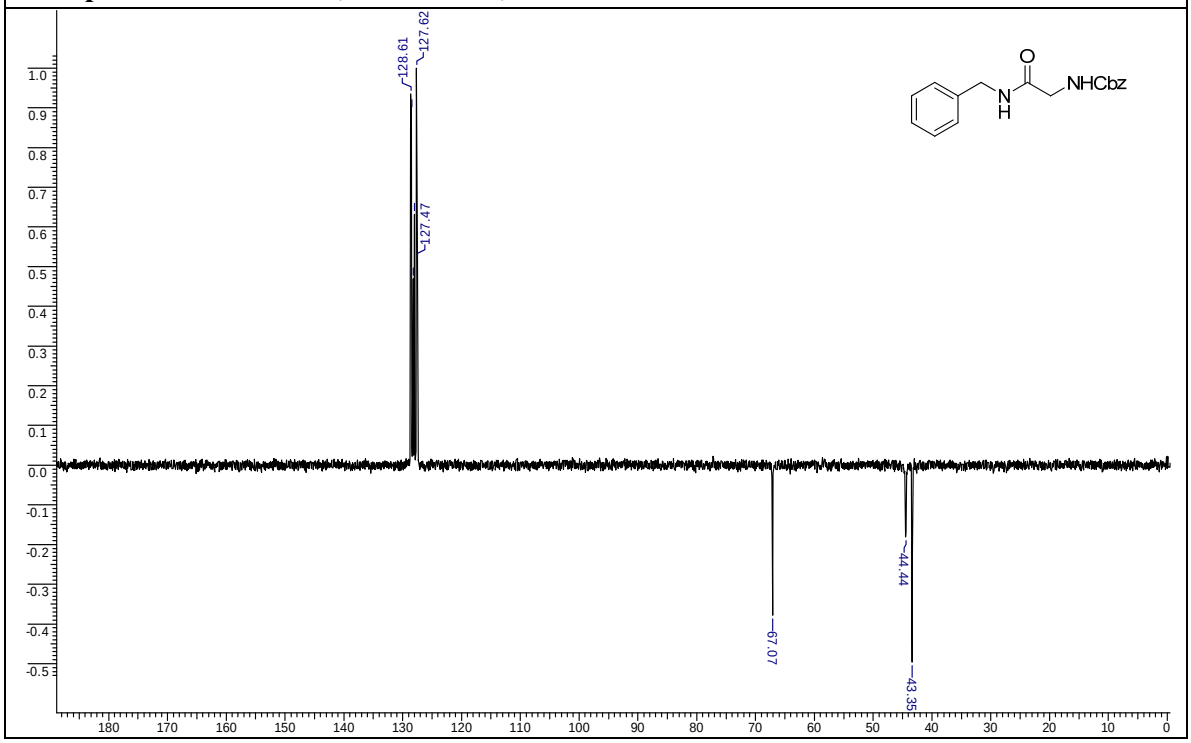
Compound 9: ^{1}H NMR, 200 MHz, $CDCl_3$



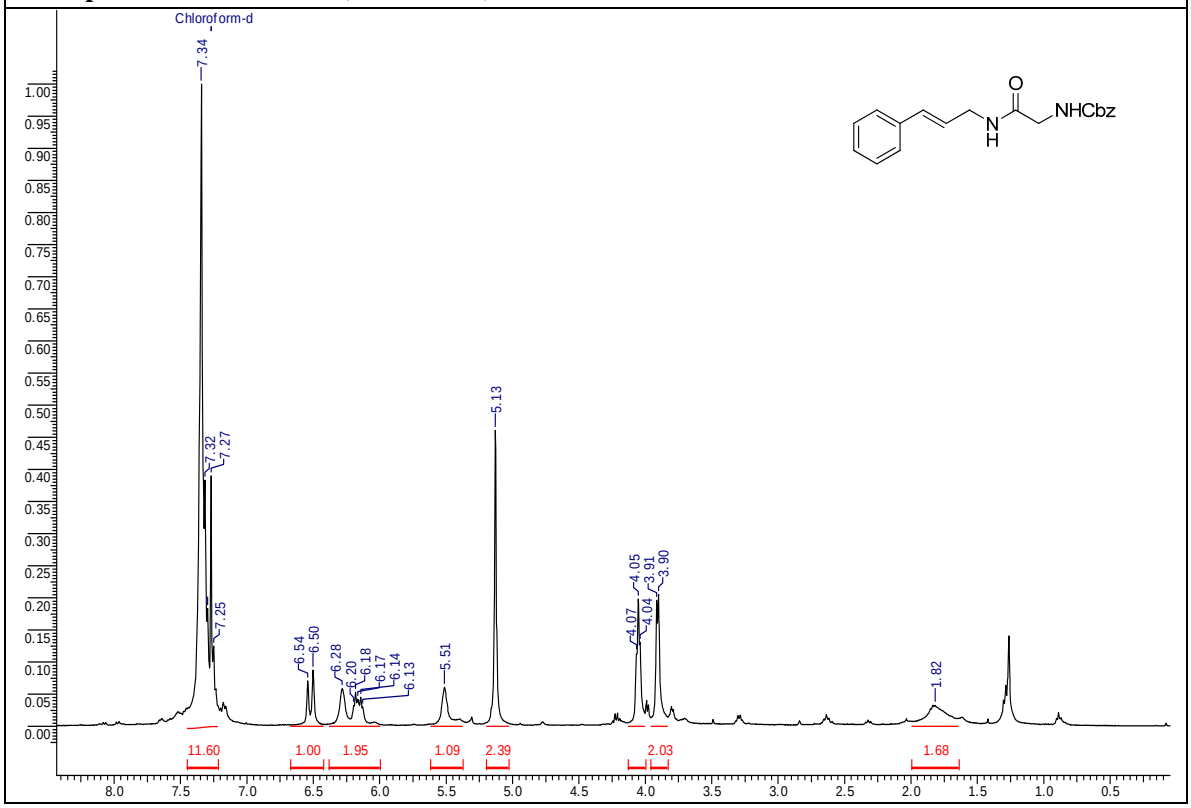
Compound 9: ^{13}C NMR, 50.32 MHz, $CDCl_3$



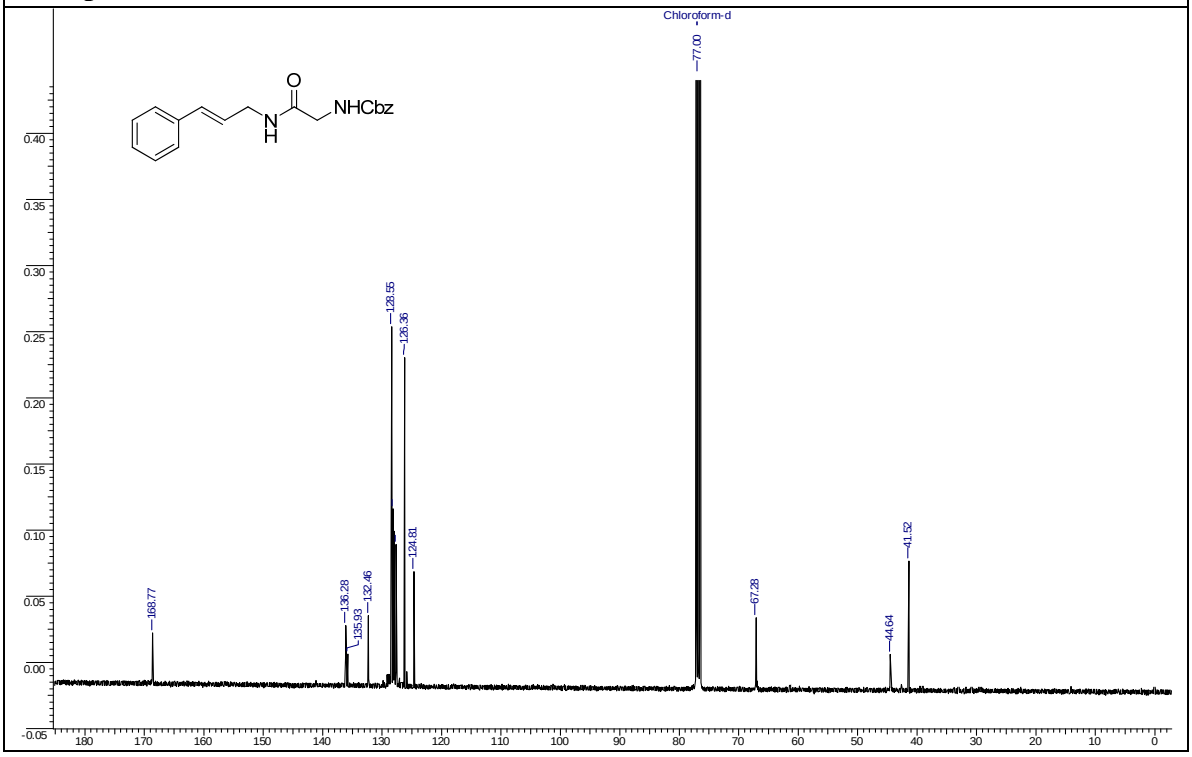
Compound 9: ^{13}C NMR, 50.32 MHz, $CDCl_3$



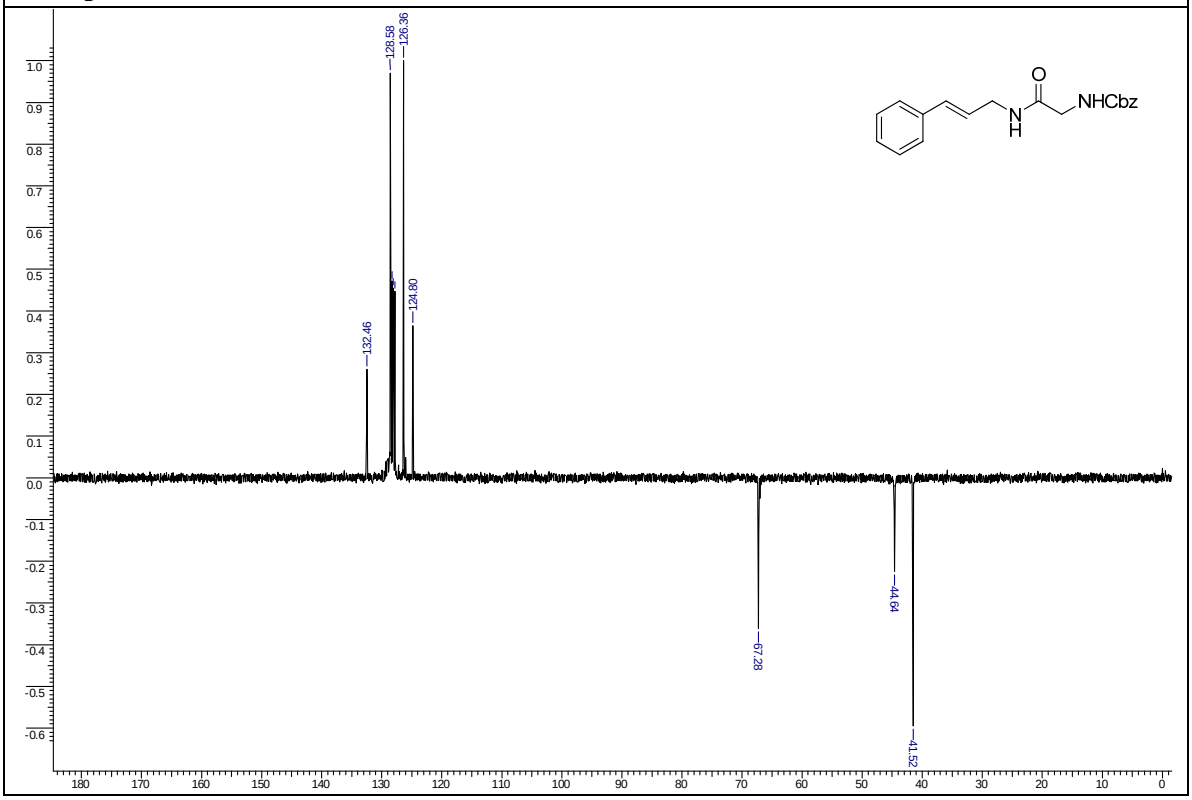
Compound 10: ^{1}H NMR, 200 MHz, $CDCl_3$



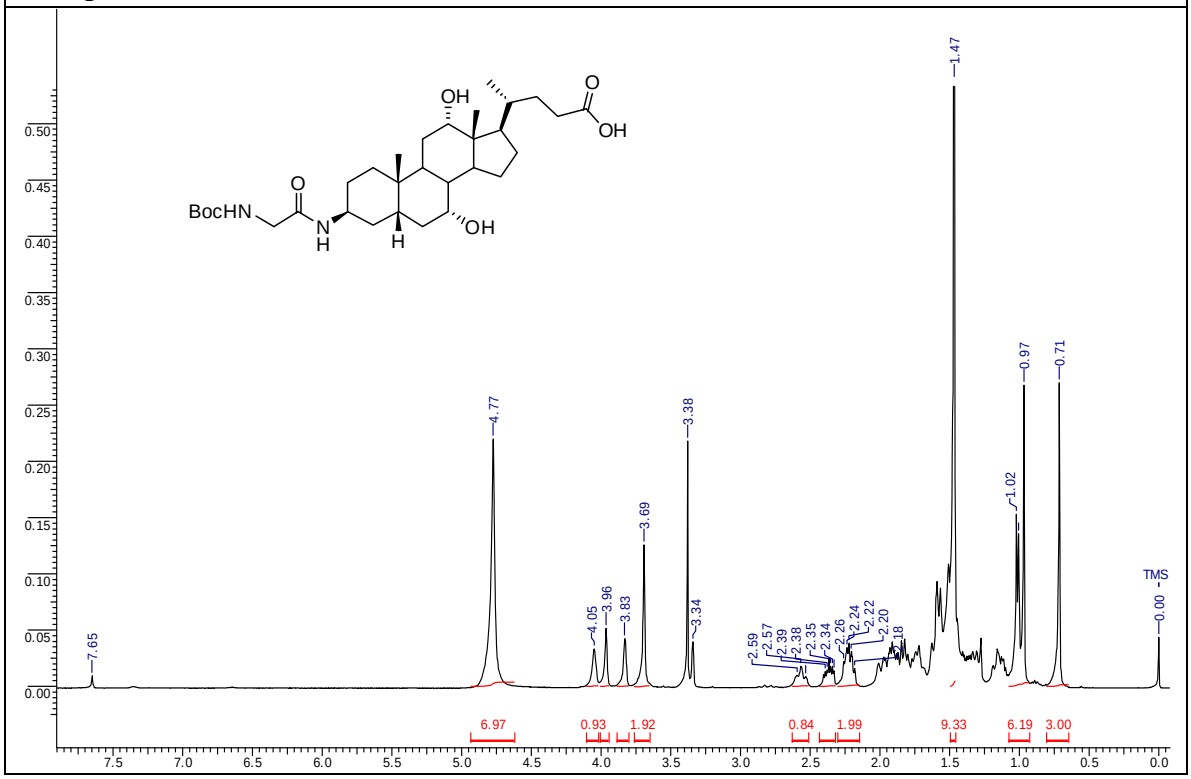
Compound 10: ^{13}C NMR, 50.32 MHz, $CDCl_3$



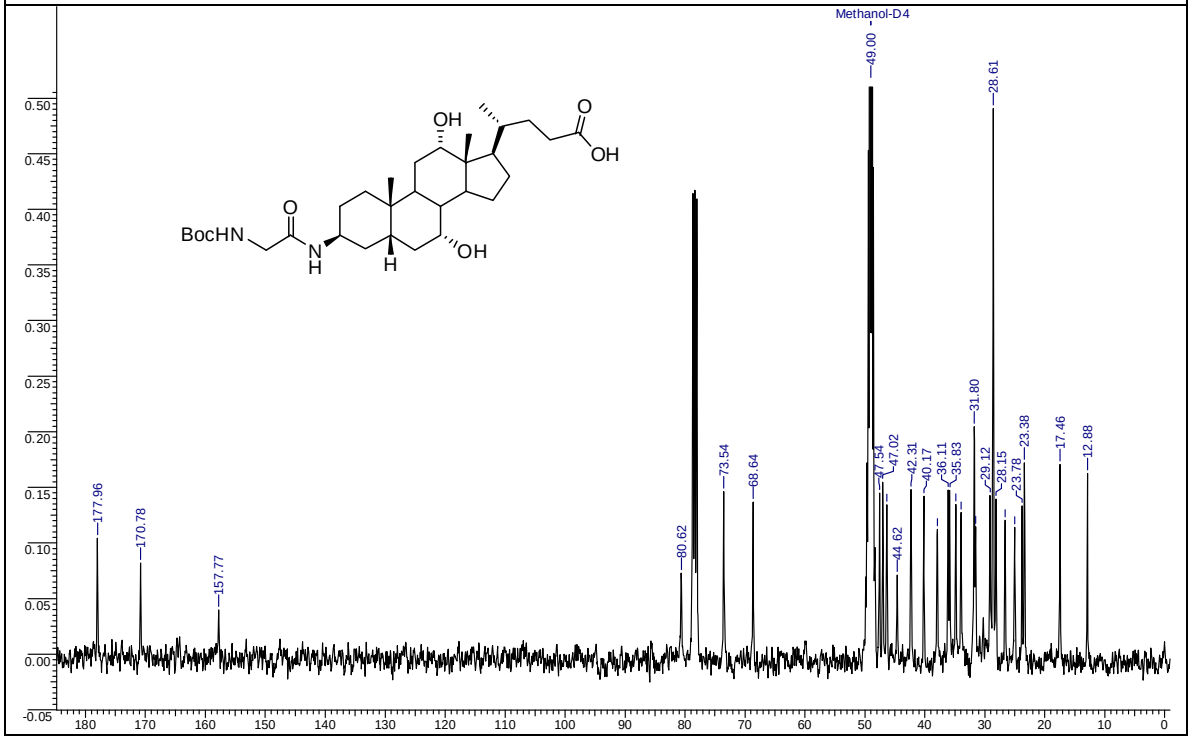
Compound 10: ^{13}C NMR, 50.32 MHz, $CDCl_3$



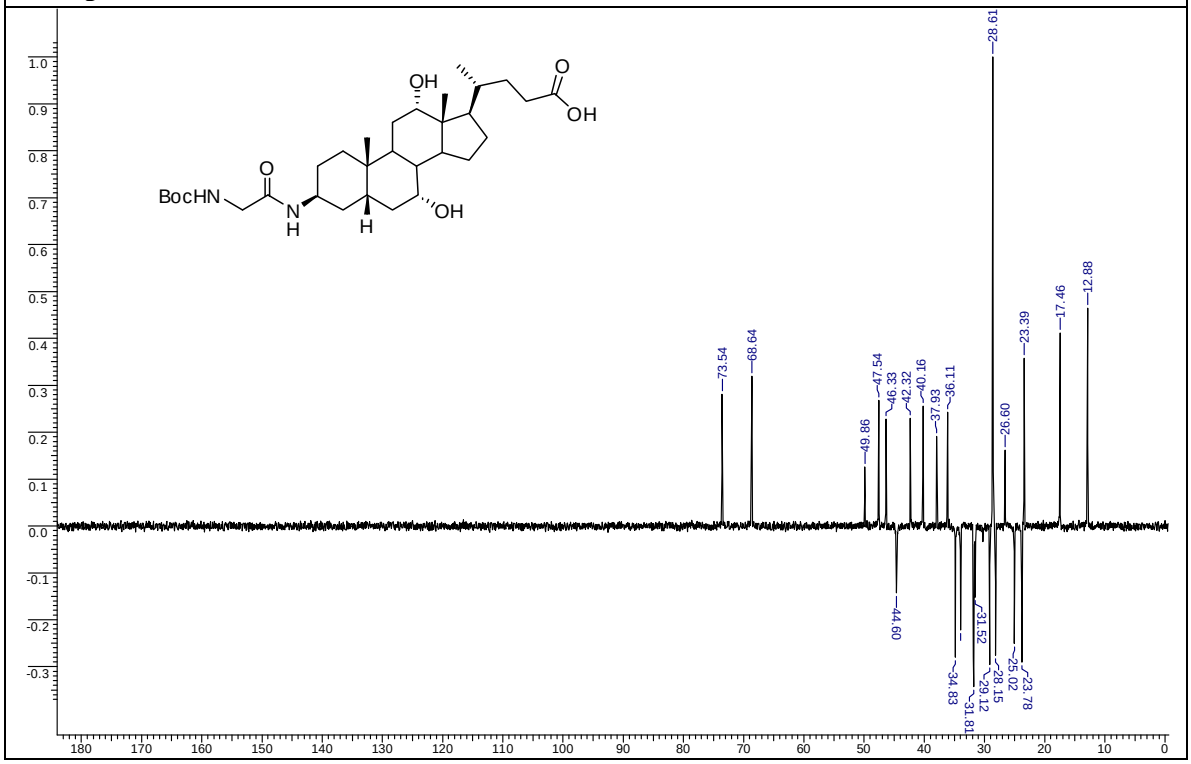
Compound 12: ^{1}H NMR, 400 MHz, $CDCl_3$+CD_3OD



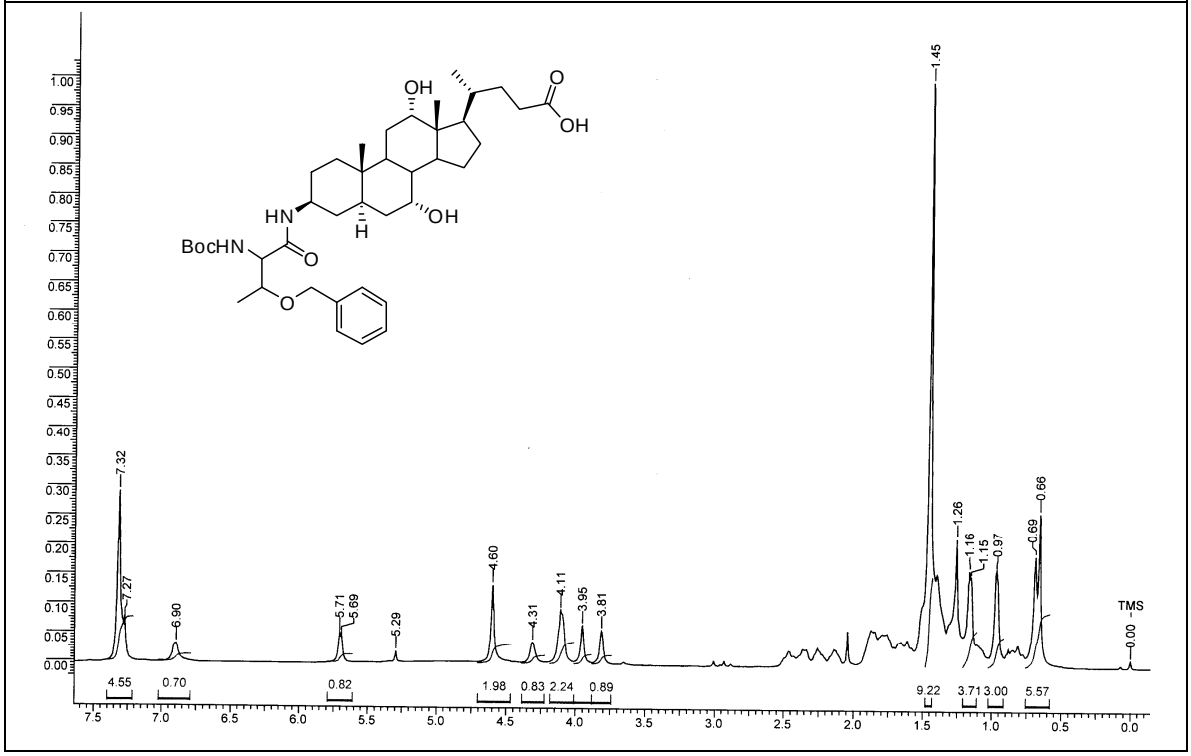
Compound 12: ^{13}C NMR, 100.61 MHz, $CDCl_3$



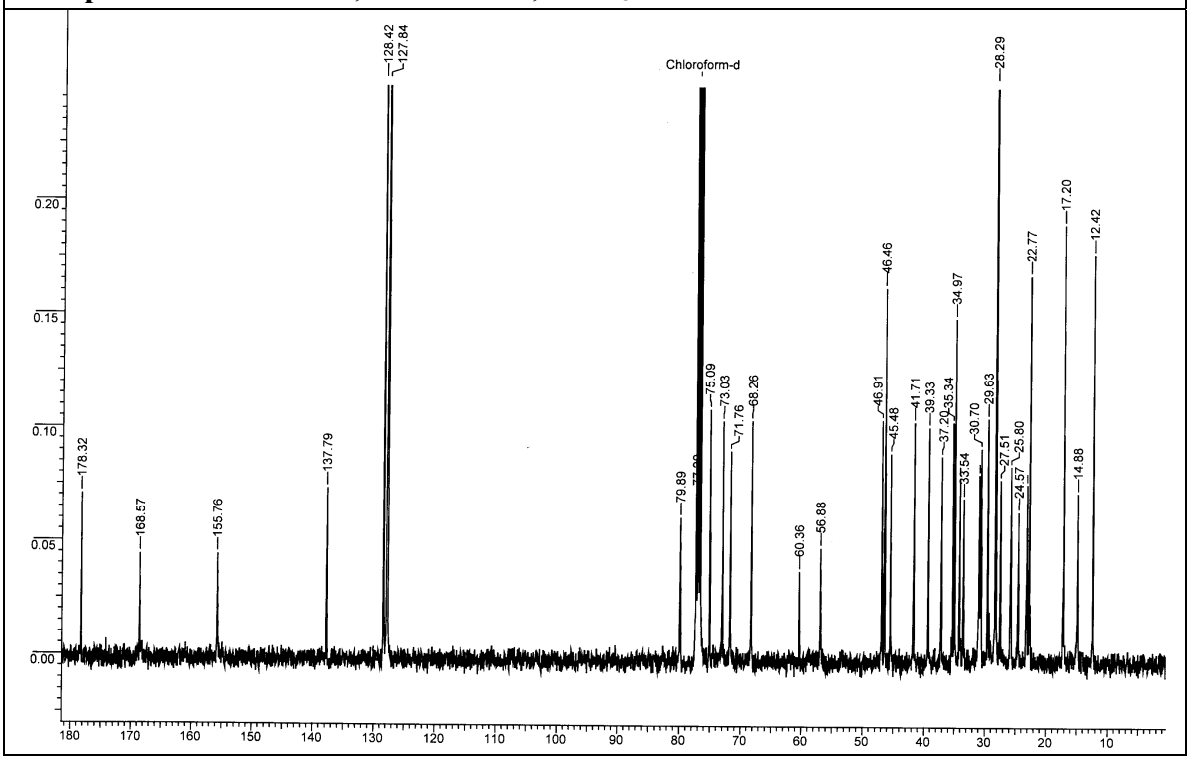
Compound 12: ^{13}C NMR, 100.61 MHz, $CDCl_3$



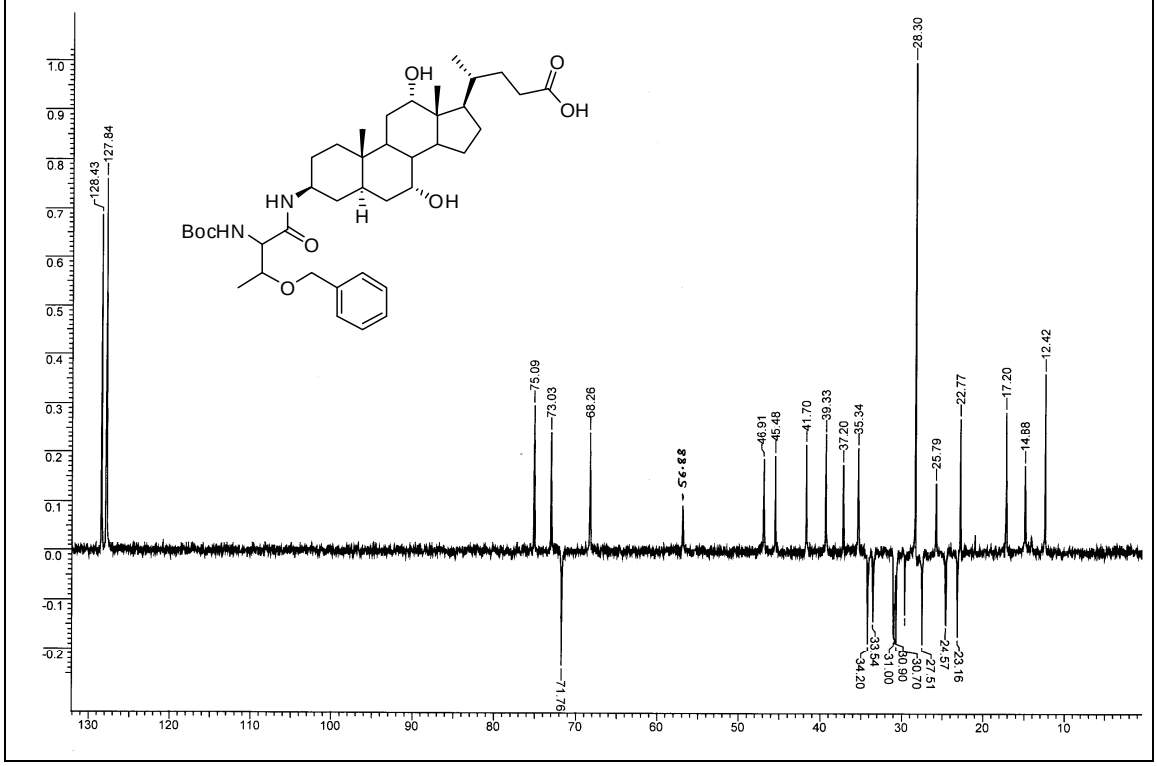
Compound 13: ^{1}H NMR, 400 MHz, $CDCl_3$



Compound 13: ^{13}C NMR, 100.61 MHz, $CDCl_3$



Compound 13: ^{13}C NMR, 100.61 MHz, $CDCl_3$



2B.8. References

1. Scriven, E. F. V.; Turanbull, K. *Chem. Rev.* **1988**, *88*, 297.
2. Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827-10852.
3. Garcia, J.; Urpf, F.; Vilarrasa, J. *Tetrahedron Lett.* **1984**, *25*, 4841-4844.
4. Bosch, I.; Urpi, F.; Vilarrasa, J. *J. Chem. Soc., Chem. Commun.* **1995**, 91-92.
5. Maunier, V.; Boullanger, P.; Lafont, D. *J. Carbohydr. Chem.* **1997**, *16*, 231-235.
6. Boullanger, P.; Maunier, V.; Lafont, D. *Carbohydr. Res.* **2000**, *324*, 97-106.
7. Damkaci, F.; DeShong, P. *J. Am. Chem. Soc.* **2003**, *125*, 4408-4409.
8. Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141-2143.
9. Inazu, T.; Kobayashi, K. *Synlett* **1993**, 869-870.
10. Mizuno, M.; Haneda, K.; Iguchi, R.; Muramoto, I.; Kawakami, T.; Aimoto, S.; Yamamoto, K.; Inazu, T. *J. Am. Chem. Soc.* **1999**, *121*, 284-290.
11. Ghosh, S. K.; Verma, R.; Ghosh, U. ; Mamdapur, V. R. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1705-1711.
12. Wu, X.; Hu, L. *J. Org. Chem.* **2007**, *72*, 765-774 and the references cited therein.
13. Kolawski, R. V.; Shangguan, N.; Sauers, R. R.; Williams, L. J. *J. Am. Chem. Soc.* **2006**, *128*, 5695-5702.
14. Barlett, K. N.; Kolakowski, R. V.; Katukojvala, S.; Williams, L. J. *Org. Lett.* **2006**, *8*, 823-826.
15. Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939-1941.
16. Bavikar, S. N.; Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J.; Shirazi, F.; Deshpande, M. V.; Srinath, K.; Chattopadhyay, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5512-5517.
17. Saito, S.; Nakajima, M.; Inaba, M.; Moriwake, T. *Tetrahedron Lett.* **1989**, *30*, 837-838.

18. Reddy, P. G.; Pratap, T. V.; Kumar, G. D. K.; Mohanty, S. K.; Baskaran, S. *Eur. J. Org. Chem.* **2002**, 3740-3743.

19. Kotsuki, H.; Ohishi, T.; Araki, T. *Tetrahedron Lett.* **1997**, *38*, 2129-2132.

20. Chandrasekhar, S.; Chandraiah, L.; Reddy, C. R.; Reddy, M. V. *Chem. Lett.* **2000,** 780-781.

21. Chandrasekhar, S.; Babu, B. N.; Reddy, C. R. *Tetrahedron. Lett.* **2003**, *44*, 2057-2059.

22. Ph. D. Theisis submitted by Deepak B. Salunke to Univercity Pune, India in March 2008

23. Madhavaiah, C.; Parvez M.; Verma, S. *Bioorg. Med. Chem.* **2004**, *12*, 5973-5982.

24. Shi, F.; Waldo, J. P.; Chen, Y.; Larock, R. C. *Org. Lett.* **2008**, *10*, 2409-2412.

25. Luo, J.; Chen, Y.; Zhu, X. X. *Synlett* **2007**, *14*, 2201-2204.

26. Jeganathan, A.; Richardson, S. K.; Mani, R. S.; Haley, B. E.; Watt, D. S. *J. Org. Chem.* **1986**, *51*, 5362.

27. (a) Lampariello, L.; Raffaella, L. *Lett. In Org. Chem.* **2005**, *3*, 265; (b) Muriel, G. *J. of Med. Chem.* **1998**, *41*, 24.

CHAPTER - 3

Cu(I) Catalyzed Alkyne-Azide "Click" Cycloaddition: Efficient Synthesis and Bioevalution of Bile Acid Bistriazole in the Presence of Base

3.1. Abstract

Cu(I) Catalysed base assisted cycloaddition reaction of propargyl ester of deoxycholic acid with benzyl azide and C-24 azide of deoxycholic acid leads to a formation of bile acid bistriazole in good yield which is an ideal addition to the family of click reaction. All the synthesized compounds were tested against a wide variety of microorganisms. 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. (Gram-negative bacteria, Gram-positive bacteria and fungi).

3.2. Introduction

Bile acids are naturally occurring compounds in the steroids family, which are essential for many physiological functions. Currently, bile acids, due to their inexpensive availability, the rigid steroid frame works, unique facial amphiphilicity, and together with viability of being chemically modified on C-3, C-7, C-12 and C-24 have attracted considerable interest for diverse significant applications, involved in pharmacology, asymmetric synthesis, molecular recognition and also polymeric materials.[1] Bile acids have been considered very useful in the preparation of new pharmaceutical drugs because of their inherent chemical nature and biological properties.[1(a),2] Bile acids and their derivatives are pharmacologically interesting as potential transporters of liver specific drugs, absorption enhancers and cholesterol lowering agents.[3] 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,[5a] membrane bilayer probes,[6] and ion complexation.[7]

3.3.1 Literature Survey of Dimeric Steroidal Conjugates

Since the discovery of Japindine **1** (Figure 1), the first example of novel sulphur containing dimeric alkaloid isolated from the root-bark of *Chonemorpha macrophylla*,[8] several examples of bis(steroid) derivatives have appeared in the literature. These dimeric and oligomeric steroids possess interesting micellar, detergent, and liquid crystal properties and many of them led to enhance pharmacological activities.[9] Among these the most pertinent with regard to their extraordinary biological activities are cephalostatins **2** and ritterazines **3**.[10] Cephalostatins are a group of complex steroidal pyrazine alkaloids that were isolated from the marine worm *Cephalodiscus gilchrist*.[11] 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.

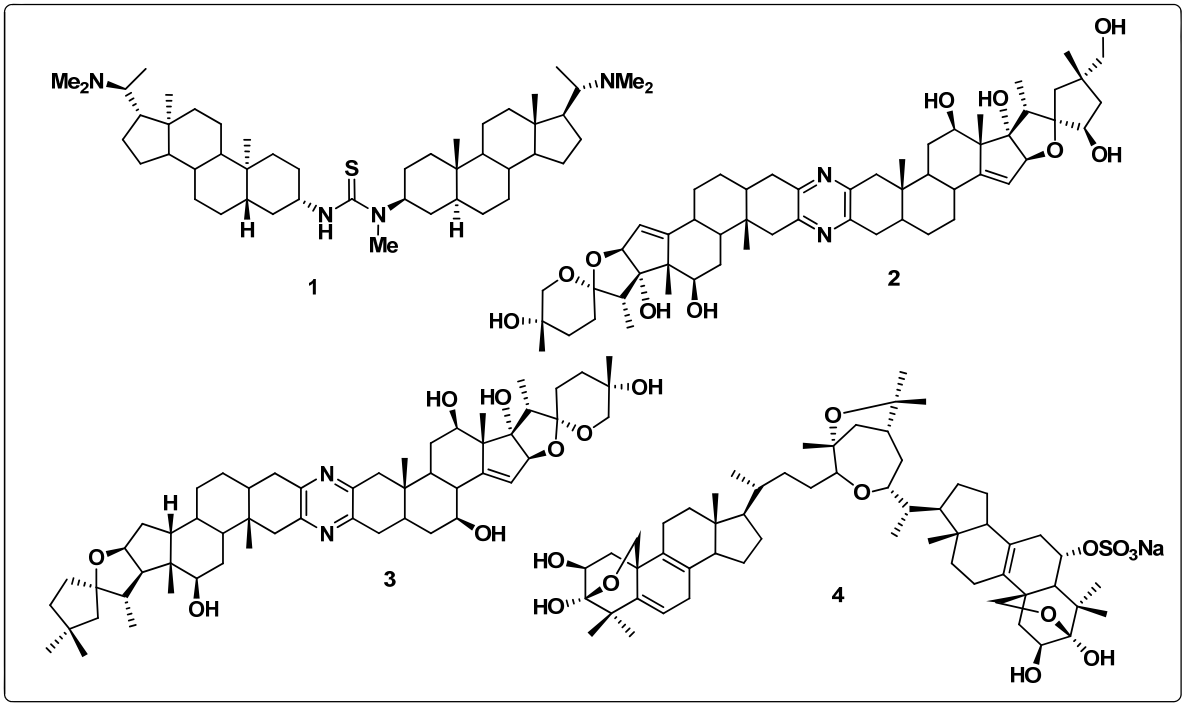


Figure 1

Isolation of crellastatin A **4** (Figure 1)[12] 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.[8]

Kobuke *et al*. have synthesized bile acid based transmembrane ion channels **5** and **6** (Figure 2) by linking two units of amphiphilic cholic acid methyl ethers by biscarbamate.[13] Both the compounds **5** and **6** showed stable single ion channel currents,

when incorporated into a planar bilayer membrane. They have synthesized voltage-dependent artificial ion channels **7** and **8**[14] 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 **7** and hydroxyl and carboxylic acid for **8**. They found that these head groups dissociate easily under basic conditions. Compounds **7** and **8** are the first stable single ion channels having a rectification property except peptidic channels. Recently Kobuke and co-workers also synthesized bischolic acid derivatives **9** (Figure 2) linked by *m*-xylylene dicarbamate unit at 3-3'-position and examined their single ion channel properties.[15]

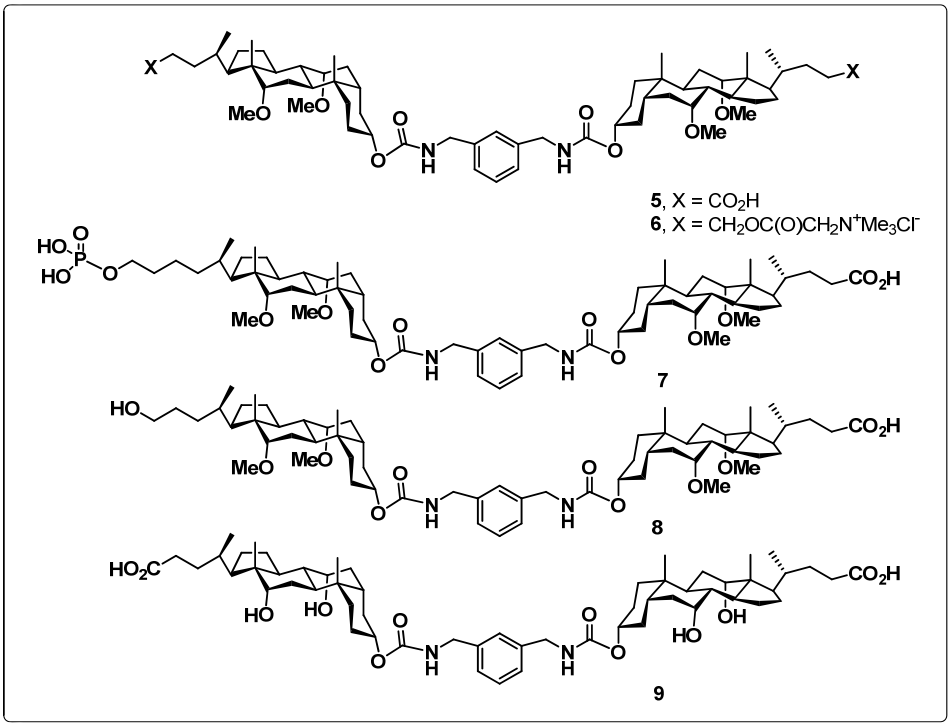


Figure 2

Bile acid based dimers and oligomers have potential applications in the area of drug design and delivery. Janout and co-workers[16] synthesized compound **10** (Figure 3). This type of molecular umbrella can cover an attached agent and shield it from an incompatible environment. Regen and co-workers synthesized[17] persulfated molecular umbrellas **11** 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 **12** bearing two sterols showed 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.[18]

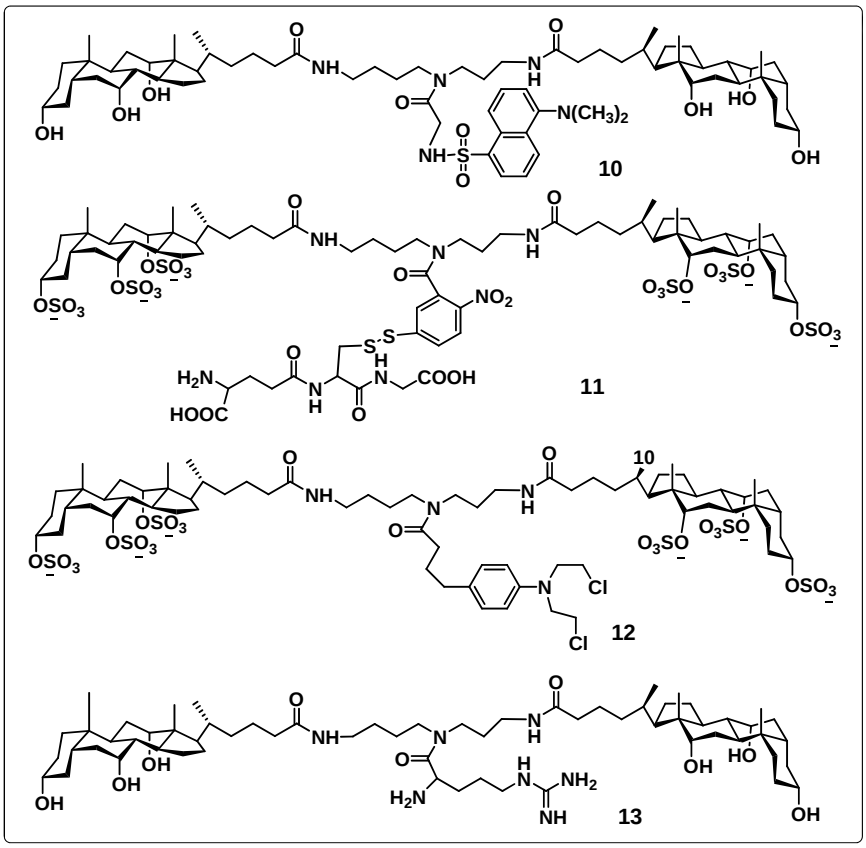


Figure 3

Burrows and Sauter reported synthesis and conformational studies of a new host system **14** (Figure 4) incorporating two molecules of cholic acid linked by a rigid diamine.[19] 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 **15**[20] and DNA binding of steroidal tetramine dimer **16**.[21] McKenna *et al*. synthesized head-to-head dimers **17** and **18** of cholic acid with a linker at C-24. This type of dimers were found to solubilise perylene in aqueous solution without micelle formation.[22]

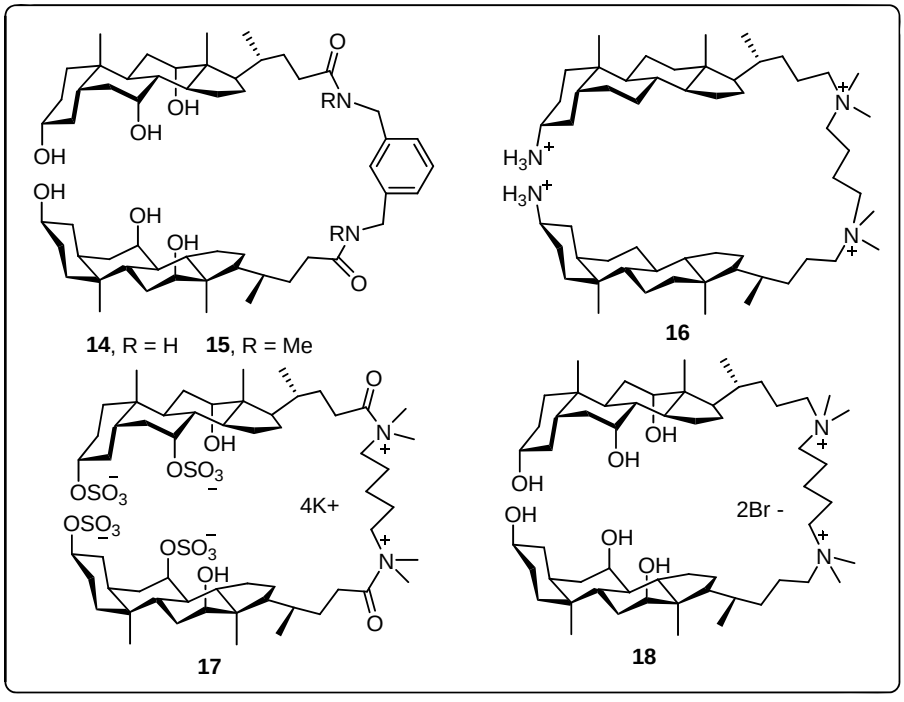


Figure 4

Wess and co-workers synthesized various bile acid dimers and trimers such as **19** and **20** (Figure 5), as bile acid reabsorption inhibitors for potential use in hypercholesterolemia.[23] 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. These compounds were further characterized pharmacologically by *in situ* ileal perfusion experiments in rats.[23]

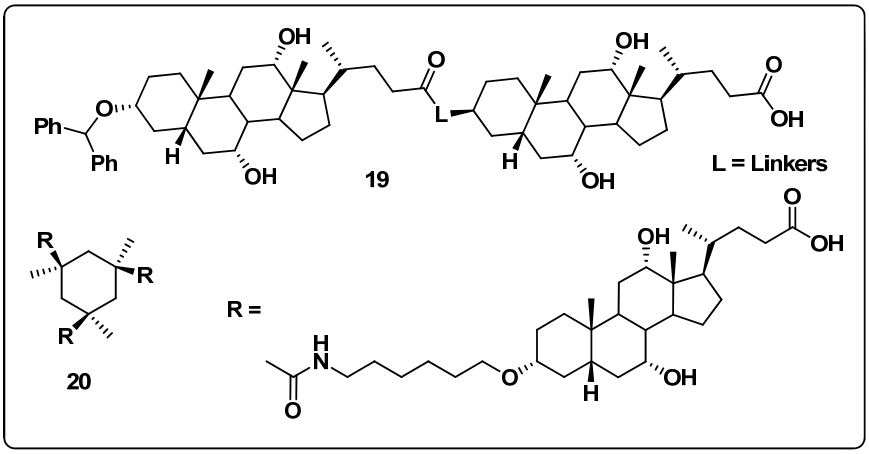


Figure 5

Zhu and co-workers[24] have reported the synthesis of 3α-dimers **21**, **22** (Figure 6) 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.

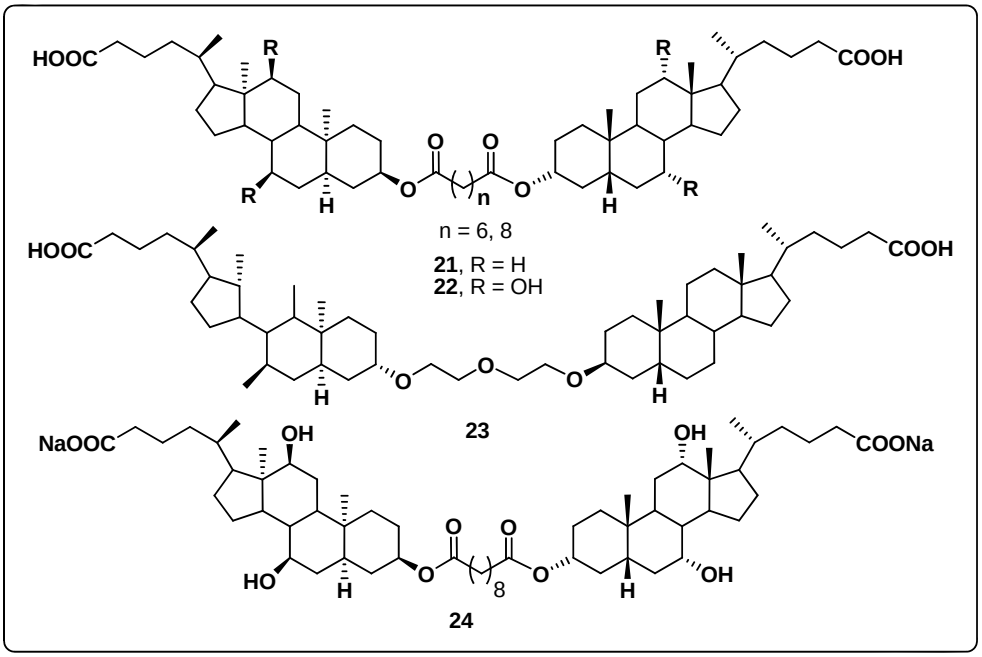


Figure 6

They also synthesized the 3β-dimer **23** 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 **24** by linking two cholic acid molecules via a spacer has been reported by the same group. The synthesized sodium cholate dimer **24** can facilitate the micellization of bile salts.[25] More recently, Yang Li *et al.* have synthesized dimeric bile acid-amino acid conjugates **25-28** (Figure 7) as the mimic of cyclic peptide. These compound shows antitumor activity against human breast cancer cell MCF-7.[26]

Taking advantage of the amphiphilic topology of bile acids, our group has reported the synthesis of various cholic acids, deoxycholic acid dimers e.g. **29** and **32** using a varity linkers (Figure 8).[27]

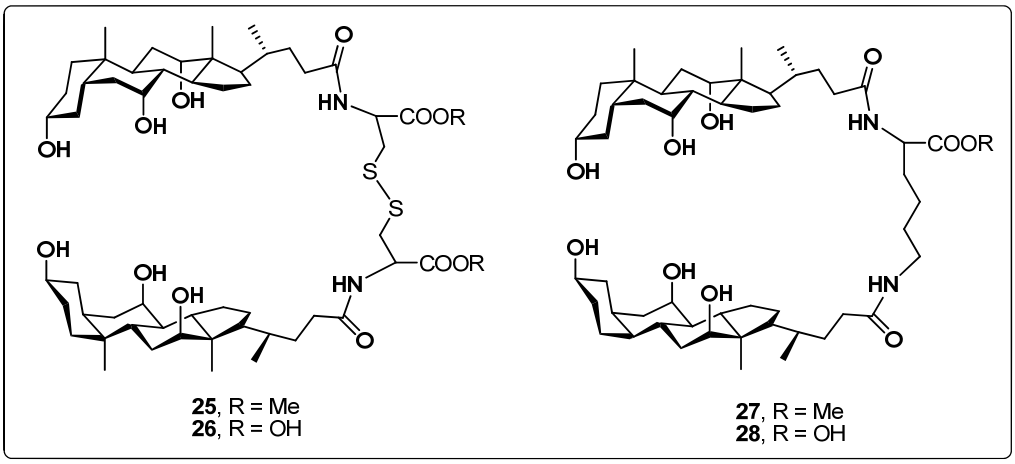


Figure 7

Dimers **29** and **30** were found to posses antifungal and antiproliferative activity. Synthesis of novel bile acid dimers **31**, **32** containing 1,2,3-triazole as a linker using click chemistry have been very recently reported form our laboratory.[28] Micellar properties of these molecules were investigated through hydrophilic dye solubilisation studies in non polar media.[29] Synthesis of bile acid dimer linked through 1,2,3-triazole and bis-β-lactam **33 (a-d)** has been recently reported from our group[30] which exhibited significant antifungal as well as antibacterial activity (Figure 9).

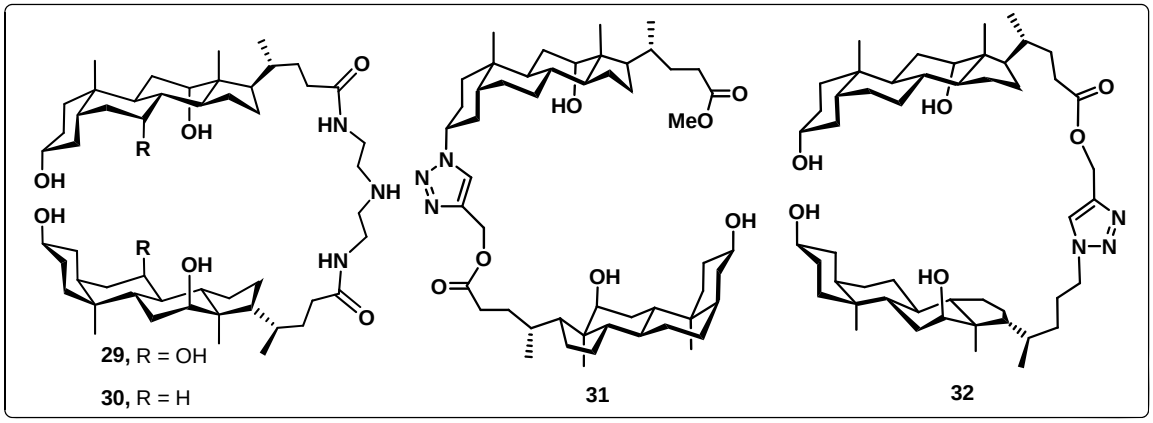


Figure 8

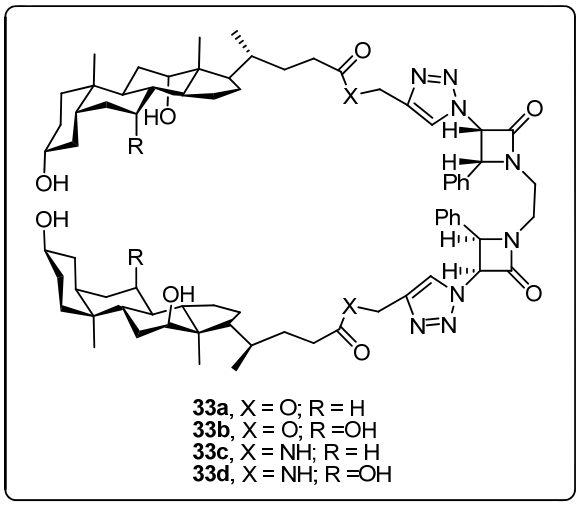


Figure 9

It can be seen from the above discussion that triazole ring moieties are attractive connecting units, since they are stable to metabolic degradation and capable of hydrogen bonding, which can be favourable in binding of biomolecular targets.

3.3.2. Literature Survey of Chemotypes with Azole as Privileged Structure

Azoles are the largest class of antifungal agents in clinical use.[31] 1,2,3-Triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole unit shows diverse biological activities including antibacterial, herbicidal, fungicidal, antiallergic, and anti-HIV.[32] Triazole nucleus has been incorporated into a wide variety of therapeutically interesting drug candidate including H_1/H_2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, antianxiety agents and sedative.[33] Triazole

moieties are attractive connecting units, since they are stable to metabolic degradation and capable of hydrogen bonding, which can be favourable in binding of biomolecular targets and for solubility.[34] The great interest in the synthesis of members of the 1,2,3-triazole family has its roots in the interesting biological properties.

If two triazoles units are linked by covalent bond or by carbon atom, then they form bis-triazole. Bistriazole based size-specific mRNA hairpin loop binding agents have been developed to target mRNAs coding for proteins,[35] which could be a promising approach in drug discovery (Figure 10).

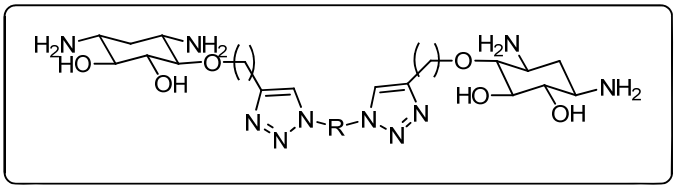


Figure 10

There are several reports on the synthesis of bistriazole derivatives and studied their antimicrobial activity. Recently there have been reports on synthesis of bitriazolyl compounds **34a-j** and **35a-j** and their activity against tobacco mosaic virus was assessed (Figure 11). Two of them compound **34e** and **35d** showed promising antiviral activity and were more potent than the reference compounds. Moreover, these compounds are predicted not to be carcinogenic or mutagenic.[36] Therefore, the bistriazolyl compounds may provide interesting new leads or scaffolds for use in further attempts to screen novel antimicrobial as well as antiviral candidates.

Being inspired by the wide range of pharmacological activities and applications, as well as continuous research work on the bile acid dimers and conjugates in our laboratery,[27-30] we report herein the synthesis of novel bile acid bistrazole **40** and **47**. These compounds have been synthesised using Cu(I) catalysed base assisted cycloaddition reaction of

propargyl ester of deoxycholic acid **37** with benzyl azide **38** and C-24 azide of deoxycholic acid **45** with propargyl ester **37** (Click Chemistry) and studied their antimicrobial activity. The syntheses of these novel deoxycholic acid dimers **40** and tetramer **4t** linked with unique pharmacophore units such as bistriazole and the bioactivity data are reported herein for the first time (Scheme 1-5).

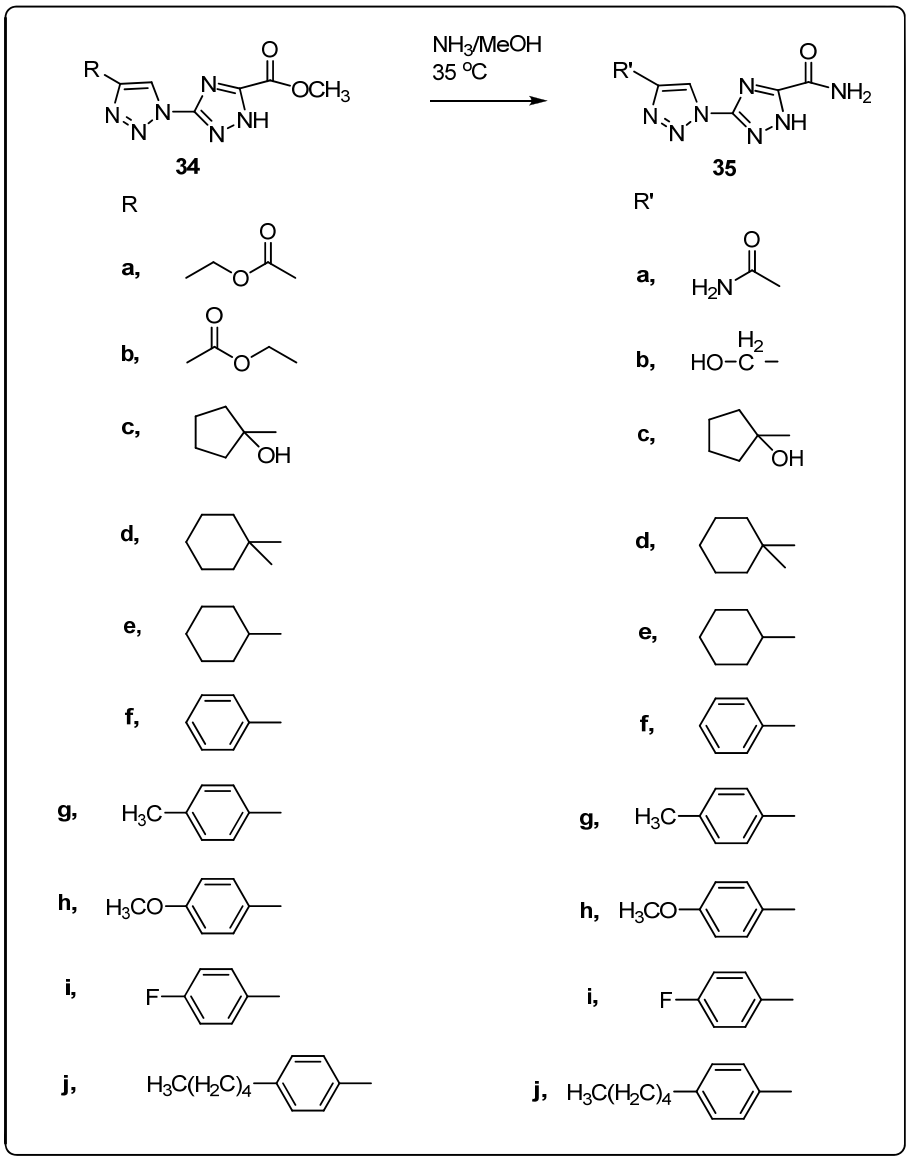


Figure 11

3.4. Results and Discussion

3.4.1. Click Chemistry

The 1,3-dipolar cycloaddition of azides to alkynes was first reported more than hundred years ago[37] and investigated by Huisgen and co-workers in detail.[38] The reaction of terminal alkynes with organic azides leads under thermal conditions to a mixture of the 1,4- and 1,5-disubstituted 1*H*-1,2,3-triazoles.[39] This 1,3-dipolar cycloaddition reaction of organic azides and alkynes has gained considerable attention in recent years due to the introduction of Cu(I) catalysis by Tornøe and Meldal[40] and by Sharpless *et al*[41] leading to a major improvement in both rate and regioselectivity of the reaction (Figure 10).[42] The Cu(I) catalysed variant of the Huisgen 1,3-dipolar cycloaddition of azide and alkynes affords regioselectively 1,4-disubstituted 1,2,3-triazoles with such efficiency and scope that the transformation has been described as "click" chemistry. The Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction has successfully fulfilled the requirement of "click chemistry" as prescribed by Sharpless and during the past few years and at present one of the most useful and premier component of synthetic organic chemistry.[41-43]

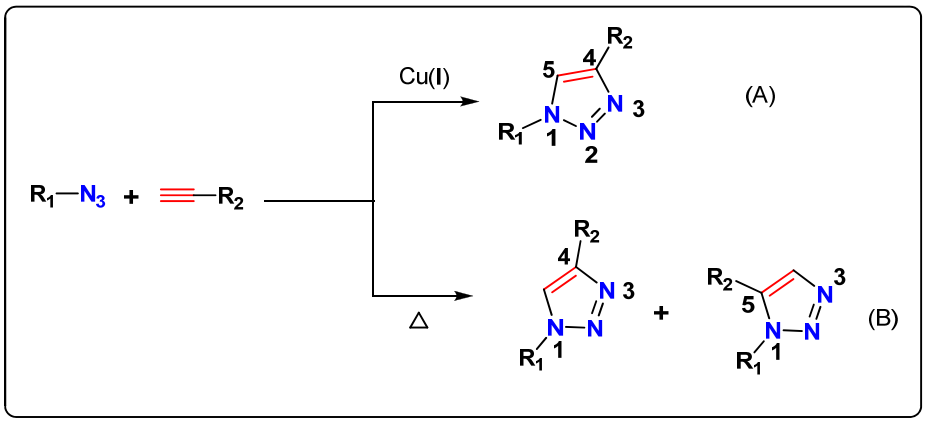


Figure 10

The great success of the Cu(I) catalyzed 1,3-dipolar cycloaddition reaction is rooted in the fact that it is a virtually quantitative, very robust, insensitive, general, and orthogonal ligation reaction, suitable for even biomolecular ligation[44] and in vivo tagging[45,46] or as a

polymerization reaction for synthesis of long linear polymers.[47] The triazole formed is essentially chemically inert to reactive conditions, e.g. oxidation, reduction, and hydrolysis, and has an intermediate polarity with dipolar moment of ~5 D.[48]

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.[49]

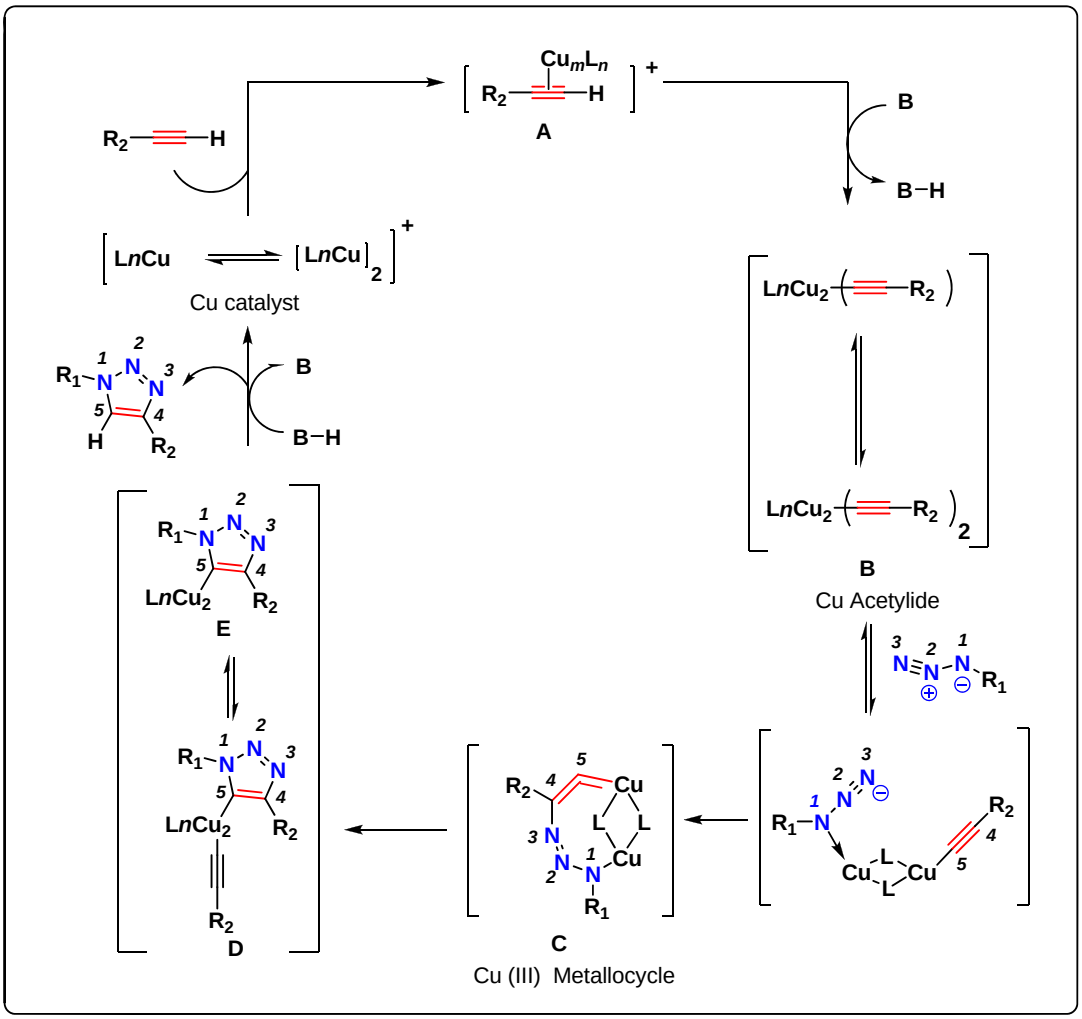


Figure 11

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.[49] 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 as much as 11.8 kcal/mol.[50] 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**) (Figure 11).[51]

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}).[51] 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 towards 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.[52] Azides and acetylenes are by definition kinetically stable entities possessing high built-in energy and are tolerant to a wide range of synthetic conditions.[49] 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

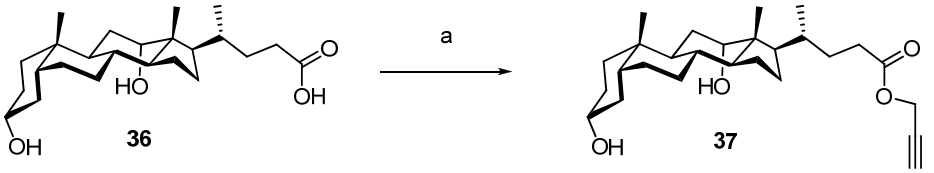
functionalized biological milieus has allowed click chemistry to become a viable bioconjugation strategy for labeling biomolecules and for *in situ* lead discovery applications.[53] The 1,2,3-triazole moiety is a potential pharmacophore owing to its moderate dipole character and rigidity and can therefore be readily incorporated into a design strategy, rather than used as passive linker between two respective fragments of structural space.[54]

Sharpless and Co-workers perceive minor amounts of undesired by-product bis-triazols by oxidative coupling during development of the aqueous $CuSO_4$/Ascorbate Huisgen cycloaddition.[41] Without reporting their characterization, they attributed these by products to the direct use of Cu(I) species.[55] Various methods have been developed for synthesis of bistriazole derivative. Amanov *et al* obtained bistriazole derivatives by reaction of diacetylenic diesters of benzoic acid derivatives with phenyl azide in a toluene.[56] Holla *et al* achieved synthesis of bis triazole derivatives from diacid.[57] Kumar *et al* synthesised sugar based unsymmetrical bis1,2,3-tiazole via Domini click approach. Addition of resonance-stabilized allenylmagnesium bromide to sugar azides, which resulted in the formation of novel 5-butynylated triazoles in good yields. These molecules, upon Cu(I) catalyzed 1,3-dipolar cycloaddition with sugar azides, generated novel unsymmetrical bis1,2,3-triazoles.[58] Benoist *et al* synthesised polydentat bi-functional chelating agent by using, bistriazole based polyamino carboxylic acid. This 1,4-disubstituted bistriazole scaffold was achieved from bis azide and corresponding propargyl compound using classical click reaction.[59] Monkowius *et al* achived synthesis of 1,4-disubstuted bistriazoles(R-bta, R = organic group) by a Cu(I) catalysed cycloaddition between 1,3-butadiyne and the organic azide.[60] Peng et al discovered novel bittriazolyl compound as novel antiviral candidates. Under mild conditions, azido-triazols readily engaged in a

Cu(I) catalysed Huisgen reaction with variety of terminal acetylenes giving regiosalevtively 1,4-disubstuted 1,2,3-triazoles products.[36] Recently very elegantly Angell and Burgess[61] reported oxidative coupling product, bistriazole which is the major product in the presence of Cu/$CuSO_4$ and carbonate base which is key variable.

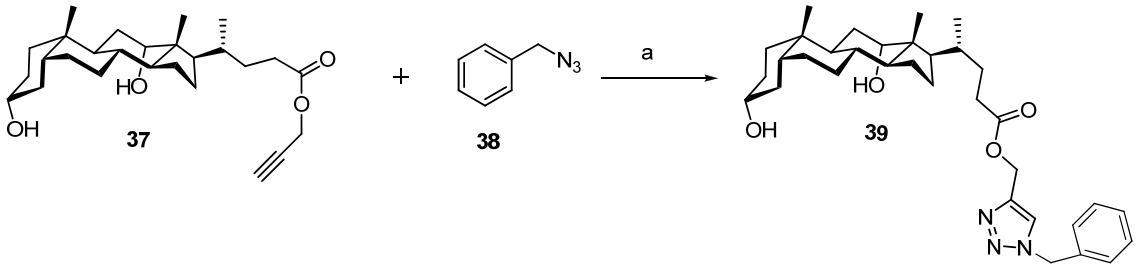
3.4.2. Chemistry

In continuation of our work on bile acid dimers, propargyl ester of deoxycholic acid **37** and benzyl azide **38** was chosen as a model alkyne and azide components. Propargyl ester of deoxycholic acid **37** was prepared by esterification of deoxycholic acid **36** using an excess of propargyl alcohol and catalytic amount of para-toluene sulfonic acid (*p*-TSA) to get compounds **37** (96%) according to earlier report (Scheme 1).[29]



Scheme 1: Reagent and condition (a) *p*-TSA (10 mol%), propargyl alcohol, 55-60 °C, 7 h, 96%

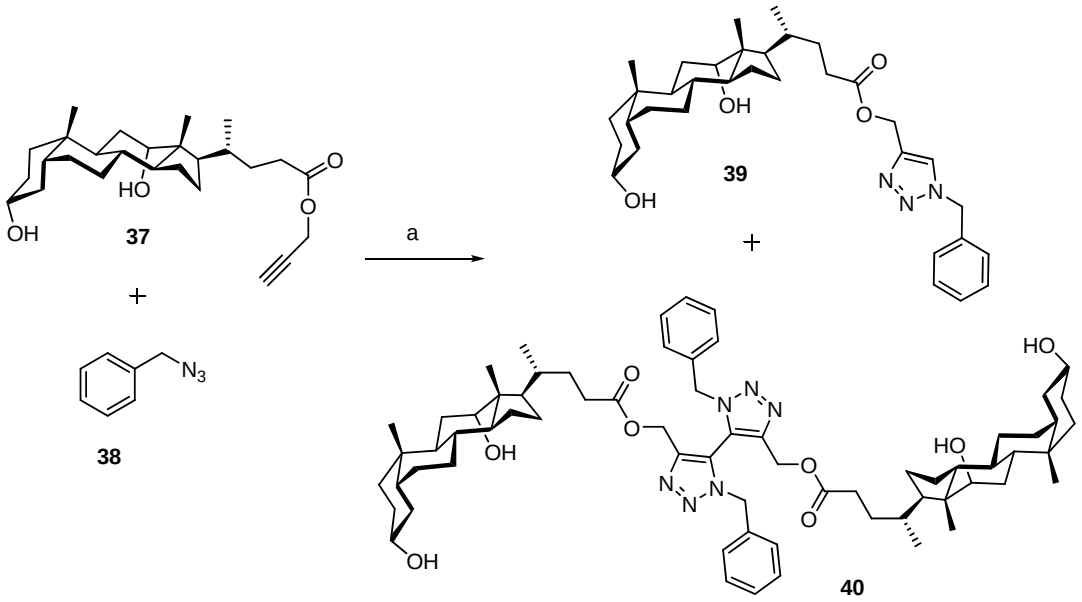
Reaction of propargyl ester of deoxycholic acid **37** and benzyl azide **38** in the presence of copper sulphate and sodium ascorbate in DMF (usual click condition) gave expected 1,2,3-triazole compound **39** exclusively in excellent yield (Scheme 2).



Scheme 2: Reagent and condition (a) $CuSO_4.5H_2O$ (0.05 eq), sodium ascorbate (0.5 eq), DMF/H_2O, 14 h, 95%.

The first successful result for the bistriazole of propargyl ester of deoxycholic acid **37** with benzyl azide **38** was obtained using Cu/$CuSO_4$ in THF in the presence of inorganic

base Na_2CO_3 (1.5M) to furnish 1,4-substituted triazole compound **39** and 1,4-disubstituted bistriazole **40** in 29 and 63% yield, respectively (Scheme 3).



Scheme 3: Reagent and condition (a) $CuSO_4.5H_2O$ (0.1 eq), Cu (powder) (1.0 eq.), Na_2CO_3 (1.5 eq), THF, 31 h, **39** (29%) and **40** (63%) (Entry 1, Table 1).

Continuous 31 h of stirring was required for the complete consumption of the starting materials at 25 °C (Entry 1, Table 1). We have noticed that in the absence of carbonate base the reaction was not completed and no formation of bistriazole **40** even stirring the mixture for 48 h of stirring.

Attempts were made to increase the amount of bistriazole by increasing concentration of base. Optimal base concentrations were found to be 1.5 to 3 M. This result encouraged us to explore other carbonate bases. We varied the reaction conditions in attempts to optimize the yield of bistriazole **40**. Oxidative dimerisation product, bistriazole **40** was a major product when K_2CO_3 base and optimal base concentration was found to be 1.5 M (Entry 4, Table 1). Whereas, in presence of nitrogenous organic bases we have noticed formation of triazole **39** took place and no formation of oxidative dimerisation product for 48 hours stirring (Entry 12-14, Table 1). Given that in presence of CuI "click" reactions

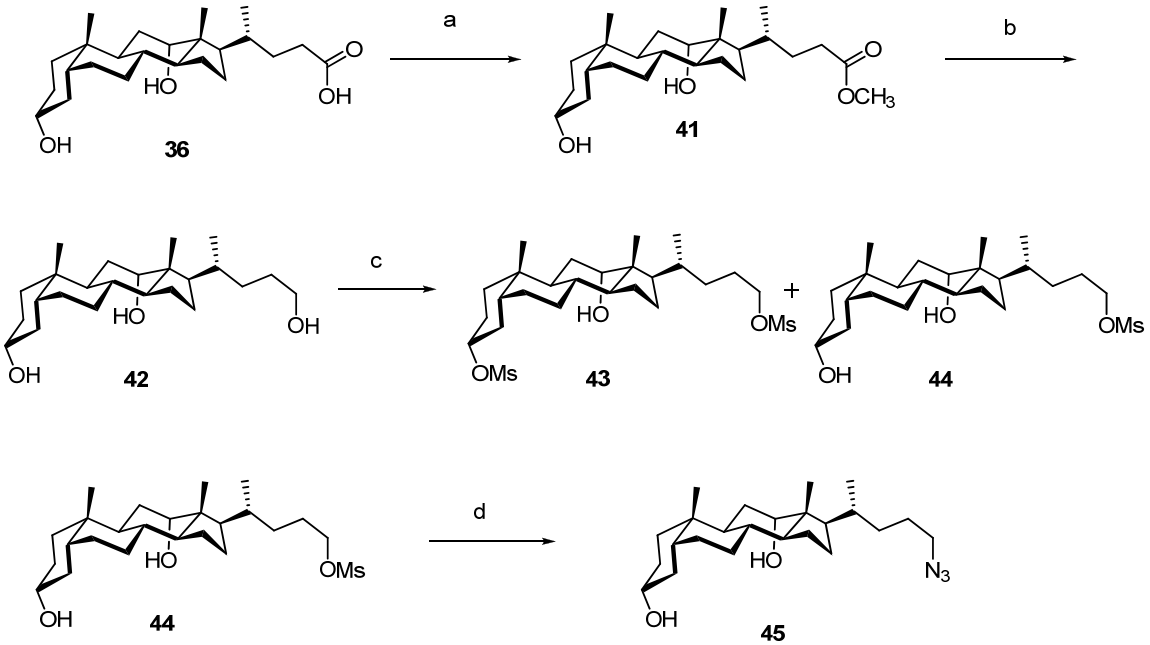
generally proceed in high yield,[62] we felt that by using CuI in combination with various organic as well as inorganic bases it might be possible to improve bistriazole **40**, but most of the attempts were not encouraging (Entry 15-24). Also it was observed that, in the presence of very strong base like KOH, NaOH hydrolysis of propargyl ester functionality occurs. Our experiments with K_2CO_3 as base indicated that the oxidative dimerization was more prevalent in THF than DMF as a solvent. The oxidative dimerisation methodology was extrapolated to different bases (both organic and inorganic) having variable concentrations for both copper catalyst ($CuSO_4$/Cu and CuI) using THF or DMF as solvent and yilds of **39** and **40** are depicted in Table 1.

Table 1

Entry	Reagent used	Solvent	Base	Conc. of base	Time [h]	Product distribution	
						39 [%]	**40** [%]
1	$CuSO_4$/Cu	THF	Na_2CO_3	1.5M	31	29	63
2	$CuSO_4$/Cu	THF	Na_2CO_3	2.0M	30	32	60
3	$CuSO_4$/Cu	THF	Na_2CO_3	3.0M	29	39	53
4	$CuSO_4$/Cu	THF	K_2CO_3	1.5M	28	29	68
5	$CuSO_4$/Cu	THF	K_2CO_3	2.0M	28	30	66
6	$CuSO_4$/Cu	THF	K_2CO_3	3.0M	27	32	60
7	$CuSO_4$/Cu	THF	$NaHCO_3$	1.5M	34	61	38
8	$CuSO_4$/Cu	THF	$NaHCO_3$	2.0M	30	60	35
9	$CuSO_4$/Cu	THF	$NaHCO_3$	3.0M	36	69	22
10	$CuSO_4$/Cu	DMF	Na_2CO_3	1.5M	26	48	43
11	$CuSO_4$/Cu	DMF	K_2CO_3	1.5M	27	51	41
12	$CuSO_4$/Cu	THF	DIPEA	2 eq.	29	83	-
13	$CuSO_4$/Cu	THF	DBU	2 eq.	31	91	-
14	$CuSO_4$/Cu	THF	TEA	2 eq.	26	90	-
15	CuI	DMF	DIPEA	2 eq.	13	67	19
16	CuI	DMF	DBU	2 eq.	14	69	21
17	CuI	DMF	TEA	2 eq.	12	68	24
18	CuI	THF	DIPEA	2 eq.	16	65	21
19	CuI	THF	DBU	2 eq.	16	69	18
20	CuI	THF	TEA	2 eq.	12	73	14
21	CuI	THF	Na_2CO_3	2 eq.	23	60	32
22	CuI	DMF	Na_2CO_3	2 eq.	23	63	35
23	CuI	THF	K_2CO_3	2 eq.	22	45	52
24	CuI	DMF	K_2CO_3	2 eq.	21	56	39

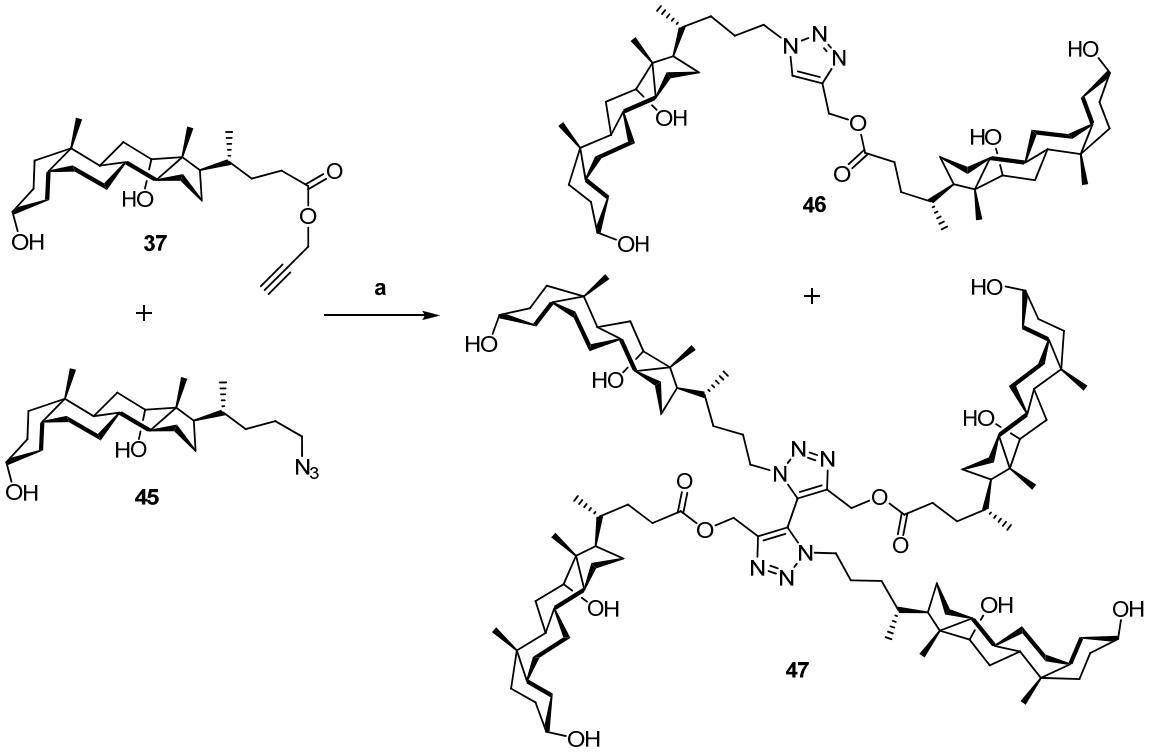
DIPEA- *N*,*N*-Diisopropylethylamine; DBU- 1,8-Diazabicycloundec-7-ene; TEA- triethylamine.

Deoxycholic acid C-24 azide of **45** was synthesized according to earlier report[29] and presented in Scheme 4. Accordingly, *p*-TSA catalyzed esterification of deoxycholic acid **36** was carried out with methanol to obtain methyl ester **41** in 95% yield. Subsequently, reduction of ester moiety in **41** with $LiAlH_4$ was carried out to provide trihydroxy compound **42** in 93% yield. Primary hydroxyl functionality in triol **42** was mesylated (Et_3N, DCM) to give C-24 monomesylate **44** and C-3, C-24 dimesylate **43** with poor regioselectivity. When reaction was carried out under high dilution and less reaction time, expected regioisomer **44** was formed as major isomer along with **43** (regioselectivity 7:3). These compounds were easily separated by flash column chromatography. Further, *mono*mesylates **44** was subjected to nucleophilic substitution of mesyl group with azide anion (NaN_3 in DMF) which gave the corresponding C-24 azides **45** (94%). Formation of azides **45** was confirmed by IR spectroscopy which showed a characteristic absorption band at 2100 cm^{-1} due to azido functionality.



Scheme 4: Reagents and conditions (a) *p*-TSA/MeOH, 25 ºC, 24 h, 95-96%; (b) LAH/THF, 25 ºC, 2 h, 93%; (c) MsCl, Et_3N, DCM, 0 ºC, 10 min; (d) NaN_3, DMF, 60 ºC, 3 h, 94%.

Our next goal was to synthesise targeted deoxycholic acid 1,4-disubstituted bistriazole **47.** The similar strategy has been applied here for the synthesis of bistriazole having four bile acids by using propargyl ester of deoxycholic acid **37** and C-24 azide of deoxycholic acid **45**. The 1,4-substituted triazole compound **46** and 1,4-disubstituted bistriazole **47** was obtained in 19 and 72% yield, respectively by using Cu/$CuSO_4$ in THF in the presence of inorganic base K_2CO_3 (2M) (Scheme 5) (Entry 5, Table 2). Also significant amount of oxidative dimerisation product bistriazole **47** was obtained when we attempted coupling reaction with $CuSO_4$/Cu, in the presence of 1.5 M Na_2CO_3 (Entry 1, Table 2).



Scheme 5: Reagents and conditions (a) $CuSO_4.5H_2O$ (0.1 eq), Cu (powder) (1.0 eq.), K_2CO_3 (2 M), THF, 12 h, **46** (19%) and **47** (72%) (Entry 5, Table 2).

Attempts were made to increase the amount of the bistriazole **47** by using CuI as Cu(I) source with inorganic bases as well as organic bases (Entry 15-24, Table 2). Most of our attempts to realize maximum yields of deoxycholic acid bistriazole **47** were unsuccessful. Under this condition major product was compound **46** (usual "click" 1,4 disubstuted 1,2,3 triazole) and minor one was bistriazole **47**. The oxidative dimerisation methodology was

extrapolated to different bases having variable concentrations for both copper catalyst ($CuSO_4$/Cu and CuI) and solvents are depicted in Table 2.

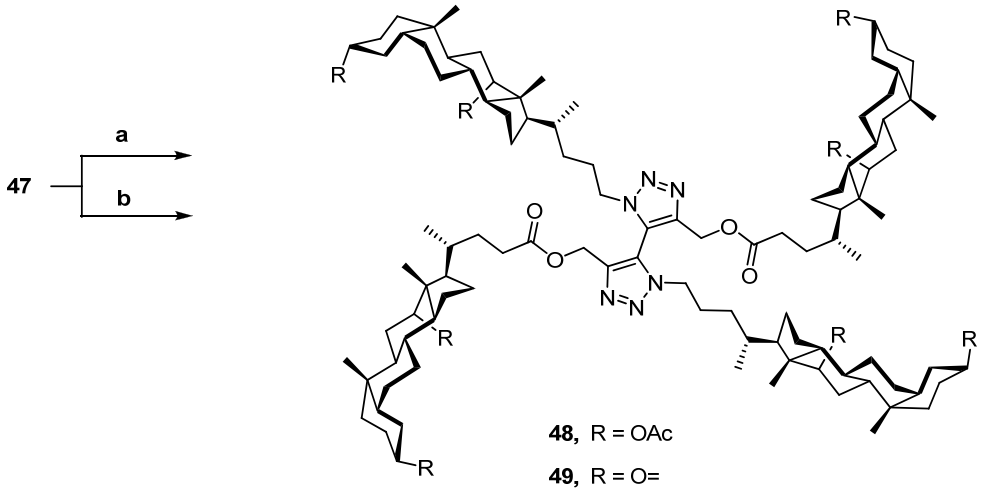
Table 2

Entry	Reagent used	Solvent	Base	Conc. of base	Time [h]	Product distrubution	
						46 [%]	**47** [%]
1	$CuSO_4$/Cu	THF	Na_2CO_3	1.5 M	16	20	72
2	$CuSO_4$/Cu	THF	Na_2CO_3	2.0M	16	21	71
3	$CuSO_4$/Cu	THF	Na_2CO_3	3.0M	15	23	68
4	$CuSO_4$/Cu	THF	K_2CO_3	1.5M	13	23	71
5	$CuSO_4$/Cu	THF	K_2CO_3	2.0M	12	19	72
6	$CuSO_4$/Cu	THF	K_2CO_3	3.0M	12	27	63
7	$CuSO_4$/Cu	THF	$NaHCO_3$	1.5M	21	34	60
8	$CuSO_4$/Cu	THF	$NaHCO_3$	2.0M	19	38	53
9	$CuSO_4$/Cu	THF	$NaHCO_3$	3.0M	18	37	55
10	$CuSO_4$/Cu	DMF	Na_2CO_3	1.5M	16	41	53
11	$CuSO_4$/Cu	DMF	K_2CO_3	1.5M	17	28	64
12	$CuSO_4$/Cu	THF	DIPEA	2 eq.	20	78	14
13	$CuSO_4$/Cu	THF	DBU	2 eq.	21	83	7
14	$CuSO_4$/Cu	THF	TEA	2 eq.	19	80	9
15	CuI	DMF	DIPEA	2 eq.	13	54	37
16	CuI	DMF	DBU	2 eq.	14	60	30
17	CuI	DMF	TEA	2 eq.	13	54	32
18	CuI	THF	DIPEA	2 eq.	17	79	12
19	CuI	THF	DBU	2 eq.	15	68	23
20	CuI	THF	TEA	2 eq.	14	72	17
21	CuI	THF	Na_2CO_3	2 eq.	20	61	28
22	CuI	DMF	Na_2CO_3	2 eq.	20	57	40
23	CuI	THF	K_2CO_3	2 eq.	20	41	52
24	CuI	DMF	K_2CO_3	2 eq.	19	60	38

DIPEA- *N*,*N*-Diisopropylethylamine; DBU- 1,8-Diazabicycloundec-7-ene; TEA- triethylamine.

We would like to have single crystal for X-ray analysis of our compound, deoxycholic acid bistriazole **47**. Compound **47** is an amorphous solid and we could not get a suitable single crystal for X-ray analysis. For this we acetylated compound **47** with excess of acetic anhydride and catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) in pyridine to get octacetyl bistriazole **48** in 62% yield. We had also oxidized compound 47 with IBX in DMSO to obtained C-3 and C-12 octaoxo derivative **49** in 73% yield (Scheme 6). Compound **48** and **49** are also amorphous solids. We have fully characterized

these two compounds **48** and **49** by IR, ^{1}HNMR, ^{13}CNMR, and mass spectrometry. All our efforts to get single crystal for both these derivatives (**48** and **49**) did not materialise. It is worth mentioning here that all the compounds are new and characterized fully by IR, ^{1}H NMR and ^{13}C NMR spectroscopy.



Scheme 6: Reagents and condition (a) Ac_2O, DMAP, Pyridine, Reflux, 18 h, 62%; (b) IBX, DMSO, 26 °C, 16 h, 73%.

3.5. Antimicrobial Activity

The entire newly synthesised compound and deoxycholic acid were examined *in vitro* for antifungal as well as antibacterial activity. The antifungal activity was tested using NCL isolate fungal strains *Candida albicans*, *Cryptococcus neoformans* (human pathogen), *Benjaminiella poitrasii*, *Yarrowia lipolytica* (saprophytes) and *Fusarium oxysporum* (plant pathogen). The antibacterial activity was evaluated against *Escheirchia* coli and *Staphylococcus aureus*. The MIC values were determined using standard broth microdilution technique described by NCCLS.[63] (Table 3). 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 3 as MIC values

Table 3: Minimum inhibitory concentration (MIC) of bileacid bistriazole conjugates.

Entry	Compound Number	Antimicrobial Activity MIC (μg/mL)						
		Fungal Strains					Bacterial Strains	
		A	***B***	***C***	***D***	***E***	***F***	***G***
1	**36**	>128	>128	128	>128	>128	>128	>128
2	**39**	16	8	8	4	4	8	28
3	**40**	24	16	4	64	16	8	26
4	**46**	8	16	32	32	16	8	12
5	**47**	16	8	4	16	32	8	24
6	**48**	32	8	8	32	8	16	16
7	**49**	8	12	16	8	2	4	14
8	**AmpB**	2	16	16	8	16	NT	NT
9	**Fluconazole**	32	32	32	64	8	NT	NT
10	**Tetracycline**	NT	NT	NT	NT	NT	8	16
11	**Erythromycin**	NT	NT	NT	NT	NT	64	32

A, *Candida albicans*; ***B,*** *Cryptococcus neoformans*; ***C,*** *Benjaminiella poitrasii*; ***D,*** *Yarrowia lipolytica*; ***E,*** *Fusarium oxysporum*; ***F,*** *Escherichia coli*; ***G,*** *Staphylococcus aureus.*

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

Negative control, DMSO (2.5% v/v), No inhibition.

"**NT**" Not tested

As seen in Table 3 most of the synthesised dimers and tetramers showed temperate to superior antifungal and antibacterial activity against all the tested fungal and bacterial strains in comparison with standard drugs. From the biological data it was observed that deoxycholic acid **36** did not show any antifungal or antibacterial effect. The MIC value for all these compounds was >128 μg/mL. Compound **49** (3,12 oxo deoxycholic acid bistriazole conjugate) showed potent antifungal and antibacterial activity against all the tested fungal and bacterial strains. The activity of compounds **39**, **40** and **46-48** was higher or comparable to that of fluconazole against *C. albicans* with MIC value of 8–32 μg/mL. The compounds **39, 47** and **48** showed significant antifungal activitiy against *C. neoformans* having MIC value of 8 μg/mL higher that of amphotericin B and fluconazole. However, all the compounds except **46** showed significant growth inhibitory activity against *B. poitrasii,* where as only compounds **40** and **49** showed promising activity

against *Y. lipolytica* and other compound shows moderate activity. Surprisingly *F. oxysporum* was adversely affected particularly by **39** and **49** was the most potent with a low MIC value of 4 μg/mL and 2μg/mL comparable to amphotericin B and fluconazole. Also surprisingly distinct antifungal activity (4-8 μg/mL) for all compounds except compound **48** against bacterial strain *E. coli* comparable to reference drug (tetracycline and erythromycin) shows strong antibacterial properties. From the overall activity results, it was observed that the activity shown by our novel compounds is due to their inherent amphiphilicity.

3.6. Conclusion

A series of novel bile acid bistriazole dimers and tetramers have been synthesized using base assisted Cu(I) catalysed cycloaddition reaction (click chemistry) of propargylester of deoxycholic acid with benzyl azide or C-24 azide of deoxycholic acid in moderate to good yields. These novel dimers and tetramers were evaluated for antifungal as well as antibacterial activities. Most of the compounds demonstrated potent antimicrobial activity against all the strains tested. Among them, compound **39**, **47**, **48** and **49** are most potent antimicrobials. The synthesis of bile acid dimers linked with pharmacophores, bistriazole can open as new horizon for the control of human and plant pathogens.

3.7. Experimental Section

Compound 37: (*Propargyl 3α,12α-dihydroxy-5β-cholan-24-oate*) To a solution of Deoxycholic acid **36** (1.960g, 5 mmol) in propargyl alcohol (10 mL), a catalytic amount (82 g, 10 mol %) of *para*-toluene sulfonic acid (*p*-TSA) was added. The reaction mixture was then heated at 50- 60 °C for 7 h. It was then poured on crushed ice and extracted with EtOAc (3x25 mL). The extract was washed with water (3x25 mL), brine (25 mL), and

dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product. Purification of the crude product by column chromatography on silica gel (2% MeOH/DCM) produced Propargyl 3α,12α-dihydroxy-5β-cholan-24-oate **37** as white solid, yield: 96%; mp 160-162 °C (lit.[29] 160-161 ºC); $[\alpha]_D^{27}$ +43.5 (c 1.5, $CHCl_3$); Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83; Found: C, 74.97; H, 9.62; IR ($CHCl_3$, cm^{-1}) 3382, 3307, 1737; ^1H NMR (200 MHz, CDCl3) δ 0.67 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.97 (d, 3H, *J*=5.9 Hz, CH_3-21), 2.47 (t, 1H, *J* =2.2 Hz), 3.60 (m, 1H, CH-3), 3.98 (br s, 1H, CH- 12), 4.68 (d, *J* =2.20 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50.32 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 $(M + Na)^+$.

Experimental Procedure for the preparation of 1,2,3-triazole using Sodium ascorbate and $CuSO_4$ (Scheme 2).

Compound 39: A solution of benzyl azide **38** (26 μL, 0.23 mmol) and propargyl ester of deoxycholic acid **37** (100 mg, 0.23 mmol) in DMF/H_2O (10:1) (5 mL) was stirred at 27 °C for 15 min. Then, $CuSO_4 \cdot 5H_2O$ (3 mg, 0.011 mmol, 5 mol% in 0.5 mL of H_2O) and sodium ascorbate (9 mg, 0.046 mmol, 20 mol% in 0.5 mL of H_2O) were added to the reaction mixture and it was stirred at 27 °C for 14 h. The solvent was evaporated under reduced pressure to afford a crude product, which on purification by column chromatography on silica gel gave pure dimeric compounds containing 1,2,3-triazole moiety in 95% yield, mp 171-173 ºC; $[\alpha]_D^{26}$ + 33.78 (*c* 1.48, $CHCl_3$); Anal. Calcd for $C_{34}H_{49}N_3O_4$: C, 72.43; H, 8.76; N, 7.45; Found: C, 72.55; H, 8.91; N, 7.22; IR ν_{max} (Nujol)/(cm^{-1}) 1305, 1377, 1496, 1511, 1728, 3350; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.63 (s, 3H), 0.89 (s, 3H), 0.91 (bs, 3H), 3.60 (m, 1H), 3.95 (bs, 1H), 5.17 (s, 2H), 5.51 (s, 2H), 7.36 (bs, 5H), 7.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.6, 17.2, 23.1, 23.6, 26.0,

27.0, 27.4, 28.5, 30.3, 30.6, 30.9, 36.3, 42.0, 46.4, 46.5, 48.1, 54.2, 57.3, 71.7, 73.1, 116.3, 123.6, 128.1 X 2, 128.8, 129.1 X 2, 143.2, 149.7, 174.1; MS (LCMS) *m/z* 564.71 $[M+H]^+$, 586.77 $[M+Na]^+$.

Experimental Procedure for the preparation of bistriazole using $CuSO_4$/Cu and 1.5 M K_2CO_3 (Scheme 3) (Entry 4, Table 1).

The propargyl ester of deoxycholic acid **37** (430 mg, 1.0 mmol) was added to a mixture of benzyl azide **38** (123 μL, 1.2 mmol) in THF 1.5 mL and aqueous K_2CO_3 solution (1.5 M, 1.5 mL). Copper sulphate (1.0 M, 0.1 mL) was added to the above suspension fallowed by copper powder (64.0 mg, 1.0 equiv) and the resulting suspension was stirred at 27 °C for 28h. After completion of reaction the reaction mixture was filtered through celite pad. The solution was concentrated and the residue was extracted with DCM using the standard work up procedure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 98:2) to furnish compound **39** and **40** in 29 and 68% yield, respectively as a white solid.

Compound 40: Mp 177-180 ºC; $[\alpha]_D^{26}$ + 32 (*c* 1.5, $CHCl_3$); Anal. Calcd for $C_{68}H_{96}N_6O_8$: C, 72.56; H, 8.60; N, 7.47; Found: C, 72.52; H, 8.57; N, 7.51; IR ν_{max} (Nujol)/(cm^{-1})1731, 1738, 3370 ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.65 (s, 6H), 0.90 (bs, 12H), 3.60 (m, 2H), 3.96 (s, 2H), 4.48 (d, 2H, *J*= 14.7 Hz), 5.02 (d, 2H, *J*= 14.7 Hz), 6.90 (m, 2H), 7.29 (s, 5H), 7.32 (s, 5H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.6, 17.2, 22.6, 23.1, 23.7, 26.1, 27.2, 27.5, 27.6, 28.4, 29.1, 29.3, 29.6, 30.5, 34.1, 35.1, 35.3, 36.0, 36.3, 36.4, 42.1, 46.5, 46.8, 48.0, 52.7, 56.4, 56.6, 71.7, 73.0, 121.5, 121.6, 128.01, 129.2, 133.5, 145.2, 173.3, 173.33; MS (LCMS**)** *m/z* 1148 $[M+Na]^+$.

Compound 41: (***Methyl 3α,12α-dihydroxy-5β-cholane-24-oate*****)** To a solution of deoxycholic acid **36** (0.3 g, 0.74 mmol) in dry methanol (10 mL) was added *p*-TSA (0.03

g, 0.17 mmol). The mixture was stirred at 28 °C for 24 hrs. Methanol was evaporated and the residue was extracted with DCM (3x50 mL). The organic extract was washed with cold H_2O (2x10 mL), 10 % $NaHCO_3$ (2x10 mL), brine (2x10 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product. Purification by column chromatography on silica gel (5 %, MeOH/$CHCl_3$) afforded compound **36** (0.3 g, 98 %) as a white solid; mp 82-105 ºC (lit.[64a] mp 70-108 ºC); IR (cm^{-1}): 3385, 1728; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.98 (d, J = 6.4 Hz, 3H, CH_3-21), 3.62 (m, 1H, CH-3), 3.67 (s, 3H) 3.98 (bs, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.6, 17.1, 23.0, 23.6, 26.0, 27.1, 27.4, 28.4, 29.6, 30.1, 30.8, 31.0, 33.4, 34.0, 35.2, 35.9, 36.2, 42.0, 46.3, 47.0, 48.0, 51.4, 71.4, 72.9, 174.7.

Compound 42: (*3α,12α,24-Trihydroxy-5β-cholane*) Compounds **42** was synthesized in overall good yield starting from methyl esters **41** of deoxycholic acid using the literature procedure.[64b] To a stirred suspention of $LiAlH_4$ (0.076g, 2mmol) in dry THF (10 mL), compound **42** (0.406 g, 1 mmol) in dry THF (20 mL) was added dropwise at 25 ºC. After 2 h saturated NH_4Cl solution was added to the ice cooled reaction mixture. It was filtered through celite and residue was washed with THF (25 mL). Solvent was evaporated under reduced pressure and it was extracted with EtOAc (3x50 mL). Extract was washed with water (2x25 mL) and brine (25 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to afford crude product which was purified by column chromatography on silica gel (5% MeOH/DCM) to produce compound **42** as white solid (0.363g, 96%); mp 123-124 ºC (lit.[64c] mp 107-114 ºC, lit.[64d] mp 123 ºC); IR (cm^{-1}) 3257; - ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, J = 6.9 Hz, 3H), 3.61 (m, 3H), 4.00 (bs, 1H), ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.7, 23.1, 23.7, 26.2, 27.2, 27.6, 28.5, 29.4, 30.4, 31.8, 33.6, 34.1, 35.3, 35.4, 36.1, 36.4, 42.1, 46.5, 47.5, 48.2, 63.4,

71.8, 73.3. Anal. Calcd for $C_{24}H_{42}O_3$: C, 76.14; H, 11.18; Found: C, 75.85 H, 10.87; MS (LCMS) *m/z*: 379.61 (M + 1).

Compound 43 and 44: To a solution of **42** (2.0 g 5.28 mmol) in dry DCM (20 mL), was added triethylamine (1.5 mL, 0.56 mmol) at 0 ºC. Methane sulfonyl chloride (0.53 mL, 6.86 mmol in 10 mL DCM) was added dropwise in 10 min at 0 ºC, ice was added to the reaction mixture immediately after addition was complete. The reaction mixture was extracted with DCM. Organic layer was washed with $NaHCO_3$, water and brine. Solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (0.5% MeOH/DCM) to obtain pure products **43** (0.615 g) and **44** (1.805 g).

Compound 43: (*3α,24-dimesyloxy-12α-hydroxy-5β-cholane*) White solid; mp 67-68 ºC; $[\alpha]_D^{27}$ ($CHCl_3$, c 2.7) = + 43.25; IR (cm^{-1}): 3566; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.92 (s, 3H, CH_3-19), 1.00 (d, *J* = 6.3 Hz, 3H, CH_3-21), 3.00 (s, 3H), 3.01 (s, 3H), 4.00 (s, 1H, CH-12), 4.21 (t, *J* = 6.7 Hz, 2H), 4.65 (m, 1H, CH-3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.6, 17.4, 22.7, 23.4, 25.7, 25.8, 26.6, 27.4, 27.5, 28.5, 31.2, 33.1, 33.4, 33.7, 34.7, 34.9, 35.7, 37.2, 38.7, 41.9, 46.3, 47.1, 47.9, 70.6, 72.7, 82.7; Anal. Calcd for $C_{26}H_{46}O_7S_2$: C, 58.39; H, 8.67; S, 11.99; Found: C, 58.22; H, 8.39; S, 12.16; MS (LCMS) *m/z*: 557.32 $(M + Na)^+$.

Compound 44: (*3α,12α-Dihydroxy 24-mesyloxy-5β-cholane*) White solid; mp 78 ºC; $[\alpha]_D^{26}$ ($CHCl_3$, c 0.2) = + 93.04; IR (cm^{-1}): 3419, 1416, 1448 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J* = 6.3 Hz, 3H, CH_3-21), 3.00 (s, 3H) 3.60 (m, 1H, CH-3), 3.97 (s, 1H, CH-12), 4.19 (t, *J* = 6.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.6, 17.4, 23.0, 23.6, 25.9, 26.0, 27.0, 27.5, 28.6, 30.3, 31.4, 33.5,

34.0, 35.0, 35.2, 35.9, 36.3, 37.3, 42.0, 46.4, 47.3, 48.1, 70.5, 71.6, 73.0; Anal. Calcd for $C_{25}H_{44}O_5S$: C, 65.75; H, 9.71; S, 7.02; Found: C, 65.45; H, 9.53; S, 7.28; MS (LCMS) *m/z*: 457.19 $(M + 1)^+$, 479.13 $(M + Na)^+$.

Compound 45: (*3α,12α-dihydroxy 24-azido-5β-cholane*) To a solution of **44** (0.300 g, 0.66 mmol) in dry DMF (10 mL), sodium azide (0.214 g, 3.28 mmol) was added and the reaction mixture was stirred at 60-65 ºC for 3-5h. The reaction mixture was then allowed to cool to room temperature, poured into ice-cold water (30 mL) and extracted with EtOAc. The organic extract was washed with cold water and brine. Solvent was evaporated under reduced pressure to afford crude product which was purified by column chromatography on silica gel (10% EtOAc/hexane) to give pure compound **45** as white solid (0.247 g, yield 94%); mp 126 ºC; $[\alpha]_D^{28}$ ($CHCl_3$, c 1.4) = + 40.57; IR (cm^{-1}): 2090, 3409; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J* = 6.7 Hz, 3H, CH_3-21), 3.24 (t, *J* = 7.1 Hz, 2H), 3.62 (m, 1H, CH-3), 4.00 (bs, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.6, 23.1, 23.6, 25.6, 26.1, 27.1, 27.5, 28.7, 30.5, 32.9, 33.7, 34.1, 35.3, 36.1, 36.5, 42.2, 46.5, 47.5, 48.3, 51.9, 71.7, 73.2; Anal. Calcd for $C_{24}H_{41}N_3O_2$: C, 71.42; H, 10.24; N, 10.41; Found: C, 71.19; H, 10.46; N, 10.38; MS (LCMS) *m/z*: 404.88 $(M + 1)^+$, 426.83 $(M + 23)^+$.

Experimental procedure for the preparation bistriazole using $CuSO_4$/Cu and 1.5 M K_2CO_3 (Scheme 5) (Entry 5, Table 2).

Compound 46 and 47: The propargyl ester of deoxycholic acid **37** (430 mg, 1.0 mmol) was added to a mixture of C-24 azide of deoxycholic acid **45** (403 mg, 1.0 mmol) in THF 1.5 mL and aqueous K_2CO_3 solution (1.5 M, 1.5 mL). Copper sulphate (1.0 M, 0.1 mL) was added to the above suspension fallowed by copper powder (64.0 mg, 1.0 equiv) and

the resulting suspension was stirred at 27 °C for 12h. After completion of reaction the reaction mixture was filtered through Celite pad. The solution was concentrated and the residue was extracted with $CHCl_3$ using the standard work up procedure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 95:5) to furnish compound **46** and **47** in 19 and 72% yield, respectively as a white solid.

Compound 46: Mp 135–138 °C (lit.[29] 136–138 ºC); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.65 (s, 3 H), 0.66 (s, 3 H), 0.90–0.98 (s, 12 H,), 3.59 (m, 2 H), 3.96 (br s, 2 H), 4.31 (t, J = 7.07 Hz, 2 H, OCH2), 5.21 (s, 2 H), 7.60 (s, 1 H, triazole H). The other spectroscopic data was consistent with that reported in literature.[29]

Compound 47: Mp 157-159 ºC; $[\alpha]_D^{26}$ + 6.25 (*c* 1. 12, MeOH); Anal. Calcd for $C_{102}H_{164}N_6O_{12}$: C, 73.52; H, 9.92; N, 5.04; Found: C, 73.32; H, 10.01; N, 5.02; IR ν_{max} (Nujol)/(cm^{-1}) 1306, 1377, 1463, 1634, 1737, 3369; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.65 (s, 12H), 0.90 (s, 12H), 0.94 (bs, 12H), 2.19 (m, 4H), 3.55 (bs, 4H), 3.94(d, 4H, J = 9.37 Hz), 4.02 (m, 4H), 5.04-5.13 (m, 4H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.5, 16.9, 17.2, 22.9, 23.6, 26.1, 26.3, 27.0, 27.02, 27.4, 27.45, 27.5, 28.2, 28.3, 28.33, 29.8, 29.9, 30.4, 30.5, 32.5, 33.3, 34.0, 35.0, 35.1, 35.2, 35.21, 35.8, 35.9, 41.9, 46.2, 46.3, 46.5, 46.6, 46.64, 46.7, 47.7, 47.8, 49.5, 49.6, 49.7, 49.8, 53.3, 56.5, 56.7, 71.3, 72.7, 72.7, 72.8, 121.9, 122.0, 143.6, 143.7 173.46, 173.49; MS (LCMS)$^+$ *m/z* 1690.09 $[M+Na]^+$.

Compound 48: A mixture of compound **47** (0.167 g, 0.1 mmol), Ac_2O (0.19 ml, 2 mmol), DMAP (20%) dry pyridine (10 mL) was refluxed for 18h. The solution was concentrated and the residue was extracted with ethyl acetate using the standard work up procedure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 99:1) to furnish compound **48** (0.124 g, 62%), as a white solid. mp 151-153 ºC; $[\alpha]_D^{26}$ + 91 (*c* 2.2, $CHCl_3$); Anal. Calcd. for $C_{118}H_{180}N_6O_{20}$: C, 70.77; H, 9.06; N,

4.20; Found: C, 70.87; H, 9.30; N, 4.31; IR ν_{max} (Nujol)/(cm^{-1}) 1028, 1243, 1377, 1460, 1736; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.69 (bs, 12H), 0.73-0.76 (m, 12H), 0.89 (s, 12H), 2.02 (s, 12H), 2.09 (d, 6H, *J*= 4.36 Hz), 2.18 (m, 2H), 3.89-4.02 (m, 4H), 4.68 (m, 4H), 5.02-5.05 (m, 8H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 12.4, 17.4, 17.7, 21.4, 23.0, 23.4 25.6, 25.8, 26.5, 26.8, 27.3, 27.4, 30.5, 30.7, 32.2, 32.6, 33.9, 34.3, 34.6, 34.7, 35.6, 41.7, 44.9, 47.6, 47.7, 49.3, 49.4, 46.7, 74.1, 75.7, 75.8, 121.9, 143.8 170.3, 170.4, 170.5, 173.1; MS (MALDI-TOF) *m/z* 2024.98 $[M+Na]^+$, 2041 $[M+K]^+$.

Compound 49: Compopund **47** (0.167 g, 0.1 mmol) and 2-Iodoxybenzoic acid (IBX) (0.57 g, 2 mmol) were dissolved in dry DMSO (2 mL) and the reaction mixture was stirred at 27 ºC for 6 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (200 mL). The organic phase was washed successively with H_2O, and brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated to afford crude product. The crude product was purified by flash chromatography on silica gel (DCM/MeOH, 99:1) to furnish compound **49** (0.120 g, 73%), as a white solid. mp 154-157 ºC; $[\alpha]_D^{26}$ + 86.88 (*c* 1.22, $CHCl_3$); Anal. Calcd. for $C_{102}H_{148}N_6O_{12}$: C, 74.23; H, 9.04; N, 5.09; Found: C, 74.66; H, 9.12; N, 4.91; IR ν_{max} (Nujol)/(cm^{-1}) 1167, 1268, 1378, 1460, 1739, 1704; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79-82 (m, 12H), 1.03 (d, 12H, *J*= 2.62 Hz), 1.10 (s, 12H), 2.59 (m, 8H), 4.02 (m, 4H), 5.01-5.11 (m, 4H); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ 11.6, 11.7, 18.5, 18.8, 22.1, 24.3, 25.4, 26.1,26.7, 26.9, 27.4, 27.6, 27.63, 29.6, 29.6, 30.1, 30.9, 31.6, 31.9, 32.3, 35.3, 35.4, 35.5, 35.6, 36.7, 36.7, 36.9, 38.2, 38.3, 42.1, 43.5, 43.6, 44.1, 46.2, 46.3, 46.4, 49.5, 53.4, 56.6, 56.7, 57.4, 57.5, 58.4, 122.0, 122.1, 143.78, 143.82, 173.16, 17317, 212.16, 212.22, 213.95, 214.08; MS (MALDI-TOF) *m/z* 1673 $[M+Na]^+$, 1689 $[M+K]^+$.

Antimicrobial activity

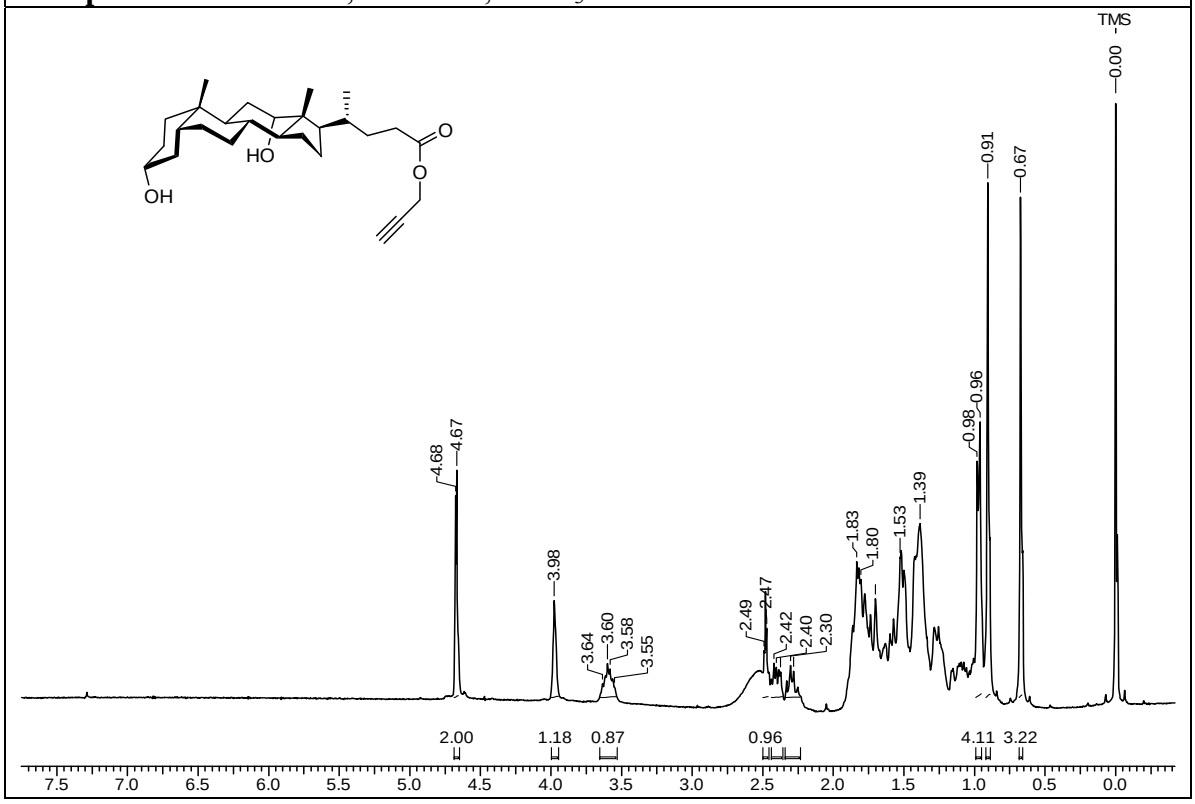
Materials and Methods. Human pathogens *C. albicans* and *C. neoformans*; saprophytes *B. poitrasii* and *Y. lipolytica* were maintained on YPG (yeast extract, 0.3%; peptone, 0.5%; and glucose, 1%) agar slants. *F. oxysporum* (plant pathogen) was maintained on PDA (potato, 20%; dextrose, 2%) agar slants at 28 ^{o}C. *E. coli* (NCIM 2574) and *S. aureus* (NCIM 2122) were maintained on NA (beef extract, 0.3%; peptone, 0.5%; sodium chloride, 0.5%) 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 ^{o}C where as *B. poitrasii* was incubated at 37 ^{o}C for 24 h. *F. oxysporum* was inoculated in potato dextrose and incubated at 28 ^{o}C for 48 h whereas bacterial strains *E. coli* and *S. aureus* in NA broth for 24 h. Compounds **36**, **39**, **40**, and **46-49** were solubilised in DMSO, and stock solutions of 1.28 mg/mL were prepared. Amphotericin B, Fluconazole, Tetracycline and Erythromycin 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* (NCIM 2574), and *S. aureus* (NCIM 2122), respectively to find out MIC (Minimum Inhibitory Concentration) and IC_{50} (50%, Inhibition of Growth) values. Experiments were performed 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.[44] 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

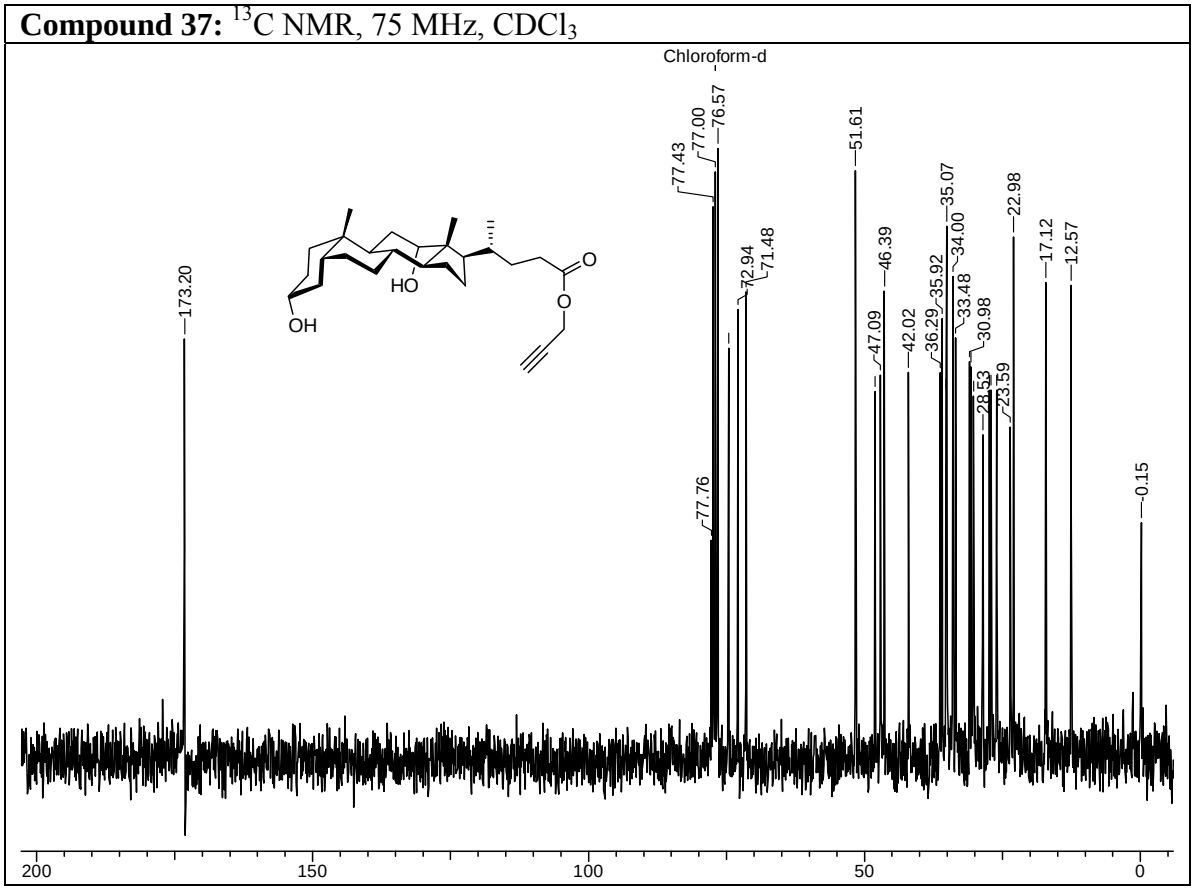
plates were incubated at 28 °C for all the microorganisms except for *B. poitrasii* (37 °C), absorbance at 600 nm were recorded to assess the inhibition of cell growth after 24 h for *B. poitrasii* and *Y. lipolytica*, 48 h for *C. albicans* and *F. oxysporum*, 72 h for *C. neoformans* and 24 h for bacterial 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.

3.8. Selected Spectras

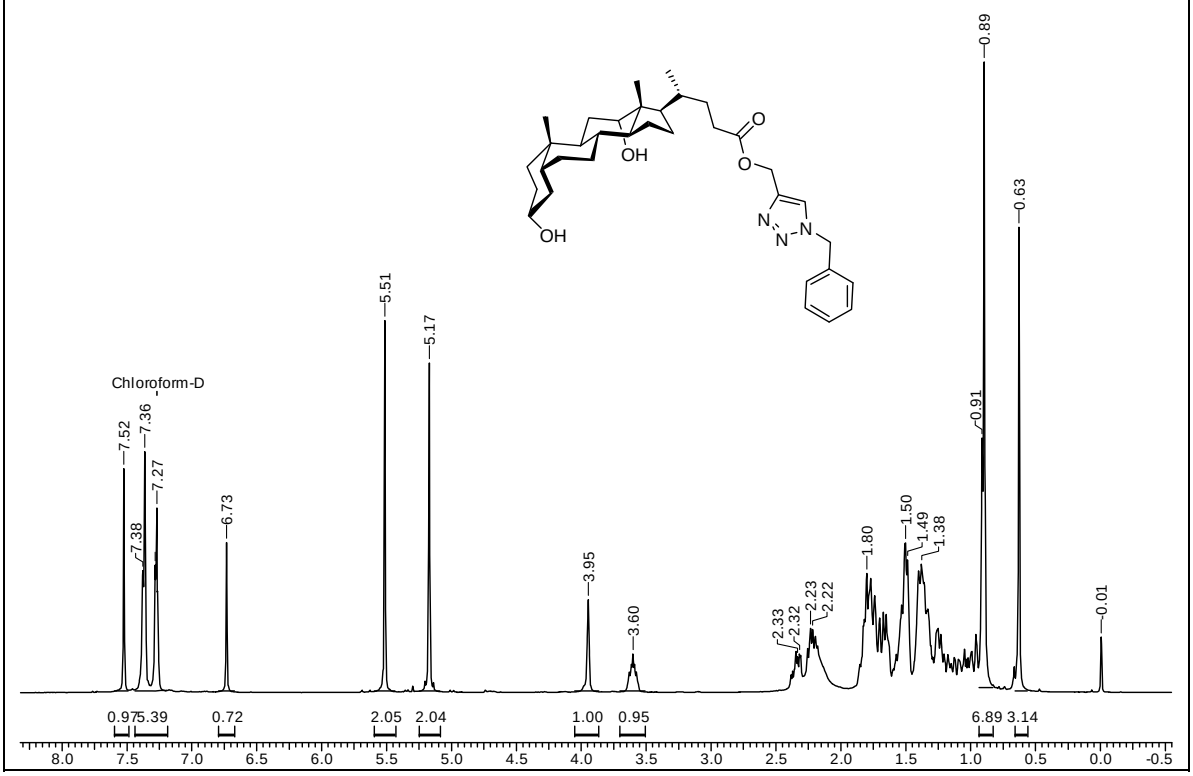
Compound 37: ^{1}H NMR, 200 MHz, $CDCl_3$



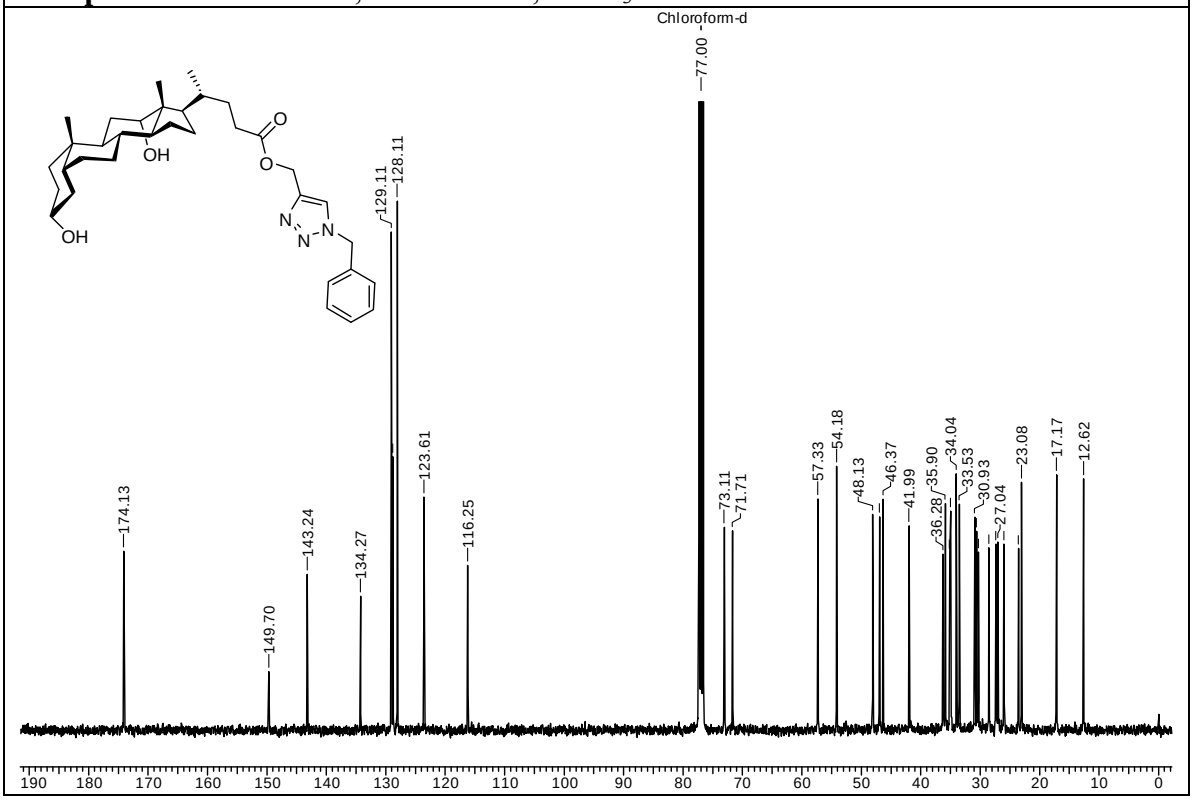
Compound 37: ^{13}C NMR, 75 MHz, $CDCl_3$



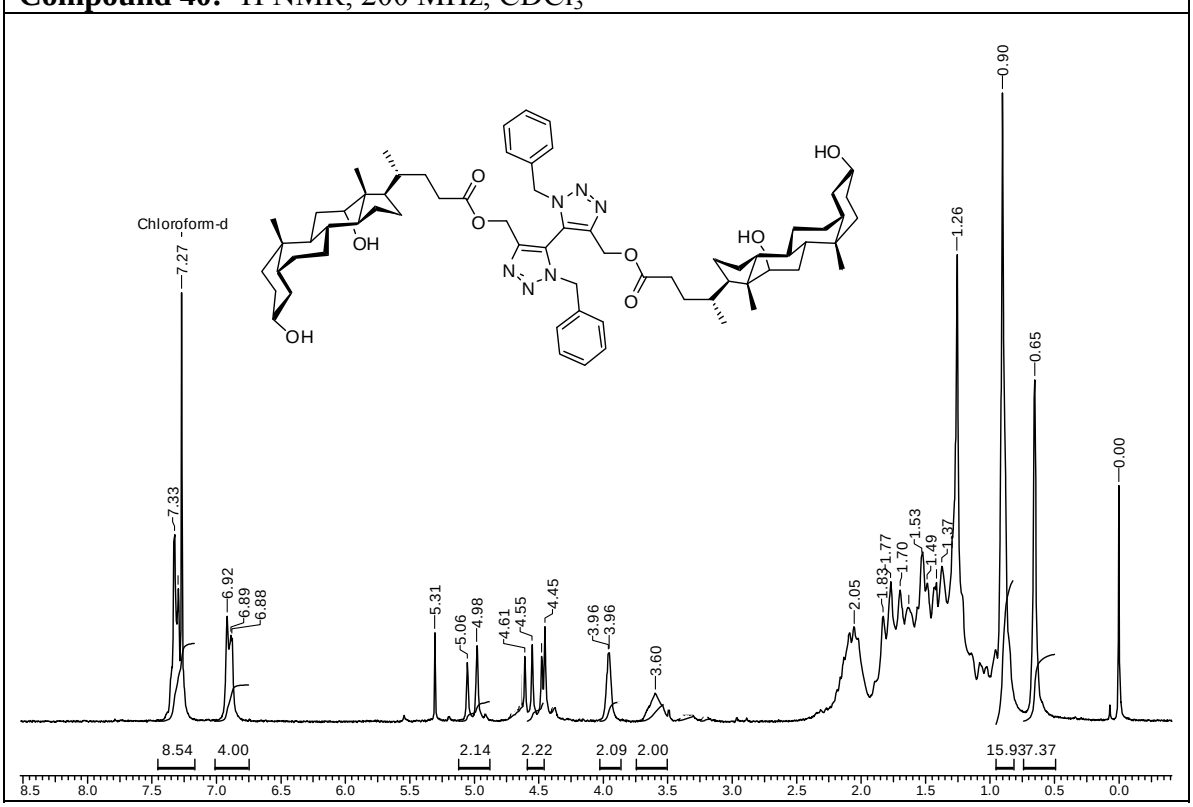
Compound 39: ^{1}H NMR, 200 MHz, $CDCl_3$



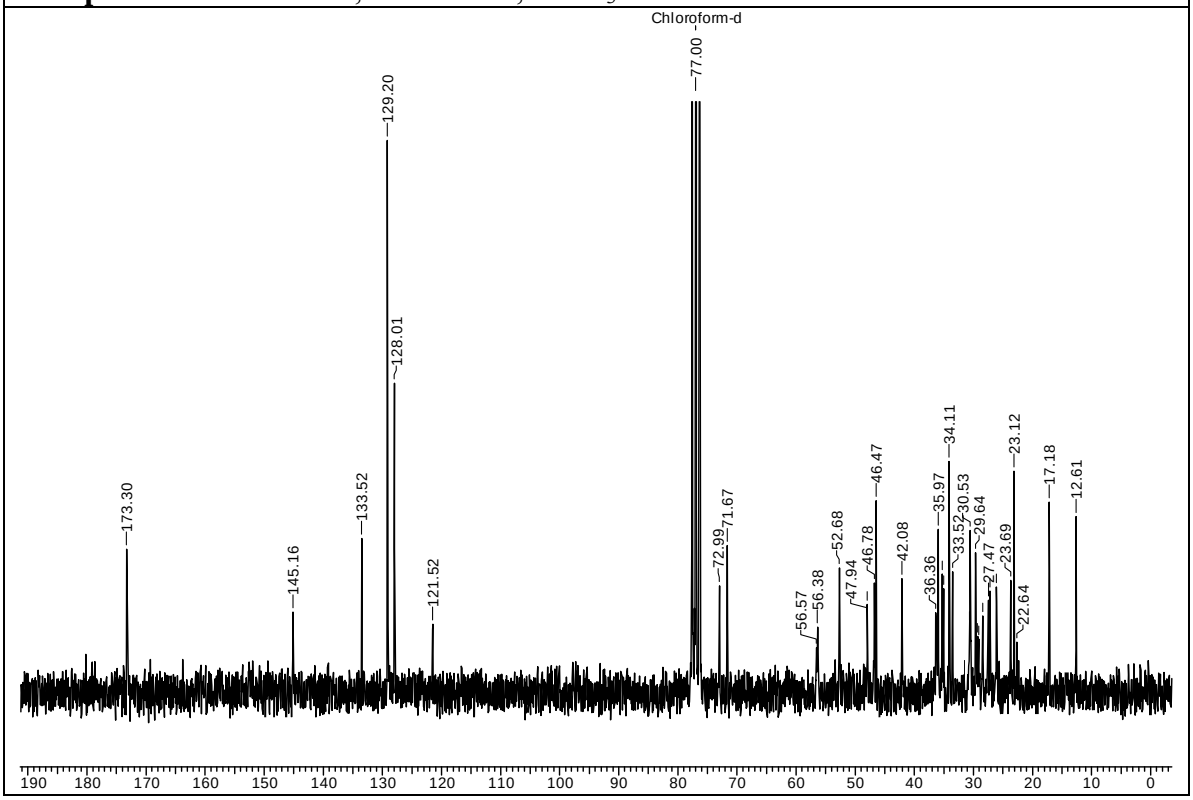
Compound 39: ^{13}C NMR, 125.76 MHz, $CDCl_3$



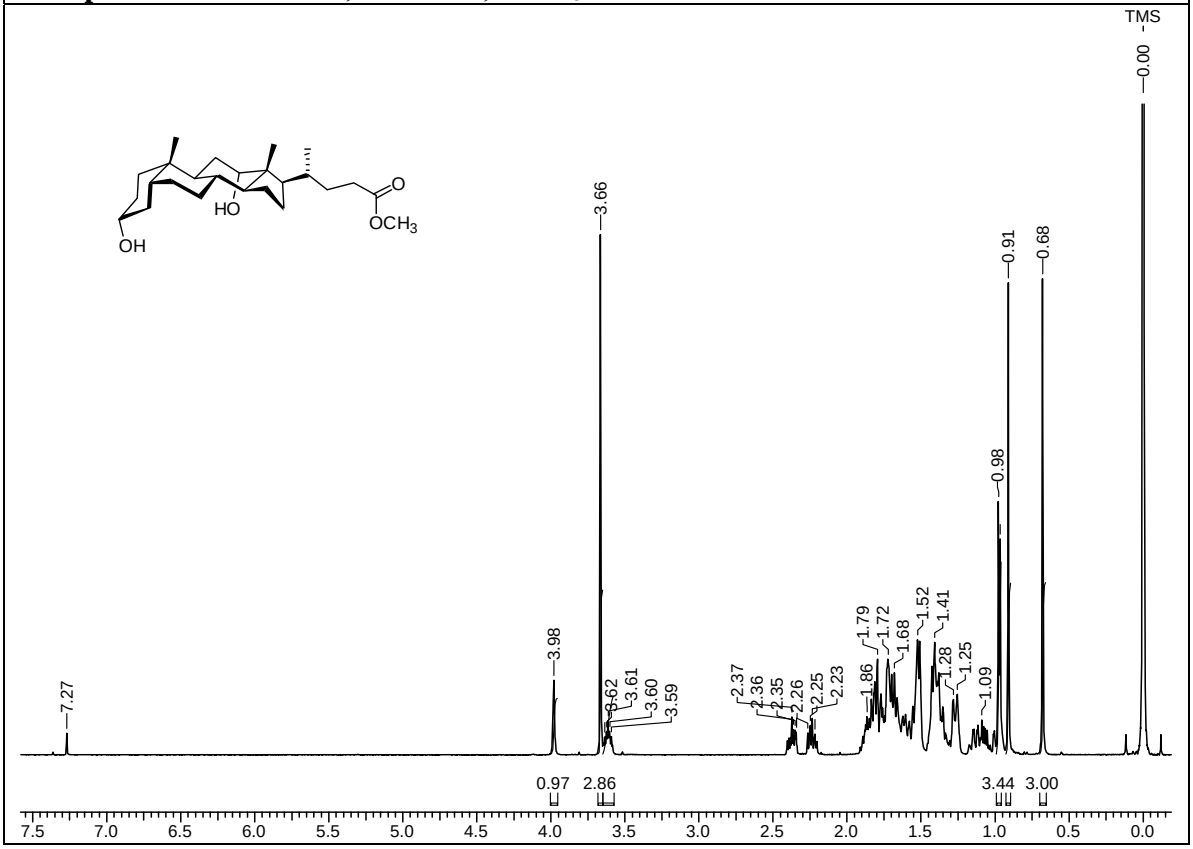
Compound 40: ^{1}H NMR, 200 MHz, $CDCl_3$



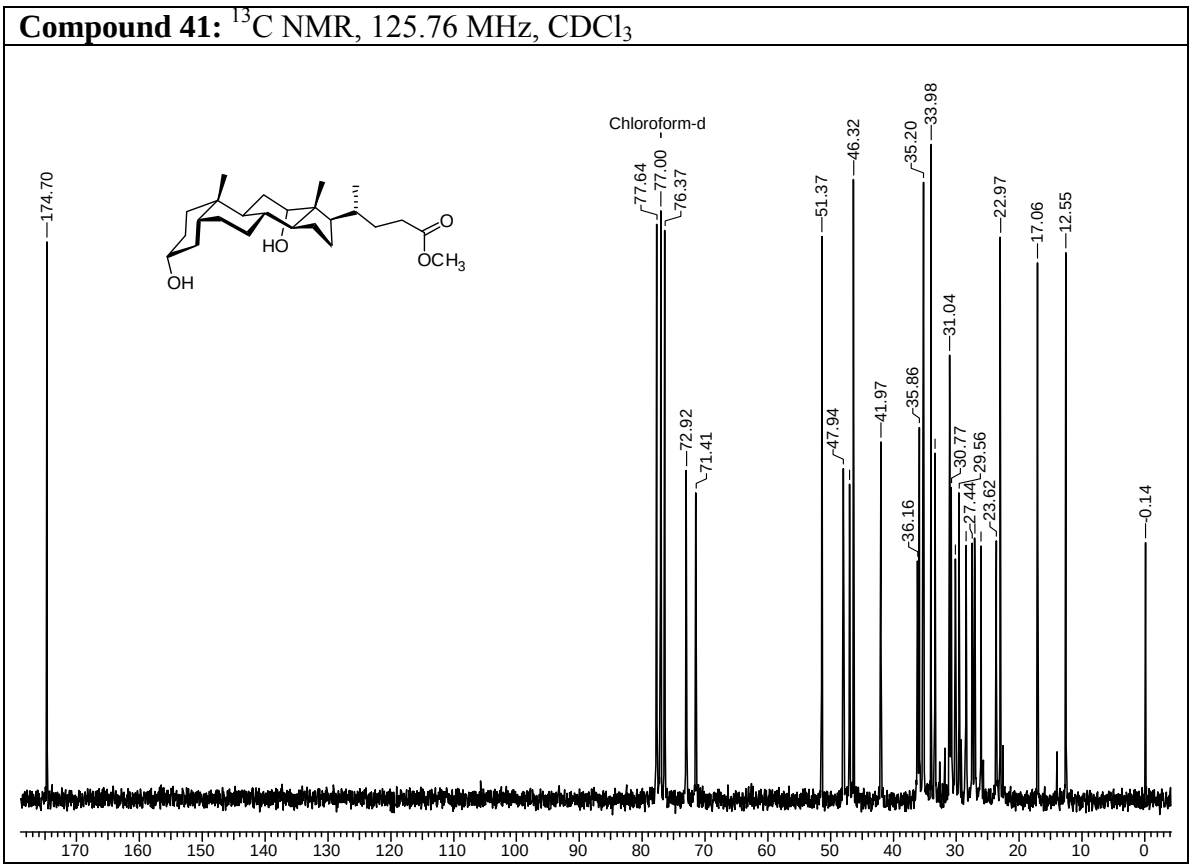
Compound 40: ^{13}C NMR, 50.32 MHz, $CDCl_3$



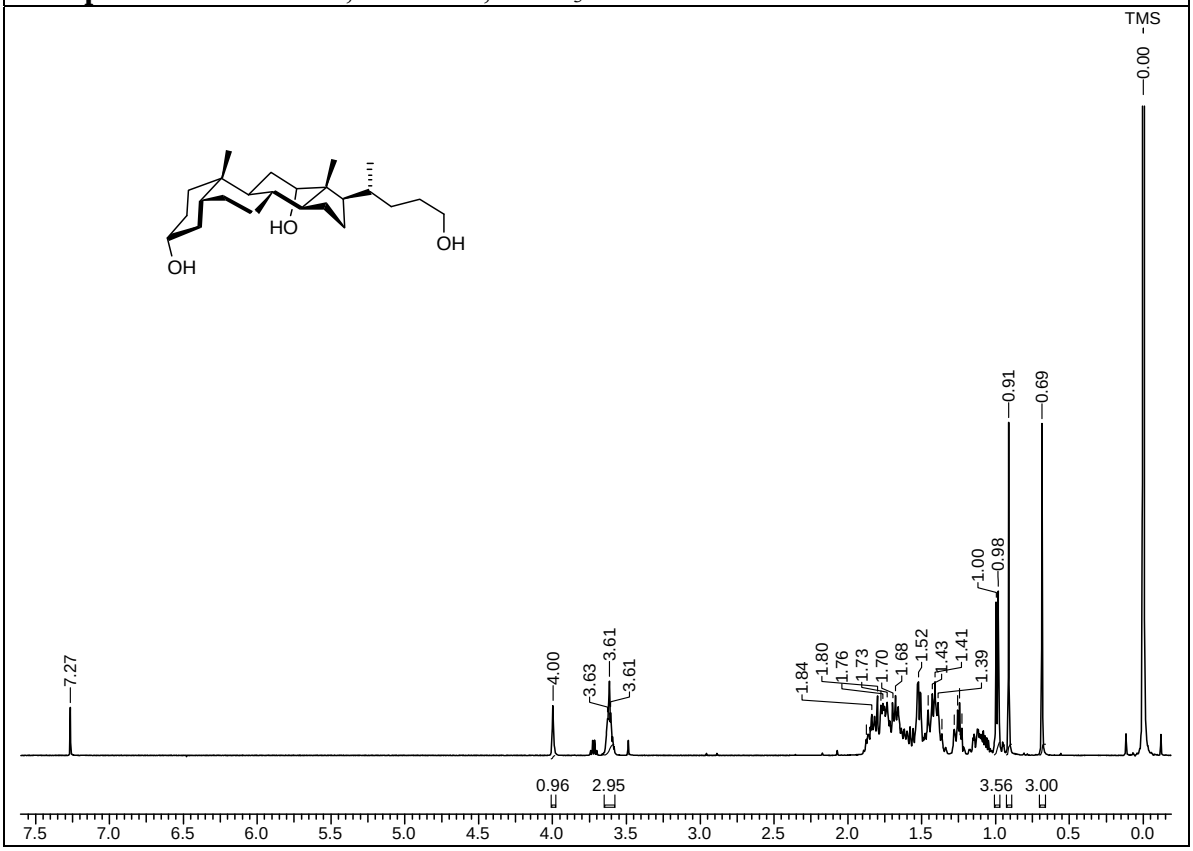
Compound 41: ^{1}H NMR, 500 MHz, $CDCl_3$



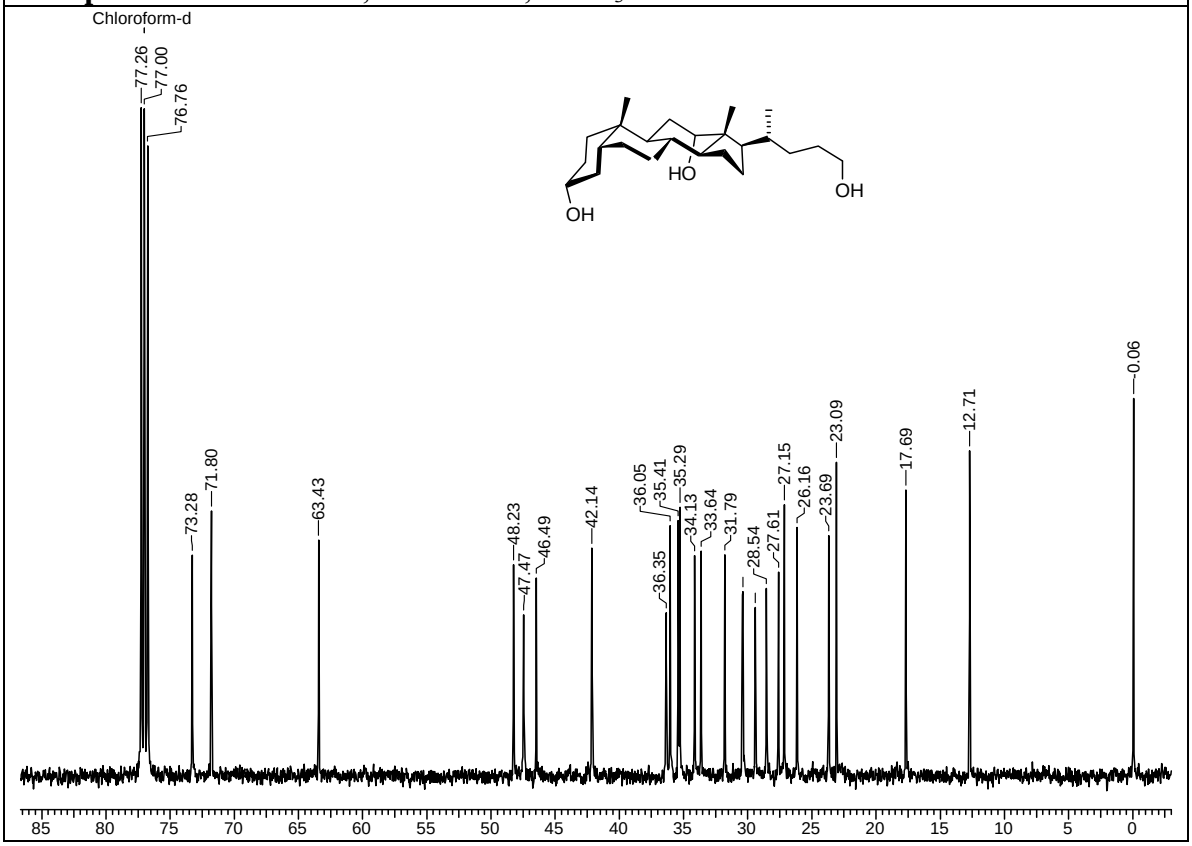
Compound 41: ^{13}C NMR, 125.76 MHz, $CDCl_3$



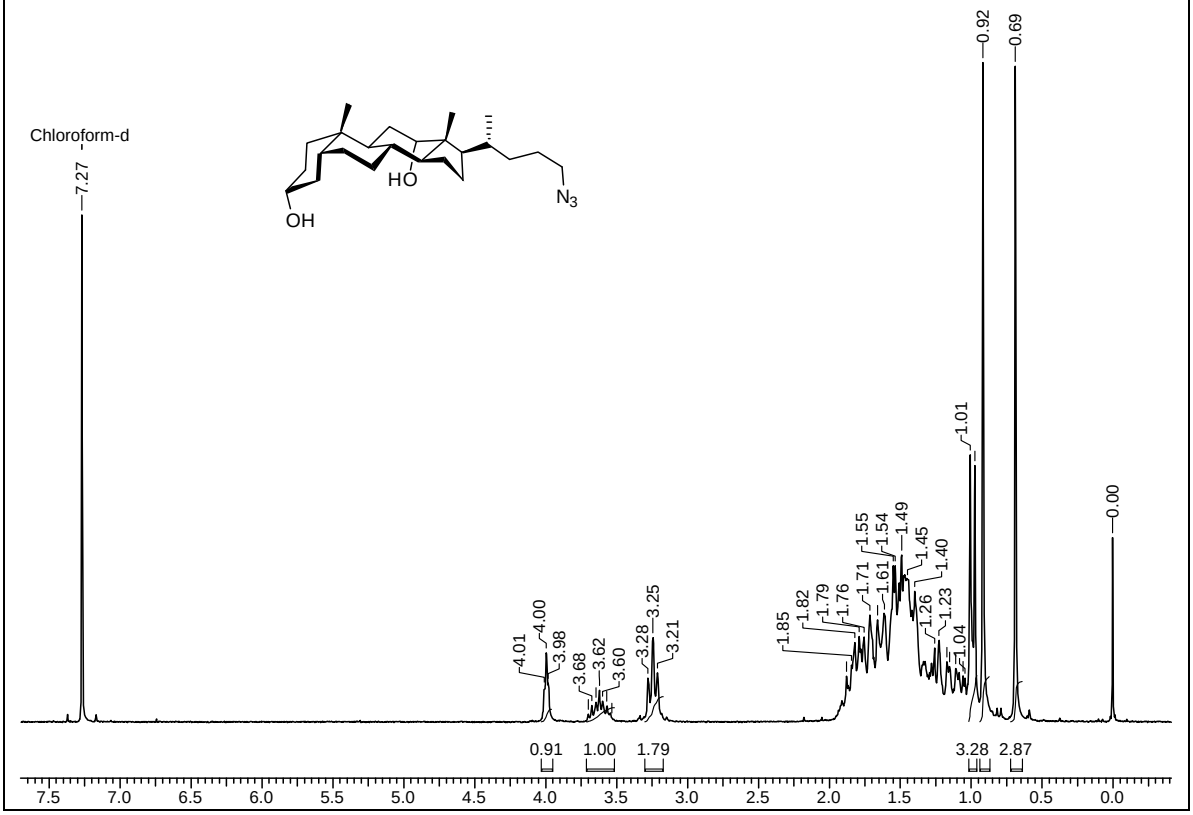
Compound 42: ^{1}H NMR, 200 MHz, $CDCl_3$



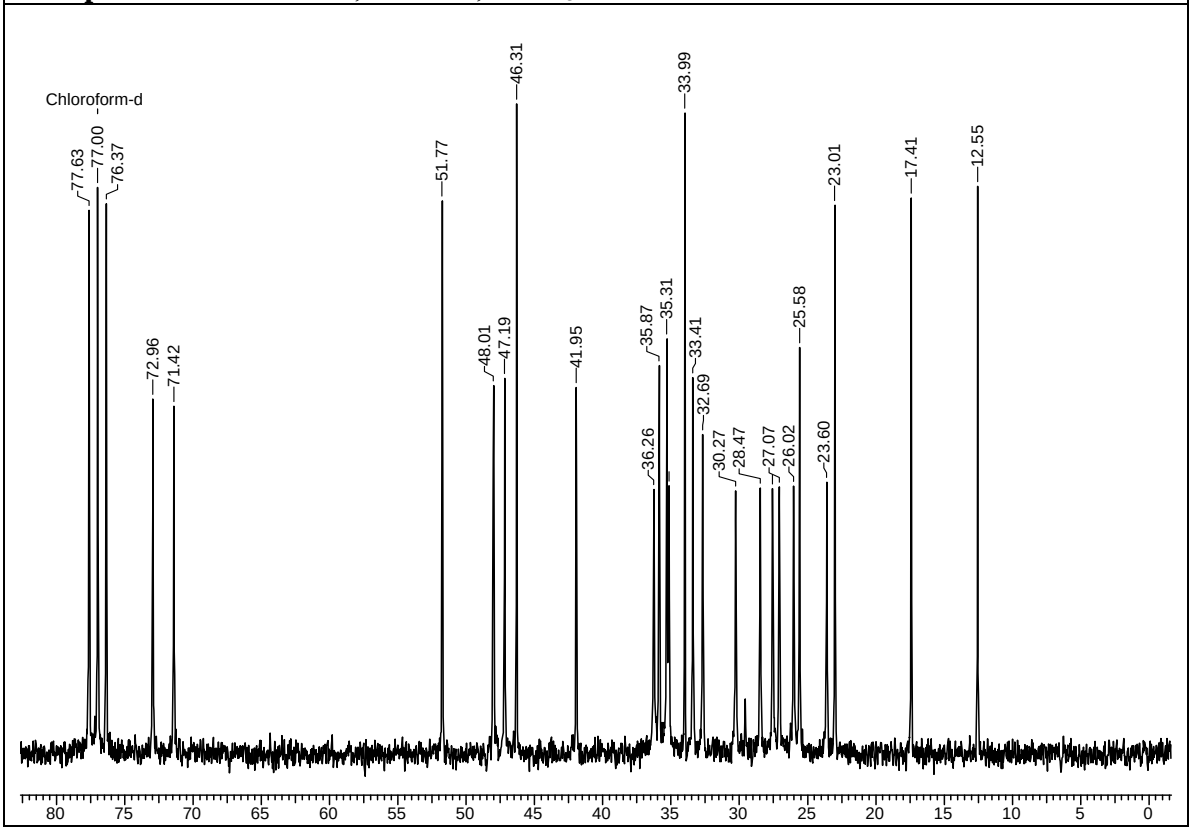
Compound 42: ^{13}C NMR, 50.32 MHz, $CDCl_3$



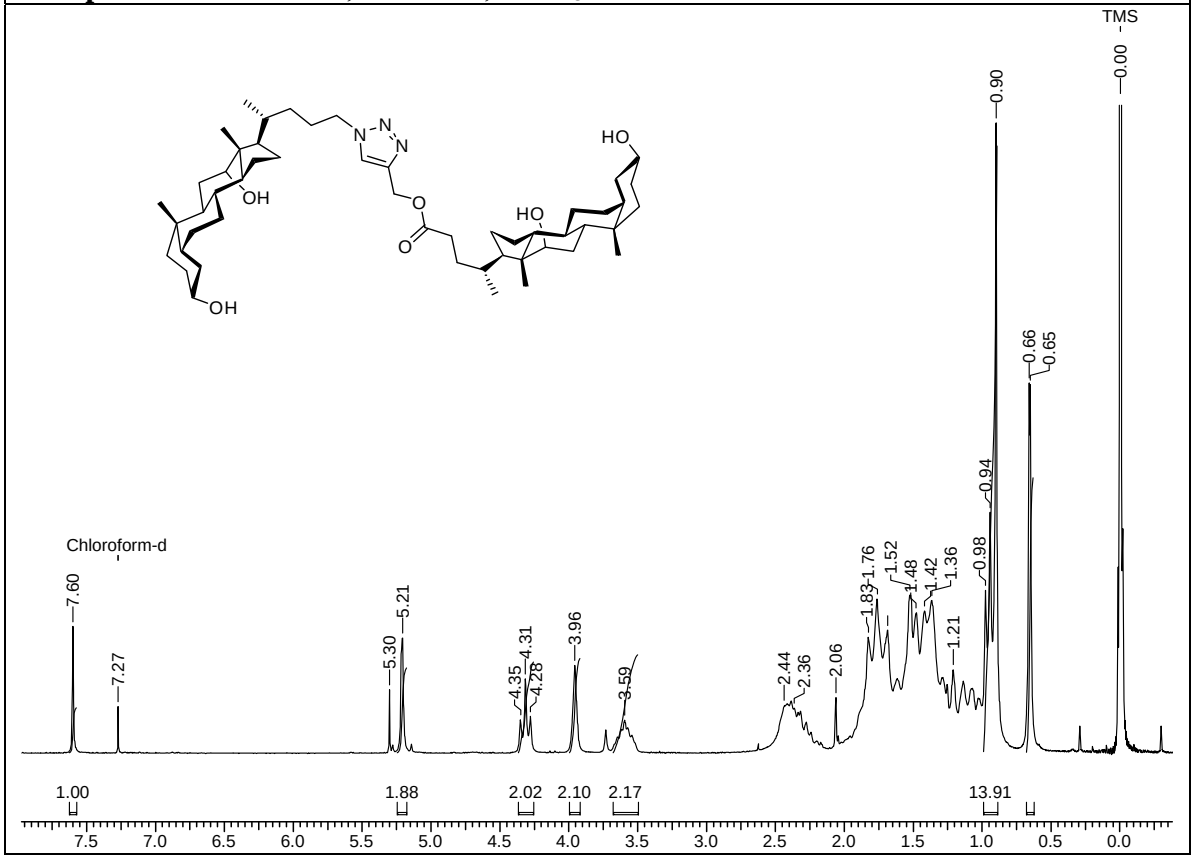
Compound 45: ^{1}H NMR, 200 MHz, $CDCl_3$



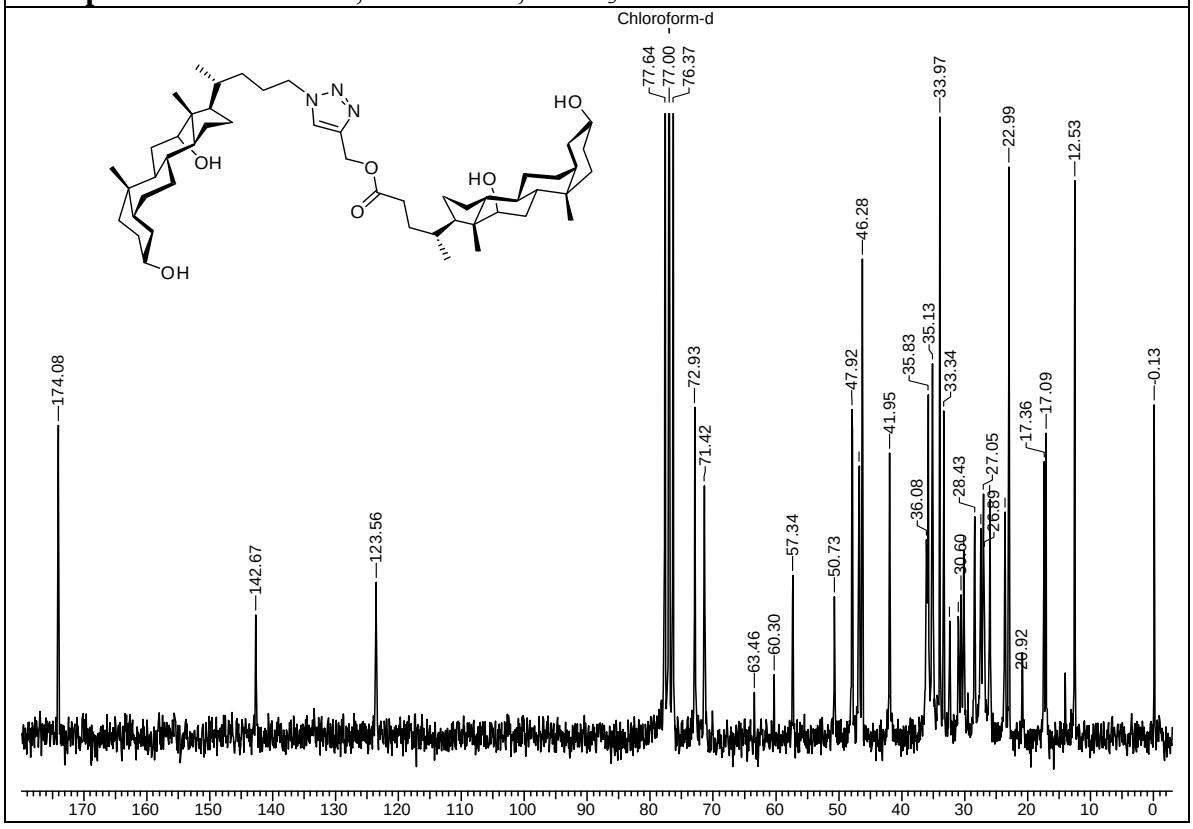
Compound 45: ^{13}C NMR, 50 MHz, $CDCl_3$



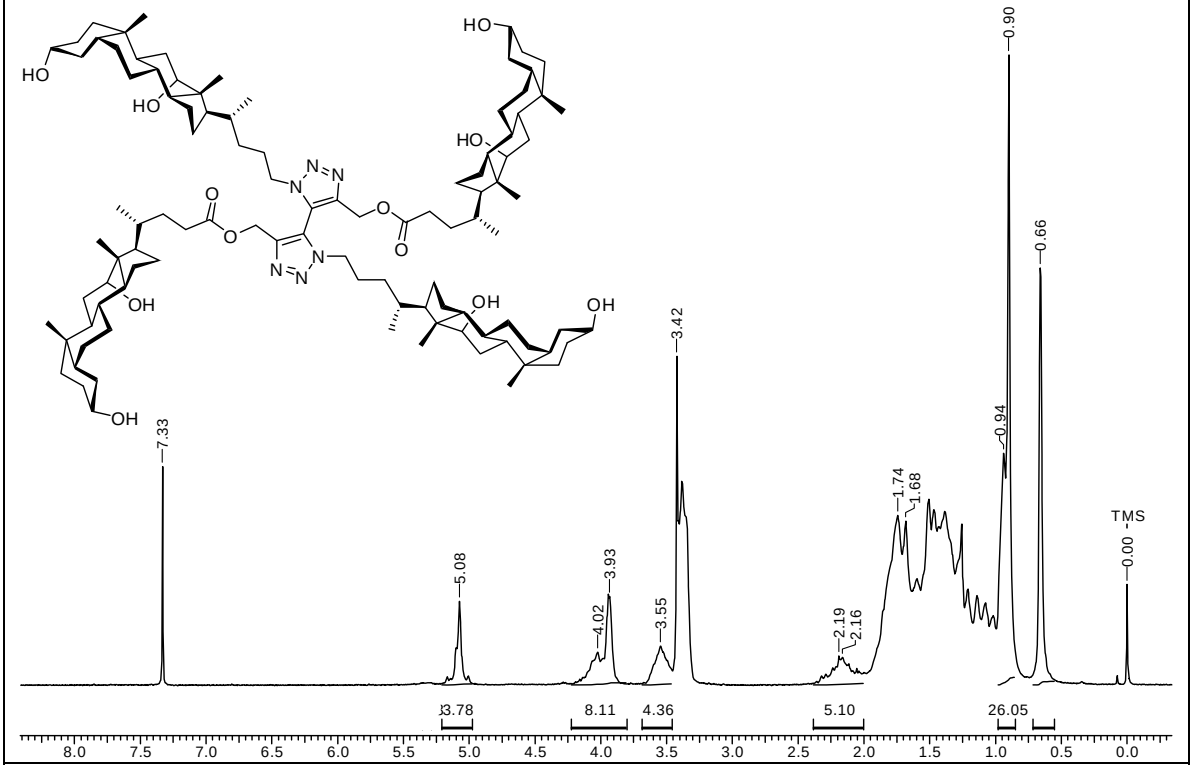
Compound 46: ^{1}H NMR, 200 MHz, $CDCl_3$



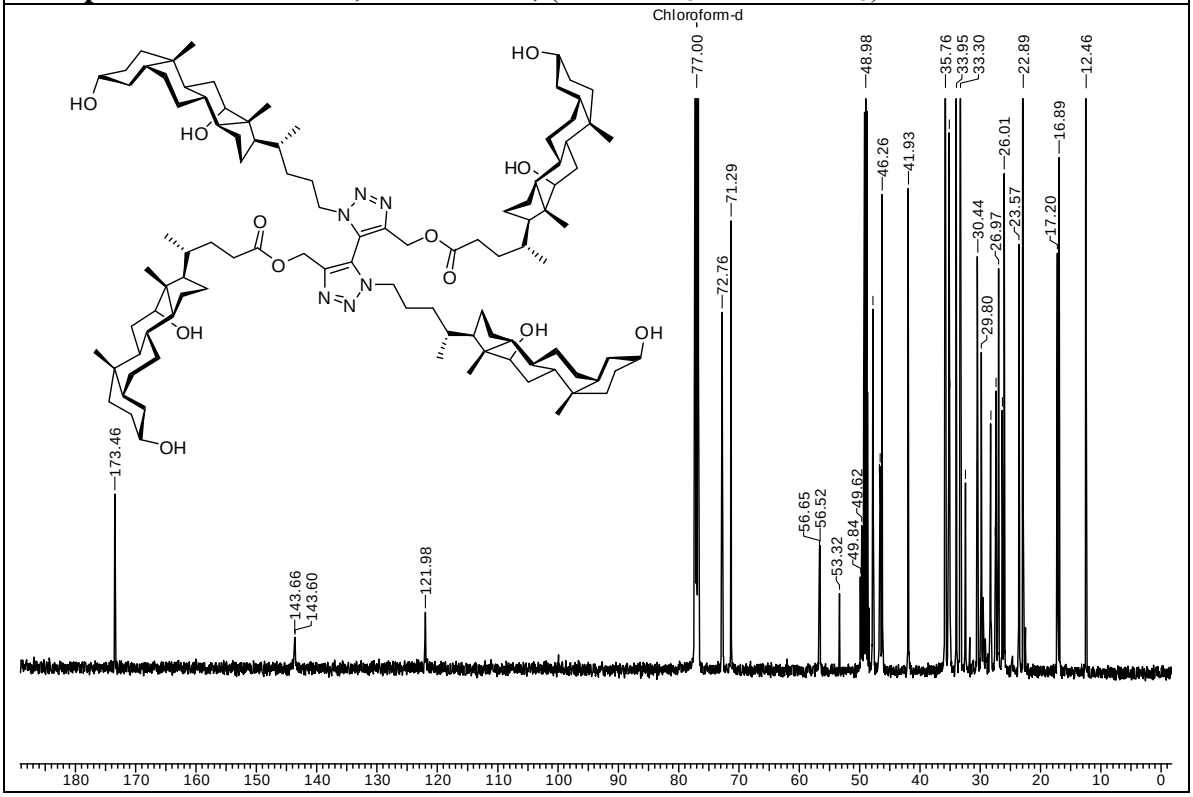
Compound 46: ^{13}C NMR, 50.32 MHz, $CDCl_3$



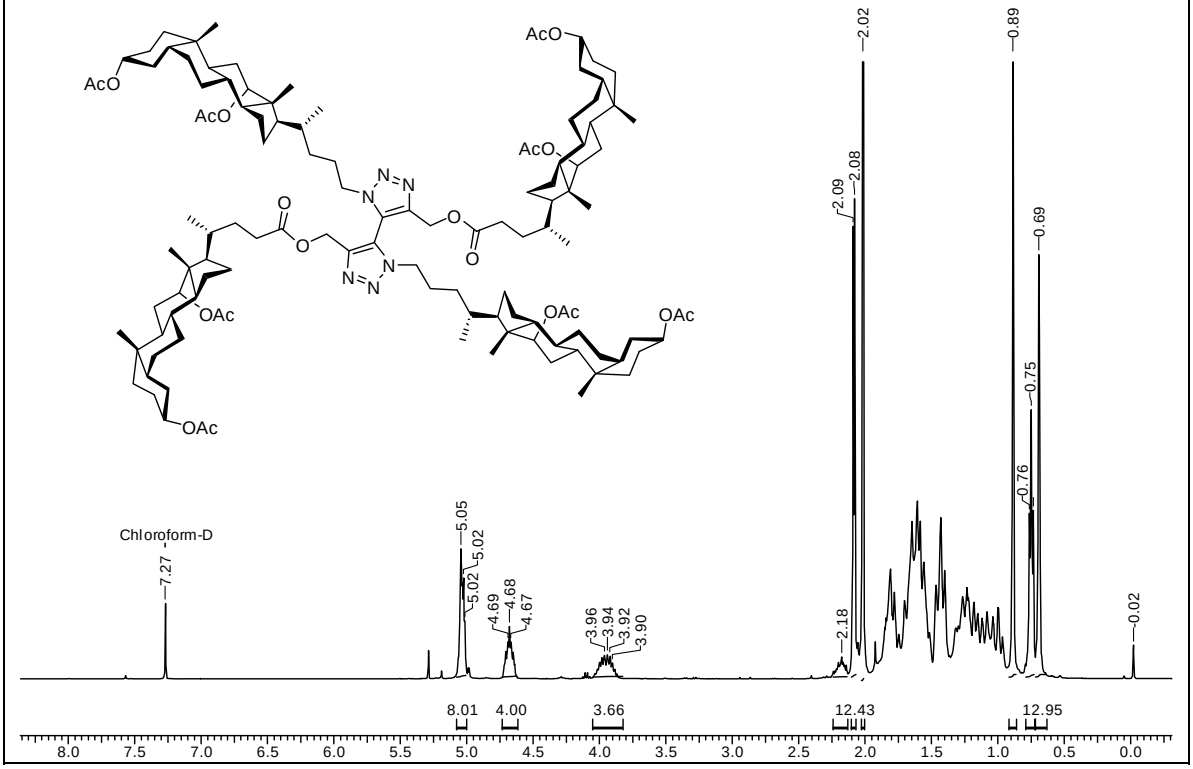
Compound 47: ^{1}H NMR, 200 MHz, $CDCl_3$



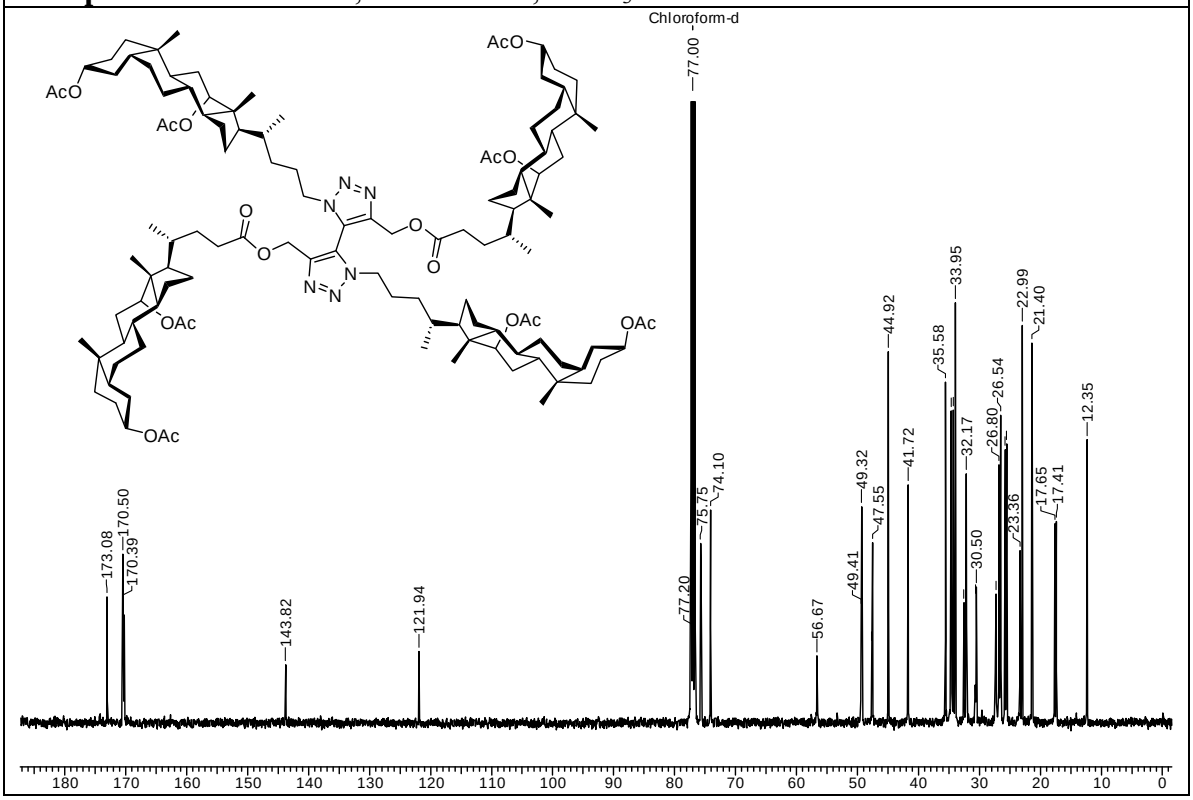
Compound 47: ^{13}C NMR, 100.61 MHz, (~30% CD_3OD in $CDCl_3$)



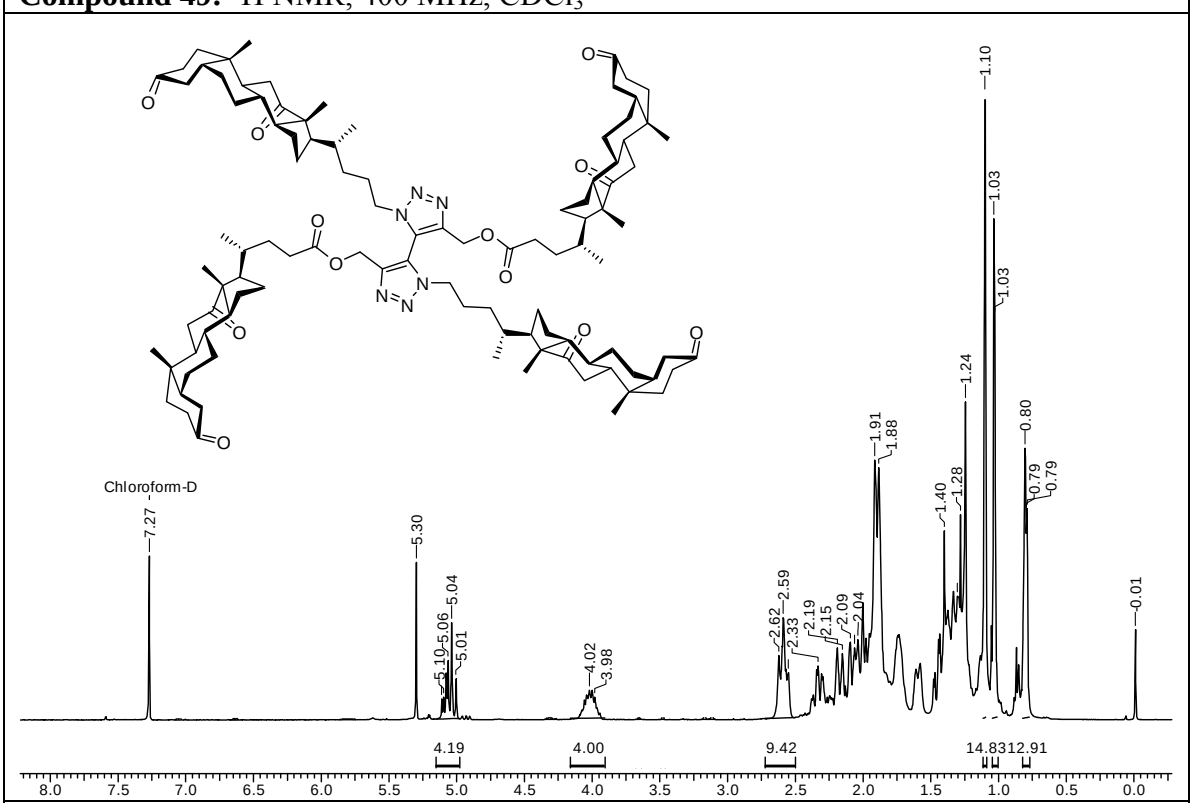
Compound 48: ^{1}H NMR, 400 MHz, $CDCl_3$



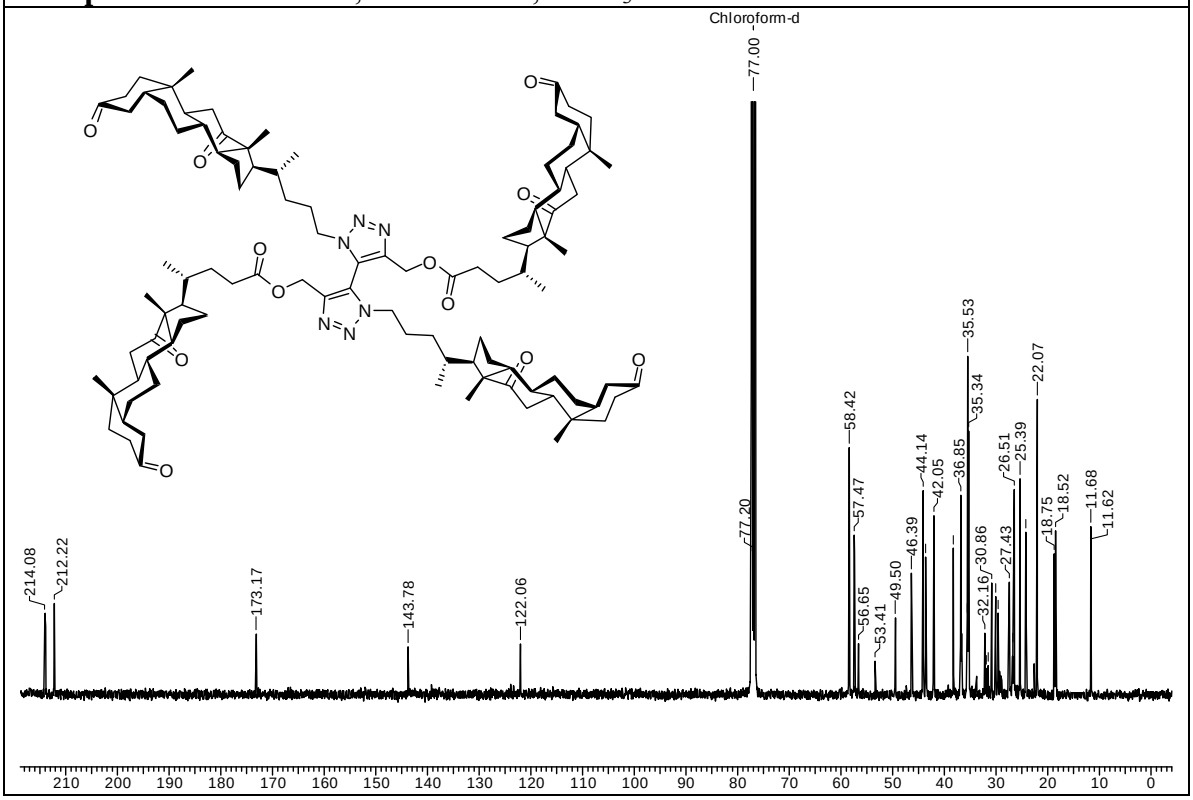
Compound 48: ^{13}C NMR, 100.61 MHz, $CDCl_3$



Compound 49: ^{1}H NMR, 400 MHz, $CDCl_3$



Compound 49: ^{13}C NMR, 100.61 MHz, $CDCl_3$



3.9. References

1. (a) Salunke, D. B.; Hazra, B. G.; Pore, V. S. *Curr. Med. Chem*. **2006**, *13*, 813; (b) Iuliano, A.; Facchetti, S.; Uccello-Barretta, G. *J. Org. Chem*. **2006**, *71*, 4943; (c) Davis, A. P. *Coord. Chem. Rev*. 2006, **250**, 2939; (d) Zhu, X.-X.; Nichifor, M. *Acc. Chem. Res*. **2002**, *35*, 539; e) Zhang, Z.; Ju, Y.; Zhao, Y. *Chem. Lett*. **2007**, *36*, 1450-1451.
2. Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem*. **2004**, 3385.
3. Enhsen, A.; Kramer, W and G. Wess, *Today* 1998, **3**, 409-418.
4. (a) Tamminen, J.; Kolehmainen, E.; Haapala, M.; Linnanto, J. *Synthesis* **2000,** 1464-1468; (b) Pandey, P. S.; Rai R.; Singh, R. B. *Tetrahedron* 2002, **58,** 355-362; (c) Davis, A. P.; Wareham, R. S. *Angew. Chem. Int. Ed.* 1999, **38**, 2978-2996.
5. (a) Yuexian, L.; Dias, J. R. *Chem*. *Rev*. 1997, **97**, 283-304 and references cited therein; (b) Davis, A. P. *Chem. Soc. Rev.* 1993**,** 243-253.
6. Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* 1989, **111**, 2900-2909.
7. Bonar-Law, R. P.; Sanders, J. K. *Tetrahedron Lett.* 1992, **33,** 2071-2074.
8. Banerji, J.; Chatterjee, A. *Indian J. Chem.* **1973**, *11*, 1056.
9. Li, Y.; Dias, J. R. *Chem. Rev.* **1997**, *97*, 283-304.
10. Gryszkiewicz-Wojtkielewicz, A.; Jastrzebska, I.; Morzycki, J.W.; Romanowska, D. B. *Curr. Org. Chem.* **2003**, *7*, 1257; (b) Flessner, T.; Jautelat, R.; Scholz, U.; Winterfeldt, E. *Prog. Chem. Org. Nat. Prod.* **2004**, *87*, 1.
11. Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006.

12. (a) D'Auria, M. V.; Giannini, C.; Zampella, A.; Minale, L.; Debitus, C.; Roussakis, C. *J. Org. Chem.* **1998**, *63*, 7382. (b) Zampella, A.; Giannini, C.; Debitus, C.; Roussakis, C.; D'Auria, M. V. *Eur. J. Org. Chem.* **1999**, 949.

13. Kobuke, Y.; Nagatani, T. *J. Org. Chem*. **2001**, *66*, 5094-5101.

14. Goto, C.; Yamamura, M.; Yoshino, N.; Satake, A.; Kobuke, Y. *J. Am. Chem. Soc*. **2001**, *123*, 12152-12159.

15. Yoshii, M.; Yamamura, M.; Satake, A.; Kobuke, Y. *Org. Biomol. Chem.* **2004**, *2*, 2619-2623.

16. Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1573-1574.

17. (a) Vijayaraghavan, S.; Jing, B.; Vrablik, T.; Chou, T. -C.; Regen, S. L. *Bioconj. Chem.* **2003**, *14*, 667-671. (b) Jing, B.; Janout, V.; Herold, B. C.; Klotman, M. E.; Heald, T.; Regen, S. L. *J. Am. Chem. Soc.* **2004**, *126*, 15930-15931.

18. (a) Janout, V.; Jing, B.; Staina, I. V.; Regen, S. L. *J. Am. Chem. Soc*. **2003**, *125*, 4436-4437.; (b) Janout, V.; Jing, B.; Regen, S. L. *J. Am. Chem. Soc*. **2005**, *127*, 15862-15870.

19. Burrows, C. J.; Sauter, R. A. *J. Inclusion Phenom*. **1987**, *5*, 117-121.

20. Kinneary, J. F.; Roy, T. M.; Albert, J. S.; Yoon, H.; Wagler, T. R.; Shen, L.; Burrows, C. J. *J. Inclusion Phenom*. **1989**, *7*, 155-168.

21. Hsieh, H. P.; Muller, J. G.; Burrows, C. J. *J. Am. Chem. Soc.* 1994, *116*, 12077-12078.

22. McKenna, J.; McKenna, J. M.; Thornthwaite, D. W. *J. Chem. Soc., Chem. Commun.* **1977**, 809-811.

23. Wess, G.; Kramer, W.; Enhsen, A.; Glombik, H.; Baringhaus, K. H.; Boger, G.; Urmann, M.; Bock, K.; Kleine, H.; Neckermann, G.; Hoffmann, A.; Pittius, C.; Falk, E.; Fehlhabr, H. W.; Kogler, H.; Friebrich, M. *J. Med. Chem.* **1994**, *37*, 873.

24. Gouin, S.; Zhu, X. X. Steroids, 1996, 61, 664-669.

25. Gouin, S.; Zhu, X. X. Langmuir, **1998**, *14*, 4025-4029.

26. Li, y.; Zhang, Z.; Ju, y.; Zhao, C-Q. *Lett. in Org. Chem.* **2007**, *4*, 414-418.

27. Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Bhat, M. K.; Nahar, P. B.; Deshpande, M. V. *J. Med. Chem.* **2004**, *47*, 1591-1594.

28. Aher, N. G.; Pore, V. S. *Synlett* **2005**, 2155-2159.

29. Aher, N. G.; Pore, V. S.; Patil, S. P. *Tetrahedron,* **2007**, *63*, 12927-12934.

30. Vatmurge, N. S.; Hazra, B. G.; Pore, V. S.; Shirazi, F.; Deashpande, M. V.; Kadreppa, S.; Chattopadhyay, S.; Gonnade, R. *Org. Biomol Chem.* **2008**, *6*, 3823.

31. Odds, F. C.; Brown, A. J. P.; Gow, N. A. R. *TRENDS in Microbiology* **2003**, *11*, 272-279.

32. (a) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, C. J.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem*. **2000**, *43*, 953-970; (b) Wamhoff, H. Comprehensive Heterocyclic Chemistry; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, **1984**; 669–732; (c) Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem*. **1986**, *29*, 2262–2267; (d) Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.; De Clercq, E.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem*. **1994**, *37*, 4185–4194.

33. Heindel, N. D.; Reid, J. R. *J. Heterocyclic Chem*. **1980**, *17*, 1087.

34. (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, *J. P. Chem. Res. Toxicol*. **2002**, *15*, 269–299; (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc*. **2004**, *126*, 15366–15367.

35. Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc*. **2005**, *127*, 12434.

36. Xia, Y.; Fan, Z.; Yao, J.; Liao, Q.; Li, W.; Qua, F.; Penga, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2693–2698.

37. Michael, A. *J. Prakt. Chem.* **1893**, *48*, 94.

38. (a) Huisgen, R. *Naturwiss. Rundschau* **1961**, *14*, 15. (b) Huisgen, R. *Proc. Chem. Soc.* **1961**, 357; (c) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*, Vol. 1; Padwa, A., Ed.; Wiley: Chichester, **1984**, 1.

39. Kirmse, W.; Horner, L. *Liebigs Ann. Chem.* **1958**, *614*, 1.

40. (a) Tornøe, C.W.; Meldal, M. *Peptidotriazoles: Copper(I)-catalyzed 1,3- dipolar cycloadditions on solid-phase, Peptides 2001, Proc. Am. Pept. Symp.;* American Peptide Society and Kluwer Academic Publishers: San Diego, 2001; 263-264; (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

41. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, B. K. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.

42. Meldal, M.; Tornoe, C. W. *Chemical Reviews*, **2008**, 108, 2952-3015.

43. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

44. Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686.

45. Beatty, K. E.; Xie, F.; Wang, Q.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 14150.

46. Deiters, A.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1521.

47. Golas, P. L.; Tsarevsky, N. V.; Sumerlin, B. S.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 6451.

48. Wu, P.; Fokin, V. V. *Aldrich Chim. Acta* **2007**, *40*, 7.

49. (a) Sustmann, R. *Tetrahedron Lett.* **1971**, *29*, 2717-2720; (b) Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem. Int. Ed.* **2005**, *44,* 5188-5240.

50. (a) Weber, L. *Drug Disc. Today* **2004**, *1*, 261-267; (b) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Zurenko, G. E.; Hamel, C. J.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953-970.

51. (a) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210-216; (b) Bock, V. D.; Hiemstra, H.; Van Maarseveen. J. H. *Eur. J. Org. Chem*. **2006**, 51-68.

52. (a) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128-1136.

53. Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952-3015.

54. (a) Oh, K.; Guan, Z. A. *Chem. Commun.* **2006**, *29*, 3069-3071; (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366-15367.

55. Meldal, M.; Tornoe, C. W. *Chemical Reviews*, **2008**, 108, 2952-3015.

56. Deiters, A.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1521.

57. Golas, P. L.; Tsarevsky, N. V.; Sumerlin, B. S.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 6451.

58. Arora, B. S.; Shafi, S.; Singh, S.; Ismail, T.; Sampath Kumar, H. M. *Carbohydrate Research* **2008**, *343*, 139-144.

59. Camp, C.; Dorbes, S.; Picard, C.; Benoist, E. *Tetrahedron Letters* **2008**, *49*, 1989-1983.

60. Monkowius, M.; Ritter, S.; konid, B.; Zabel, M.; Yersin, H. *Eur. J. Inorg. Chem*. **2007**, 4597-4606.

61. Angell, Y.; Burgess, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 3649.

62. Tornøe, C. W.; Christensen, C.; Meldal M. *J. Org. Chem.* **2002,** *67,* 3057-3064.

63. (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, 1997; (b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, 1998; (c) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard, 5th ed.; NCCLS: Villanova, PA, 2000; M7–A5.

64. (a) Chang, F. C.; Wood, N. F.; Holton, W. G. *J. Org. Chem*. **1965**, *30*, 1718-1723.; (b) Li, C.; Peters A. S.; Meredith E. L.; Allman G. W.; Savage P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961-2962.; (c) Blickenstaff, R. T.; Chang, F. C. *J. Am. Chem. Soc.* **1959**, *81*, 2835-2838.; (d) Ruzicka, L.; Goldberg, M. W. *Monatsh* **1951**, *82*, 437.

List of Publication

1. **Bavikar, S. N.**; Salunke D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J.; Shirazi, F.; Deshpande, M. V.; Kadreppa, S.; Chattopadhyay, S. Synthesis of Chimeric Tetrapeptide Linked Cholic Acid Derivatives : Impending Synergistic Agents *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 5512-5517.
2. **Bavikar, S. N.**; Hazra, B. G.; Shirazi, F.; Deshpande, M. V. Design Synthesis and Antimicrobial Evolution of 3β polyamino Conjugates Derived from Cholic Acid (Communicated to *Bioorg. Med. Chem., Oct **2009***).
3. **Bavikar, S. N.**; Hazra, B. G.; Shirazi, F.; Deshpande, M. V. Cu(I) Catalyzed Alkyne-Azide "Click" Cycloaddition: Efficient Synthesis and Bioevalution of Bile Acid Bistriazole in the Presence of Base (*To be communicated*).
4. **Bavikar, S. N;** Salunke, D. B.; Hazra, B. G.; Pore, V. S.; Dodd, R. H.; Thierry, J. Pd Catalyzed One-pot Chemoselective Protocol for the Preparation of Carboxamides Directly from Azides (*To be communicated*).
5. **Bavikar, S. N.**; Hazra, B. G.; Shirazi, F.; Deshpande, M. V.; Design Synthesis and Antimicrobial Evolution of Chimeric Diapeptide and Monopeptide Linked Cholic Acid Derivatives : Impending Synergistic Agents (Manuscript under preparation).

Poster Presentation

1. **Bavikar, S. N.**; Hazra, B. G.; Pore, V. S. Synthesis of Squalamine and its Analogs from Cholic and Deoxycholic Acid. Poster presented at CRSI's 7th National Symposium in Chemistry, **Feb 2005**, IACS, Kolkata, India.
2. **Bavikar, S. N.**; Hazra, B. G.; Pore, V. S. Synthesis of 3β and 3α-polyaminocholic Acid Derivatives: Analogs of Squalamine. Poster presented at CRSI's 8th National Symposium in Chemistry, **Feb 2006**, IIT, Mumbai, India.
3. **Bavikar, S. N.**; Hazra, B. G.; Synthesis and Antimicrobial Evaluation of 3β-polyamino Conjugates Derived from Cholic Acid. Poster presented at CRSI's 11th National Symposium in Chemistry, **Feb 2009**, NCL, Pune, India.

4. Participated in 4th INSA-KOSEF International Symposium in Organic Chemistry, Contemporary Organic Chemistry and its Future Directions. (January 12-13, **2009**) at National Chemical Laboratory, Pune.

List of Publication Other than Thesis

1. Bandgar, B. P.; Kamble, V. T.; **Bavikar, S. N.**; Dhavane, A. Sodium tetrafluoroborate as a new and highly efficient catalyst for one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones and thiones *J. Chinese Chem. Soc.*, **2007**, *54*, 263-266.
2. Kamble, V. T.; Bandgar, B. P.; **Bavikar, S. N.** Highly efficient synthesis of bis(indolyl)methanes catalyzed by sodium tetrafluoroborate *Chinese J. Chem.* **2007**, *25*, 13-15.
3. Kamble, V. T.; Bandgar, B. P.; **Bavikar, S. N.**; Suryawanshi, S. B. Green protocol for synthesis of bis-indolylmethanes and bis-indolylglycoconjugates in the presence of iron(III) fluoride as a heterogeneous, reusable, and eco-friendly catalyst *Aus. J. Chem,* **2006**, *59*, 837-840.
4. Bandgar, B. P.; Kamble, V. T.; **Bavikar, S. N.** Magnesium perchlorate: An efficient catalyst for selective sulfonylation of arenes under neutral conditions *J. Chem. Res.(S),* **2003,** *5*, 287-289.

ERRATUM