Synthesis of Tubuphenylalanine

M.Sc. Thesis

by

Priyanka

DISCIPLINE OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY INDORE

JUNE 2018

Synthesis of Tubuphenylalanine

A THESIS

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

by
Priyanka

DISCIPLINE OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY INDORE
JUNE 2018

INDIAN INSTITUTE OF TECHNOLOGY INDORE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Synthesis of Tubuphenylalanine** 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.

Priyanka

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

Dr. Venkatesh Chelvam

Priyanka has successfully given her M.Sc. Oral Examination held on **June , 2018**

Signature of Supervisor of M.Sc. thesis
Date:

Convener, DPGC
Date:

Signature of PSPC Member
Date:

Signature of PSPC Member
Date:

ACKNOWLEDGEMENTS

With great pleasure, I want to express my deep sense of gratitude to my supervisor **Dr. Venkatesh Chelvam**, for the wonderful opportunity to pursue research and believing in my research abilities. His constant guidance, support, and motivation has been immensely helpful to complete this M.Sc. project. His enthusiasm and dedication has always inspired me. Further, I would like to thank my PSPC members Dr. Biswarup Pathak and Dr. Sudeshna Chattopadhyay for their valuable suggestions and support.

With great pleasure, I express my respect to Prof. Pradeep Mathur (Director, Indian Institute of technology Indore) for his unending encouragement and providing all the facilities at Indian Institute of Technology Indore.

I am grateful to Dr. Amrendra Kumar Singh (Head, Discipline of Chemistry) for his suggestions and guidance in various aspects. I am also grateful to Dr. Anjan Chakraborty, Dr. Tridib Kumar Sarma, Dr. Suman Mukhopadhyay, Dr. Apurba Kumar Das, Dr. Sampak Samantha, Dr. Biswarup Pathak, Dr. Sanjay Singh, Dr. Tushar Kanti Mukherjee, Dr. Rajneesh Misra and Dr. Satya S. Bulusu for their guidance and help during various activities.

I would like to extend my deep and sincere thanks to my seniors Ramesh B. Reddy and Premansh Dudhe for their guidance, encouragement, and support.

I extend my deep thanks to my group members Sagnik Sengupta, Amit Pandit, A. V. R. Krishna Rao, Mena Asha Krishnan and Konika Thukral for their selfless co-operation and help to make my work successful.

I would also like to thank technical staff from Sophisticated Instrumentation Center (SIC), IIT Indore, Ms. Sarita Batra, Mr. Kinny Pandey, Mr. Ghanshyam Bhavsar and Mr. Manish Kushwaha for their

patience and timely technical support without which it was impossible to continue with my work. I would also like to thank Ms. Anjali Bandiwadekar, Mr. Rajesh Kumar, Mr. Lala Ram Ahirwar and other library staff.

I personally want to extend my thanks to my friend Mitali Chhabra and my all batch mates who were always there and never let me down during these M.Sc. days.

Most importantly none of this would have been possible without the support of my family. I deeply express my love and gratitude to my father Late Sh. Dilbag Singh and my mother Mrs. Sheela Devi. I want to extend my deep love and thanks to my sister Sonia Chauhan, my brothers Pardeep Chauhan and Parveen Chauhan.

Finally, I would like to express my thanks to IIT Indore for infrastructure and all others who helped and supported me directly or indirectly.

Priyanka

DEDICATION

This thesis is dedicated to my lovely Mom and Dad

Abstract

In this work, we have developed an efficient synthetic route for gram-scale synthesis of *N*-Boc-tubuphenylalanine benzyl ester (*N*-Boc-Tup-OBn) and *N*-Boc-*epi*-tubuphenylalanine benzyl ester (*N*-Boc-*epi*-Tup-OBn). These γ-aminoacid intermediates are key components for synthesis of anticancer tetrapeptides, tubulysins and can be used to derive novel tubulysin architectures with better therapeutic indices. A regio- and stereoselective hydroboration of (*R*)-4-methyl-*N*-(4-methyl-1-phenylpent-4-en-2yl)benzenesulfonamide and subsequent *in situ* oxidation in alkaline medium was used as the key step to provide corresponding *N*-tosyl 1,4-amino alcohols, which were effectively transformed into the desired γ-aminoacid intermediates in overall excellent yields.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SYMBOLS/UNITS

λ	Wavelength
δ	Chemical shift
nm	Nanometer
° C	Degree Celsius
mmol	Millimole
M	Molar
g	Gram
h	Hour
J	Coupling constant
nM	Nano molar
mL	Milli litre
dd	Doublet of doublet
Hz/MHz	Hertz/Mega Hertz
R_f	Retention factor
ppm	Parts per million

ACRONYMS

TMS	Tetramethylsilane
NMR	Nuclear Magnetic Resonance
DCM	Dichloromethane
ACN	Acetonitrile
HRMS	High Resolution Mass Spectroscopy
$CHCl_3$	Chloroform
CH_3	Methyl
$CDCl_3$	Chloroform-d
Et_3N	Triethylamine
TsCl	*p*-Toluenesulfonyl chloride
LAH	Lithium aluminum hydride
THF	Tetrahydrofuran
PDC	Pyridinium dichromate
DMF	*N*,*N*-Dimethyl formamide
9-BBN	9-Borabicyclo[3.3.1]nonane
H_2O	Water
Na_2SO_4	Sodium sulphate
OH	Hydroxyl
DMAP	4-Dimethylaminopyridine
D-Mep	*N*-Methyl-D-pipecolic acid
Ile	Isoleucine
Tup	Tubuphenylalanine
Tut	Tubutyrosine
SAR	Structure Activity Relationship
RT	Room temperature
s	Singlet
d	Doublet
t	Triplet

m	Multiple
brs	Broad singlet

Chapter 1

Introduction

1.1. General introduction

Cancer is the uncontrolled growth of the malignant cells in various tissues and organs of the body. It is one of the leading causes of mortality in India [1]. According to the survey conducted by Indian Council of Medical Research [2], approximately 25 lakhs cases of cancer are registered in India, and this number is rapidly increasing by diagnosis of ten lakh new cases per year. Cancer related mortality is estimated to be more than three lakhs per year in India. The leading sites of cancer are oral cavity, lungs, oesophagus and stomach among men and cervix, breast and oral cavity among women. The study also states that the most common causes of cancer-related mortality are due to cancers of oral cavity and lungs in males and cervix and breast in females [3]. This accounts for more than 50% of all cancer-related deaths in India.

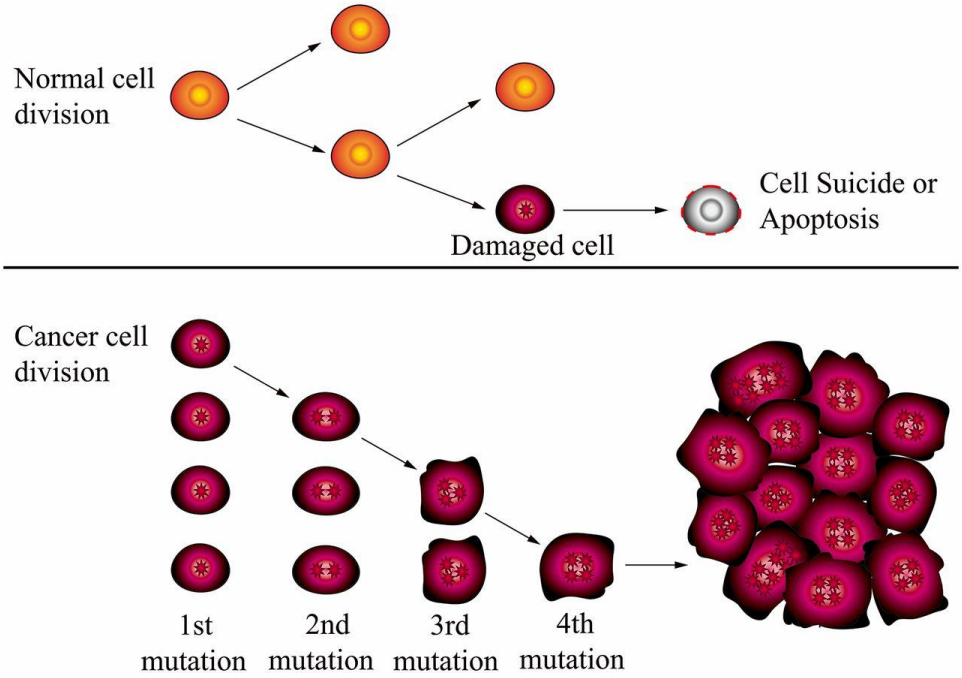


Figure 1. Division of normal cells and cancer cells [4]

1.2. Anticancer drugs for the treatment of cancer

Natural products have played a vital role in the development of important drug candidates. Natural product chemistry is a rapidly expanding research domain which explores new natural products with better therapeutic indices. Currently, more than 30 compounds of natural origin are in different phases of clinical studies for the treatment of various types of cancers. Moreover, most of the currently marketed drugs are synthetic or semi-synthetic derivatives of natural compounds. These natural products arrest cancerous growth either by inhibition of cell proliferation or by induction of apoptosis [5, 6].

Several plant-derived compounds viz., vincristine, irinotecan, etoposide, and paclitaxel are currently employed in cancer treatment [7]. Dactinomycin, bleomycin, and doxorubicin fall into a different category of anticancer agents that are derived from microbial sources. Cytarabine, bryostatin-1, aplidine, dolastatin and ET-743 are anticancer agents of marine origin and these are recently being investigated in phase I and II clinical trials [8]. Nature is one of the best inspirations to design new drugs, with microtubules as one of the favorite targets of natural products. Especially, vinca alkaloids like vinblastine, vincristine or vinorelbine have been used as a treatment for a significant number of human cancer types in past few decades [9]. Recently taxanes, with paclitaxel and docetaxel as lead compounds, joined this selected group of drugs to make a benchmark revolution in anticancer therapy. Both vinca alkaloids and taxanes, disrupt microtubule polymerization dynamics by targeting the cellular protein tubulin [10]. According to the current success rates of these drugs, microtubules are certainly the best target for treatment of cancer.

Table 1. Microtubule targeting agents in cancer treatment and their mechanism

Drugs	Cancer type	Mechanism of action
Vinca alkaloids	Breast, leukemia, head and neck cancers	Natural destabilizer; Depolymerises microtubule polymers; Disrupts mitotic spindle at high dosage; Blocks mitosis, low effective dosage without any significant microtubule depolymerization; Causes apoptosis [11]
Dolastatin	Extrahepatic bile duct, gall bladder, and liver cancers	Natural destabilizer; Causes mitotic arrest; Inhibits microtubule polymerization; Cause multi-polarity; Suppress natural decay of tubulin [12, 13]
Colchicine	Non-neoplastic cancers	Natural destabilizer; Delays microtubule growth at a low dosage and suppresses microtubule dynamics; Depolymerizes microtubule at high dosage [14, 15]
Tubulysin	Breast, lung, leukemia, ovarian and prostate cancers	Inhibits tubulin polymerization; Potent cell-growth inhibitors; Shows antiangiogenic properties; Possesses high selectivity for cancer cells than normal cells [16−18]

1.3 Tubulysins: Potent antimiototic peptides for treatment of cancer

Hofle and co-workers first isolated tubulysins from myxobacteria culture broths as antimitotic tetrapeptides [19]. Later, Muller and co-workers identified a gene cluster in myxobacteria with mixed non-ribosomal peptide synthetase-polyketide synthase system that is responsible for biosynthesis of tubulysin [20, 21]. Interestingly, tubulysin was found to surpass cell growth inhibition activity of all the established anticancer natural products such as epothilones, vinblastine, and taxol by a factor of 20 to 1000 [16]. Tubulysin interacts with the eukaryotic cytoskeleton and prevents microtubule formation by binding with tubulin protein leading to apoptosis of cells [22, 23]. The interesting biological activities of tubulysins and their derivatives justify the rapidly growing interest in the development of novel chemosynthetic methods to access more potent tubulysin derivatives with cytotoxicity in the range of low nanomolar to picomolar concentrations.

1.4 Structure activity relationships of tubulysins

Tubulysins are tetrapeptides comprised of four amino acid fragments; *N*-methyl pipecolic acid (D-Mep), L- isoleucine (Ile), and two unusual amino acids, tubuvaline (Tuv) and tubutyrosine (Tut) or tubuphenylalanine (Tup), which are connected to each other through amide linkage. *N*-Methylpipecolic acid is a carboxylic acid derivative of piperidine and is biosynthesized from L-lysine [24]. In most of the cases with minimal loss of activity, Mep ring was successfully replaced with *N*,*N*-dimethyl-D-alanine and *N*-methyl-D-proline, though tertiary amine at the *N*-terminus is important for good activity [25]. For better activity, D-configuration, and tertiary amine are required on Mep residue. Removal of Mep ring and replacement of Mep with an acetyl group results in loss of activity. Isoleucine is a natural amino acid, which provides necessary hydrophobic interactions in the tubulin protein binding pocket. Tubulysins of A-H series possess greater cytotoxicity with longer and more lipophilic Tuv-

N,O-diacyl or *N,O*-acetal side chains. The changes in *N,O*-acetyl group of Tuv unit are quite tolerated but at the C11-acetyl group of Tuv minimum variations are tolerated. In contrast to this, the C-terminus of (Tup or Tut) allows us to introduce various modifications [26].

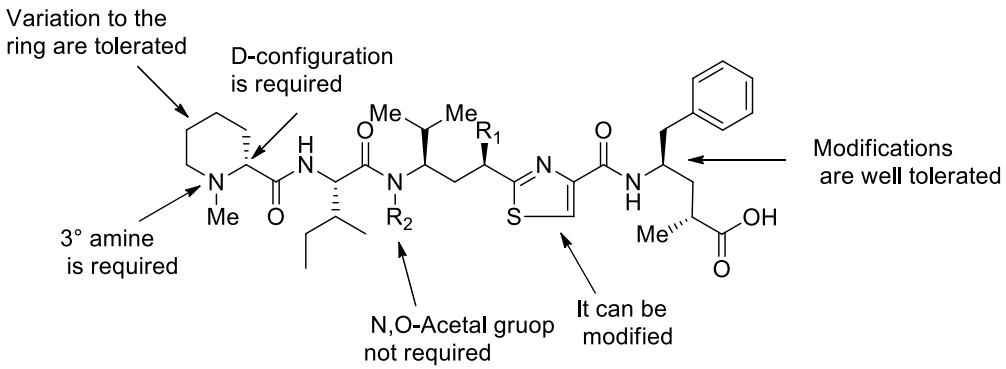


Figure 2. Summary of Structure activity relationship (SAR)

1.5 Importance of tubuphenylalanine

A broad range of modification can be made at the C-terminus fragment of tubulysins i.e. Tup and Tut. For optimal biological activity, the α-methyl group is required in both Tup and Tut residues. The stereochemistry of the tubuphenylalanine residue is less vital than the tubuvaline fragment, only a small decrease in activity is observed on inversion of C4-benzyl or C2-methyl groups [26(b), 28]. For targeted drug delivery approaches tubuphenylalanine is the most common site for introducing conjugation. According to SAR studies, Tup is in appropriate position for novel derivatization to attach fluorescence agents and targeting ligands [29]. Therefore, researchers are interested in developing novel synthetic strategies towards synthesis of Tup and *epi*-Tup. Natural tubulysins with tubuphenylalanine counterparts at C-terminus are comparatively more active and lipophilic than the tubutyrosine residue at the C-terminus [16].

1.6 Objective of the project

Tubulysins have sparked a great deal of interest in the development of novel anticancer molecules with better efficacy and multimodal theranostic tools. As per above discussion, Tup is well tolerated for a broad range of modifications and it is also a common site of conjugation for targeted drug delivery approaches which makes Tup one of the most important fragment of tubulysin.

The present thesis is focused on the up-scale synthesis of tubuphenylalanine (Tup) and its stereoisomer *epi*-tubuphenylalanine (*epi*-Tup). These unnatural amino acids will be further modified to prepare cytotoxic warheads against cancer.

In this work, a novel and practical route for the gram-scale synthesis of *N*-Boc-tubuphenylalanine benzyl ester (*N*-Boc-Tup-OBn) and *N*-Boc-*epi*-tubuphenylalanine benzyl ester (*N*-Boc-*epi*-Tup-OBn) have been developed using diastereoselective hydroboration-oxidation of (*R*)-4-methyl-*N*-(4-methyl-1-phenylpent-4-en-2yl)benzenesulfonamide without using any chiral auxiliary. The large-scale synthesis performed in the current study has explored a new horizon of possibilities to develop interesting tubulysin architectures.

Chapter 2

Literature review

Tup and Tut are the most versatile fragments of tubulysins; therefore the C-terminus is an ideal position for introducing foreign entities in this molecule to develop theranostic aids for cancer [29(a)]. To realize this goal, highly flexible stereoselective synthesis of tubuphenylalanine is desirable. Many research groups have made important contribution for the synthesis of Tup and *epi*-Tup, key building blocks of the natural and synthetic tubulysins. The synthesis of tubuphenylalanine begins with the natural chiral amino acid precursor (*S*)-phenylalanine [30], or its derivatives (*S*)-phenylalninol [31, 32], 2-benzylaziridine [33] and *γ*-benzyl lactam [34].

The existing methods for the synthesis of Tup and *epi*-Tup mostly employ chiral auxiliary controlled alkylation using oxazolidinones [35], pseudoephedrine [33], and *tert*-butanesufinamide [36], along with substrate controlled diastereoselective methylation of chiral lactam [34], diastereoselective hydrogenation [37−40], epoxide ring-opening [41] and Ireland-Claisen rearrangement of allyl ester of *β*-amino acid [42]. Recently, Park and co-workers have reported a chiral auxiliary-free direct synthetic pathway for the synthesis of tubuphenylalanine by Lewis acid-catalyzed Mukaiyama aldol condensation reaction [43].

Although a variety of methods have been developed till date, but there is enough scope to introduce refined synthetic protocols for large-scale synthesis of these biologically active entities.

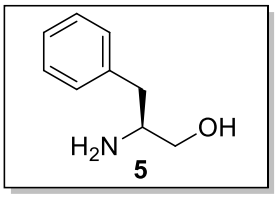
Chapter 3

Experimental Section

3.1. Materials and methods

All the reactions were performed in oven-dried glass wares under an inert atmosphere with magnetic stirring. Air and moisture sensitive liquids and solutions were transferred by using glass syringes. Flash chromatography was performed on 230−400 μm or 100−200 μm mesh silica gel. All reagents were obtained from commercial sources and used as received unless otherwise stated. Solvents were distilled using suitable drying agents (CaH_2 or Na wire, Mg turnings) under nitrogen atmosphere. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AV 400MHz NMR spectrometer with TMS as an internal reference. $CDCl_3$ was used as NMR solvent. Chemical shift is reported in delta (δ) units, expressed in parts per million (ppm) downfield from tetramethylsilane (TMS). The HRMS spectra of the compounds were recorded by using Bruker Daltonics MicroTOF-Q II mass spectrometer using acetonitrile as solvent. FT-IR spectra of samples dissolved in CH_2Cl_2 were recorded using Fourier Transform Infrared-Attenuated Total reflection (FTIR-ATR) Spectrometer, Bruker (Tensor-27) over a range of 500-4000 cm^{-1}.

3.2. Synthesis of (*S*)-2-Amino-3-phenylpropan-1-ol (5)

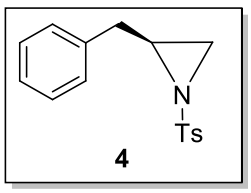


5

In an oven dried 100 mL round bottom flask, under inert atmosphere L-phenylalanine (2 g, 12.10 mmol) was added by dissolving in dry THF (15 mL). The reaction mixture was cooled to 0 °C. A solution of 1M $LiAlH_4$ (37 mL, 36.32 mmol) in THF was added dropwise to the reaction mixture with stirring. The reaction mixture was further heated to reflux on a preheated oil bath for 24 h. After complete consumption of **6**, the reaction mixture was cooled to room temperature

and 4 mL MQ water and 4 mL 15 % NaOH was added slowly to hydrolyze excess $LiAlH_4$. The solvent was evaporated under reduced pressure and the resulting white paste was dissolved in 2M NaOH (50 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford L-phenylalaninol (1.75 g, 95%) as a white solid, which was used in the next step without further purification. TLC: R_f 0.24 (1: 9, MeOH/CH_2Cl_2). IR (CH_2Cl_2): 3352 (O−H) 3279 (N−H), 3083, 3061, 3027, (=C−H), 2923, 2855(C−H), 1649 (N−H), 1575–1520 (C=C) 1494–1454 (C−H), 1054 (C−O), 701 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.25–7.11 (m, 5H), 3.56 (dd, J = 10.8, 2.8 Hz, 1H), 3.36–3.27 (dd, J = 10.8, 8.0 Hz, 1H), 3.04 (brs, 1H), 2.72 (dd, J = 13.6, 4.8 Hz, 1H), 2.45 (dd, J = 13.6, 8.8 Hz, 1H), 1.97 (brs, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.72, 129.24, 128.61, 126.45, 66.26, 54.22, 40.82. HRMS (ESI) m/z $[M+H]^+$ calcd. for $C_9H_{13}NO$ 152.1070, found 152.1101.

3.3. Synthesis of (*S*)-2-benzyl-1-tosylaziridine (4)

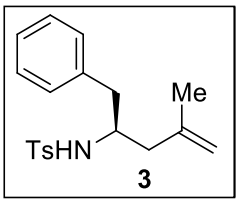


4

4 Å molecular sieves (2 g) were flame dried in a 100 mL two neck round bottom flask under reduced pressure for 15-min or more until there is no sign of appearance of water droplets or moisture in the flask. The heat dried molecular sieves were cooled to room temperature under inert atmosphere. CH_3CN (50 mL), magnetic bar and L-phenylalaninol **5** (2.8 g, 18.5 mmol) were charged to this flask. The reaction mixture was briefly cooled to 0 °C and Et_3N (7.7 mL, 55.55 mmol) and tosyl chloride (3.52 g, 18.5 mmol) were added sequentially via syringe to the reaction mixture. The reaction mixture was warmed to room temperature and further stirred for 1 h. After complete consumption of L-phenylalaninol, as confirmed by TLC, acetonitrile was evaporated under reduced

pressure using rotary evaporator and the residue was dissolved in EtOAc (50 mL). The resultant precipitate and molecular sieves were filtered using Buchner funnel and washed with EtOAc (3 × 50 mL). The organic solvent was concentrated by rotary evaporator under reduced pressure and the crude mixture was purified by silica gel column chromatography (1:24 EtOAc/hexane) to afford **4** (4 g, 85%) as a white solid. m.p. 91–93 °C. TLC: R_f 0.25 (9:1, hexane/EtOAc). IR (CH_2Cl_2): 3029, 3001 (=C−H), 2949, 2922 (C−H), 1699–1539 (C=C), 1317 (N−S=O), 1157 (S=O), 712 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 7.16–7.14 (m, 3H), 7.05–7.03 (m, 2H), 2.97–2.91 (m, 1H), 2.81 (dd, J = 14.4, 5.2 Hz, 1H), 2.70 (d, J = 6.8 Hz, 1H), 2.68 (dd, J = 14.4, 7.2 Hz, 1H), 2.42 (s, 3H), 2.16 (d, J = 4.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.32, 137.02, 134.88, 129.60, 128.73, 128.46, 127.88, 126.51, 41.19, 37.50, 32.83, 21.62. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{16}H_{17}NO_2S$ 310.0872, found 310.0883.

3.4. Synthesis of (*R*)-4-methyl-*N*-(4-methyl-1-phenylpent-4-en-2-yl) benzenesulfonamide (3)



3

A 250 mL two-neck round-bottom flask was charged with catalytic amount of CuCN (0.2 equiv.) under an inert atmosphere. *N*-Tosyl-benzylaziridine **4** (2.5 g, 8.7 mmol) dissolved in dry THF (50 mL) was added to the reaction mixture at 0 °C. Isopropenyl magnesium bromide (34.8 mL, 17.4 mmol, 0.5 M in THF) was added dropwise to the reaction mixture over a period of 20 minutes with stirring. The reaction mixture was allowed to warm to room temperature and further stirred for 2 h. After the consumption of *N*-Tosyl-benzylaziridine **4,** as confirmed by TLC, the reaction mixture was quenched with saturated NH_4Cl (30 mL) solution and further diluted with EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL) and the combined organic extracts was

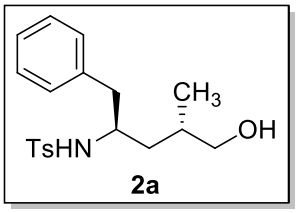
washed with brine (100 mL) and dried over anhydrous Na_2SO_4. The organic layer was filtered, evaporated under reduced pressure, and the crude residue was purified over silica gel column chromatography (32:1 hexane/EtOAc) to afford the alkene **3** (2.1 g, 90%) as colorless liquid. TLC: R_f 0.27 (9:1, hexane/EtOAc). IR (CH_2Cl_2): 3284 (N−H), 3064, 3027 (=C−H), 2923, (C−H), 1716–1520 (C=C) 1494–1452 (−C−H), 1342, 1157 (S=O), 701 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 8.0 Hz, 2H), 7.26–7.19 (m, 5H), 7.07–7.05 (m, 2H), 4.76−4.67 (m, 2H), 4.32 (d, J = 5.6 Hz, 1H), 3.49–3.41 (m, 1H), 2.85, (dd, J = 13.6, 5.6 Hz, 1H), 2.76, (dd, J = 13.6, 6.8 Hz, 1H) 2.41 (s, 3H), (dd, J = 14.0, 6.4 Hz, 1H), (dd, J = 14.0, 8.4 Hz, 1H), 1.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.22, 141.66, 137.21, 137.20, 129.58, 129.55, 128.47, 127.18, 126.54, 114.51, 52.66, 43.07, 41.20, 21.55, 21.51. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{19}H_{23}NO_2S$ 352.1342, found 352.1345.

3.5. General procedure for hydroboration reactions

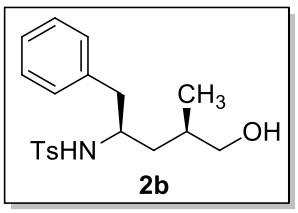
9-BBN (3 equiv., 0.5 M THF solution) was added to a solution of alkene **3** (1.0 equiv.) in dry THF at room temperature. The resulting solution was further stirred for 12 h at the same temperature under nitrogen atmosphere. 2M NaOH (3.5 equiv.) and 30% H_2O_2 (4 equiv.) were added at 0 °C to the reaction mixture and stirring was continued for 12 h at room temperature. The reaction mixture was quenched by adding saturated aq. NaCl (20 mL) at room temperature and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified using hexane:EtOAc as eluent over silica gel column chromatography.

3.6. Synthesis of *N*-((2*R*,4*S* or 2*R*,4*R*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (2a and 2b)

According to the general procedure, 9-BBN (38.2 mL, 19.1 mmol, 0.5 M THF solution) was added to alkene **3** (2.1 g, 6.37 mmol) in dry THF (20 mL) and stirred at room temperature for 12 h. 2M NaOH (11.0 mL, 22.29 mmol) and 30% H_2O_2 (9.0 mL, 89.14 mmol) were added at 0 °C to the reaction mixture and stirred for 12 h at room temperature and worked up as mentioned in general procedure. The residue was purified over silica gel using column chromatography (3:1 hexane/EtOAc), to afford **2a** and **2b** as colorless liquid and pale yellow liquid respectively (9.1 g, combined yield 90 %, (**2a:2b** 69:31).



2a: TLC: R_f 0.3 (1:1, hexane/EtOAc). IR (CH_2Cl_2): 3504 (O−H), 3061, 3028 (=C−H), 2925–2872 (C−H), 1598 (C=C), 1494, 1453, (C−H), 1320, 1152 (S=O), 1090 (C−O), 700 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 8.0 Hz, 2H), 7.26–7.19 (m, 5H), 6.69–6.97 (m, 2H), 4.66 (d, J = 7.2 Hz, 1H), 3.70–3.61 (m, 1H), 3.44 (dd, J = 10.4, 5.2 Hz, 1H), 3.33 (dd, J = 10.4, 6.8 Hz, 1H), 2.64–2.62 (m, 2H), 2.41 (s, 3H), 1.87–1.79 (m, 1H), 1.55–1.49 (m, 1H), 1.30–1.23 (m, 1H), 0.84 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.23, 137.82, 136.92, 129.66, 129.50, 128.53, 127.01, 126.61, 67.89, 52.94, 41.19, 39.10, 31.79, 21.52, 17.58. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{19}H_{25}NO_3S$ 370.1447, found 370.1448.



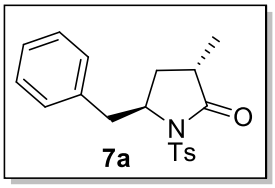
2b: TLC: R_f 0.25 (1:1, hexane/EtOAc). IR (CH_2Cl_2): 3506 (O−H), 3061, 3028 (=C−H), 2927, 2873 (C−H) 1598 (C=C), 1495, 1454, (C−H), 1322, 1154 (S=O), 1092 (C−O), 733 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.71 (d, J = 8.0 Hz, 2H), 7.27–7.18 (m, 5H), 7.02–7.01 (m, 2H), 4.85 (brs,

1H), 3.56–3.48 (m, 1H), 3.43 (dd, J = 10.4, 5.2 Hz, 1H), 3.28 (dd, J = 10.4, 7.2 Hz, 1H), 2.73 (dd, J = 13.6, 4.8 Hz 1H), 2.63 (dd, J = 13.6, 7.2 Hz 1H) 2.41 (s, 3H), 1.69 (brs, 1H), 1.64–1.57 (m, 1H), 1.55–1.48 (m, 1H), 1.22–1.16 (m, 1H), 0.68 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.24, 137.95, 137.27, 129.67, 129.55, 128.48, 127.07, 126.53, 68.11, 53.24, 42.37, 38.55, 32.34, 21.54, 16.42. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{19}H_{25}NO_3S$ 370.1447, found 370.1443.

3.7. General procedure for the synthesis of *N*-Ts-*γ*-lactams 7a and 7b

Dry DMF (10 mL) was added to 1,4-amino alcohol (**2a** or **2b,** 1.0 equiv.) taken in a 100 mL round-bottom flask charged with a magnetic bead and the mixture was stirred at room temperature. Pyridinium dichromate (5.0 equiv.) was added to above reaction mixture in one portion and stirred for 24 h at the same temperature. After the complete consumption of 1,4-amino alcohol, the reaction mixture was quenched with cold water (10 mL). The aqueous layer was extracted with EtOAc (3 × 75 mL) and the combined organic layers were washed with cold brine (3 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified over silica gel column chromatography.

3.8. Synthesis of (3*S*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (7a)

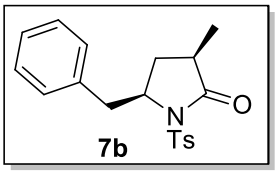


7a

According to the general procedure, to a solution of 1,4-amino alcohol **2a** (500 mg, 1.43 mmol) in dry DMF (10 mL) taken in a round bottom flask (50 mL), PDC (2.7 g, 7.19 mmol) was added to the reaction mixture and stirred for 24 h at room temperature. The work up was performed as mentioned in the general procedure and the residue was purified over silica gel column chromatography (6:1 hexane/EtOAc) to afford lactam **7a** as colorless sticky liquid, (379 mg, 77%). TLC: R_f 0.53

(2:1, hexane/EtOAc). IR (CH_2Cl_2): (O−H), 3062, 3028, (=C−H), 2931, 2874 (C−H) 1729 (C=O), 1650 (C=C), 1494, 1453 (C−H), 1352, 1159 (S=O), 711 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.0 (d, J = 8.4 Hz, 2H), 7.35–7.21 (m, 7H), 4.54 (td, J = 8.8, 3.2 Hz, 1H), 3.36 (dd, J = 13.2, 3.2 Hz, 1H), 2.81 (dd, J = 13.2, 9.6 Hz, 1H), 2.44 (s, 3H), 2.34–2.26 (m, 1H), 2.11–2.04 (m, 1H), 1.67–1.58 (m, 1H), 1.02 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.82, 145.06, 136.72, 136.08, 129.59, 129.50, 128.81, 128.39, 127.04, 58.88, 40.25, 35.70, 31.86, 21.69, 14.90. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{19}H_{21}NO_3S$ 366.1134, found 366.1138.

3.9. Synthesis of (3*R*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (7b)



7b

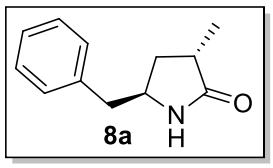
According to the general procedure, to a solution of 1,4-amino alcohol **2b** (457 mg, 1.31 mmol) in dry DMF (10 mL) taken in a round bottom flask (50 mL), PDC (2.5 g, 6.57 mmol) was added to the reaction mixture and stirred for 24 h at room temperature. After workup, the residue was purified over silica gel column chromatography (6:1 hexane/EtOAc) to afford γ-lactam **7b** as white solid, (340 mg, 77 %). m.p. 122-124 °C. TLC: R_f 0.53 (2:1, hexane/EtOAc). IR (CH_2Cl_2): 3061, 3028, (=C−H), 2930, 2875 (C−H) 1732 (C=O), 1650 (C=C), 1494, 1454 (C−H), 1355, 1164 (S=O), 702 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 8.4 Hz, 2H), 7.35–7.22 (m, 7H), 4.48–4.41 (m, 1H), 3.84 (dd, J = 12.8, 3.6 Hz, 1H), 2.63 (dd, J = 12.8, 10.0 Hz, 1H), 2.44 (s, 3H), 2.41–2.35 (m, 1H), 2.20–2.12 (m, 1H), 1.46–1.39 (m, 1H), 1.09 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.82, 145.01, 136.44, 136.14, 129.59 (2C*), 128.72, 128.32, 126.96, 59.61, 42.63, 36.90, 31.68, 21.69, 16.15. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{19}H_{21}NO_3S$ 366.1134, found 366.1140.

*higher intensity carbon

3.10. General procedure for tosyl deprotection

In a 100 mL round-bottom flask charged with magnetic bead, Mg turnings (10.0 equiv.) and NH_4Cl (5.0 equiv.), dry methanol was added under an inert atmosphere with stirring. A solution of Ts-*γ*-lactam (**7a** or **7b,** 1.0 equiv.) in dry methanol was added to the above suspension and stirred at room temperature for 10 minutes. The reaction mixture was further refluxed at 70 °C for 2 h. After complete consumption of Ts-*γ*-lactam (**7a** or **7b**), methanol was concentrated under reduced pressure in rotary evaporator and the crude residue was purified over silica gel column chromatography using hexane:EtOAc as eluent.

3.11. (3*S*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one (8a)

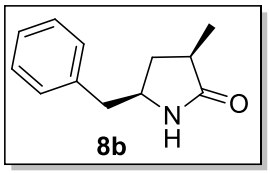


8a

According to the general procedure, *N*-tosyl-*γ*-lactam **7a** (350 mg, 1.02 mmol) in dry methanol (7 mL) was added to a suspension of Mg turnings (244 mg, 10.2 mmol) and NH_4Cl (273 mg, 5.0 mmol) in methanol (5 mL) taken in a 100 mL round bottom flask. The suspension was refluxed at 70 °C for 2 h under inert atmosphere and worked up as mentioned in the general procedure. The crude residue was purified over silica gel column chromatography (1:1 hexane/EtOAc) to afford **8a** (161 mg, 83%) as colorless liquid. TLC: R_f 0.11 (1:3, hexane/EtOAc). IR (CH_2Cl_2): 3230 (N−H), 3028 (=C−H), 2928, 2871 (C−H), 1693 (C=O), 1557, 1493, 1456 (C−H), 701 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.23 (m, 3H), 7.21–7.15 (m, 2H), 5.87 (brs, 1H), 3.85–3.78 (m, 1H), 2.81 (dd, J = 13.2, 5.6 Hz, 1H), 2.71 (dd, J = 13.2, 8.4 Hz, 1H), 2.50–2.40 (m, 1H), 2.15–2.08 (m, 1H), 1.93–1.86 (m, 1H), 1.18 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.36, 137.68,

129.12, 128.80, 126.84, 53.41, 42.81, 35.12, 34.92, 16.23. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{12}H_{15}NO$ 212.1046, found 212.1069.

3.12. (3*R*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one (8b)



8b

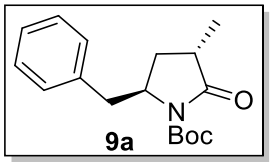
According to the general procedure, a solution of *N*-tosyl-*γ*-lactam **7b** (200 mg, 0.58 mmol) in dry methanol (5 mL), was added to suspension of Mg turnings (140 mg, 5.82 mmol) and NH_4Cl (156 mg, 3 mmol) in methanol (5 mL) in a round bottom flask. The suspension was refluxed at 70 °C with stirring for 2 h under inert atmosphere and worked up as mentioned in the general procedure. The crude residue was purified over silica gel column chromatography (1:1 EtOAc/hexane) to afford **8b** (80 mg, 79%) as colorless liquid. TLC: R_f 0.11 (3:1, EtOAc/hexane).IR (CH_2Cl_2): 3232 (N−H), 3029 (=C−H), 2928, 2875 (C−H), 1695(C=O), 1557, 1492, 1456 (C−H), 700 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.22 (m, 3H), 7.20–7.12 (m, 2H), 5.96 (brs, 1H), 3.85–3.78 (m, 1H), 2.81 (dd, J = 13.6, 6.0 Hz, 1H), 2.72 (dd, J = 13.6, 8.0 Hz, 1H), 2.49–2.40 (m, 1H), 2.14–2.08 (m, 1H), 1.92–1.85 (m, 1H), 1.18 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.69, 137.66, 128.88, 128.84, 126.95, 53.78, 43.38, 37.00, 36.81, 15.97. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{12}H_{15}NO$ 212.1046, found 212.1055.

3.13. General procedure for synthesis of 9a and 9b

Dry CH_2Cl_2 (10 mL) was added to *γ*-lactam (**8a** or **8b**, 1.0 equiv.) taken in a two-neck round bottom flask (100 mL) under inert atmosphere. The reaction mixture was cooled to 0 °C and solid DMAP (0.2 equiv.) was added to the reaction mixture in single portion with stirring followed by dropwise addition of Boc_2O (2.0 equiv.) using syringe. The reaction mixture was allowed to warm to room temperature and stirred for 2.5 h. After the consumption of *γ*-

lactam (**9a** or **9b**), the solvent was evaporated under reduced pressure using rotary evaporator. The crude mixture was purified over silica gel column chromatography using hexane:EtOAc as eluent.

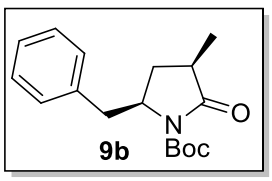
3.14. (3*S*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate (9a)



9a

According to the general procedure, to a solution of *γ*-lactam **8a** (125 mg, 0.68 mmol) in dry CH_2Cl_2 (10 mL) in a round bottom flask (100 mL), DMAP (16.6 mg, 0.13 mmol) and Boc_2O (0.3 mL, 1.36 mmol) were added to the reaction mixture at 0 °C and further stirred at room temperature for 2.5 h. The solvent was evaporated under reduced pressure using rotary evaporator and the crude mixture was purified over silica gel column chromatography (8:1 hexane/EtOAc) to afford **9a** (190 mg, 96%) as colorless liquid. TLC: R_f 0.72 (1:1, hexane/EtOAc). IR (CH_2Cl_2): 3028 (=C−H), 2928, 2873 (C−H), 1746, 1711 (C=O), 1650 (C=C), 1494, 1455, 1305 (C−H), 1150 (C−O), 702 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.35–7.23 (m, 3H), 7.22–7.16 (m, 2H), 4.28 (td, J = 8.8, 3.2 Hz, 1H), 3.13 (dd, J = 13.2, 3.2 Hz, 1H), 2.71 (dd, J = 13.2, 9.2 Hz, 1H), 2.44–2.35 (m, 1H), 2.04 (dd, J = 12.8, 8.4 Hz, 1H), 1.58 (s, 9H), 1.54–1.42 (m, 1H), 1.15 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.64, 150.13, 137.45, 129.39, 128.74, 126.84, 82.92, 56.95, 39.21, 36.20, 30.78, 28.16, 15.36. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{17}H_{23}NO_3$ 312.1570, found 312.1657.

3.15. (3*R*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate (9b)



9b

According to the general procedure, to a solution of *γ*-lactam **8b** (70 mg, 0.26 mmol) in dry CH_2Cl_2 (10 mL) in a round bottom flask

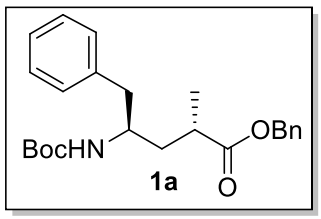
(100 mL), DMAP (6.37 mg, 0.05 mmol) and Boc_2O (0.12 mL, 0.52 mmol) were added to the reaction mixture at 0 °C and further stirred at room temperature for 2.5 h. The solvent was evaporated under reduced pressure using rotary evaporator and the crude reaction mixture was purified over silica gel column chromatography (8:1 hexane/EtOAc) to afford **9b** (109 mg, 99%) as colorless liquid. TLC: R_f 0.72 (1:1, hexane EtOAc). IR (CH_2Cl_2): 3029 (=C−H), 2928, 2873 (C−H), 1740, 1711 (C=O), 1652 (C=C), 1491, 1453, 1305 (C−H), 1148 (C−O), 702 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.23 (m, 3H), 7.22–7.15 (m, 2H), 4.24–4.17 (m, 1H), 3.49 (dd, J = 13.2, 3.2 Hz, 1H), 2.60 (dd, J = 13.2, 10 Hz, 1H), 2.54–2.44 (m, 1H), 2.17–2.09 (m, 1H), 1.60 (s, 9H), 1.40–1.33 (m, 1H), 1.18 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.22, 150.49, 137.00, 129.53, 128.65, 126.78, 83.12, 57.33, 41.32, 37.37, 30.53, 28.16, 16.67. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{17}H_{23}NO_3$ 312.1570, found 312.1574.

3.16. General procedure for the synthesis of 1a and 1b

Dry THF (5 mL) was added to *N*-Boc-*γ*-lactam (**9a** or **9b,** 1.0 equiv.) taken in a round-bottom flask (100 mL) with a magnetic bead. An aq.solution of LiOH (5.0 equiv.) and 30% aq.H_2O_2 (5.0 equiv.) were added to the reaction mixture at room temperature with constant stirring. The reaction mixture was stirred for further 12 h at room temperature, acidified with an aq. solution of 5% $KHSO_4$ (5 mL), and extracted with EtOAc (20 × 3 mL). The combined organic layers was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, evaporated under reduced pressure to afford the intermediate carboxylic acid which was used as such for benzylation reaction without further purification. The intermediate crude carboxylic acid was dissolved in dry CH_2Cl_2 and Et_3N (2.0 equiv.) was added to the mixture with stirring at room temperature. BnBr (1.5 equiv.) was

added to the reaction mixture via a syringe and stirred for 24 h at room temperature. The reaction mixture was quenched with cold water (5 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure using rotary evaporator. The crude residue was purified over silica gel column chromatography using hexane:EtOAc as eluent to afford **1a** or **1b**.

3.17. (2*S*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate (1a)

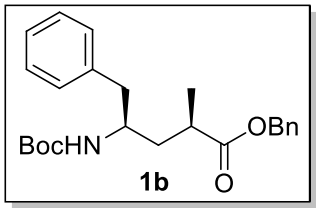


1a

According to the general procedure, to a solution of *N*-Boc-*γ*-lactam **9a** (75 mg, 0.25 mmol) in THF (5 mL) in a round bottom flask, an aq.solution of LiOH (31 mg in 2 mL of H_2O, 1.29 mmol), 30% aq.H_2O_2 (1 mL) were added and the reaction mixture was stirred at room temperature for 12 h. After workup, as mentioned in general procedure, the solvent was evaporated under reduced pressure using rotary evaporator to afford the intermediate carboxylic acid. The crude carboxylic acid (75 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and Et_3N (0.06 mL, 0.48 mmol) was added to the mixture with stirring followed by addition of BnBr (0.04 mL, 0.36 mmol). The reaction mixture was stirred at room temperature for 24 h, worked up as mentioned in the general procedure. The crude residue was purified over silica gel column chromatography (11:1 hexane/EtOAc) to afford **1a** (75 mg, 75%) as pale yellow liquid. TLC: R_f 0.44 (4:1, hexane/EtOAc). IR (CH_2Cl_2): 3366 (N−H), 3062, 3029 (=C−H), 2973, 2931 (C−H), 1731, 1700 (C=O), 1651 (C=C), 1498, 1455 (C−H), 1167 (C−O), 698 (=C−H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.34–7.13 (m, 10H), 5.15-5.07 (m, 2H), 4.30 (d, J =

8 Hz, 1H), 3.89 (m, 1H), 2.77 (m, 2H), 2.65 (s, 1H), 1.95–1.88 (m, 1H), 1.48–1.38 (m, 1H), 1.38 (s, 9H), 1.17 (d, J = 8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.06, 155.23, 137.84, 136.09, 129.49, 128.54, 128.34, 128.12, 126.36, 79.11, 66.28, 49.76, 41.34, 37.99, 36.47, 28.37, 17.69. HRMS (ESI) m/z $[M+Na]^+$ calcd. For $C_{24}H_{31}NO_4$ 420.2145, found 420.2160.

3.18. (2*R*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl) amino)-2-methyl-5-phenylpentanoate (1b)



1b

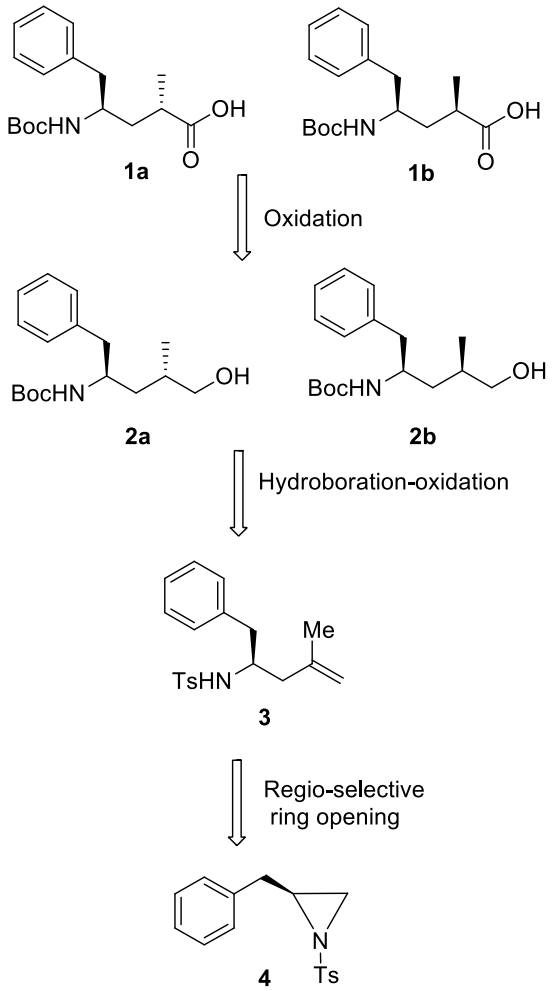
According to the general procedure, to a solution of *N*-Boc-γ-lactam **9b** (75 mg, 0.25 mmol) in THF (5 mL) in a round bottom flask, an aq.solution of LiOH (31 mg in 2 mL of H_2O, 1.29 mmol), 30% aq.H_2O_2 (1 mL) were added and the reaction mixture was stirred at room temperature for 12 h. After workup, as mentioned in general procedure, the solvent was evaporated under reduced pressure using rotary evaporator to afford the intermediate carboxylic acid. The crude carboxylic acid (75 mg, 0.24 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and Et_3N (0.06 mL, 0.48 mmol) was added to the mixture with stirring followed by addition of BnBr (0.04 mL, 0.36 mmol). The reaction mixture was stirred at room temperature for 24 h, worked up as mentioned in the general procedure. The crude residue was purified over silica gel column chromatography (11:1 hexane/EtOAc) to afford **1b** (70 mg, 72%) as colorless liquid. TLC: R_f 0.44 (4:1, hexane/EtOAc). IR (CH_2Cl_2): 3366 (N−H), 3063, 3029 (=C−H), 2973, 2931 (C−H), 1729, 1699 (C=O), 1651 (C=C), 1496, 1454 (C−H), 1164 (C−O), 697 (=C−H) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.13 (m, 10H), 5.11 (s, 2H), 4.28 (d, J = 6.8 Hz 1H), 3.90–3.84 (m, 1H), 2.79–2.72 (m, 2H), 2.55–2.68 (m, 1H), 1.96–1.84 (m, 1H), 1.48–1.41 (m, 1H), 1.38 (s, 9H), 1.17 (d, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 175.01, 154.21, 136.80, 135.06, 128.46, 127.50, 127.33, 127.31, 127.08, 125.32, 78.08, 65.24, 48.73, 40.32, 36.96, 35.44, 27.34, 16.65. HRMS (ESI) m/z $[M+Na]^+$ calcd. for $C_{24}H_{31}NO_4$ 420.2145, found 420.2254.

Chapter 4

Results and Discussion

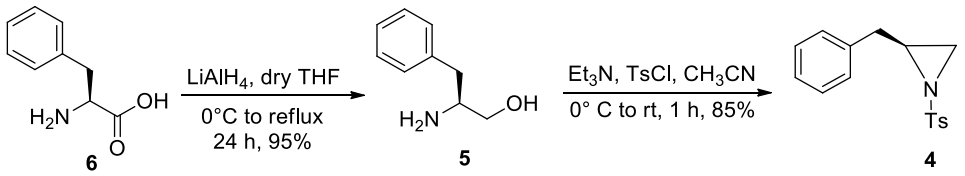
Tubuphenylalanine **1a** (Tup) and its stereoisomer *epi*-tubuphenylalanine **1b** (*epi*-Tup) are important components of cytotoxic peptides of tubulysin class. According to our retrosynthetic analysis (Scheme 1), **1a** and **1b** can be easily synthesized from their corresponding alcohols **2a** and **2b**.



Scheme 1. Retrosynthetic analysis of *N*-Boc-Tup-OBn (**1a**) and *N*-Boc-*epi*-Tup-OBn (**1b**)

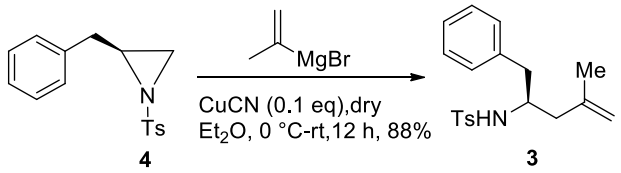
Amino alcohols **2a** and **2b** directly correspond to a common alkene precursor **3** which can be easily obtained by regioselective aziridine ring opening of **4** by Grignard reagent, isopropenyl magnesium bromide. In literature, there are several reports for aziridine ring formation from their amino acid precursors in two successive steps. The acid group of natural amino acid can be reduced into corresponding alcohol by using strong reducing agent, $LiAlH_4$ and the *O*-tosylated derivative of this amino-alcohol can furnish the desired aziridine in quantitative yield via S_N2 mechanism.

We executed our synthetic strategy starting with reduction of C-terminus of natural amino acid, L-phenyl alanine **6**. In refluxing tetrahydrofuran (THF) the natural amino acid **6** was smoothly transformed into phenyl alaninol **5** in excellent yield. Crude product **5** was directly subjected to tosylation using *p*-toluenesulfonyl chloride in acetonitrile. The intermediate bis-tosylated amino-alcohol was transformed into *N*-Ts benzylaziridine **4** in almost quantitative yields (Scheme 2).



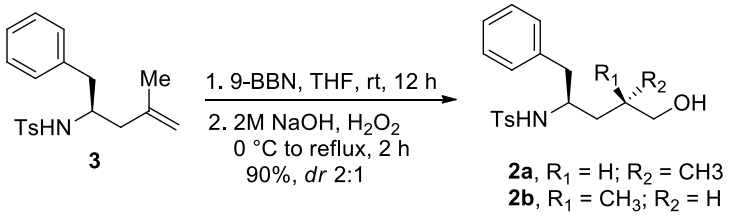
Scheme 2. Synthesis of (*S*)-2-benzyl-1-tosylaziridine (**4**)

Aziridine rings are reactive intermediates and prone to ring opening reactions due to their strained nature. Grignard reagent has been used predominantly in the literature for C−C bond formation reactions. The nucleophilic opening of aziridine ring gives higher homologues of the series by increasing chain length by two carbon atoms. Isopropenyl magnesium bromide in presence of Cu(I)CN facilitates nucleophilic ring opening of (*S*)-2-benzyl-1-tosylaziridine **4** through the less hindered methylene carbon resulting in the formation of *N*-tosyl amino alkene **3** in 88% yield (Scheme 3).



Scheme 3. Regioselective ring opening of (*S*)-2-benzyl-1-tosylaziridine (**4)**

Since its inception, hydroboration has been proved to be an instrumental reaction in the synthesis of alkylboranes. 9-BBN is a bulky reagent and has been proved useful to regioselectively prepare alcohols thereby avoiding the formation mixture of regioisomers in comparison to other conventional boronating agents. The regioselective hydroboration of alkene and subsequent *in situ* oxidation results in formation of alcohol. The crucial hydroboration-oxidation reaction of *N*-tosylamino alkene **3** was performed using the reagent 9-BBN/2MNaOH/30% H_2O_2 to provide a separable 2:1 diastereomeric mixture of 1,4-amino alcohols (**2a**, **2b**) in a combined yield of 90% (Scheme 4).

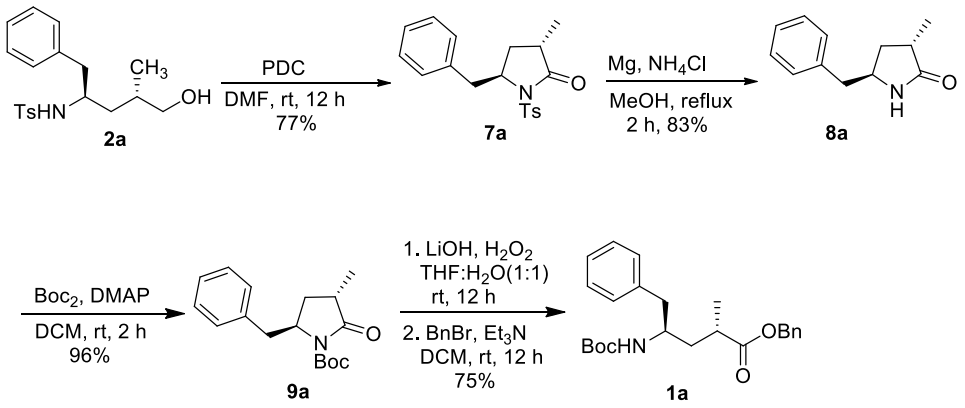


Scheme 4. Synthesis of *N*-tosyl-1,4-amino alcohols (**2a** and **2b**)

Both these amino alcohols **2a** and **2b** are precursors of biologically important synthetic targets, Tup and *epi*-Tup. Moreover the amino-alcohols **2a** and **2b** can be separately converted to **1a** and **1b** under similar reaction conditions (Schemes 5 and 6).

1,4-amino alcohol **2a** was subjected to oxidation using pyridinium dichromate (PDC). During the oxidation of primary hydroxyl group in **2a**,

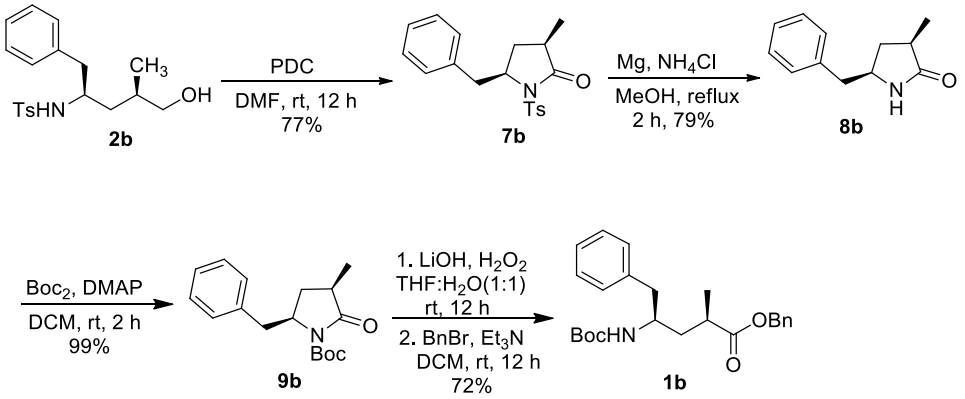
the intermediate chromate ester (not shown) underwent *in situ* cyclisation to furnish *N*-tosyl-*γ*-lactam **7a** as the only product.



Scheme 5. Synthesis of *N*-Boc-Tup-OBn (**1a**)

The *N*-tosyl *γ*-lactam **7a** was detosylated via a single electron transfer mechanism using Mg/NH_4Cl under reflux conditions in methanol to provide **8a**. The Boc-protection was performed using Boc_2O and DMAP to give **9a** in 96% yield. The Boc-protected *γ*-lactam **9a** was treated with LiOH in presence of H_2O_2 to open the lactam ring to afford a carboxylic acid intermediate. Without isolation of the carboxylic acid intermediate our desired product *N*-Boc-Tup-OBn **1a** was formed by reaction with benzyl bromide in the presence of mild base Et_3N in 75% yield over two steps (Scheme 5).

Zanda and coworkers have reported that modification of tubuphenylalanine stereochemistry does not affect the cytotoxicity of tubulysin molecule. This makes diastereomer of tubuphenylalanine i.e. *epi*-tubuphenylalanine also an important synthetic target. This molecule has been a part of many synthetic tubulysins with similar activity profile. Therefore, in our synthetic strategy, we have subjected amino-alcohol **2b** to similar reaction conditions as for the preparation of **1a** to obtain **1b** in an overall moderate yield (Scheme 6).



Scheme 6. Preparation of *N*-Boc-*epi*-Tup-OBn (**1b**)

The final products **1a** and **1b** were fully characterized using various spectroscopic techniques such as ^{1}H, ^{13}C NMR, IR and HRMS.

Chapter 5

Conclusions

In this work, we have successfully synthesized two well studied C-terminal fragments of natural and synthetic tubulysins, tubuphenylalanine (Tup) and *epi*-tubuphenylalanine (*epi*-Tup) in good yield through regio- and stereoselective hydroboration-oxidation of 1,1-disubstituted terminal alkene **3** with 9-BBN. Besides this, we have developed a straight forward synthetic pathway for preparation of *N*-tosylamino alkene **3** using regioselective ring opening of aziridine intermediate by organocuprate reagent which is the key step of Tup synthesis.

All compounds are well characterized by NMR, IR, and mass spectroscopy techniques. The synthesized tubuphenylalanine (Tup) and *epi*-tubuphenylalanine (*epi*-Tup) will be further modified to develop novel cytotoxic warheads against cancer.

APPENDIX-A

^{1}H NMR, ^{13}C NMR and Mass Spectra

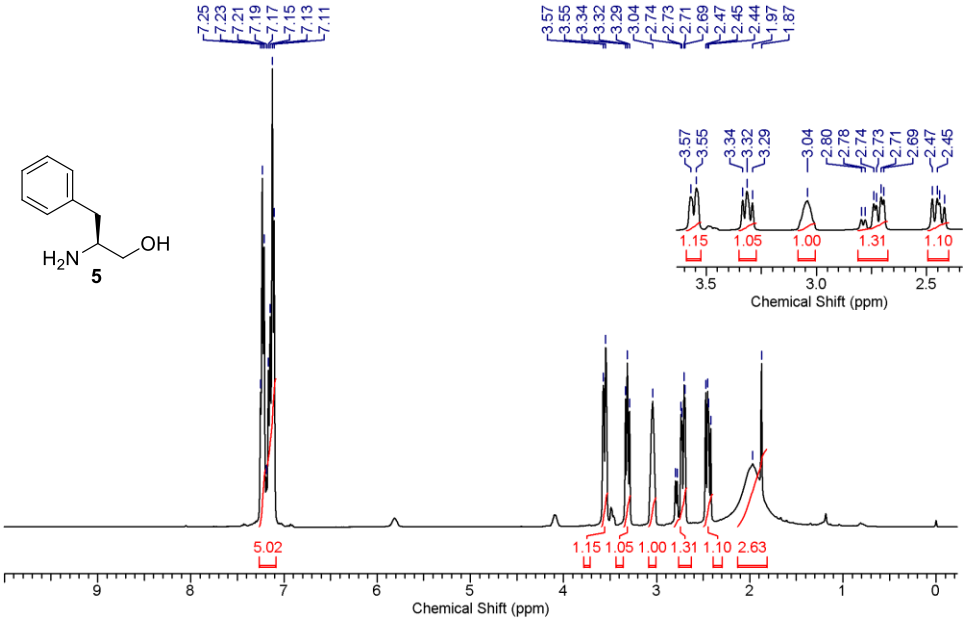


Figure 3. ^{1}H-NMR spectrum of (*S*)-2-amino-3-phenylpropan-1-ol (**5)** in $CDCl_3$

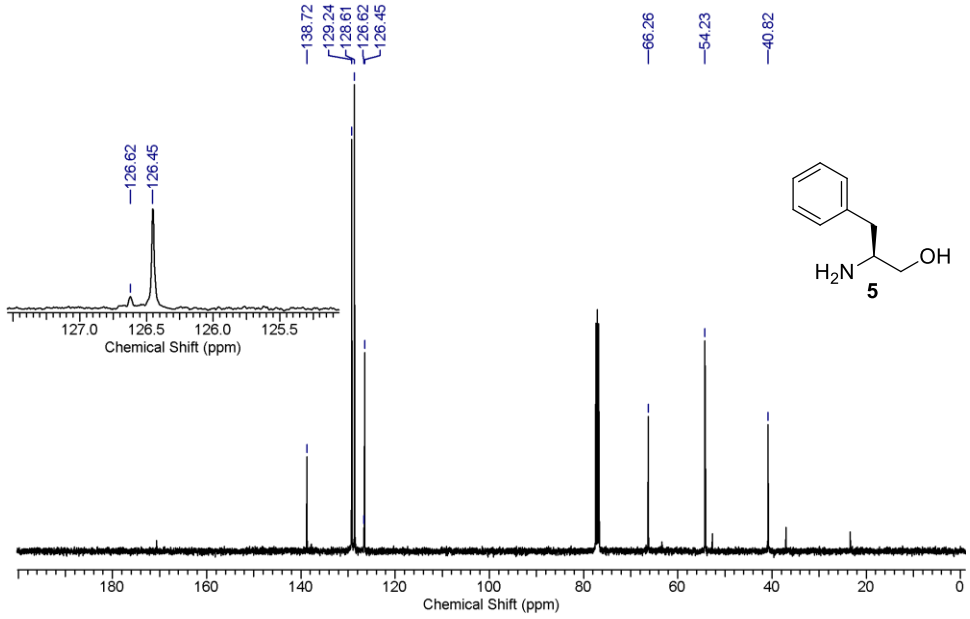


Figure 4. ^{13}C-NMR spectrum of (*S*)-2-amino-3-phenylpropan-1-ol (**5)** in $CDCl_3$

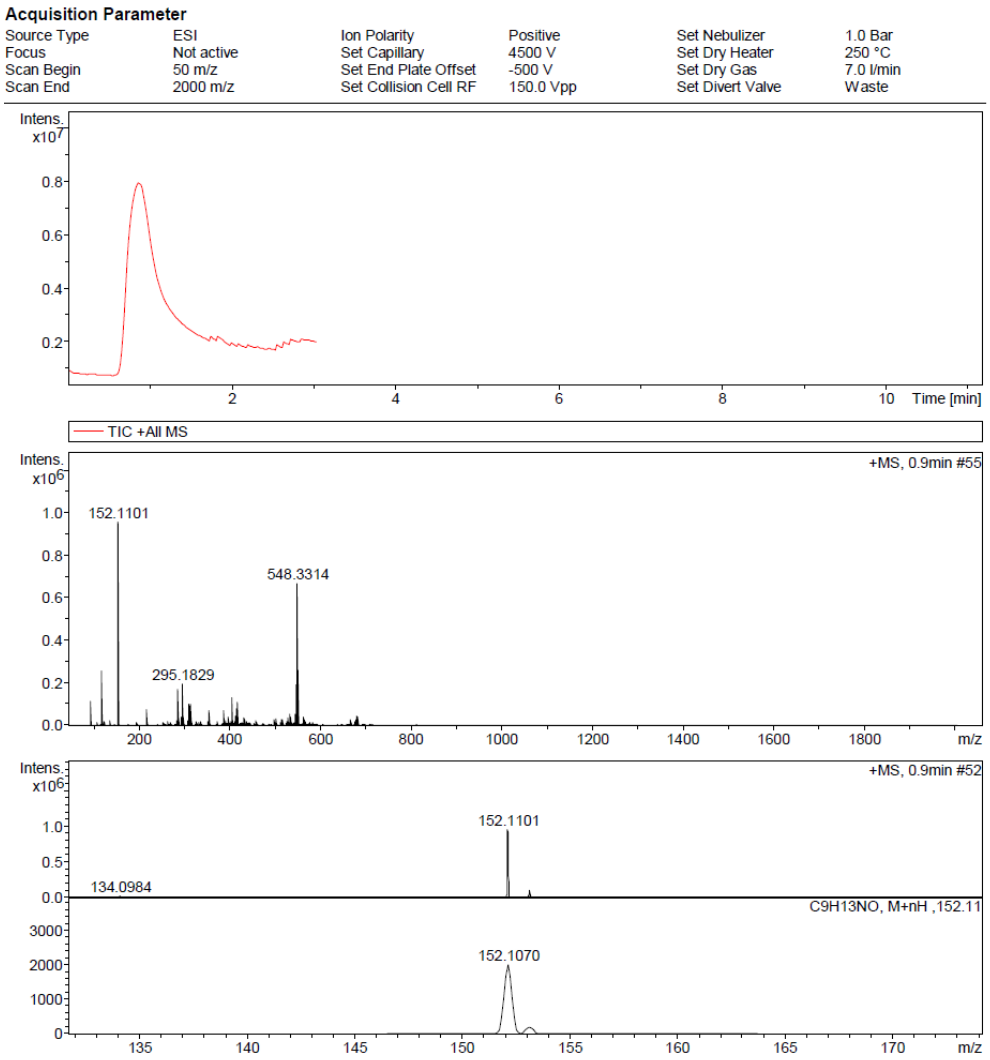


Figure 5. HRMS of (*S*)-2-amino-3-phenylpropan-1-ol (**5)**

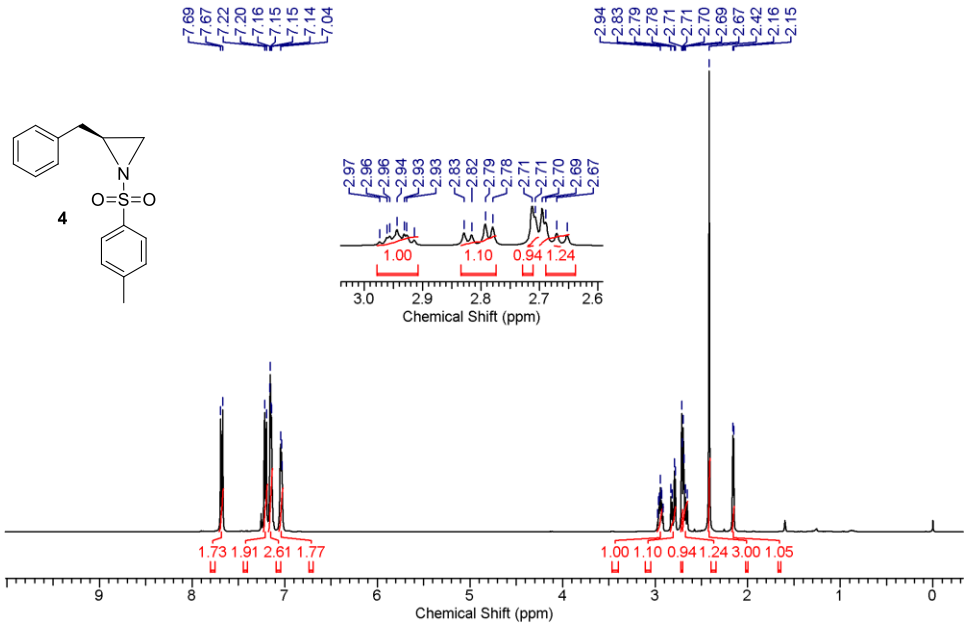


Figure 6. ^{1}H-NMR spectrum of (*S*)-2-benzyl-1-tosylaziridine (**4)** in $CDCl_3$

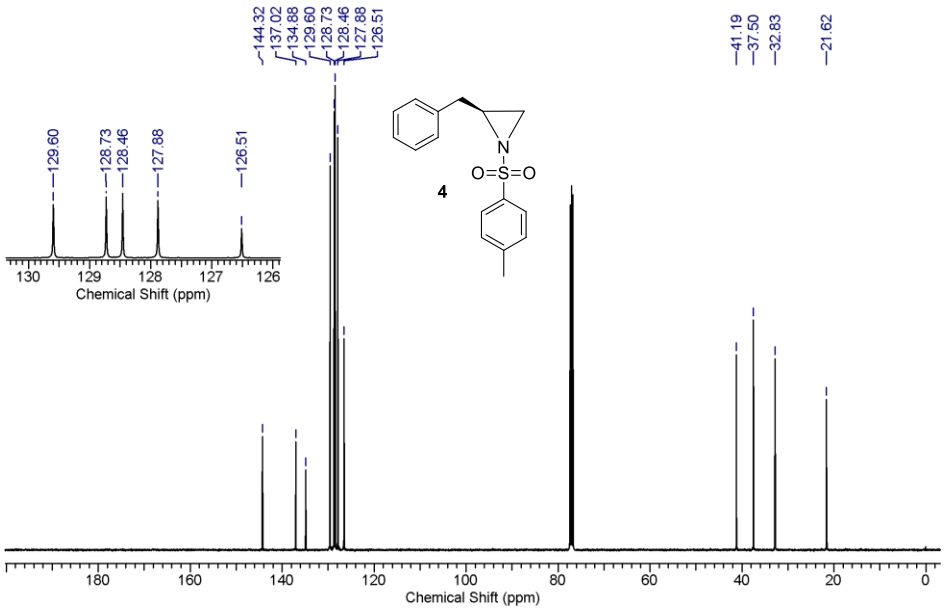


Figure 7. ^{13}C-NMR spectrum of (*S*)-2-benzyl-1-tosylaziridine (**4)** in $CDCl_3$

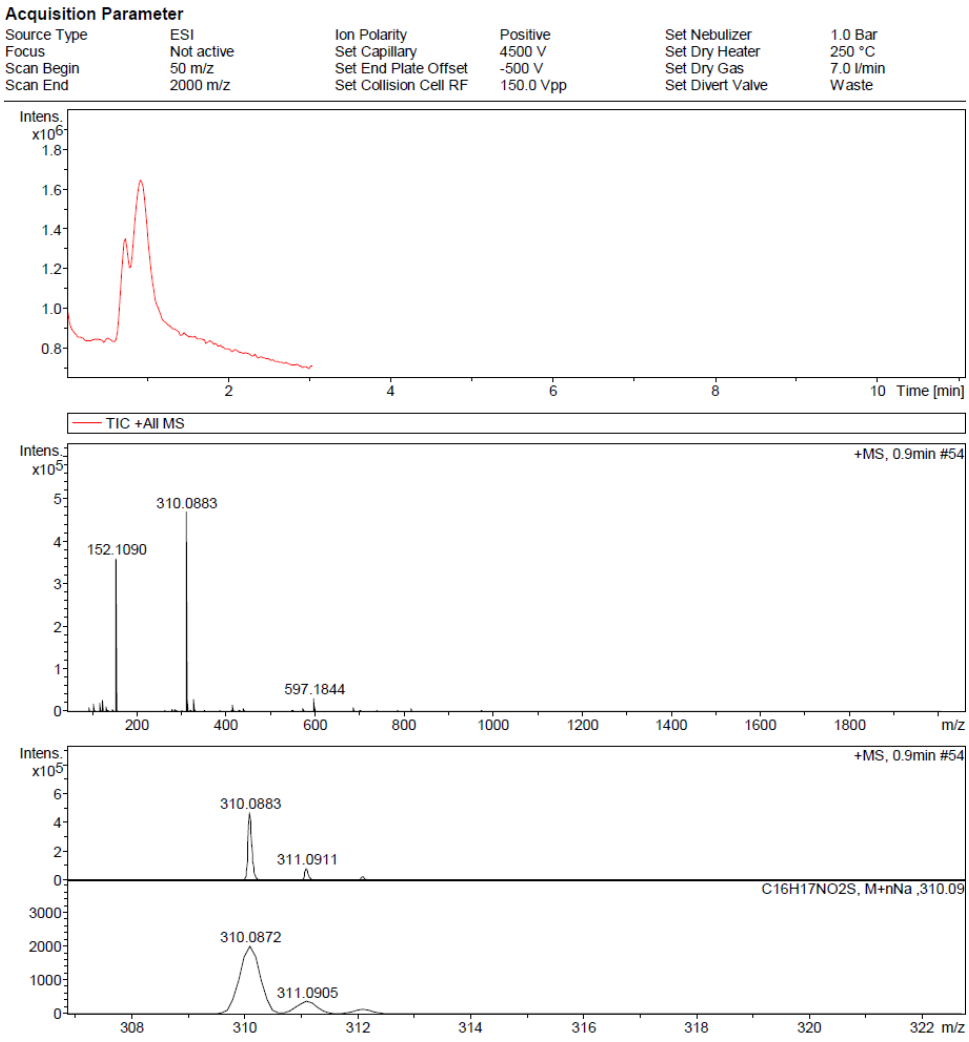


Figure 8. HRMS of (*S*)-2-benzyl-1-tosylaziridine (**4)**

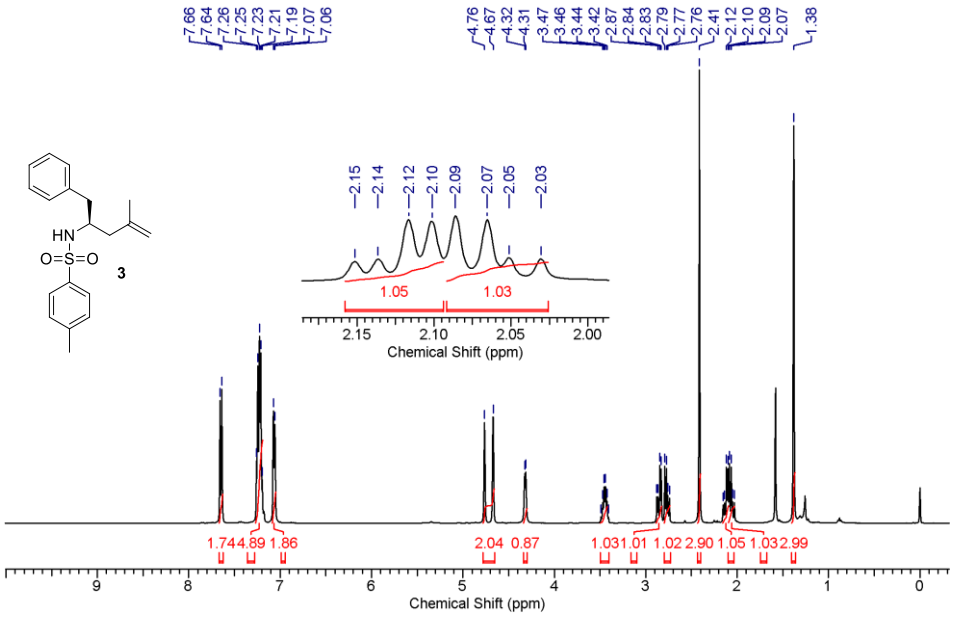


Figure 9. ^{1}H-NMR spectrum of (*R*)-4-methyl-*N*-methyl-*N*-(4-methyl-1-phenylpent-4-en-2-yl) (**3)** in $CDCl_3$

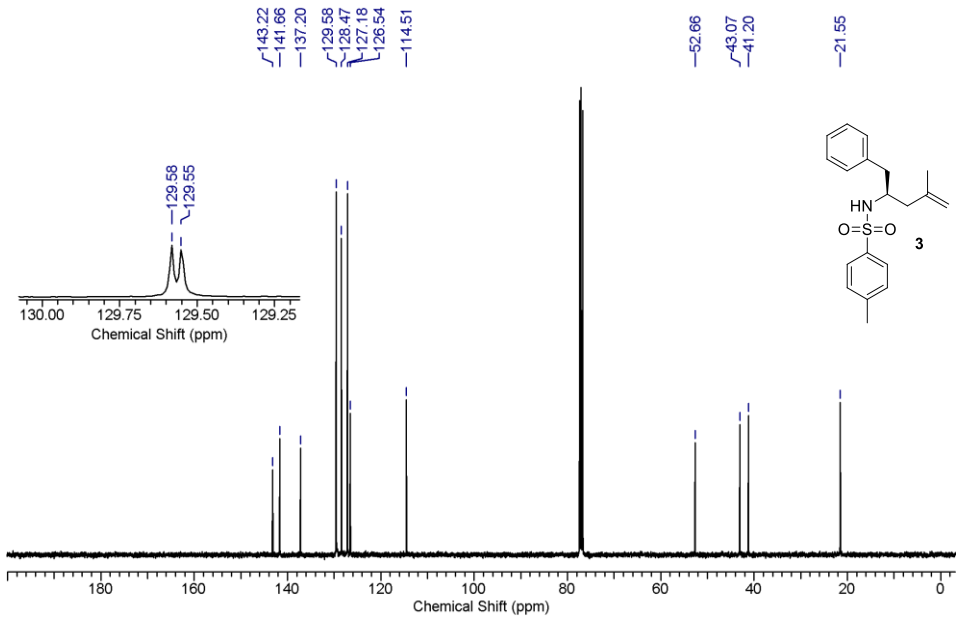


Figure 10. ^{13}C-NMR spectrum of (*R*)-4-methyl-*N*-methyl-*N*-(4-methyl-1-phenylpent-4-en-2-yl) (**3)** in $CDCl_3$

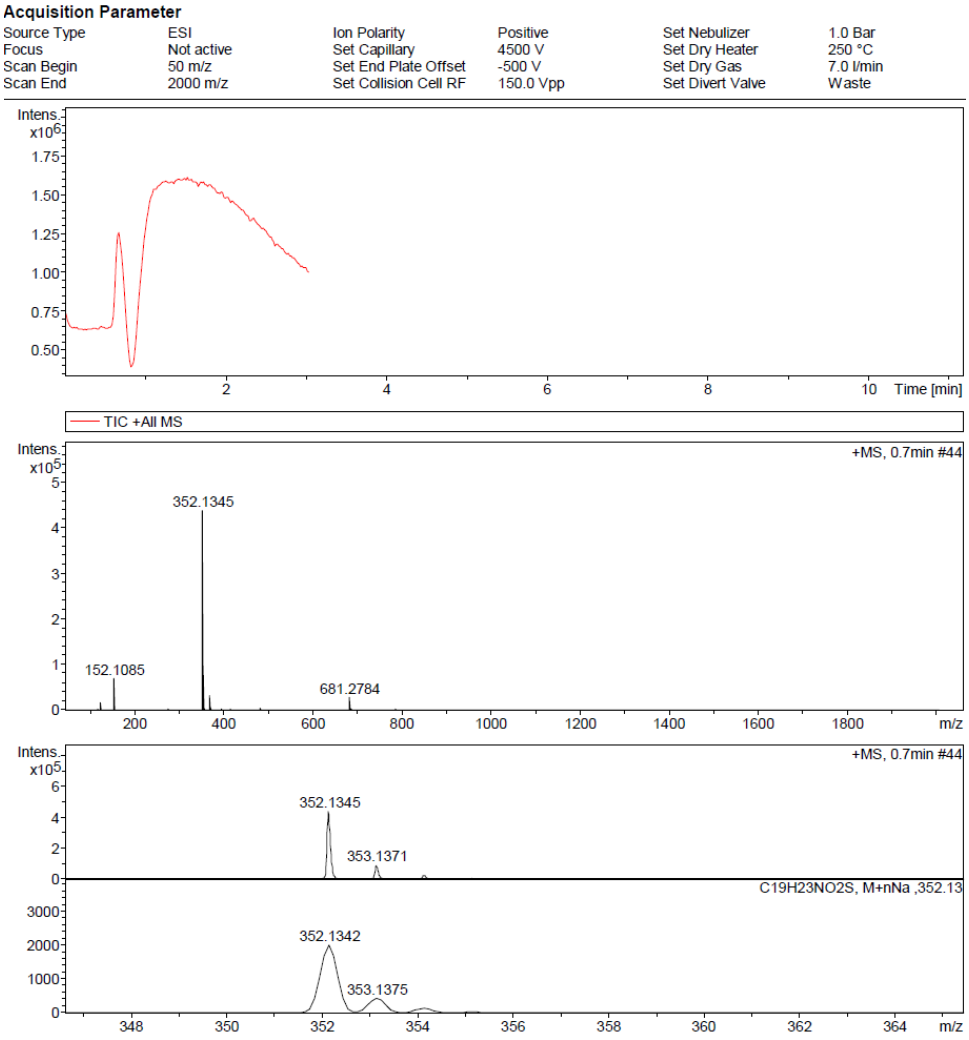


Figure 11. HRMS of (*R*)-4-methyl-*N*-methyl-*N*-(4-methyl-1-phenylpent-4-en-2-yl) (**3)**

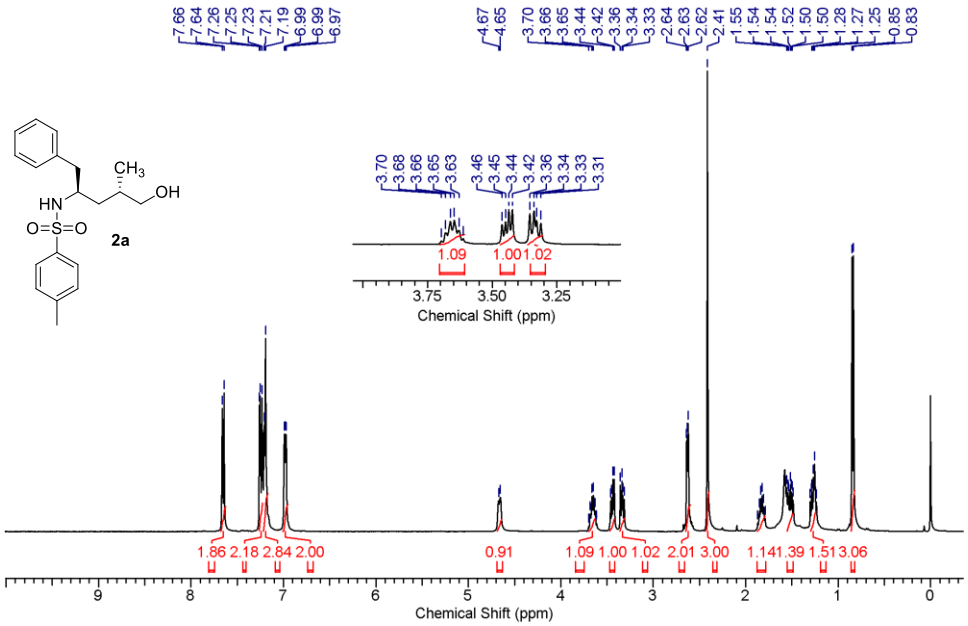


Figure 12. ^{1}H-NMR spectrum of *N*-((2*R*,4*S*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2a)** in $CDCl_3$

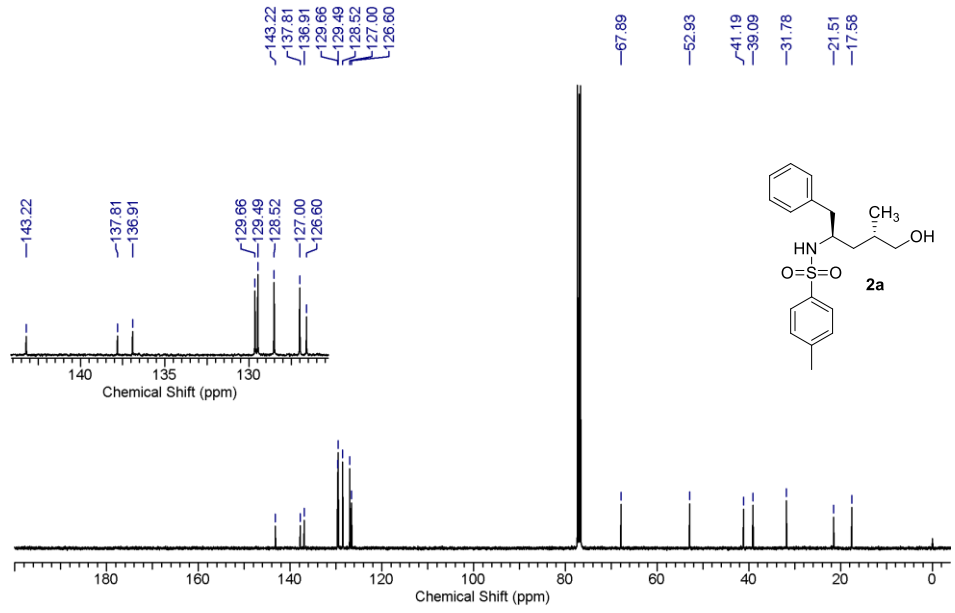


Figure 13. ^{13}C-NMR spectrum of *N*-((2*R*,4*R*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2a)** in $CDCl_3$

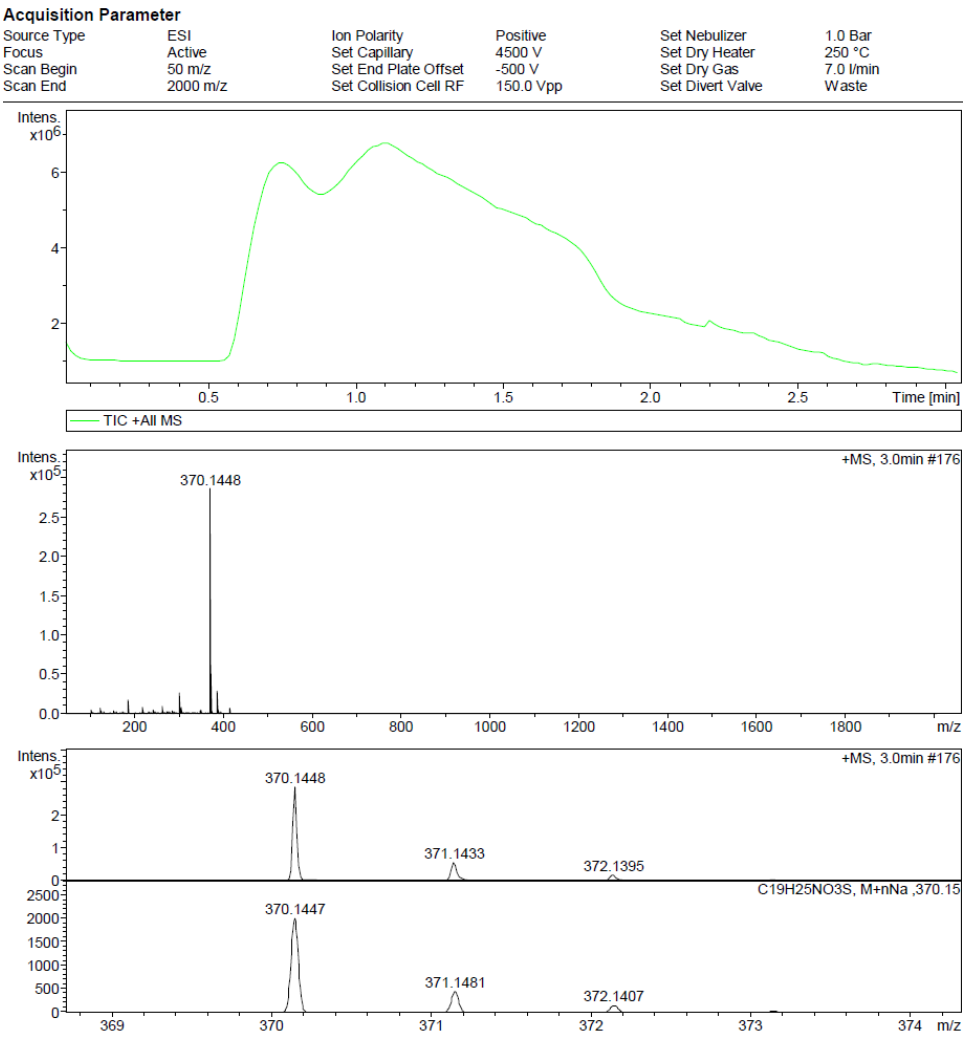


Figure 14. HRMS of *N*-((2*R*,4*S*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2a)**

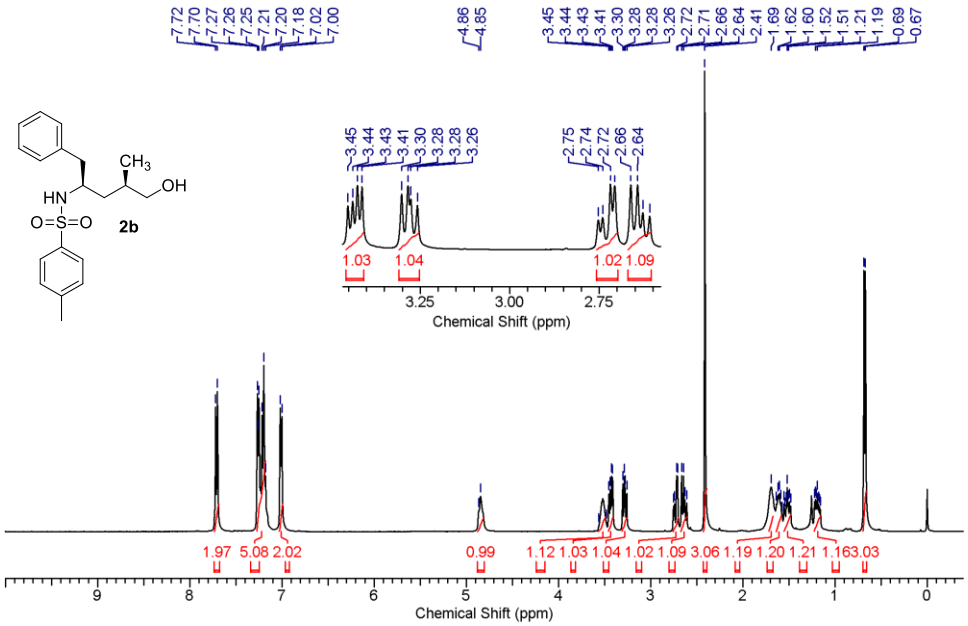


Figure 15. ^{1}H-NMR spectrum of *N*-((2*R*,4*R*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2b)** in $CDCl_3$

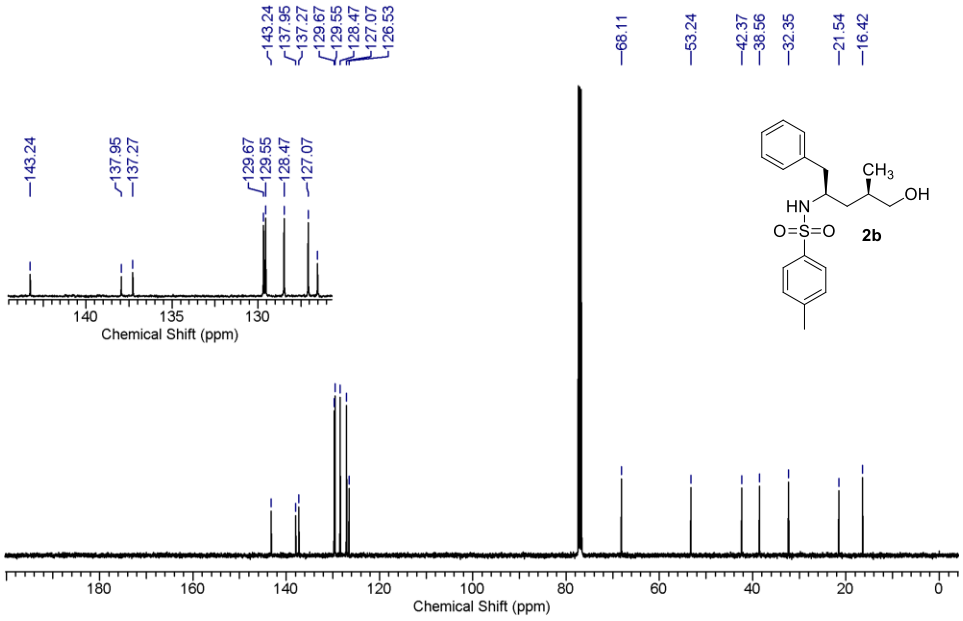


Figure 16. ^{13}C-NMR spectrum of *N*-((2*R*,4*R*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2b)** in $CDCl_3$

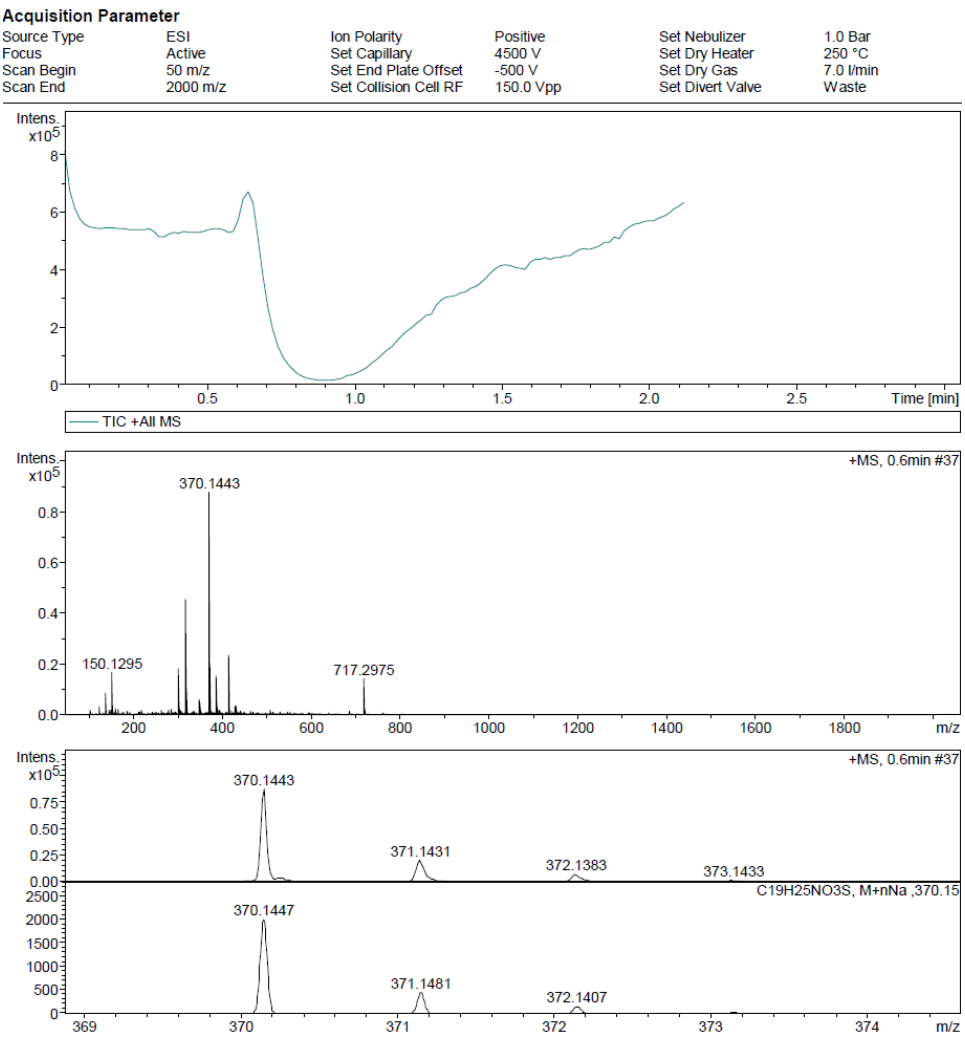


Figure 17. HRMS of *N*-((2*R*,4*R*)-5-Hydroxy-4-methyl-1-phenylpentan-2-yl)-4-methylbenzenesulfonamide (**2b)**

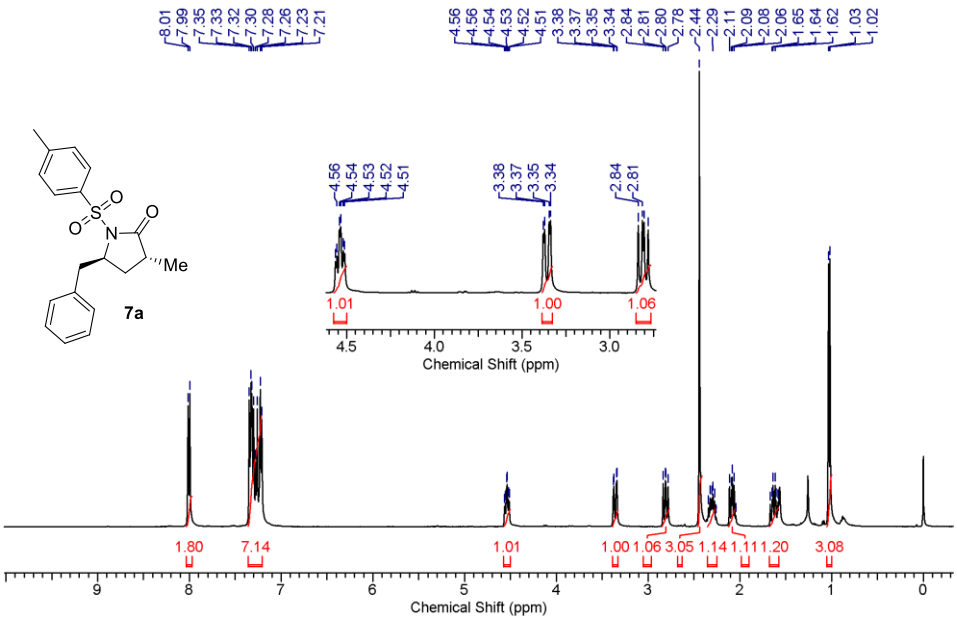


Figure 18. ^{1}H-NMR spectrum of (3*S*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one **(7a)** in $CDCl_3$

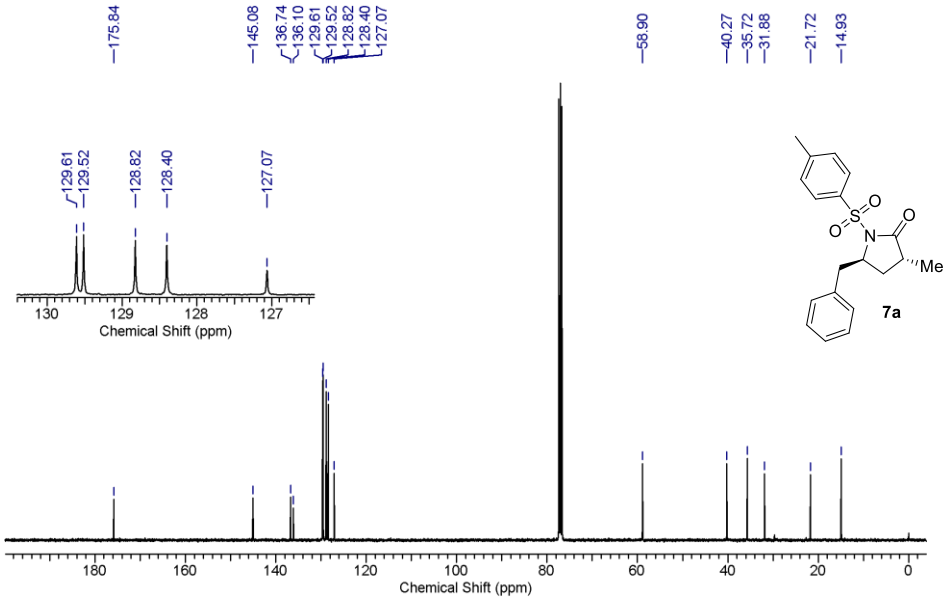


Figure 19. ^{13}C-NMR spectrum of (3*S*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one **(7a)** in $CDCl_3$

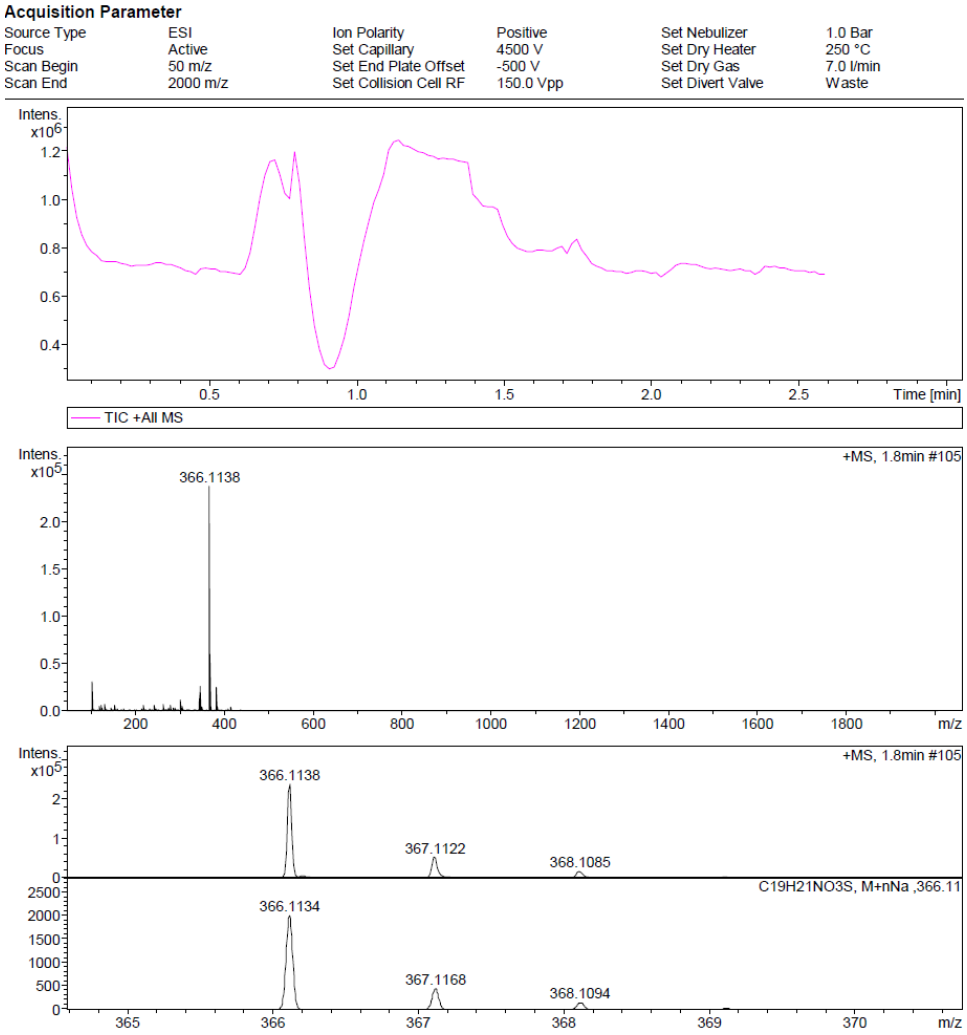


Figure 20. HRMS of (3*S*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (**7a)**

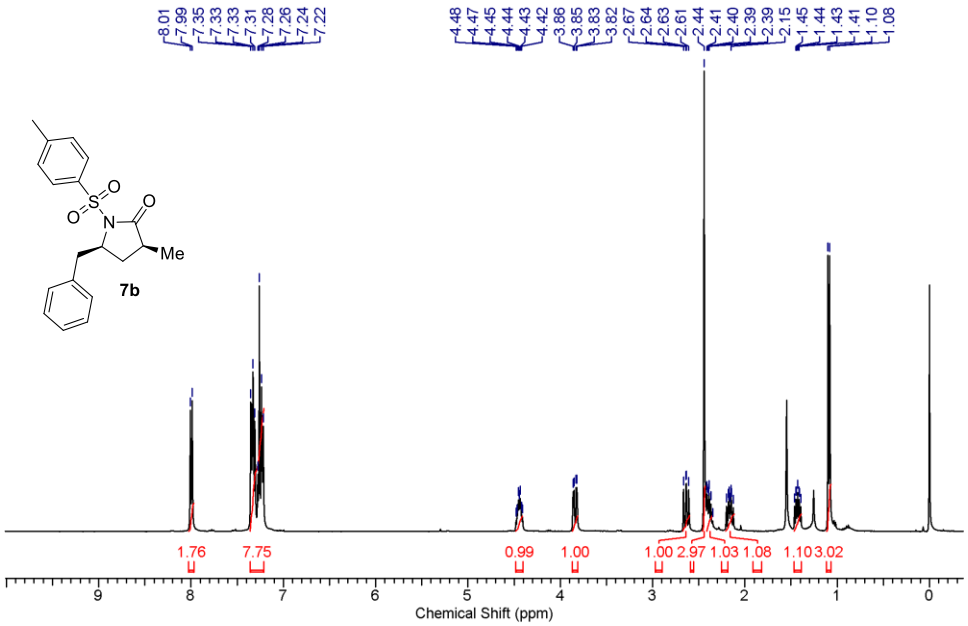


Figure 21. ^{1}H-NMR spectrum of (3*R*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (**7b)** in $CDCl_3$

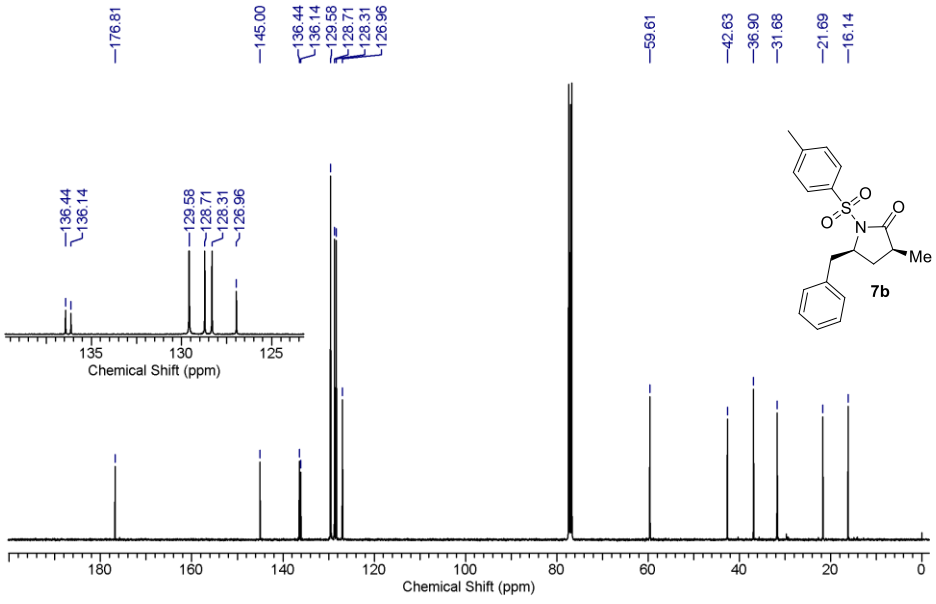


Figure 22. ^{13}C-NMR spectrum of (3*R*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (**7b)** in $CDCl_3$

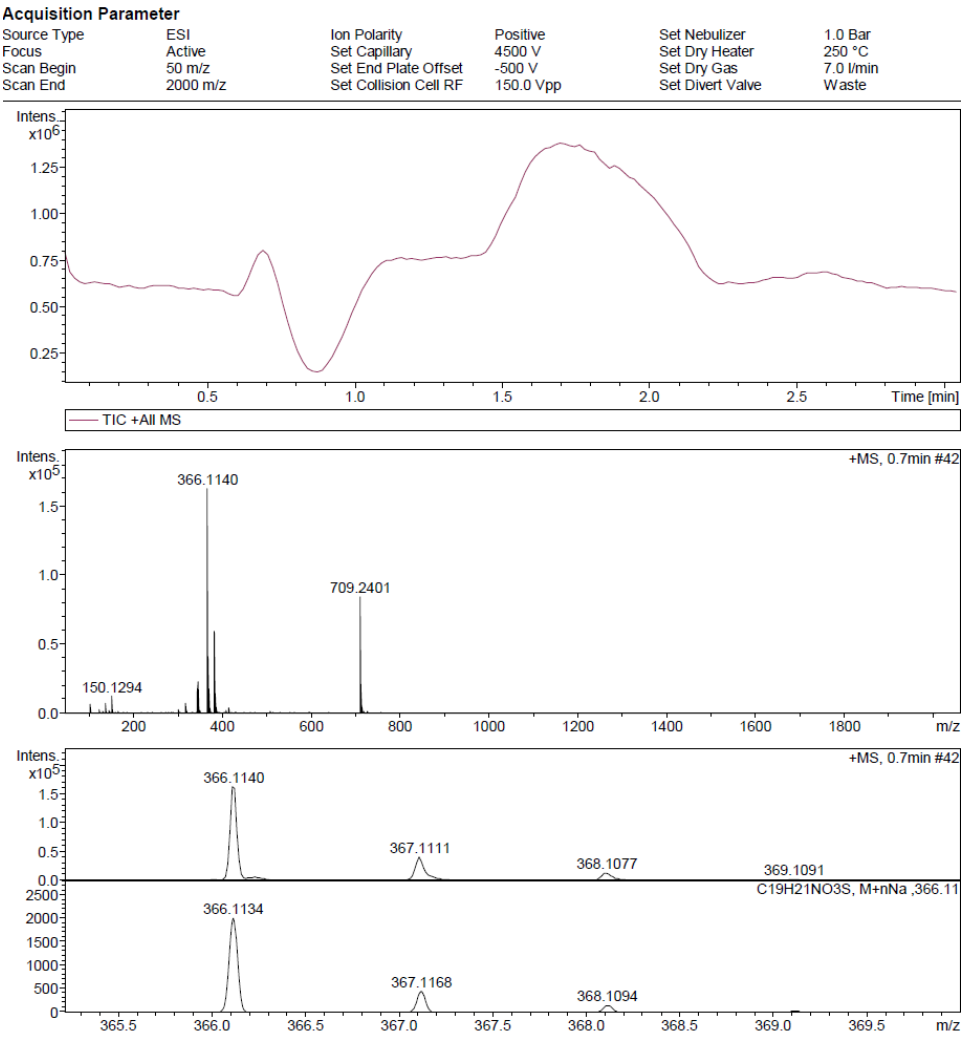


Figure 23. HRMS of (3*R*,5*R*)-5-benzyl-3-methyl-1-tosylpyrrolidin-2-one (**7b)**

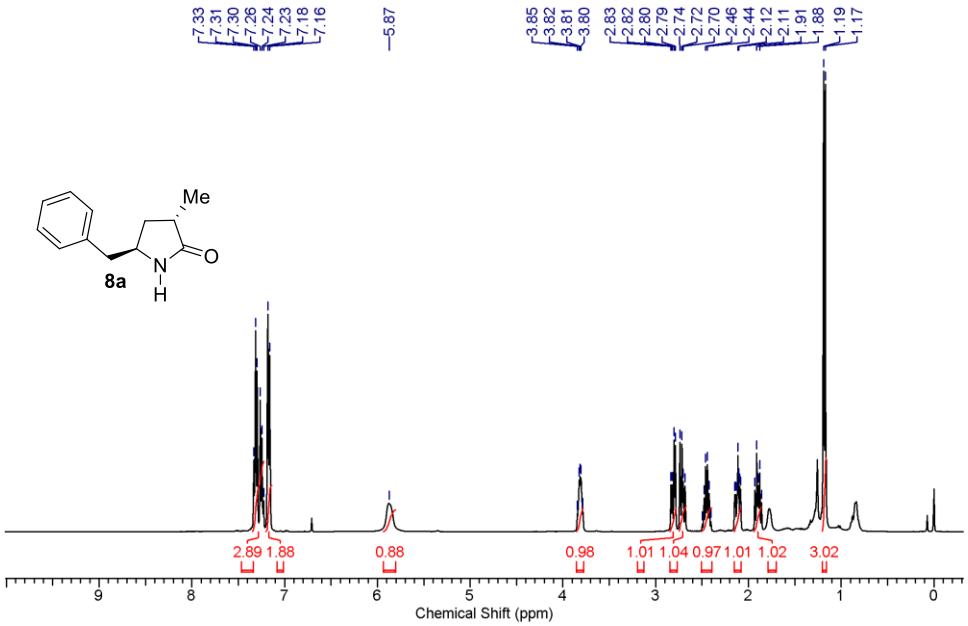


Figure 24. ^{1}H-NMR spectrum of (3*S*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one (**8a)** in $CDCl_3$

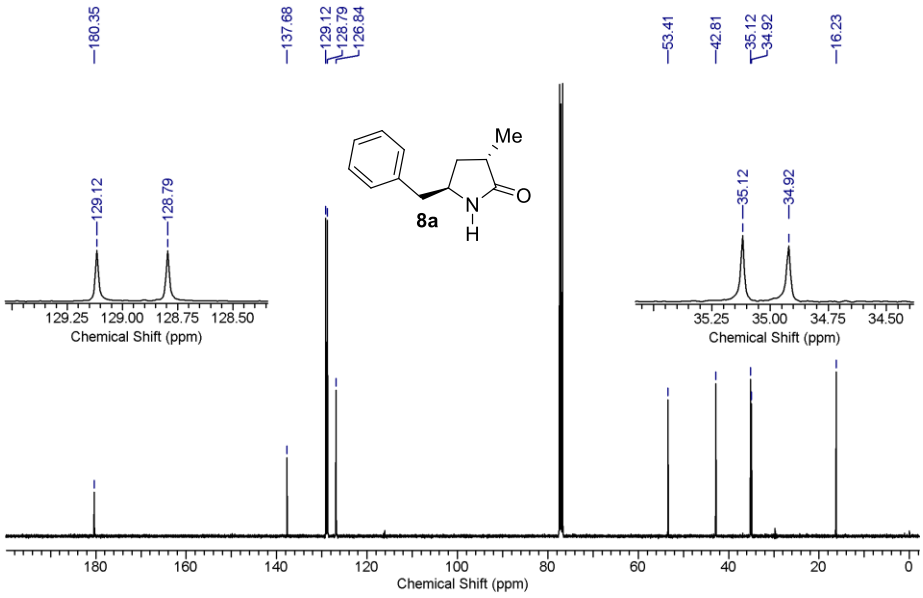


Figure 25. ^{13}C-NMR spectrum of (3*S*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one **(8a)** in $CDCl_3$

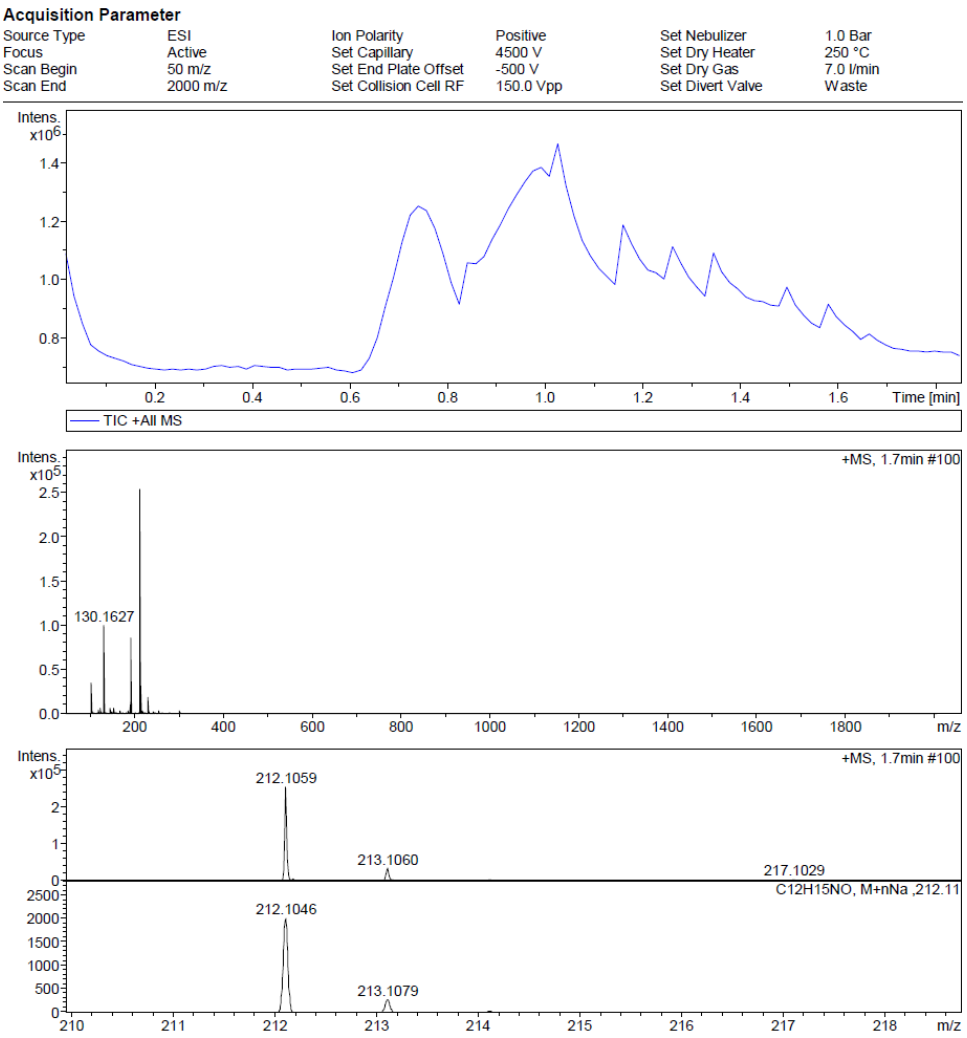


Figure 26. HRMS of (3*S*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one (**8a)**

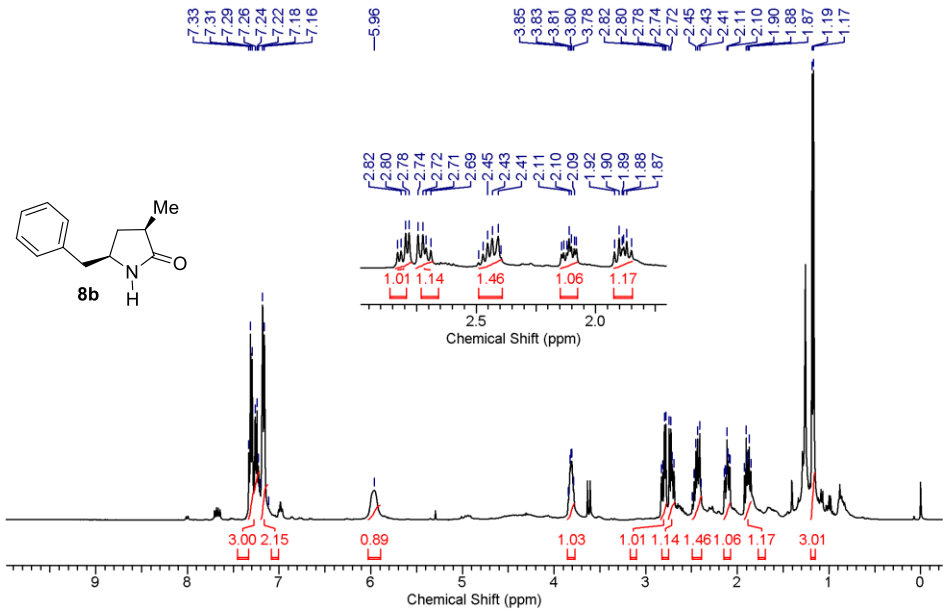


Figure 27. ^{1}H-NMR spectrum of (3*R*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one **(8b)** in $CDCl_3$

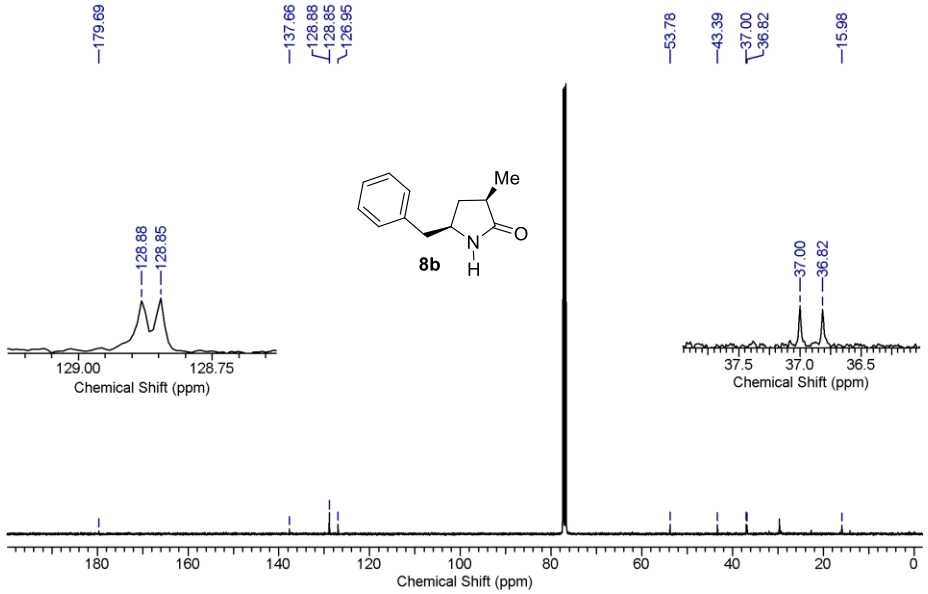


Figure 28. ^{13}C-NMR spectrum of (3*R*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one **(8b)** in $CDCl_3$

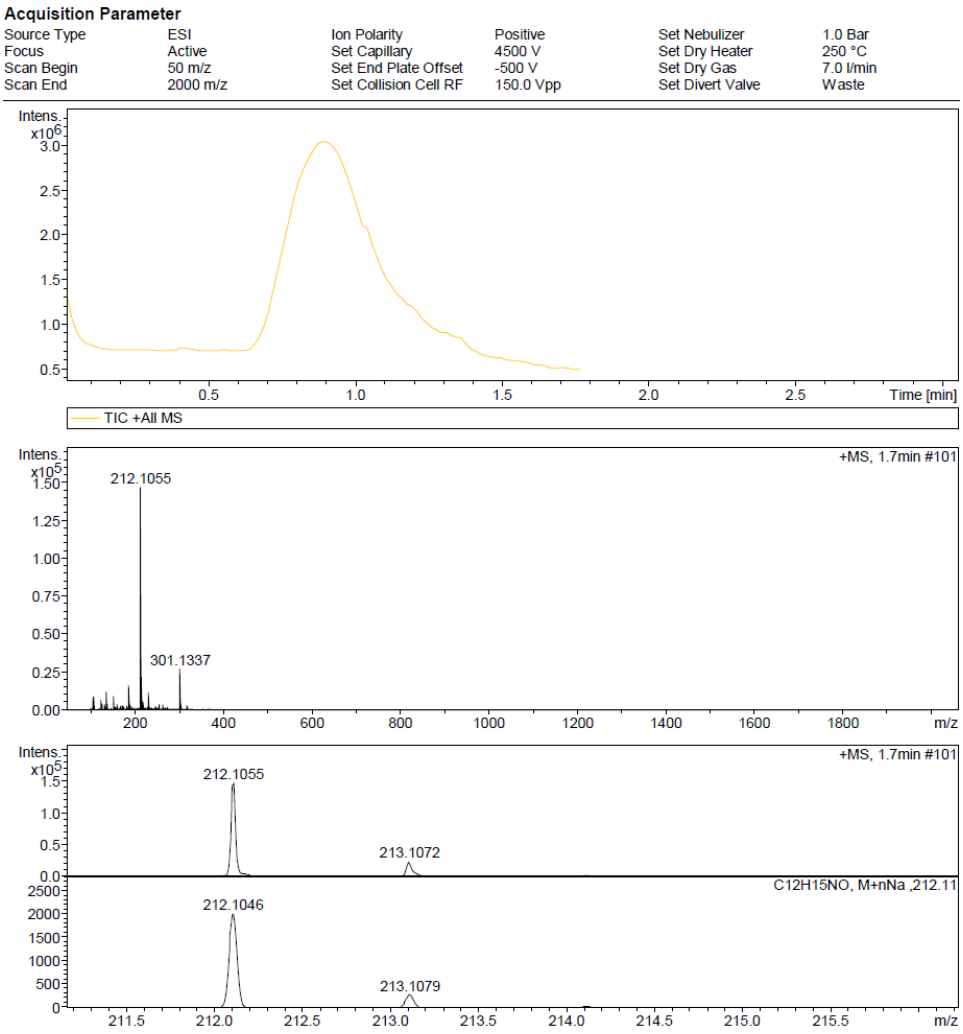


Figure 29. HRMS of (3*R*,5*R*)-5-Benzyl-3-methylpyrrolidin-2-one (**8b)**

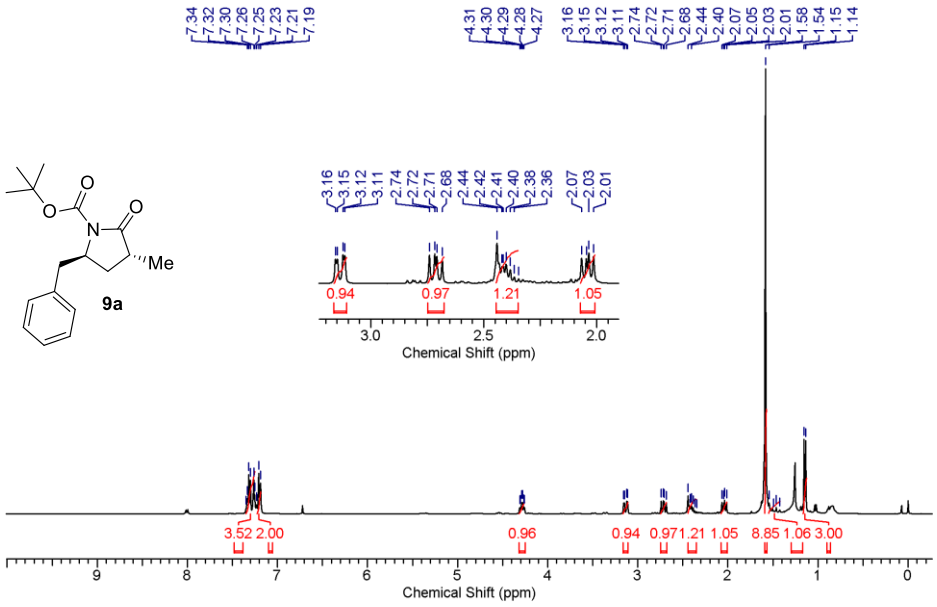


Figure 30. ^{1}H-NMR spectrum of (3*S*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate (**9a)** in $CDCl_3$

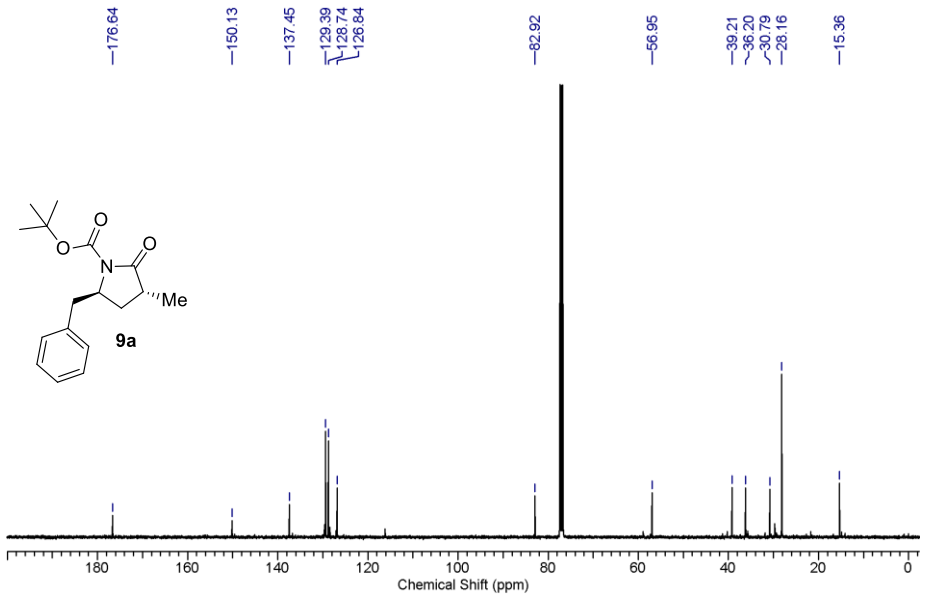


Figure 31. ^{13}C-NMR spectrum of (3*S*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate (**9a)** in $CDCl_3$

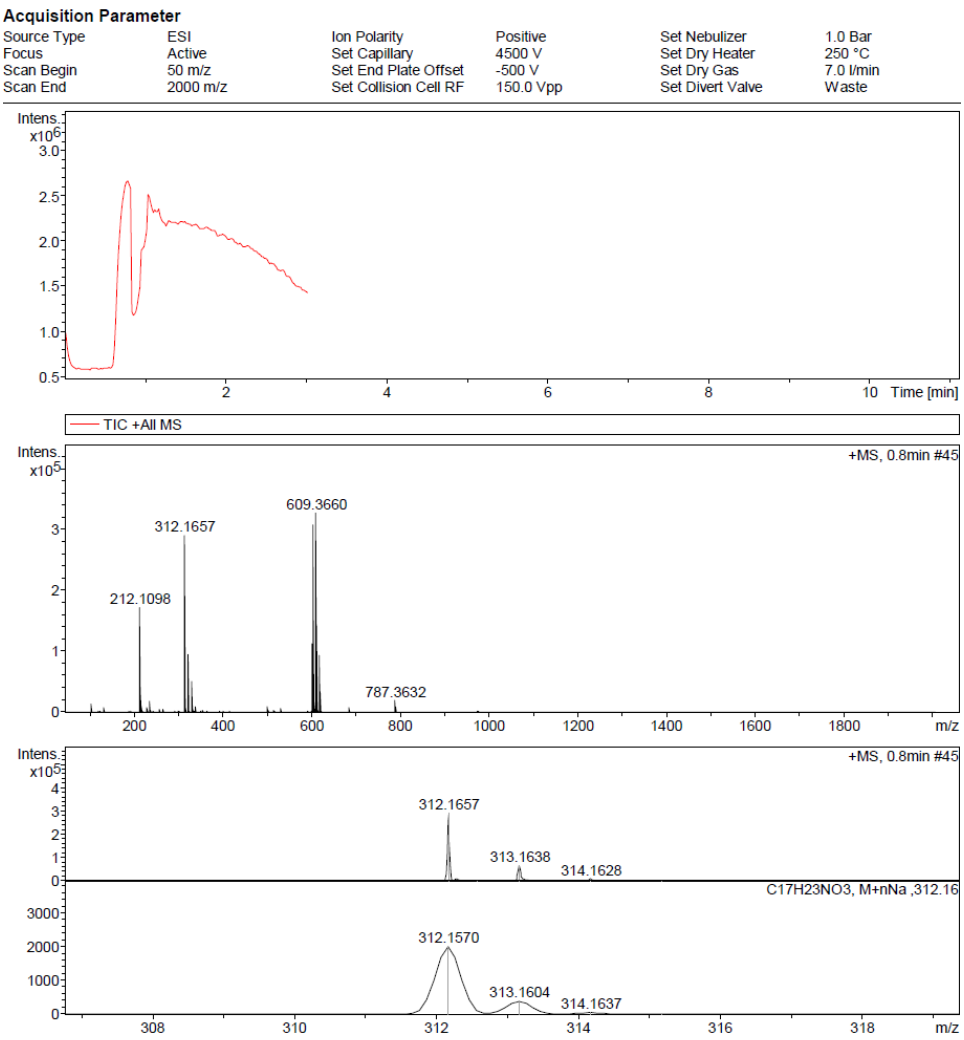


Figure 32. HRMS of (3*S*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate (**9a)**

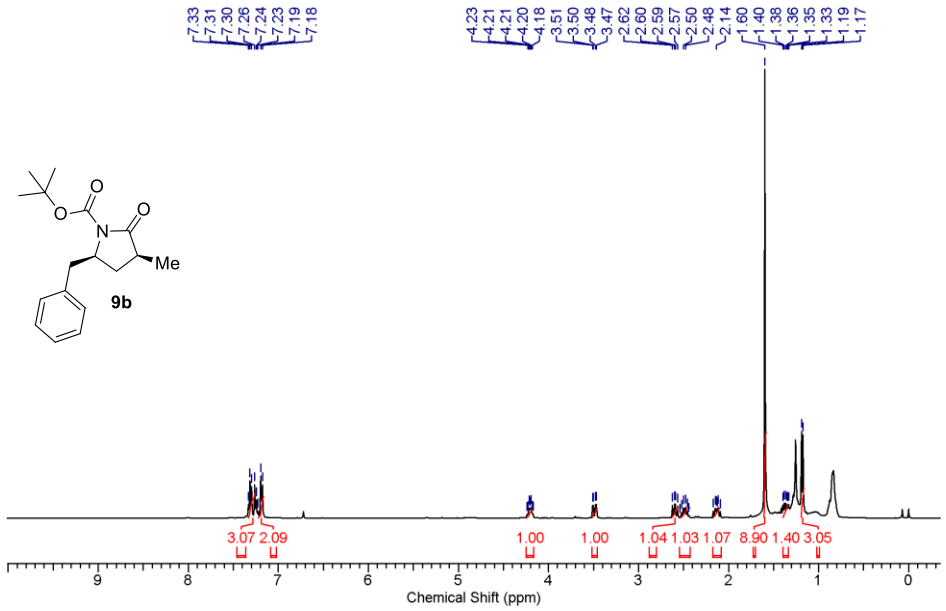


Figure 33. ^{1}H-NMR spectrum of (3*R*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate **(9b)** in $CDCl_3$

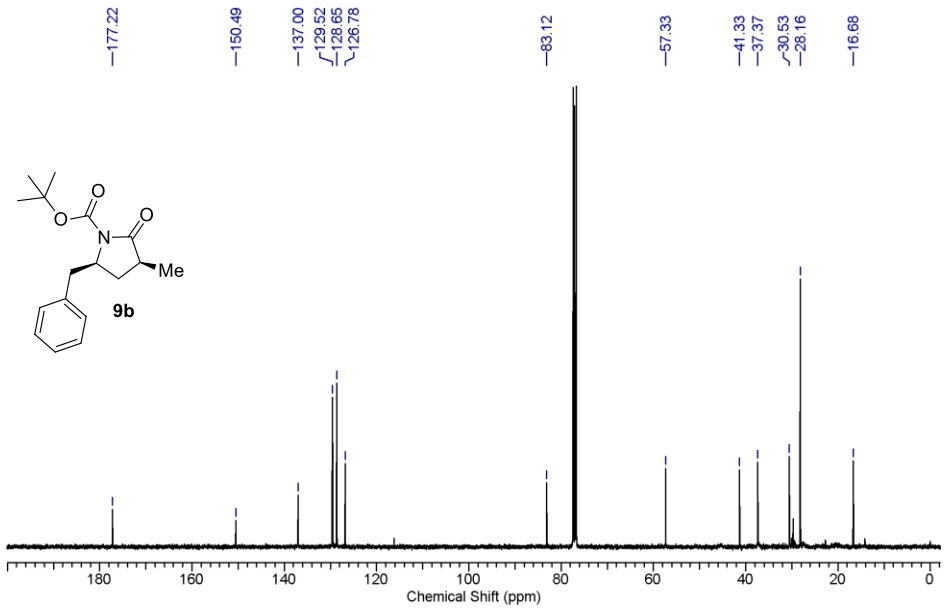


Figure 34. ^{13}C-NMR spectrum of (3*R*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate **(9b)** in $CDCl_3$

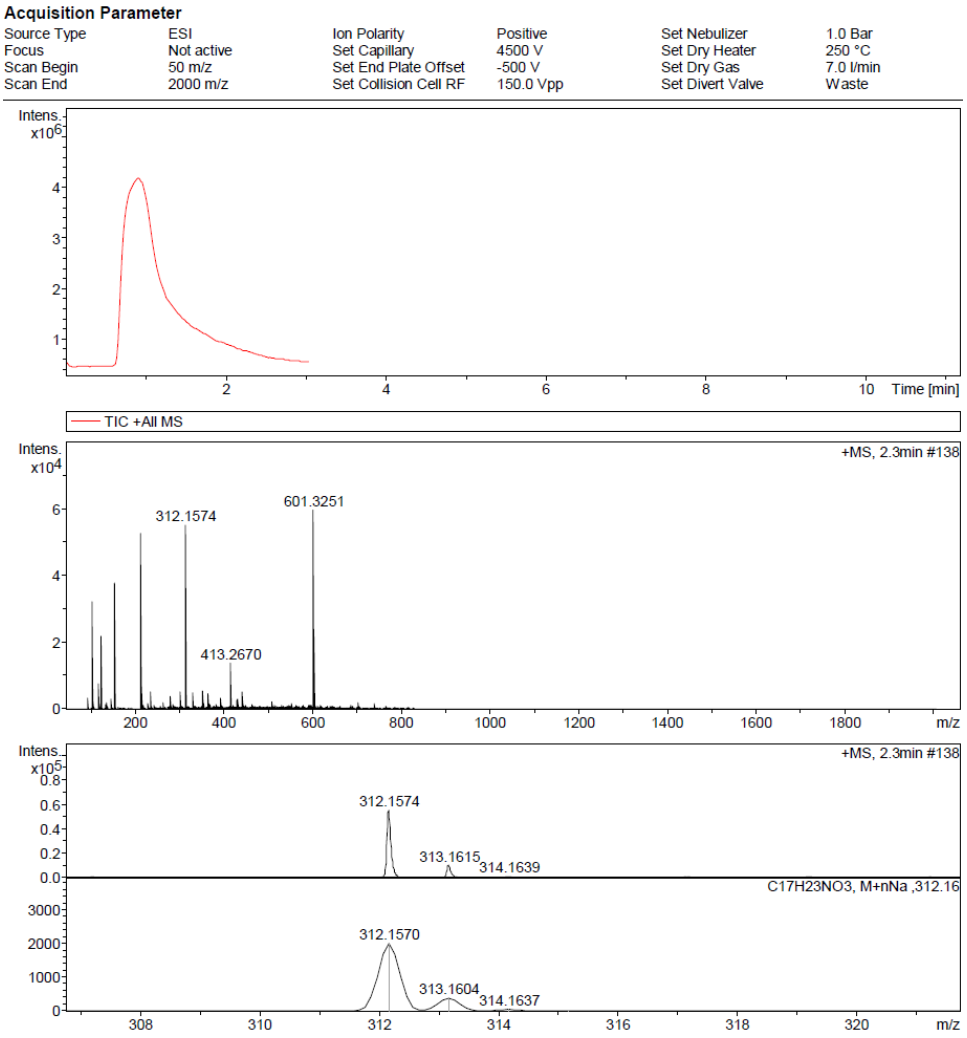


Figure 35. HRMS of (3*R*,5*R*)-*tert*-Butyl 5-benzyl-3-methyl-2-oxopyrrolidine-1-carboxylate **(9b)**

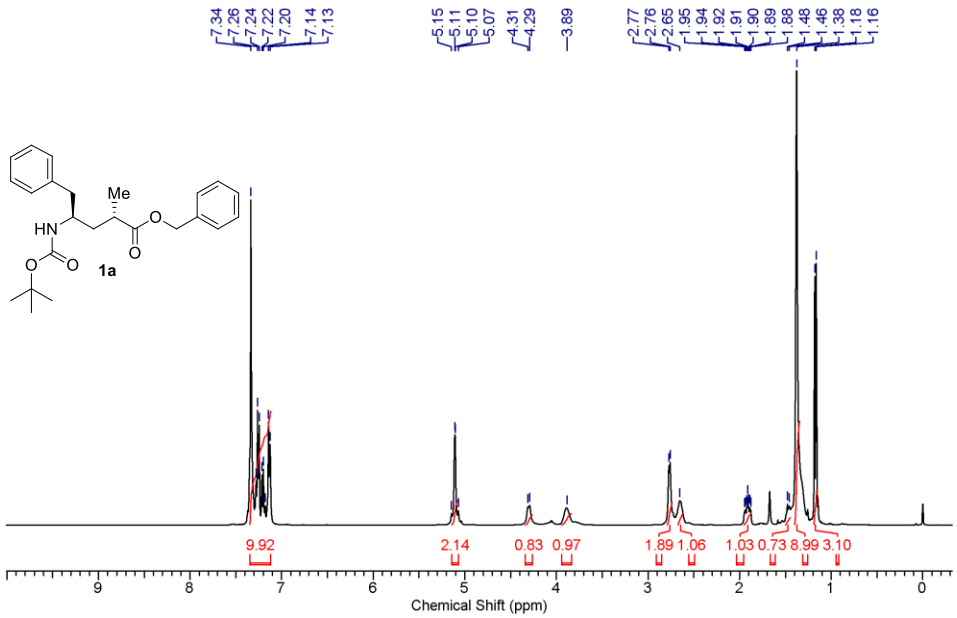


Figure 36. ^{1}H-NMR spectrum of (2*S*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate **(1a)** in $CDCl_3$

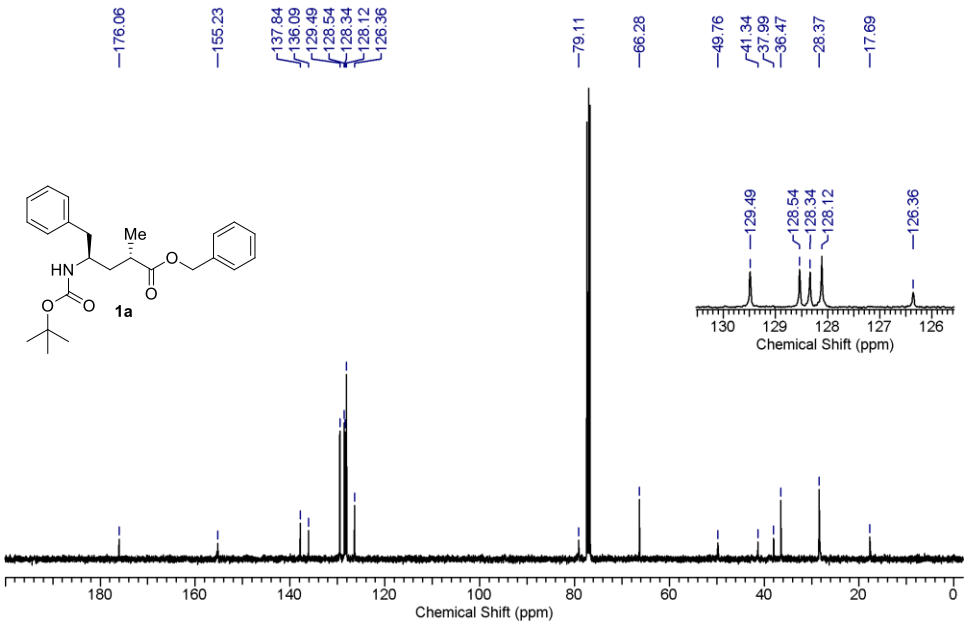


Figure 37. ^{13}C-NMR spectrum of (2*S*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate **(1a)** in $CDCl_3$

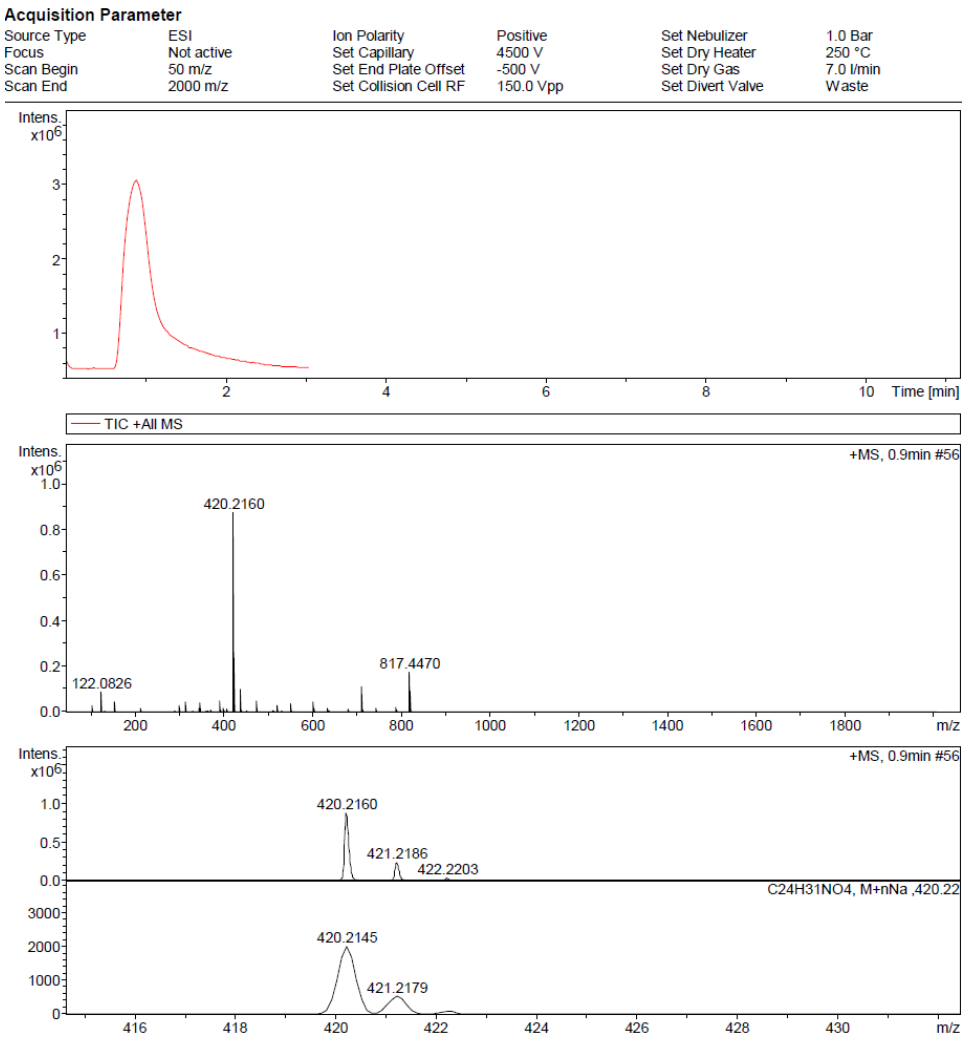


Figure 38. HRMS of (2*S*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl)amino)-2-methyl-5-phenylpentanoate **(1a)**

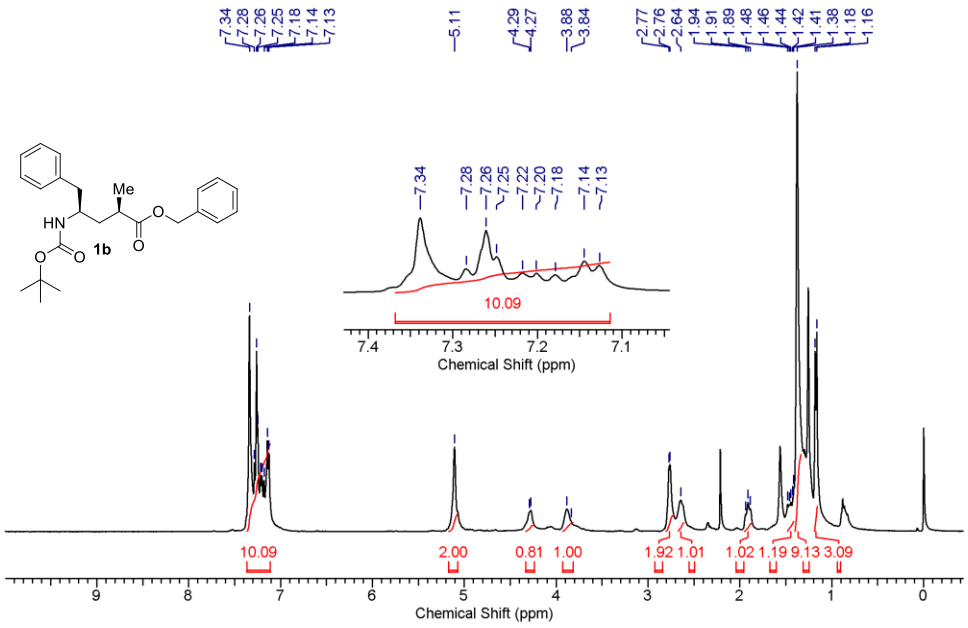


Figure 39. ^{1}H-NMR spectrum of (2*R*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl) amino)-2-methyl-5-phenylpentanoate **(1b)** in $CDCl_3$

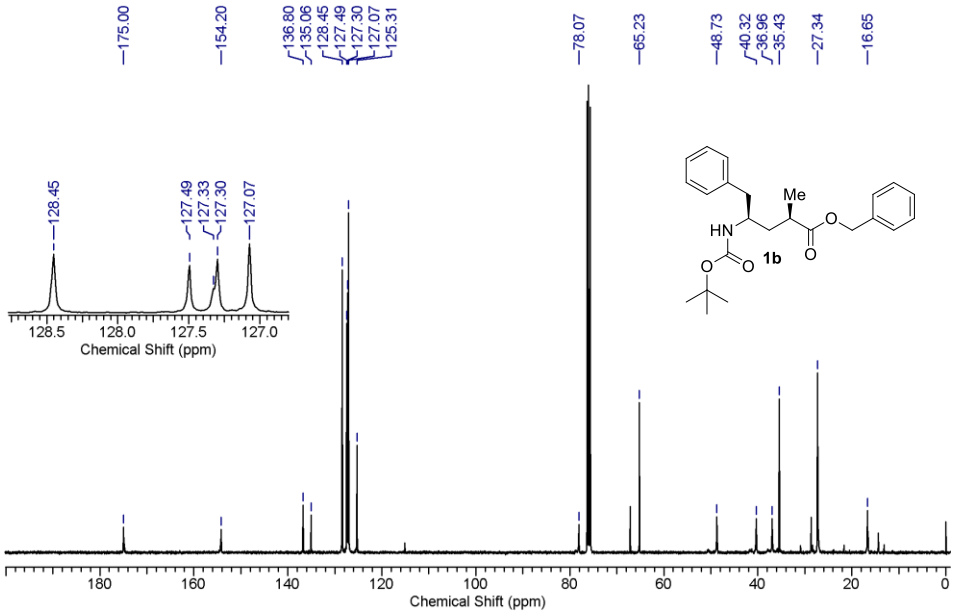


Figure 40. ^{13}C-NMR spectrum of (2*R*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl) amino)-2-methyl-5-phenylpentanoate **(1b)** in $CDCl_3$

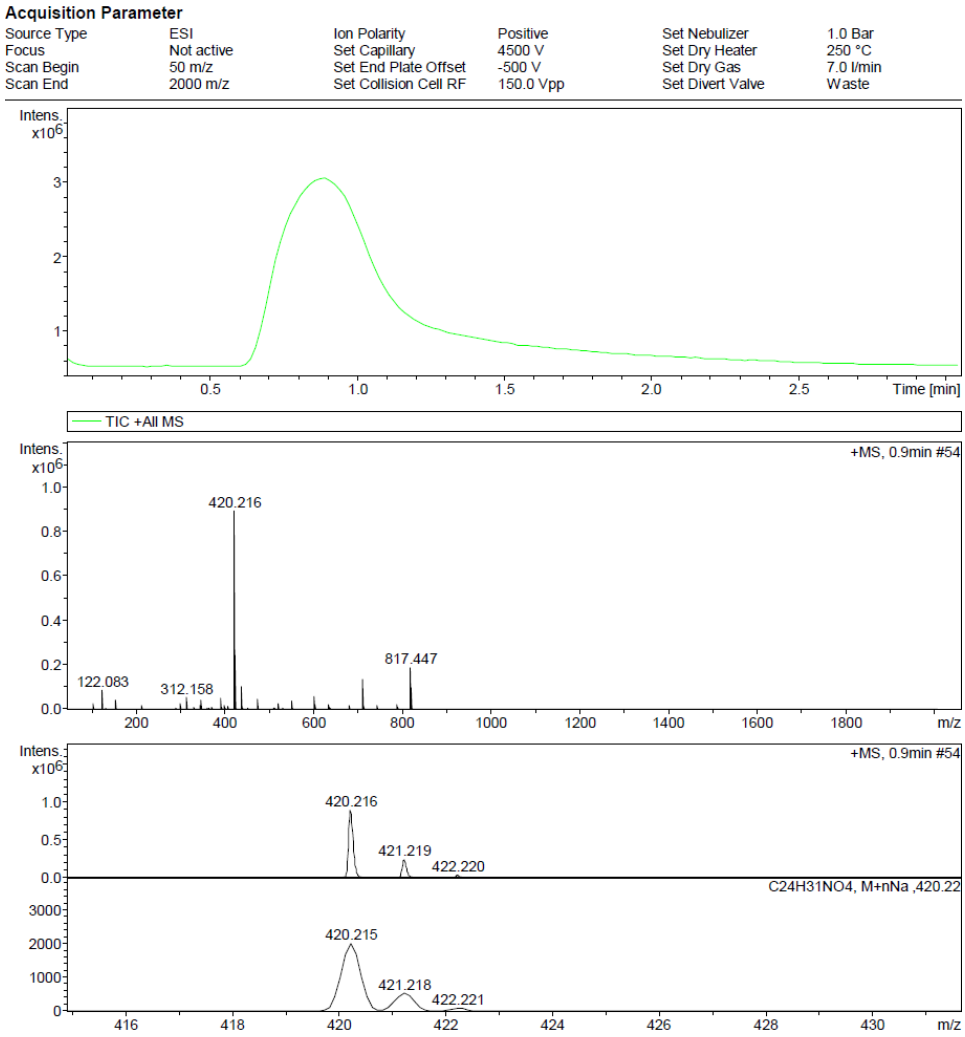


Figure 41. HRMS of (2*R*,4*R*)-Benzyl 4-((*tert*-butoxycarbonyl) amino)-2-methyl-5-phenylpentanoate **(1b)**

REFERENCES

[1] Upadhyay R. P., (2012), An overview of the burden of Non-Communicable diseases in India, Iran. J. Public Health, 41, 1–8.

[2] Mehrotra R., Kaushik R., (2018), A stitch in time saves nine: Answer to the cancer burden in India, Indian J. Med. Res., 147, 121–124.

[3] Malvia S., Bagadi S. A., Dubey U. S., Saxena S., (2017), Epidemiology of breast cancer in Indian women, Asia Pac. J. Clin. Oncol., 13, 289–295.

[4] Lun Z-R., Lai D-H., Wen Y-Z., Zheng L-L., Shen J-L., Yang T-B., Zhou W-L., Qu L-H., Hide G., Ayala F. J., (2015), Cancer in the parasitic protozoans trypanosoma brucei and toxoplasma gondii, Proc. Natl. Acad. Sci. U. S. A., 112, 8835–8842.

[5] Rayan A., Raiyn J., Falah M., (2017), Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity, PLoS One, 12, 1–12.

[6] Greenwell M., Rahman P. K. S. M., (2015), Medicinal plants: Their use in anticancer treatment, Int. J. Pharm. Sci. Res., 6, 4103–4112.

[7] Iqbal J., Abbasi B. A., Mahmood T., Kanwal. S., Ali B., Khalil A. T., (2017), Plant-derived anticancer agents: A green anticancer approach, Asian Pac. J. Trop. Biomed., 7, 1129−1150.

[8] Nastrucci1 C., Cesario1 A., Russo P., (2012), Anticancer drug discovery from the marine environment, Recent Pat. Anticancer Drug Discov., 7, 218–232.

[9] Moudi M., Go R., Yien C. Y. S., Nazre M., (2013), Vinca alkaloids, Int. J. Prev. Med., 4, 1231–1235.

[10] Bates D., Eastman A., (2017), Microtubule destabilising agents: Far more than just antimitotic anticancer drugs, Br. J. Clin. Pharmacol., 83, 255–268.

[11] Jordan M. A., Thrower D., Wilson L., (1991), Mechanism of inhibition of cell proliferation by vinca alkaloids, Cancer Res., 51, 2212–2222.

[12] Hu Z-B., Gignac S. M., Quentmeier H., Pettit G. R., Drexler H.G., (1993), Effects of dolastatins on human B-lymphocytic leukemia cell lines, Leuk. Res., 17, 333–339.

[13] Lopus M., (2013), Mechanism of mitotic arrest induced by dolastatin 15 involves loss of tension across kinetochore pairs, Mol. Cell Biochem., 382, 93–102.

[14] Ravelli R. B., Gigant B., Curmi P. A., Jourdain I., Lachkar S., Sobel A., Knossow M., (2004), Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, Nature, 428, 198–202.

[15] Mukhtar E., Adhami V. M., Mukhtar H., (2014), Targeting microtubules by natural agents for cancer therapy, Mol. Cancer Ther., 13, 275–284.

[16] Steinmetz H., Glaser N., Herdtweck E., Sasse F., Reichenbach H., Höfle G., (2004), Isolation, crystal and solution structure determination, and biosynthesis of tubulysins-powerful inhibitors of tubulin polymerization from myxobacteria, Angew. Chem. Int. Ed., 43, 4888−4892.

[17] Kaur G., Hollingshead M., Holbeck S., Schauer-Vukasinovic V., Camalier R. F., Dömling A., Agarwal S., (2006), Biological evaluation of tubulysin A: A potential anticancer and antiangiogenic natural product, Biochem. J., 396, 235−242.

[18] Floyd W. C., Datta G. K., Imamura S., Kieler-Ferguson H. M., Jerger K., Patterson A. W., Fox M. E., Szoka F. C., Frchet J. M. J., Ellman J. A., (2011), Chemotherapeutic evaluation of a synthetic tubulysin analogue–dendrimer conjugate in C 26 tumor bearing mice, Chem. Med. Chem., 6, 49–53.

[19] Sasse F., Steinmetz H., Heil J., Höfle G., Reichenbach H., (2000), Tubulysins, new cytostatic peptides from myxobacteria acting on microtubuli production, isolation, physico-chemical and biological properties, J. Antibiot., 53, 879−885.

[20] Sandmann A., Sasse F., Muller M., (2004), Identification and analysis of the core biosynthetic machinery of tubulysin, a potent cytotoxin with potential anticancer activity, Chem. Biol., 11, 1071–1079.

[21] Murray B. C., Peterson M. T., Fecik R. A., (2015), Chemistry and biology of tubulysins: Antimitotic tetrapeptides with activity against drug resistant cancers, Nat. Prod. Rep., 32, 654–662.

[22] Kaur G., Hollingshead M., Holbeck S., Schauer-Vukasinovic V., Camalier R. F., Dömling A., Agarwal S. S., (2006), Biological evaluation of tubulysin A: A potential anticancer and antiangiogenic natural product, Biochem. J., 396, 235–242.

[23] Eirich J., Burkhart J. E., Ullrich A., Rudolf G. C., Vollmar A., Zahler S., Kazmaier U., Sieber S. A., (2012), Pretubulysin derived probes as novel tools for monitoring the microtubule network via activity-based protein profiling and fluorescence microscopy. Mol. BioSyst., 8, 2067−2075.

[24] Gatto G. J., Boyne M. T., Kelleher N. L., Walsh C. T., (2006), Biosynthesis of pipecolic acid by RapL, a lysine cyclodeaminase encoded in the rapamycin gene cluster, J. Am. Chem. Soc., 128, 3838−3847.

[25] Balasubramanian R., Raghavan B., Begaye A., Sackett D. L., Fecik R. A., (2009), Total synthesis and biological evaluation of tubulysin U, tubulysin V, and their analogues, J. Med. Chem., 52, 238–240.

[26] (a) Balasubramanian R., Raghavan B., Steele J. C., Sackett D. L., Fecik R. A., (2008), Tubulysin analogs incorporating desmethyl and dimethyl tubuphenylalanine derivatives, Bioorg. Med. Chem. Lett., 18, 2996–2999. (b) Patterson A. W., Peltier H. M., Sasse F., Ellman J. A., (2007), Design, synthesis, and biological properties of highly potent tubulysin D analogues, Chem. Eur. J., 13, 9534–9541. (c) Vlahov I. R., Wang Y., Vetzel M., Hahn S., Kleindl P. J., Reddy J. A., Leamon C. P., (2011), Acid mediated formation of an N-acyliminium ion from tubulysins: A new methodology for the synthesis of natural tubulysins and their analogs, Bioorg. Med. Chem. Lett., 21, 6778–6781.

[27] Pando O., Dörner S., Preusentanz R., Denkert A., Porzel A., Richter W., Wessjohann L., (2009), First total synthesis of tubulysin B, Org. Lett., 11, 5567–5569.

[28] Shankar P. S., Bigotti S., Lazzari P., Manca L., Spiga M., Sani M., Zanda M., (2013), Synthesis and cytotoxicity evaluation of diastereoisomers and N-terminal analogues of tubulysin-U, Tetrahedron Lett., 54, 6137–6141.

[29] (a) Eirich J., Burkhart J. L., Ullrich A., Rudolf G. C., Vollmar A., Zahler S., Kazmaier U., Sieber S. A.., (2012), Pretubulysin derived probes as novel tools for monitoring the microtubule network via activity-based protein profiling and fluorescence microscopy, Mol. Biosyst., 8, 2067–2075. (b) Reddy J. A., Dorton R., Dawson A., Vetzel M., Parker N., Nicoson J. S., Westrick E., Klein P. J., Wang Y., Vlahov I. R., Leamon C. P., (2009), In vivo structural activity and optimization studies of folate-tubulysin conjugates, Mol. Pharm., 6, 1518–1525.

[30] Ullrich A., Chai Y., Pistorius D., Elnakady Y. A., Herrmann J. E., Weissman K. J., Kazmaier U., Muller R., (2009), Pretubulysin, a potent and chemically accessible tubulysin precursor from *Angiococcus disciformis*, Angew. Chem. Int. Ed., 48, 4422−4425.

[31] Neri D., Fossat G., Zanda M., (2006), Efforts toward the total synthesis of tubulysins: New hopes for a more effective targeted drug delivery to tumors, Chem. Med. Chem., 1, 175–180.

[32] Sani M., Fossati G., Huguenot F., Zanda M., (2007), Total synthesis of tubulysins U and V, Angew. Chem. Int. Ed., 46, 3526–3529.

[33] Dömling A., Beck B., Eichelberger U., Sakamuri S., Menon S., Chen Q-Z., Lu Y., Wessjohann L. A., (2006), Total synthesis of tubulysin U and V, Angew. Chem. Int. Ed., 45, 7235–7239.

[34] Raghavan B., Balasubramanian R., Steele J. C., Sackett D. L., Feecik R. A., (2008), Cytotoxic simplified tubulysin analogues, J. Med. Chem., 51, 1530–1533.

[35] Shibue T., Hirai T., Okamoto I., Morita N., Masu H., Azumaya I., Tamura O., (2009), Stereoselective synthesis of tubuvaline methyl ester and tubuphenylalanine, components of tubulysins, tubulin polymerization inhibitors, Tetrahedron Lett., 50, 3845–3848.

[36] Peltier H. M., McMahon J. P., Patterson A. W., Ellman J. A., (2006), The total synthesis of tubulysin D, J. Am. Chem. Soc., 128, 16018–16019.

[37] Shankar S. P., Jagodzinska M., Malpezzi L., Lazzari P., Manca I., Greig I. R., Sani M., Zanda M., (2013), Synthesis and structure-activity relationship studies of novel tubulysin U analogues-effect on cytotoxicity of structural variations in the tubuvaline fragment, Org. Biomol. Chem., 11, 2273–2287.

[38] Ullrich A., Herrmann J., Müller R., Kazmaier U., (2009), Synthesis and biological evaluation of pretubulysin and derivatives, Eur. J. Org. Chem., 6367–6378.

[39] Ullrich A., Chai Y., Pistorius D., Elnakady Y. A., Herrmann J. E., Weissman K. J., Kazmaier U., Müller R., (2009), Pretubulysin, a potent and chemically accessible tubulysin precursor from *Angiococcus disciformis*, Angew. Chem. Int. Ed., 48, 4422–4425.

[40] Wipf P., Takada T., Rishel M., (2004), Synthesis of the tubuvaline-tubuphenylalanine (Tuv-Tup) fragment of tubulysin, Org. Lett., 6, 4057−4060.

[41] Chandrasekhar S., Mahipal B., Kavitha M., (2009), Toward tubulysin: Gram-scale synthesis of tubuvaline-tubuphenylalanine fragment, J. Org. Chem., 74, 9531–9534.

[42] Becker D., Kazmaier U., (2013), Synthesis of tubuphenylalanines via Ireland−Claisen rearrangement, J. Org. Chem., 78, 59–65.

[43] Park Y., Sim M., Chang T-S., Ryu J-S., (2016), A concise synthesis of tubuphenylalanine and *epi*-tubuphenylalanine via a diastereoselective Mukaiyama aldol reaction of silyl ketene acetal, Org. Biomol. Chem., 14, 913–919.