SYNTHESIS OF PIPECOLIC ACID: AN INTEGRAL FRAGMENT OF ANTIMITOTIC TUBULYSINS

M.Sc. Thesis

by

KONIKA

DISCIPLINE OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY INDORE

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SYNTHESIS OF PIPECOLIC ACID: AN INTEGRAL FRAGMENT OF ANTIMITOTIC TUBULYSINS

A THESIS

Submitted in partial fulfillment of the requirements for the award of the degree of

Master of Science

by

KONIKA

DISCIPLINE OF CHEMISTRY

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **SYNTHESIS OF PIPECOLIC ACID: AN INTEGRAL FRAGMENT OF ANTIMITOTIC TUBULYSINS** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DISCIPLINE OF CHEMISTRY, Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the period from July 2016 to June 2018 under the supervision of Dr. Venkatesh Chelvam, Assistant Professor, IIT Indore.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

Konika

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Dr. Venkatesh Chelvam

Konika has successfully given her M.Sc. Oral Examination held on

Signature of Supervisor of M.Sc. thesis
Date:

Convener, DPGC
Date:

Signature of PSPC Member
Date:

Signature of PSPC Member
Date:

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DEDICATED TO MY LOVABLE FAMILY

Abstract

Cancer is the second savage cause of deaths worldwide. Currently, finding a cure to such a deadly disease is a matter of concern. Antimitotic compounds provide promising solution to the aforementioned problem. The primary aim of antimitotic compounds is to interfere with the polymerization of tubulin components of microtubules. This results in hindrance of cell division, an essential process of cell life cycle, leading to apoptosis. Therefore, inhibiting tubulin polymerization directly leads to cell death, which makes them crucial targets by cytotoxic compounds. Tubulysin family of natural products is one such example of those prominent antimitotic compounds, isolated from myxobacteria culture extracts. Due to their commendable cytotoxic activity, tubulysins have become a center of attraction for research in the field of chemotherapy.

Structurally, tubulysins are tetrapeptides consisting of four unusual amino acids, *N*-methyl pipecolic acid (Mep), isoleucine (Ile), tubuvaline (Tuv) and tubutyrosine (Tut) or tubuphenylalanine (Tup). Among these, pipecolic acid is an interesting fragment to analyze since tubulysin family of compounds bind to tubulin protein primarily through pipecolic acid and it is least tolerant to modifications. All these factors provide a growing opportunity for discovering new and better methodologies for synthesis of pipecolic acid.

In this work, we have reported two different synthetic routes which might be used for the gram scale synthesis of pipecolic acid. We started our synthesis using naturally occurring L-serine as the chiral source and successfully synthesized advanced intermediates which can be used for furnishing the final target pipecolic acid in future.

All the intermediates are well characterized using various spectroscopic techniques.

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SYMBOLS/UNITS

α	Alpha
β	Beta
δ	Delta
Å	Angstrom
M	Molar
g	Gram
h	Hour
J	Coupling constant
nM	Nano molar
mM	Milli molar
mL	Milli litre
dd	Doublet of doublet
Hz/MHz	Hertz/Mega Hertz
R_f	Retention Factor
IC_{50}	Inhibition concentration
ppm	Parts per million
brs	Broad singlet

ACRONYMS

Abbreviations used for amino acids, substituents, reagents etc. are largely in accordance with the recommendations of the IUPAC-IUB commission on Biochemical Nomenclature, 1974, Pure and Applied Chemistry, 40, 315-331. All amino acids have L-configuration. Standard three letter coding is used for all amino acids. Additional abbreviations used in this thesis are listed below.

ACN	Acetonitrile
Asp	Aspartic Acid
CaH_2	Calcium hydride
$CDCl_3$	Chloroform-d
$CHCl_3$	Chloroform
CH_3	Methyl
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethyl formamide
D_2O	Deuterium oxide
Et_3N	Triethylamine
EtOAc	Ethyl acetate
H_2O	Water
HCl	Hydrochloric acid
Ile	Isoleucine

$KMnO_4$	Potassium permanganate
Mep	Methylpipecolic acid
$NaBH_4$	Sodium borohydride
$NaHCO_3$	Sodium bicarbonate
Na_2SO_4	Sodium sulphate
NMR	Nuclear Magnetic Resonance
PPh_3	Triphenylphosphine
Ser	Serine
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
TMS	Tetramethylsilane
TLC	Thin Layer Chromatography
THF	Tetrahydrofuran
Tup	Tubuphenylalanine
Tut	Tubutyrosine
Tuv	Tubuvaline
UV	Ultra-Violet
s	Singlet
d	Doublet
t	Triplet
m	Multiplet

Chapter 1

Introduction

1.1 General introduction

Cancer, being one of the most deadly diseases in the world, is responsible for millions of deaths per year. In 2012, 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer have been reported worldwide [1]. With 1.1 million new cancer cases estimated every year, India burdened the world with 7.8% of new total cancer cases and 8.33% of global cancer deaths [2].

Cancer occurs when cells grow abnormally in an uncontrolled manner. Unlike normal cells, cancerous cells multiply in a haphazard way forming lumps and tumors, which can spread to other parts of the body.

1.2 Treatment of cancer

Anticancer drugs are used for cancer treatment; however cancer develops resistance and is defiant [3]. Anticancer drugs can either be isolated from natural sources or can be synthesized in laboratories. Disrupting micro tubular assembly plays a major role in anticancer therapy. Microtubules are of utter importance for sustenance of cellular life. They play a crucial role in cell division, which make them an alluring target for anticancer drugs.

Natural products such as taxol or tubulysin target microtubules, mechanistically inhibit depolymerization of microtubules or polymerization of tubulin-building blocks respectively and induces apoptosis in cells [4, 5]. Microtubules consist of α and β-tubulins heterodimers that undergo both polymerization and depolymerization in a process called polymerization dynamics as shown in figure 1 [6].

Therefore, microtubule targeting drugs can be classified into two categories: one that stabilize microtubule assembly (e.g. paclitaxel) and other that destabilize microtubule assembly (e.g. tubulysins).

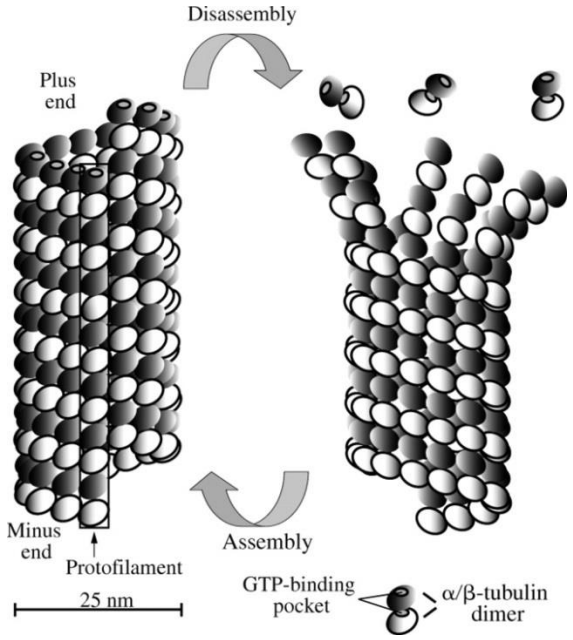


Figure 1. Polymerization dynamics of microtubules

1.3 Tubulysins as potent anticancer drugs

Tubulysins were reported for the first time in the year 2000. Isolated from myxobacterial cultures [7], tubulysins are indispensable cytotoxic compounds that act by inhibiting tubulin polymerization process leading to apoptosis [8]. Tubulysin family of natural products show commendable cytostatic activity against several mammalian cells with IC_{50} values in nano to picomolar range [7, 9].

In addition to their outstanding antitumor activity, tubulysins and their analogues exhibit dynamic activity against several multidrug resistant carcinoma cell lines [10]. Surprisingly, they are 20 to 10000-fold more cytotoxic than clinically approved anticancer drugs such as epothilone, vinblastine and paclitaxel [11, 12]. Impressive biological activity coupled

with their natural scarcity has spurred research in exploring new routes for synthesis of tubulysins and their structurally modified analogs.

On the structural aspects, tubulysins are linear tetrapeptides comprising of four amino acids: D-*N*-methyl pipecolic acid (Mep), L-isoleucine (Ile), tubuvaline (Tuv), which is itself is based on two condensed amino acids and tubutyrosine (Tut) or tubuphenylalanine (Tup) as shown in figure 2. In addition, an unusual tertiary amide *N*, *O*-acetal ester is present in the most active tubulysins [12].

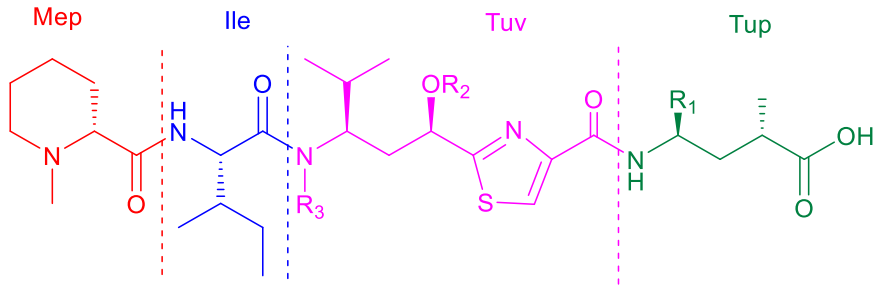


Figure 2. Structure of tubulysin family of natural products

Table 1. Structure of tubulysin family of natural products

Tubulysin	R_1	R_2	R_3
A	CH_2PhOH	$C(O)CH_3$	$CH_2OC(O)CH_2CH(CH_3)_2$
B	CH_2PhOH	$C(O)CH_3$	$CH_2OC(O)CH_2CH_2CH_3$
C	CH_2PhOH	$C(O)CH_3$	$CH_2OC(O)CH_2CH_3$
D	CH_2Ph	$C(O)CH_3$	$CH_2OC(O)CH_2CH(CH_3)_2$
E	CH_2Ph	$C(O)CH_3$	$CH_2OC(O)CH_2CH_2CH_3$
F	CH_2Ph	$C(O)CH_3$	$CH_2OC(O)CH_2CH_3$
G	CH_2PhOH	$C(O)CH_3$	$CH_2OC(O)CH=C(CH_3)_2$
H	CH_2Ph	$C(O)CH_3$	$CH_2OC(O)CH_3$
I	CH_2PhOH	$C(O)CH_3$	$CH_2OC(O)CH_3$
U	CH_2Ph	$C(O)CH_3$	H

Tubulysins belong to a class of anticancer drugs called vinca domain ligands. These are the mictrotubule inhibitors which bind to the longitudinal interface formed by two tubulin heterodimers. On binding, tubulysins cause two tubulin subunits to bend and twist slightly. The aftermath of this binding and twisting leads to conformational shift of consecutive α,β-tubulins heterodimer from a linear to curved form resulting in microtubule disassembly [13].

1.4 Pipecolic acid: an integral fragment of tubulysins

Pipecolic acid, being least tolerant to modifications, is an interesting molecule of research. The tertiary amine present in the left domain of tubulysin structure plays an inevitable role in its binding with vinca domain of tubulin protein. This amino acid residue snuggly fits into the narrowest channel between two tubulin subunits and utilize its backbone to interact with the other parts of tubulin protein.

The basic nitrogen present in the ring forms hydrogen bonding with oxygen of Asp179 of β subunit of tubulin protein. Several studies have shown that even trivial modifications in its structure can bring loss to the biological activity [14].

Dorin et al. studied various transformations in pipecolic acid structure and its effects on biological activities. Their research concluded that *R*-configuration on the chiral center in the ring is a requisite for its proper orientation in the vinca domain. Moreover, replacement of methyl group with acyl or any other groups proved to be detrimental to its activity [15].

Another report by Nicolaou and coworkers highlighted significant loss in potency on replacement of pipecolic acid moiety with its five membered proline counterpart, thus, providing strict evidence for the requirement of pipecolic acid binding site within the tubulin receptor [16].

Chapter 2

Review of past work

2.1 Literature review

Prior studies have reported various synthetic routes for pipecolic acid and its substituted derivatives. Pal et al. synthesized pipecolic acid via photocatalytic redox process using α-amino acid as the chiral source [17]. Nazabadioko and coworkers reported a chemoenzymatic process to obtain enantiopure pipecolic acid in good yields [18]. Ring closing metathesis has also been exploited for synthesizing pipecolic acid and their derivatives [19]. Recently, a short and facile synthesis has been reported for synthesis of pipecolic acid and derivatives using stereoselective aldol reaction [20].

C-3 substituted pipecolic acid derivatives are also reported via asymmetric induction using imine and β-amino alcohol as precursors [21, 22]. In addition to this, interesting desymmetrization reactions have been used for the synthesis of C-4 substituted derivatives, e.g., 4-hydroxypipecolic acid, which is an important substrate for preparation of substituted pipecolic acids [23].

On top of this, polysubstituted pipecolic acid derivatives have been synthesized via an intramolecular reaction between an iminium ion and an allylsilane moieties with complete control on diastereoselectivity [24].

Numerous work have been directed towards the synthesis of mono or polysubstituted pipecolic acid derivatives. Nonetheless, enantioenriched synthesis of such compounds remains a center of interest for chemists.

2.2 Objective of thesis

In this work, we have reported new synthetic route for the synthesis of pipecolic acid which might exhibit high efficacy for gram scale synthesis of pipecolic acid. It is a ten step total synthesis starting from L-serine aminoacid as the chiral source.

Chapter 3

Experimental Section

3.1 General Information and methods

All moisture sensitive reactions were conducted in oven-dried glassware under a nitrogen or argon atmosphere. Analytical thin-layer chromatography (TLC) was performed on SiO_2 60 F-254 plates. Visualization was accomplished by UV irradiation at 254 nm or by staining with any one of the following reagents: iodine, ninhydrin or $KMnO_4$. All compounds were purified by column chromatography which was performed using 100-200 μm or 230–400 μm mesh silica gel. Distilled hexane and distilled ethyl acetate were used as eluents in column chromatography. All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Solvents were distilled using suitable drying agents under nitrogen atmosphere. IR spectra were recorded using an Agilent FTIR spectrophotometer. ^{1}H and ^{13}C NMR were recorded by using Bruker AV 400 MHz NMR spectrometer with TMS as internal reference. Deuterated solvents like $CDCl_3$, D_2O were used as solvents for preparing NMR samples. Mass data were recorded on Bruker micro TOF-Q II by positive and negative mode electrospray ionization method.

3.2 Drying of solvents

Various common organic solvents employed for carrying out reactions were dried using drying agents such as CaH_2, P_2O_5, $MgSO_4$ and Na_2SO_4.

3.2.1 Drying of THF

Required amount of THF was taken in a round bottom flask. Sodium wire and benzophenone were added to the same flask. The mixture was heated at reflux until the solvent turned deep blue in colour. This indicates that the solvent is completely dry and moisture free. After cooling down the flask to room temperature, required amount of THF was distilled off and collected in another round bottom flask containing flame dried 4Å molecular sieves.

3.2.2 Drying of DCM/ACN

Required amount of DCM/ ACN was taken in a round bottom flask. A pinch of CaH_2 was added to the same flask and was stirred overnight. Finally, the solvent was distilled off under reflux conditions and collected in another round bottom flask containing flame dried 4Å molecular sieves.

3.2.3 Drying of methanol/ethanol

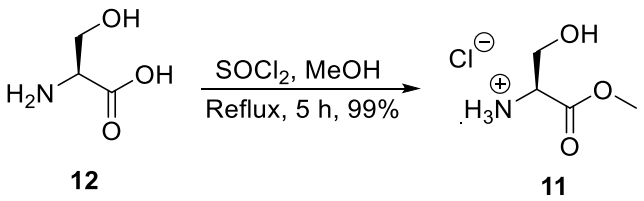
Required amount of methanol/ ethanol (50−100 ml) was taken in a round bottom flask. Magnesium turnings (250 mg) and iodine (25 mg) were added to the same flask. The mixture was heated to reflux until all of the magnesium had reacted. After cooling down the flask to room temperature, the solvent was distilled off and collected in another round bottom flask containing flame dried 4Å molecular sieves.

3.2.4 Drying of DMF

Required amount of DMF was taken in a round bottom flask. A pinch of CaH_2 was added to the same flask and was stirred overnight. Finally, the solvent was distilled off via vacuum distillation under reflux conditions and collected in another round bottom flask containing flame dried 4Å molecular sieves.

3.3 Synthesis of (*R*)-*N*-(1-((*tert*-butyldiphenylsilyl)oxy)-3-hydroxypropan-2-yl-4-methylbenzenesulfonamide 8

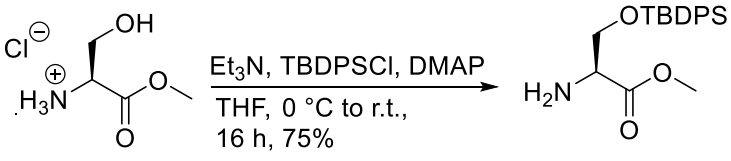
3.3.1 Synthesis of methyl L-serinate hydrochloride 11



12 **11**

Thionyl chloride (0.67 mL, 9.41 mmol) was added dropwise to a suspension of L-serine (1.0 g, 9.51 mmol) in methanol (15 mL). The solution was heated at reflux with stirring for 5 h and then cooled to ambient temperature. The solvent was removed under reduced pressure to afford methyl L-serinate hydrochloride (1.47 g, 99%) as brown solid, which was used in the next step without further purification. TLC: R_f 0.1 (1:20, MeOH/CH_2Cl_2). IR (neat): 3348 (O−H), 2922 (C−H), 1747 (C=O), 1591 (N−H), 1247 (C−N), 1092 (C−O) cm^{-1}. ^1H NMR (D_2O, 400 MHz) δ: 4.22 (t, 1H, *J* = 4.0 Hz), 4.05 (dd, 1H, *J* = 4.0 Hz, 12.0 Hz), 3.95 (dd, 1H, *J* = 4.0 Hz, 12.0 Hz), 3.79 (s, 3H); ^{13}C NMR (D_2O, 100 MHz) δ: 168.91, 59.20, 54.67, 53.66. MS (ESI) *m/z* $(M+H)^+$ Calculated for $C_4H_9NO_3$: 120.0655; Observed: 120.0632.

3.3.2 Synthesis of methyl *O*-(*tert*-butyldiphenylsilyl)-L-serinate 10

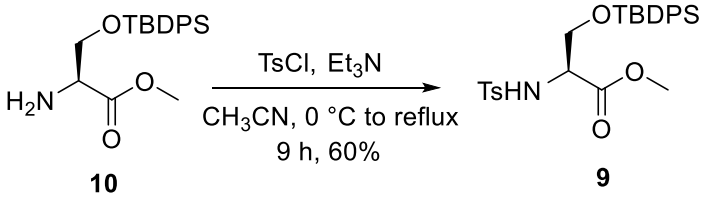


11 **10**

To a suspension of methyl L-serinate hydrochloride (100 mg, 0.64 mmol) in THF (2 mL), was added Et_3N (0.26 mL, 1.92 mmol) under inert atmosphere. After cooling to 0 °C, DMAP (0.73 mg, 0.006 mmol) was added as solid and TBDPSCl (0.17 mL, 0.67 mmol) was added via glass

syringe to the same flask. The solution was warmed to room temperature and stirred for 16 h. The reaction mixture was filtered off and washed with EtOAc (3 × 10 mL). The filtrate was concentrated to give yellow crude oil, which was purified through column chromatography using 20% ethyl acetate and hexane. Column was neutralized using 0.5 mL Et_3N in 100 mL hexane. The pure compound was obtained as yellow oil (170 mg, 75%). TLC: R_f 0.48 (100% EtOAc). IR (neat): 3365 (N−H), 2933 (C−H), 1746 (C=O), 1680 (N−H), 1430 (C=C), 1217 (C−N), 1108 (C−O), 700 (=C−H) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.65−7.62 (m, 4H), 7.45−7.37 (m, 6H), 3.99 (dd, 1H, *J* = 4.0 Hz, 8.0 Hz), 3.89 (dd, 1H, *J* = 4.0 Hz, 12.0 Hz), 3.71 (s, 3H), 3.56 (t, 1H, *J* = 8.0 Hz), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 173.50, 134.53, 134.51, 132.06, 132.01, 128.78, 126.73, 126.70, 65.15, 55.38, 50.96, 25.69, 18.25. MS (ESI) *m/z* $(M+Na)^+$ Calculated for $C_{20}H_{27}NO_3Si$: 380.1652; Observed: 380.1796.

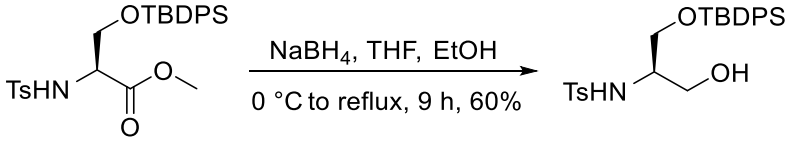
3.3.3 Synthesis of methyl *O*-(*tert*-butyldiphenylsilyl)-*N*-tosyl-L-serinate 9



4Å molecular sieves were flame dried in a 50 mL two neck round bottom flask under reduced pressure for about 15 minutes. After cooling down the flask to room temperature, tosyl chloride (138.6 mg, 0.727 mmol) was added as solid to the flask containing MS and CH_3CN (5 ml) was added to the reaction flask followed by addition of Et_3N (0.19 mL, 1.39 mmol). The suspension was stirred for 10 minutes and a solution of **10** (200 mg, 0.55 mmol) in CH_3CN (2 mL) was added to the reaction mixture. The progress of reaction was monitored by TLC. After complete consumption of **10**, the reaction mixture was quenched by adding 2−5 mL of water and extracted with EtOAc (3 × 20 mL). The organic layer was

dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure using rotatory evaporator. The crude mixture was purified by column chromatography using 15% ethyl acetate and hexane. The pure product was obtained as white solid (258 mg, 90%). TLC: R_f 0.48 (1:10 EtOAc/hexane). IR (neat): 3332 (N−H), 2959 (C−H), 1740 (C=O), 1596 (N−H), 1431(C=C), 1345 (N−S=O), 1253 (C−N), 1163 (S=O), 1092 (C−O), 705 (=C−H) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.71 (d, 2H, *J* = 8.0 Hz), 7.58 (t, 4H, *J* = 8.0 Hz), 7.46−7.34 (m, 6H), 7.27−7.25 (m, 2H), 5.45 (d, 1H, *J* = 8.0 Hz, NH), 4.06−4.02 (m, 1H), 3.97 (dd, 1H, *J* = 4.0 Hz, 8.0 Hz), 3.83 (dd, 1H, *J* = 4.0 Hz, 12.0 Hz), 3.55 (s, 3H), 2.41 (s, 3H), 1.00 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.10, 143.58, 137.08, 135.49, 132.54, 132.51, 129.94, 129.64, 127.82, 127.78, 127.14, 65.18, 57.45, 52.48, 26.64, 21.54, 19.21. MS (ESI) *m/z* $(M+Na)^+$ Calculated for $C_{27}H_{33}NO_5SSi$: 534.1741; Observed: 534.1915.

3.3.4 Synthesis of (*R*)-*N*-(1-((tert-butyldiphenylsilyl)oxy)-3-hydroxypropan-2-yl-4-methylbenzenesulfonamide 8



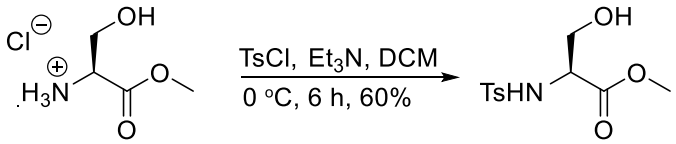
9 **8**

9 (300 mg, 0.586 mmol) was dissolved in dry THF (5 mL) and dry ethanol (5 mL) in a 50 mL two neck round bottom flask under inert atmosphere. After cooling the flask to 0 °C, $NaBH_4$ (88.67 mg, 2.34 mmol) was added portion-wise to the reaction flask. After stirring the reaction at room temperature for 1 h, the flask was fitted with double surface reflux condenser and heated to reflux for 8−9 h. After the completion of reaction, the pH of the reaction mixture was adjusted to 7.0 using 0.5 N HCl (5 mL) and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was

purified over silica gel column chromatography by using 20% ethyl acetate and hexane to afford white solid of **8** (170 mg, 60%). TLC: R_f 0.24 (1:5 EtOAc/hexane). IR (neat): 3511 (O−H), 3283 (N−H), 2932 (C−H), 1598 (N−H), 1428 (C=C), 1330 (N−S=O), 1159 (S=O), 1084 (C−O), 701 (=C−H) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.70 (d, 2H, *J* = 8.0 Hz), 7.55−7.52 (m, 4H), 7.46−7.43 (m, 2H), 7.39−7.35 (m, 2H), 7.24 (d, 2H, *J* = 8.0 Hz), 5.08 (d, 1H, *J* = 8.0 Hz, NH), 3.72−3.63 (m, 2H), 3.56−3.50 (m, 2H), 3.32−-3.27 (m, 1H), 2.40 (s, 3H), 1.96−1.94 (m, 1H, OH), 1.02(s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 143.55, 137.27, 135.47, 135.46, 132.51, 132.48, 130.08, 130.05, 129.77, 127.92, 127.89, 127.09, 63.32, 62.49, 55.77, 26.83, 21.55, 21.53, 19.17. MS (ESI) *m/z* $(M+Na)^+$ Calculated for $C_{26}H_{33}NO_4SSi$: 506.1792; Observed: 506.2003.

3.4 Synthesis of methyl (*S*)-1-tosylaziridine-2-carboxylate 14

3.4.1 Synthesis of methyl tosyl-L-serinate 13

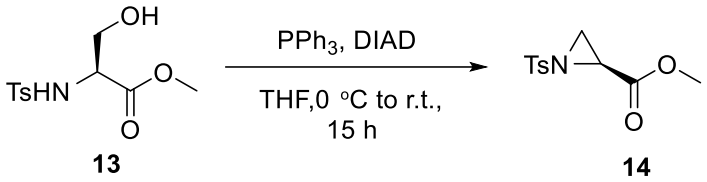


11 **13**

To a suspension of **11** (200 mg, 1.285 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C, Et_3N (0.89 mL, 6.425 mmol) and a solution of tosyl chloride (367.38 mg, 1.92 mmol) in dry CH_2Cl_2 (3 mL) were added. After stirring the reaction mixture for 6 h at 0 °C, the white precipitate was filtered off under suction and the filtrate was evaporated under reduced pressure to yield a white solid. The solid was then dissolved in EtOAc (20 mL) and washed with $NaHCO_3$ (10 mL), citric acid (10% w/v, 10 mL) and H_2O (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography using 20% ethyl acetate and hexane. The pure product **13** was obtained as white solid (204 mg, 58%). TLC: R_f 0.65 (1:20

MeOH/ CH_2Cl_2). IR (neat): 3495 (O−H), 3273 (N−H), 2956 (C−H), 1748 (C=O), 1436 (C=C), 1328 (N−S=O), 1211 (C−N), 1159 (S=O), 1090 (C−O), 683 (=C−H) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.76 (d, 2H, *J* = 8.0 Hz), 7.32 (d, 2H, *J* = 8.0 Hz), 5.59 (d, 1H, *J* = 8.0 Hz, NH), 3.99−3.97 (m, 1H, OH), 3.90−3.89 (m, 2H), 3.63 (s, 3H), 2.43 (s, 3H), 2.27−2.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.21, 143.95, 136.47, 129.81, 127.24, 63.72, 57.64, 52.96, 21.58. MS (ESI) *m/z* $(M+Na)^+$ Calculated for $C_{11}H_{15}NO_5S$: 296.0563; Observed: 296.0621.

3.4.2 Synthesis of methyl (*S*)-1-tosylaziridine-2-carboxylate 14



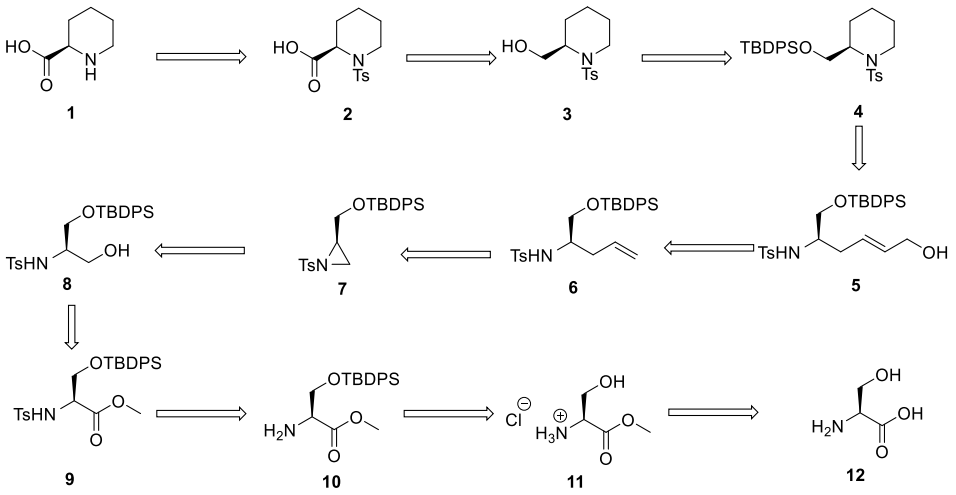
13 **14**

13 (70 mg, 0.25 mmol) was dissolved in dry THF (1 mL) at 0 °C in 50 mL two neck round bottom flask. A solution of PPh_3 (200 mg, 0.768 mmol) in dry THF (2 mL) and DIAD (0.15 mL, 0.768 mmol) were added via syringe. The resulting mixture was warmed to ambient temperature with constant stirring over a period of 15 h. After complete consumption of **13**, the solvent was evaporated under reduced pressure using rotatory evaporator. The mixture was purified by column chromatography using 20% ethyl acetate and hexane. **14** could not be isolated due to the overlapping R_f value of the compound **14** and the reduced by-product of DIAD formed during the course of reaction. TLC: R_f 0.33 (1:5 EtOAc/hexane). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (d, 2H, *J* = 8.0 Hz), 7.37 (d, 2H, *J* = 8.0 Hz), 3.74 (s, 3H), 3.36−3.33 (m, 1H), 2.77−2.76 (m, 1H), 2.57−2.56 (m, 1H), 2.46 (s, 3H).

Chapter 4

Results and Discussion

For designing an efficient route to obtain enantio enriched pipecolic acid in gram scale, a simple retrosynthetic analysis is shown in scheme 1.



Scheme 1. Retrosynthetic analysis of pipecolic acid **1**

According to retrosynthetic analysis, six membered piperidine ring of **4** can be obtained from *N*-tosylated alkenol **5** via reduction of double bond and subsequent ring closing reaction through the Mitsunobu reaction of hydroxyl group with NH group. *N*-Tosylated alkene **6** can undergo olefin metathesis with propenol in presence of Grubb's catalyst to afford *N*-tosylated alkenol **5**. Further, a highly strained three membered aziridine ring **7**, on regioselective ring opening by vinyl Grignard reagent in S_N^2 fashion can easily afford corresponding amino alkene **6**.

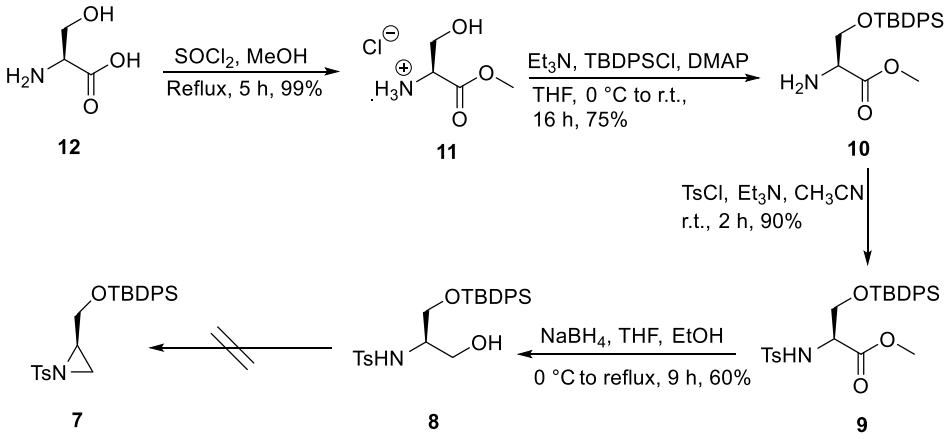
N-tosylated amino alcohol **8**, in presence of a mild base such as Et_3N and TsCl can easily undergo *O*-tosylation and yield *N*-tosylated aziridine ring **7** via nucleophilic attack of nitrogen atom on OTs group which acts as a better leaving group. Protected amino ester **9** can be reduced using

reducing agents such as $NaBH_4$ to afford amino alcohol **8.** *N*-tosylated amino ester **9** can easily be obtained from amino ester **10** via simple tosylation using TsCl and Et_3N as a mild base. *O*-Silylation of **11** can be achieved in presence of a mild base like Et_3N and TBDPSCl as the protecting group to afford corresponding *O*-protected product **10** in good yield. Naturally occurring L-serine **12** probably acts as the most suitable amino acid to furnish methyl L-serinate salt **11**.

Earlier, Lothar and coworkers have reported a short and efficient synthetic route to furnish optically active *N*-tosyl aziridines from 2-amino alcohols in one pot procedures [25]. In the aforementioned scheme, *N*-tosylated amino alcohol **8**, obtained via *N*-protection and subsequent reduction of amino ester **10**, can serve as a competent substrate to obtain desired aziridine ring **7**.

One of the important chiral sources for the preparation of pipecolic acid and derivatives are α-amino acids. Thus we began our synthesis using naturally occurring L-serine as the chiral inductor (Scheme 2). L-serine **12** on reaction with thionyl chloride and methanol gave the corresponding methyl serinate salt **11** in excellent yield. The hydrochloride salt **11** was *in situ* neutralized using mild base, Et_3N and the alcohol functionality was subsequently protected with TBDPSCl to give *O*-silylated amino alcohol **10** in good yield.

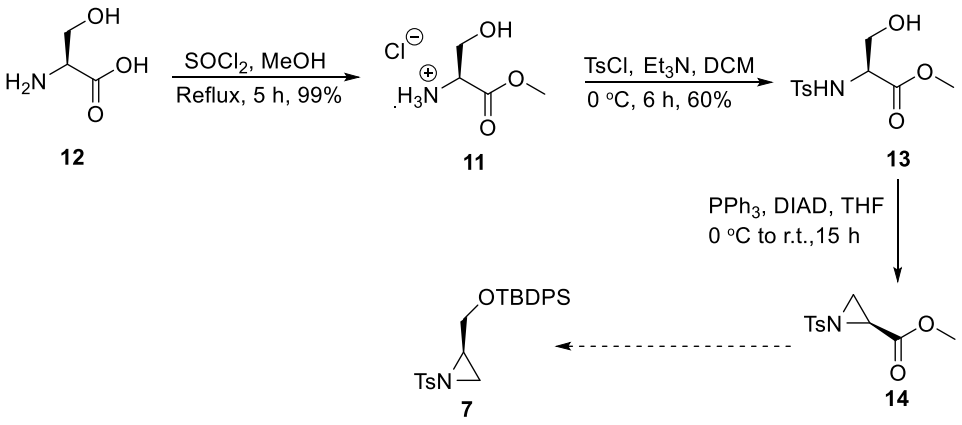
In consecutive two steps, **10** was *N*-tosylated and ester functionality was reduced to corresponding alcohol using $NaBH_4$ as the reducing agent to afford *N*-tosylated amino alcohol **8** in 55−60% yield. The conversion of amino alcohol **8** to corresponding aziridine ring **7** proved to be quite challenging. We tried to synthesize aziridine ring **7** using TsCl and Et_3N as mild base. Even after several attempts, our desired product **7** could not be obtained possibly due to the steric bulkiness of silyl protecting group (Scheme 2).



Scheme 2. Synthesis of pipecolic acid **1** from L-serine via aziridine ring formation of **7**

After several unsuccessful attempts to prepare pipecolic acid **1** via scheme **2**, we turned our attention towards scheme 3 which provides another similar route to obtain desired *N*-tosyl aziridine **7**.

Accordingly, esterification of serine was easily achieved to give corresponding methyl serinate **11** in excellent yield. The methyl ester salt **11** was *in situ* neutralized using Et_3N as base and simultaneously treated with tosyl chloride to furnish *N*-tosylated ester **13** in 60% yield (Scheme 3). The resultant *N*-tosyl ester **13** was subjected to the Mitsunobu reaction to provide *N*-tosylated aziridine **14** in poor yield of 15%. Moreover, we couldn't purify the *N*-tosylated aziridine **14** because of overlapping R_f of the reduced product from DIAD employed during the Mitsunobu reaction. The Mitsunobu reaction of **13** will be attempted with other reagents in future.



Scheme 3. Synthesis of *N*-tosylaziridine **7** from L-serine via *N*-tosylmethylserinol **13**

Chapter 5

Conclusion and scope of work

In this work, we have reported two different synthetic routes to synthesize pipecolic acid **1**. We started our synthesis using L-serine **12** as the chiral source. We have successfully prepared advanced intermediates which can be used to achieve the final target, pipecolic acid **1,** in future. All the intermediates are well characterized using various spectroscopic techniques.

APPENDIX A

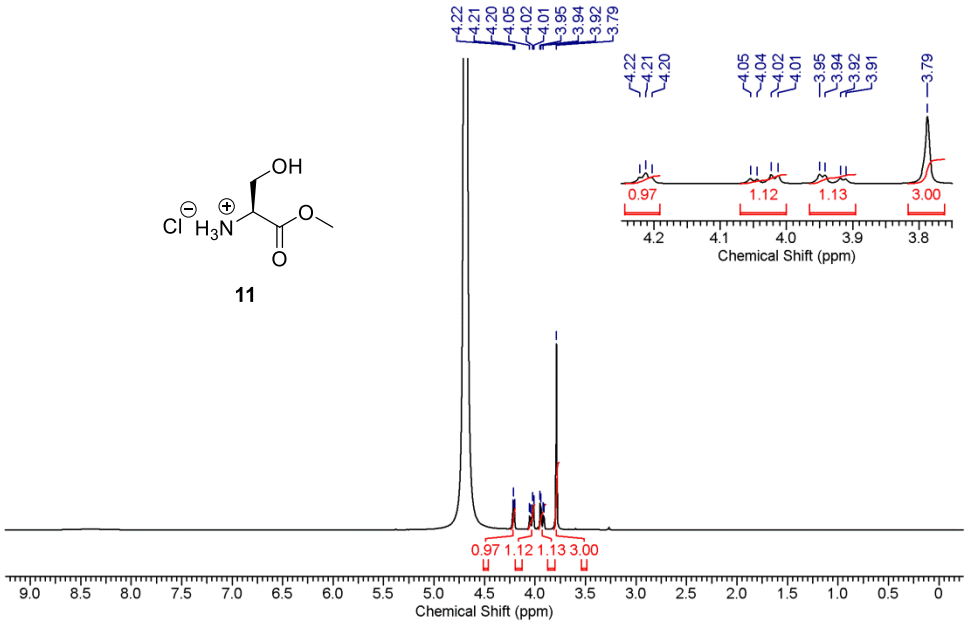


Figure 3. ^{1}H NMR spectrum of **11** in D_2O

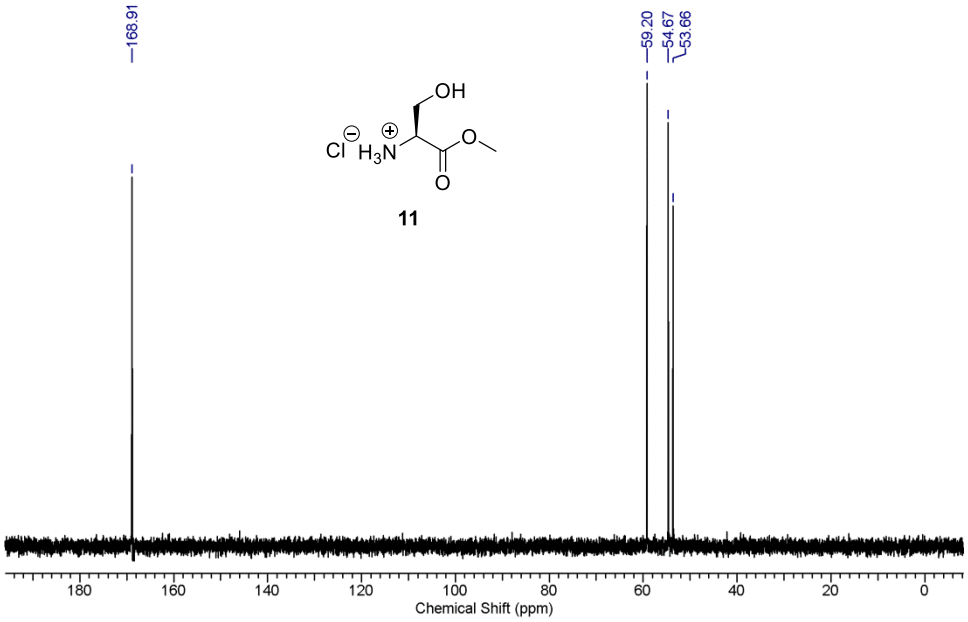


Figure 4. ^{13}C NMR spectrum of **11** in D_2O

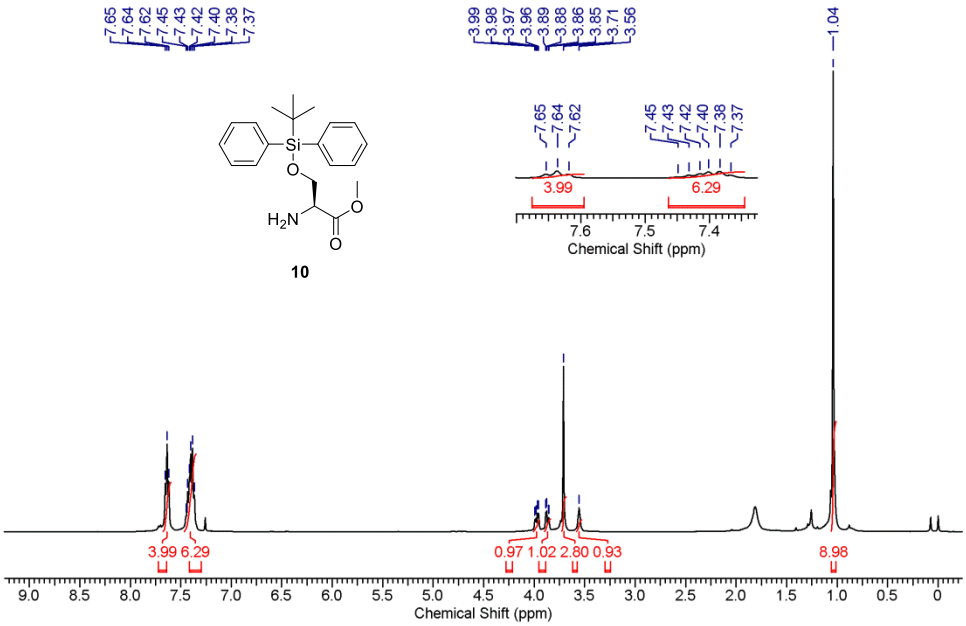


Figure 5. ^{1}H NMR spectrum of **10** in $CDCl_3$

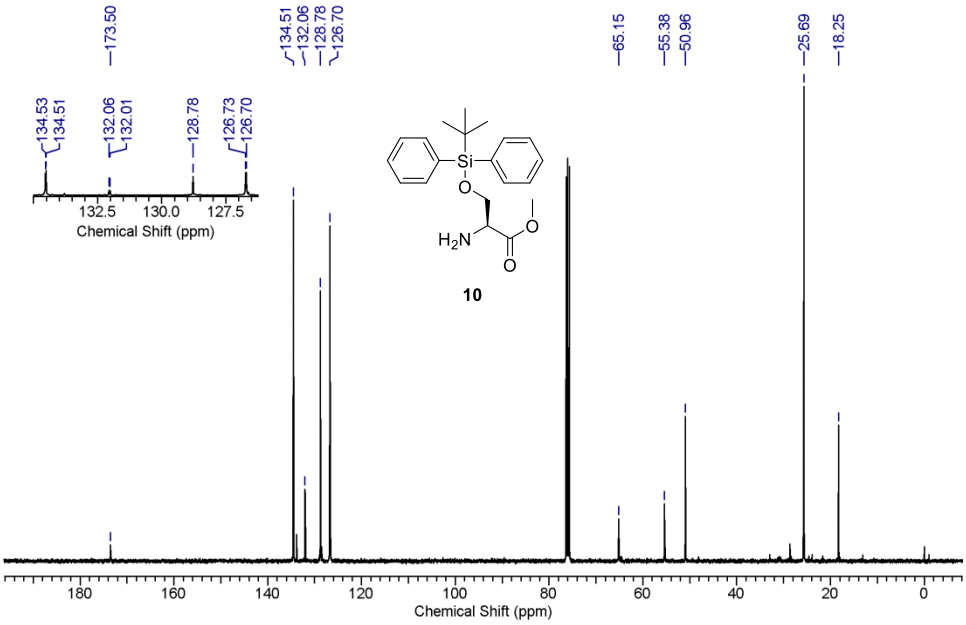


Figure 6. ^{13}C NMR spectrum of **10** in $CDCl_3$

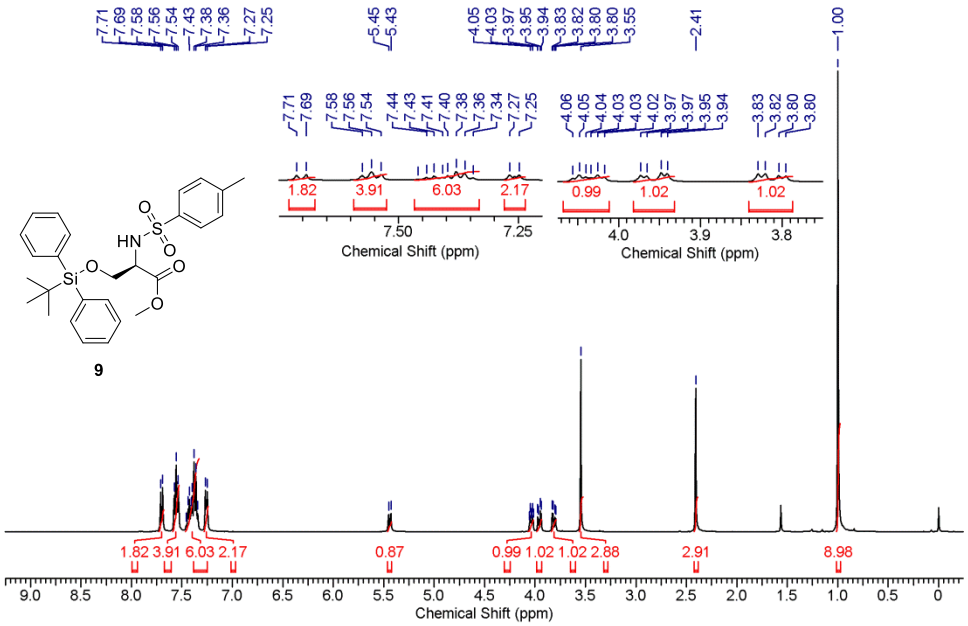


Figure 7. ^{1}H NMR spectrum of **9** in $CDCl_3$

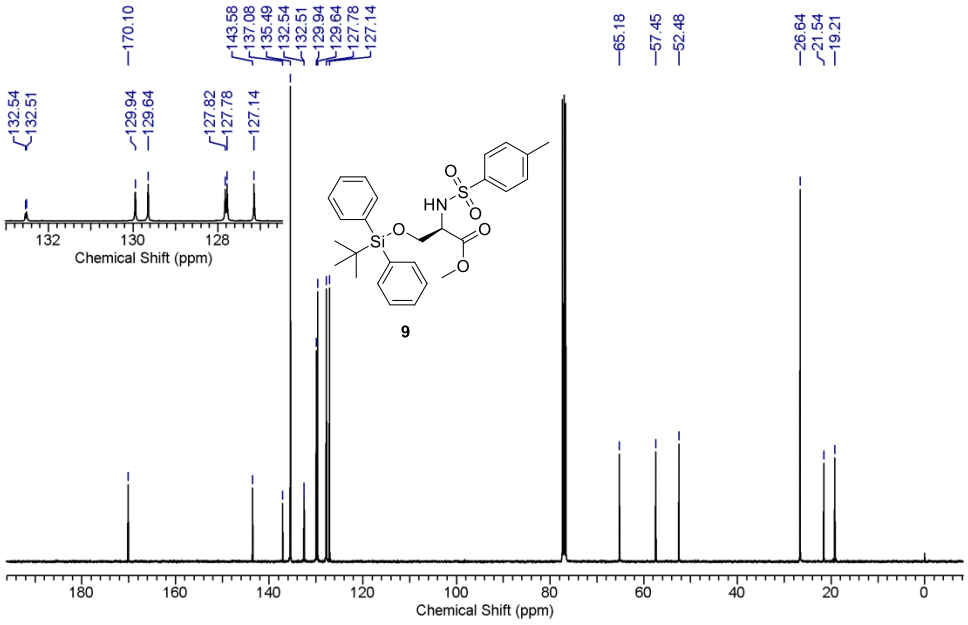


Figure 8. ^{13}C NMR spectrum of **9** in $CDCl_3$

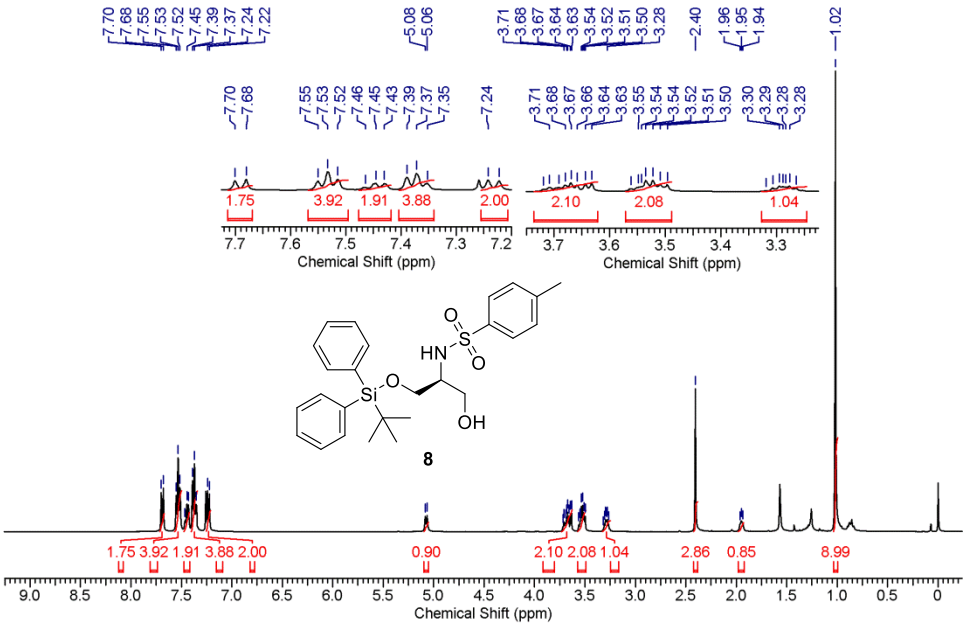


Figure 9. ^{1}H NMR spectrum of **8** in $CDCl_3$

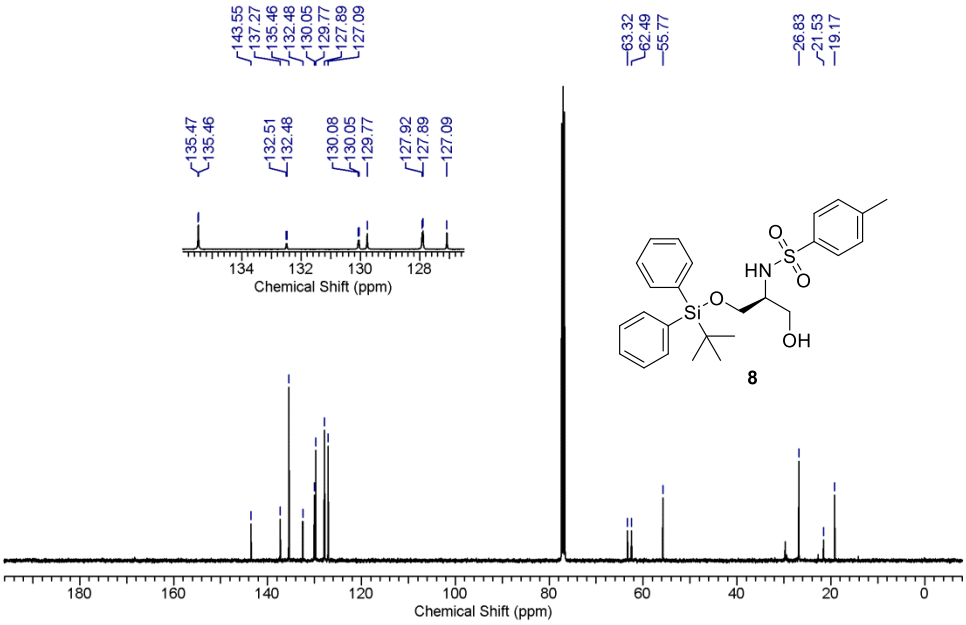


Figure 10. ^{13}C NMR spectrum of **8** in $CDCl_3$

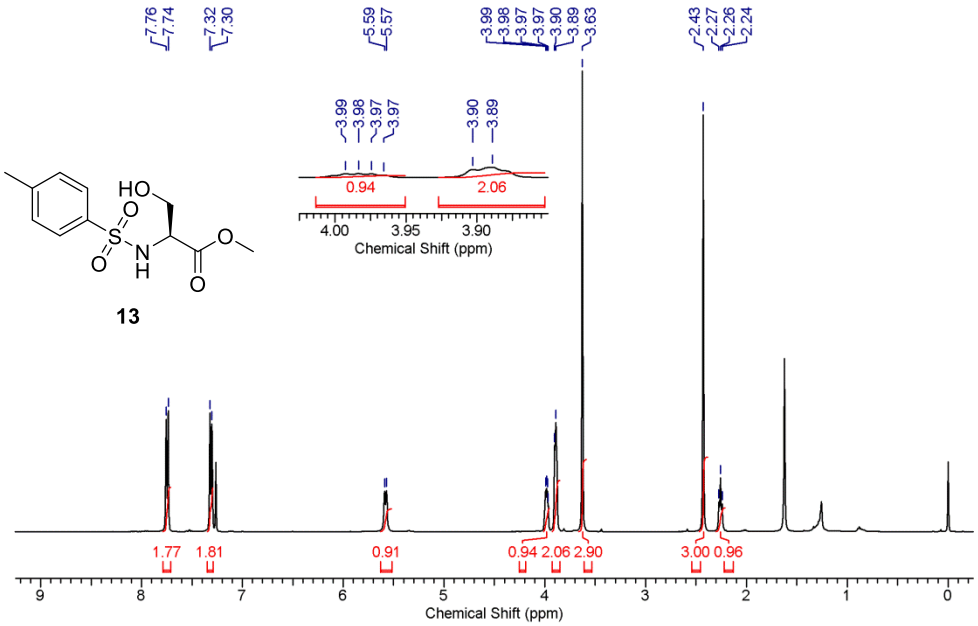


Figure 11. ^{1}H NMR spectrum of **13** in $CDCl_3$

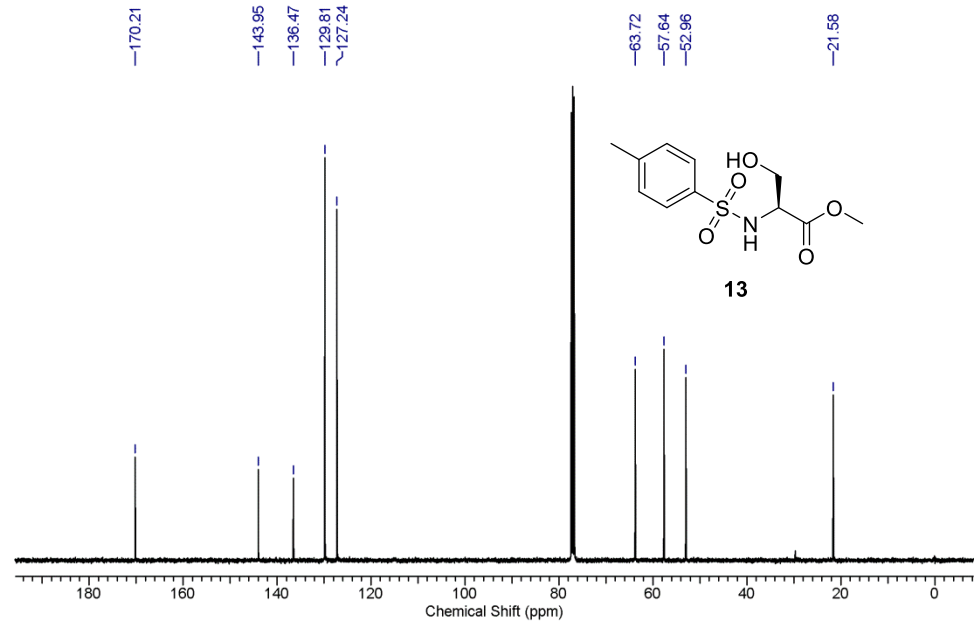


Figure 12. ^{13}C NMR spectrum of **13** in $CDCl_3$

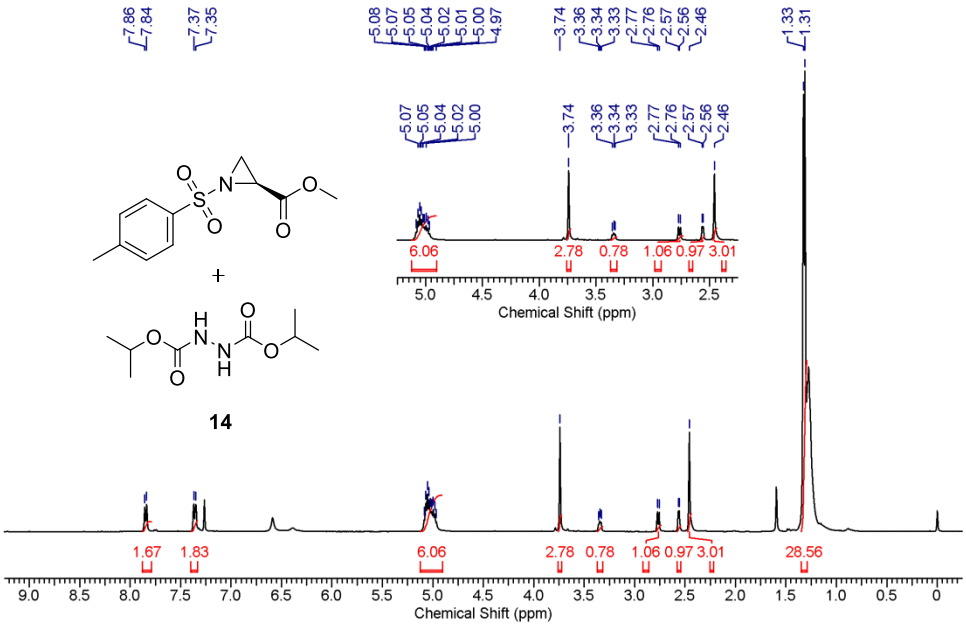


Figure 13. ^{1}H NMR spectrum of **14** in $CDCl_3$

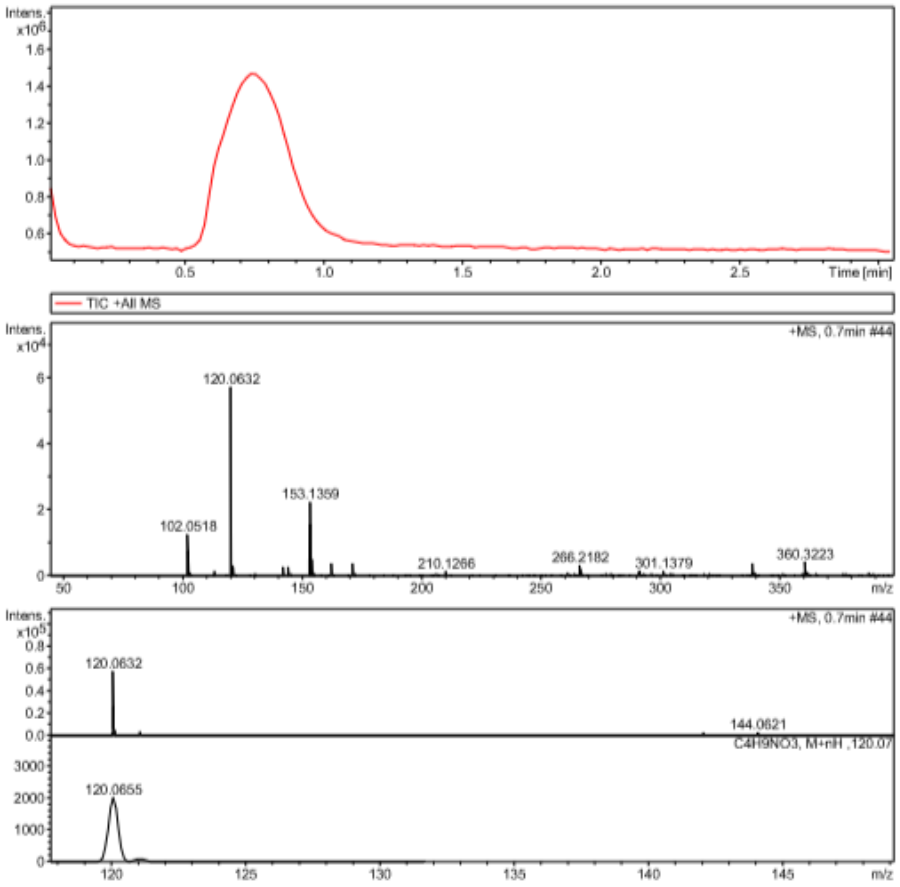


Figure 14. Mass spectrum of **11** in MeOH

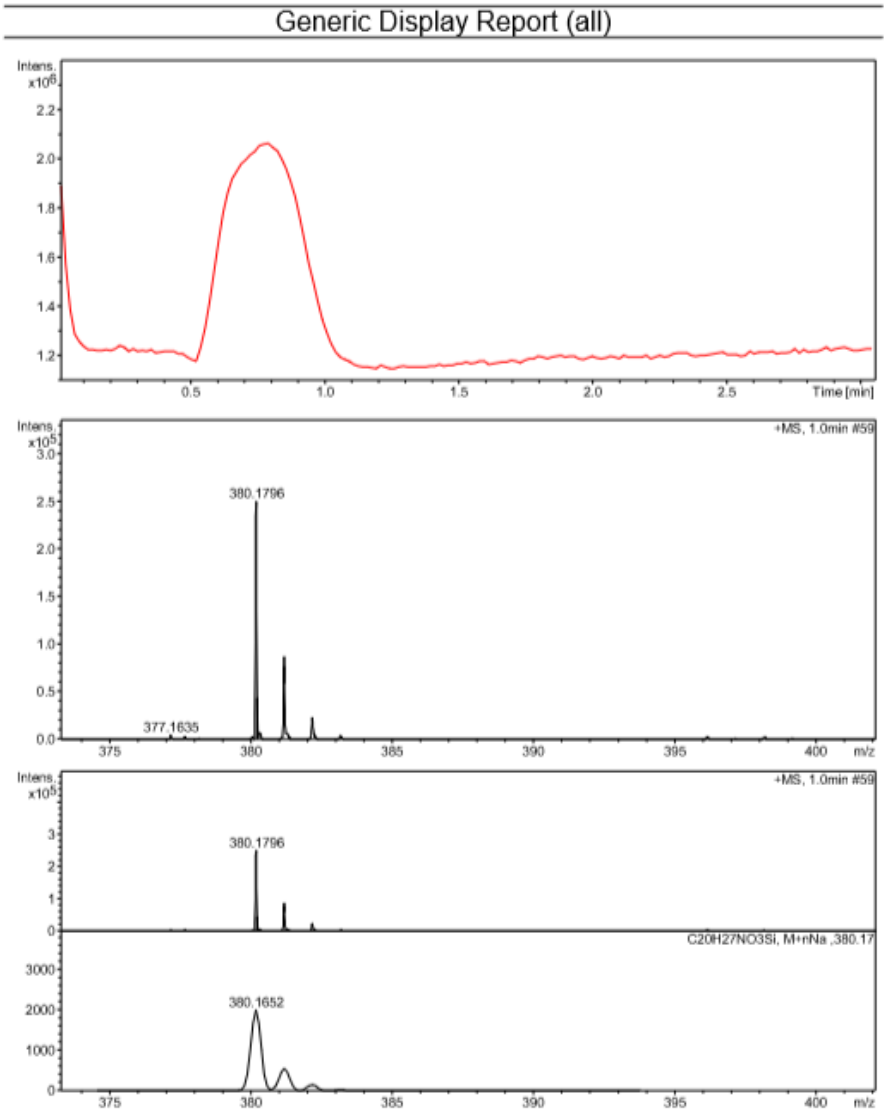


Figure 15. Mass spectrum of **10** in $CHCl_3$

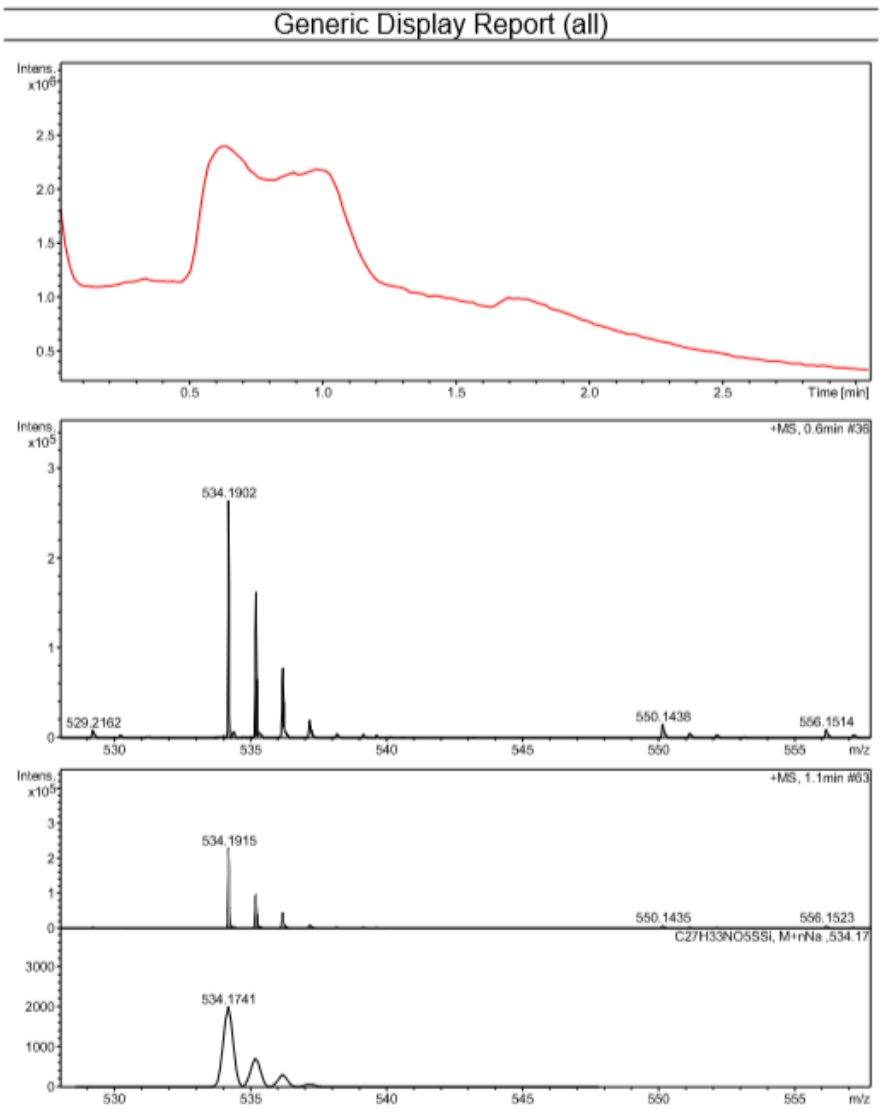


Figure 16. Mass spectrum of **9** in $CHCl_3$

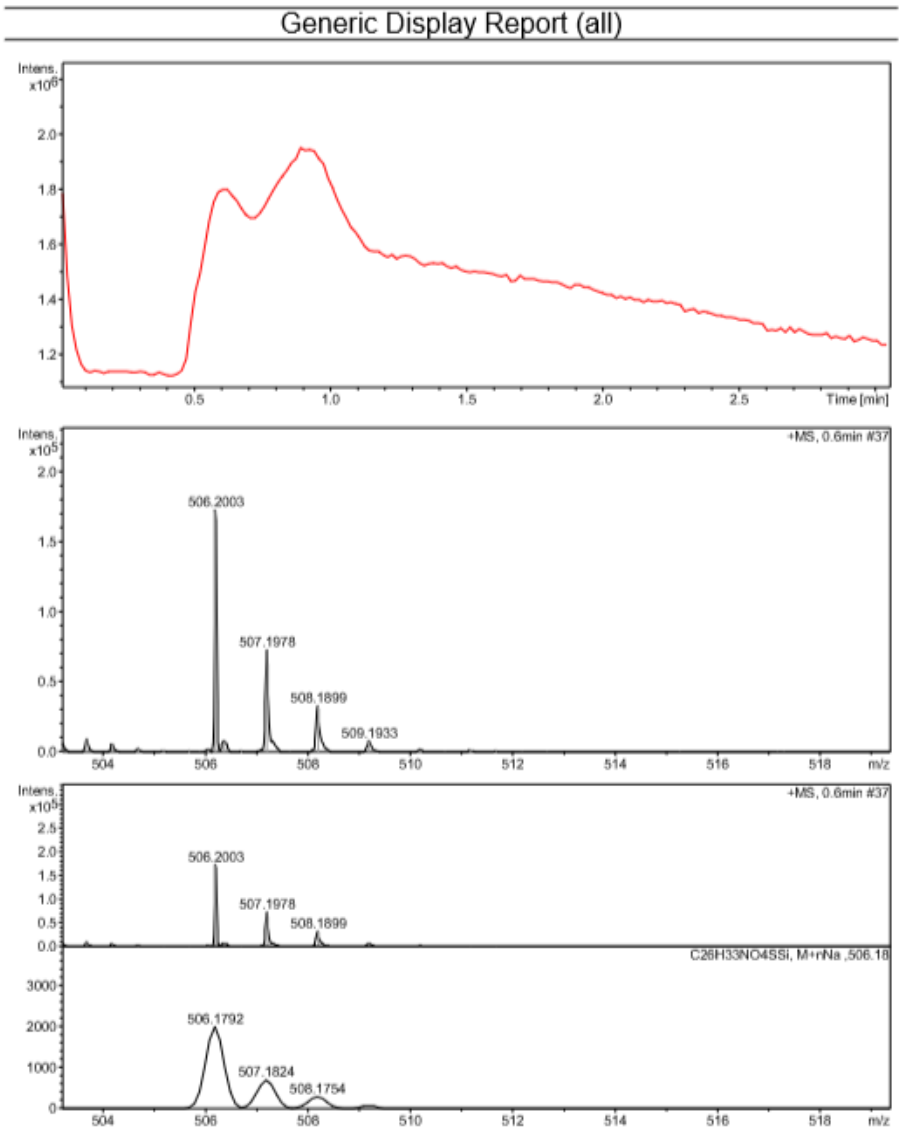


Figure 17. Mass spectrum of **8** in $CHCl_3$

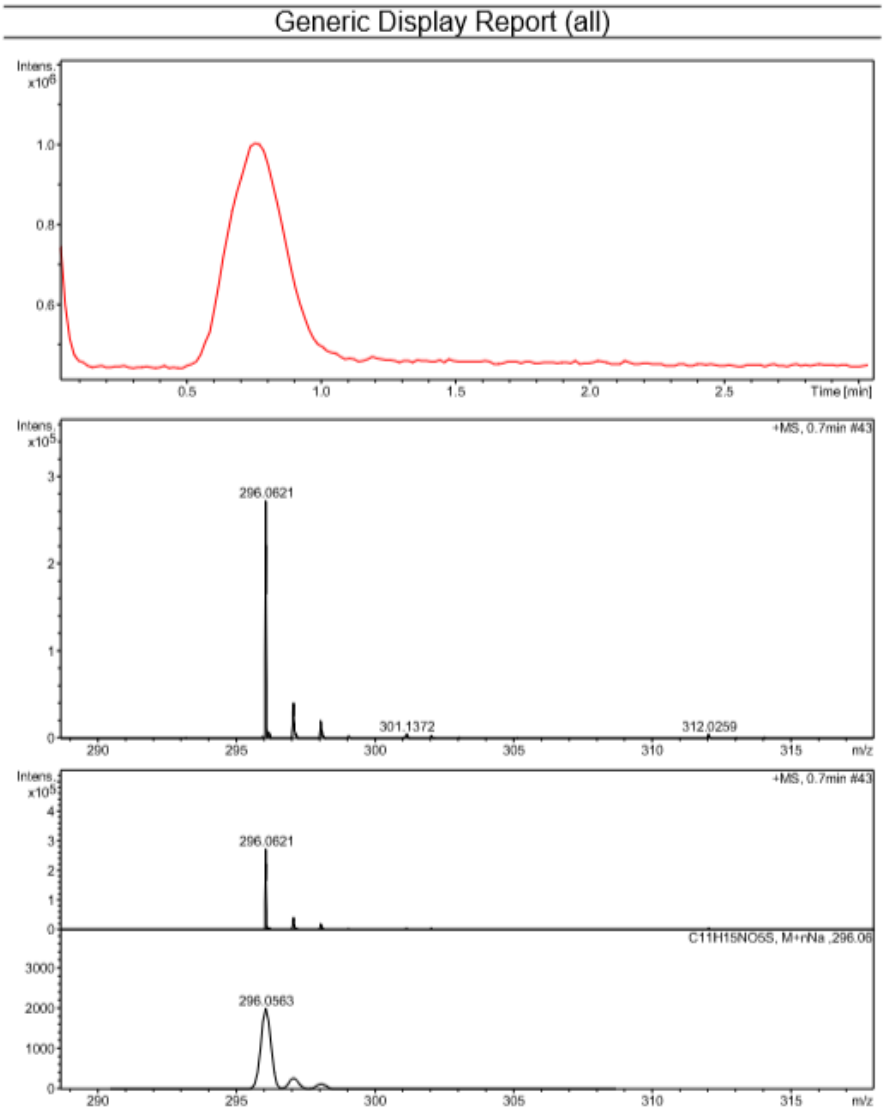


Figure 18. Mass spectrum of **13** in $CHCl_3$

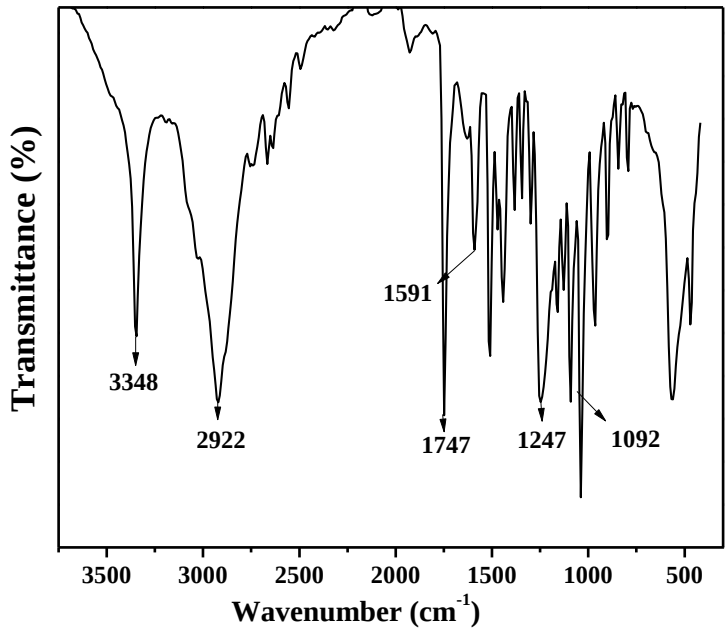


Figure 19. IR spectrum of **11**

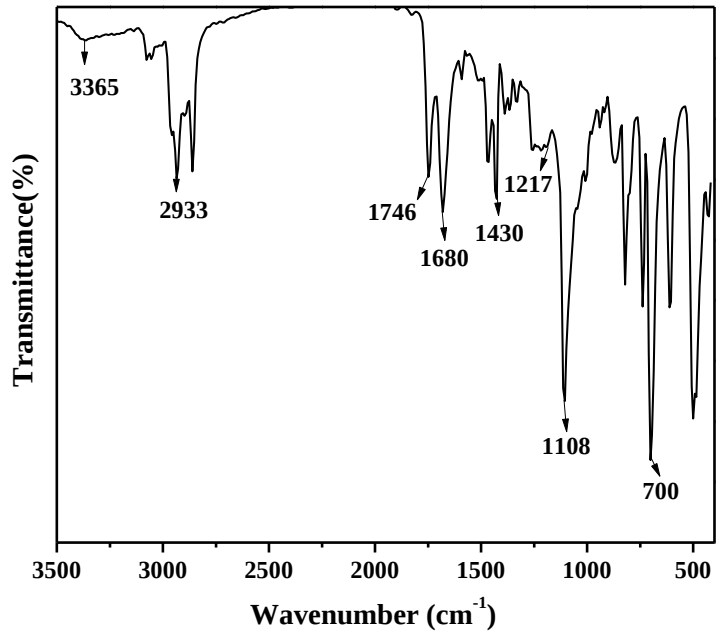


Figure 20. IR spectrum of **10**

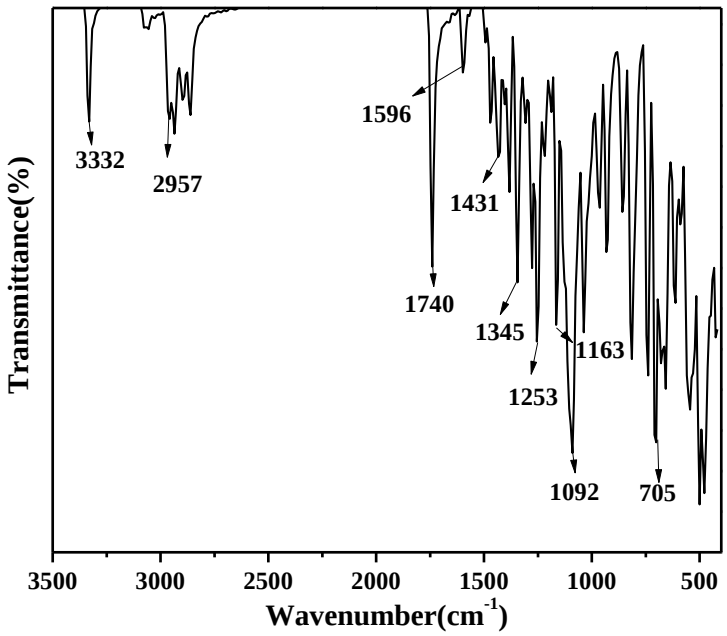


Figure 21. IR spectrum of **9**

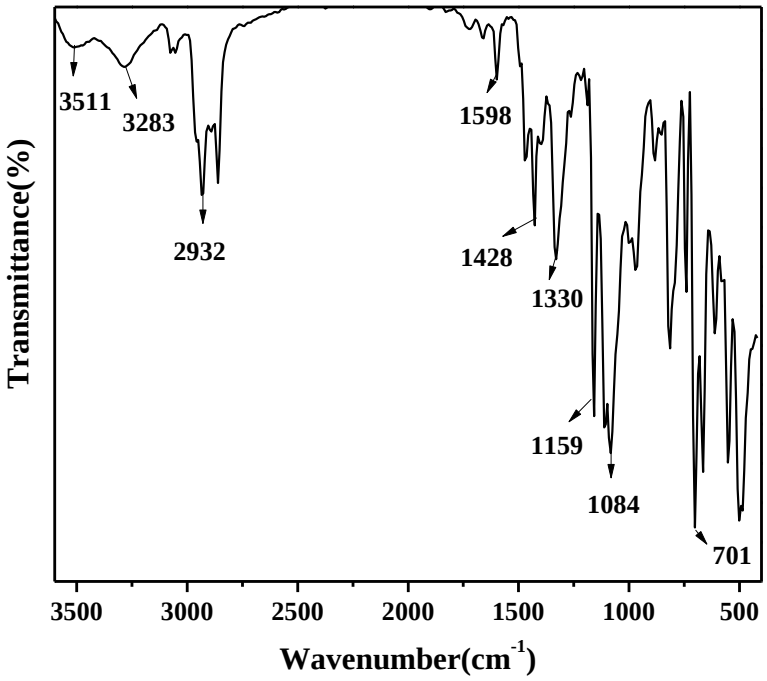


Figure 22. IR spectrum of **8**

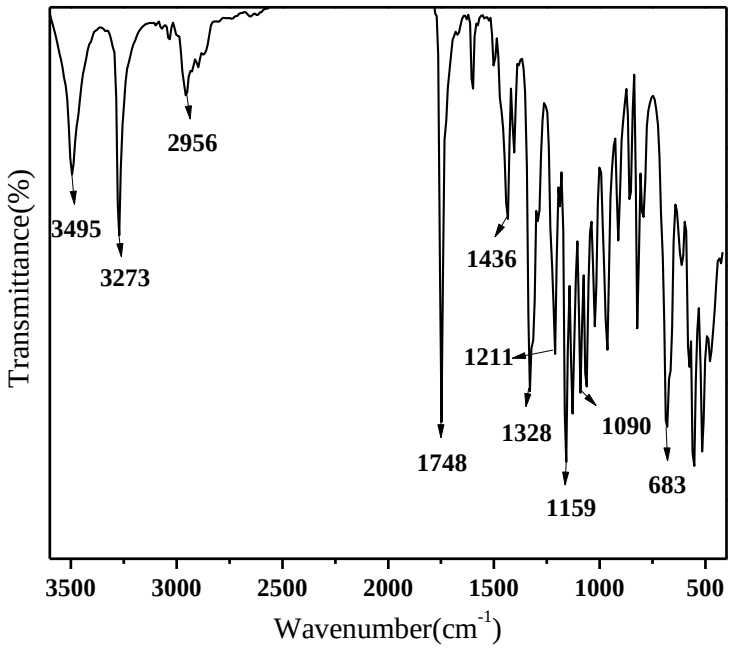


Figure23. IR spectrum of **13**

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