SYNTHESIS OF PEPTIDE-BASED 1,2-BENZOSELENAZOL-3-ONE DERIVATIVES

M.Sc. Thesis

By

SARYU BHARDWAJ

DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY INDORE

May 2023

SYNTHESIS OF PEPTIDE-BASED 1,2-BENZOSELENAZOL-3-ONE DERIVATIVES

A THESIS

Submitted in partial fulfillment of the

requirements for the award of the degree

of

Master of Science

by

SARYU BHARDWAJ

DEPARTMENT OF CHEMISTRY

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May 2023

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Candidate's Declaration

I hereby certify that the work shown in the report entitled "**Synthesis of Peptide-Based 1,2-Benzoselenazol-3-one Derivatives**" in the partial fulfillment of the requirements for the award of the degree of **Master of Science** and submitted to the Department of Chemistry, **Indian Institute of Technology Indore**, is an authentic record of my work carried out from 17/07/2022 to 16/05/2023 under the supervision of **Prof. Apurba K. Das**, Professor, Department of Chemistry, Indian Institute of Technology 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.

Saryu 16/5/23 Saryu Bhardwaj

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

16/5/2023
Prof. Apurba K. Das

SARYU BHARDWAJ has successfully given his M. Sc. Oral Examination held on May 16, 2023.

Signature of Supervisor of M.Sc. Thesis
Prof. Apurba K. Das
Date: 16/5/2023

Signature of PSPC Member
Debayan Sar
Dr. Debayan Sarkar
Date: 19/5/2023

S. Selva 19.05.2023
Convenor, DPGC (Dr. Selvakumar Acting DPGC)
Dr. Umesh A. Kshirsagar
Date:

Signature of PSPC Member
Dr. Umesh A. Kshirsagar
Date:

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Saryu Bhardwaj

Dedicated to my family

ABSTRACT

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is one of the most popular candidates for drug research due to its low toxicity. Ebselen-based 1,2-benzoselenazol-3-one are also under clinical trials for various diseases like stroke, cardiovascular disorders, etc. In this work, we have synthesized a peptide-based 1,2-benzoselenazol-3-one compound by subjecting β-alanine and L-phenylalanine to simple peptide coupling reactions using EDC.HCl and HOBt which were done at low temperature, KSeCN-based cyclization reflux reaction. $SOCl_2$ mediated esterification and, LiOH was used in hydrolysis reactions. We have used ^{1}H and ^{13}C-NMR and mass spectrometric techniques to characterize all the synthesized substrates.

TABLE OF CONTENT

NOMENCLATURE

δ	Chemical shift
˚C	Degree Celsius
g	Grams
Hz	Hertz
K	Kelvin
ml	Millilitre
mg	Milligrams
nm	Nanometer
ppm	Parts per million

ACRONYMS

Cs_2CO_3	Caesium carbonate
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
GSH	Glutathione
GPx	Glutathione peroxidase
GR	Glutathione reductase
HOBt	Hydroxybenzotriazole
LiOH	Lithium hydroxide
MeOH	Methanol
NMR	Nuclear Magnetic Resonance
DMF	N, N-dimethylformamide
EDC.HCl	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride
TLC	Thin Layer Chromatography
Et_3N	Triethyl ammine
$SOCl_2$	Thionyl chloride

LIST OF FIGURES

Chapter 1

1.1 Introduction

Selenium was thought to be a toxic element after its discovery, but as of now, it is identified as an essential micronutrient. Selenium is involved in the proper functioning of immune cells and protecting cells from oxidative stress. It is essential to know that even in high quantity selenium can show its adverse effects on human health. It is essential to note that the safe consumption of selenium on a daily basis is 55µg-70µg, and it is pretty imperative to consume selenium in the diet daily for healthy metabolism in humans.[1,2] Selenium and organoselenium compounds have shown great potential in anti-tumor research as well. Although several selenoproteins are identified as antioxidants, there are several other selenium-containing compounds that are prooxidants. Various selenium-based compounds are currently under investigation as anti-tumor and anti-inflammatory agents. Ebselen, ethaselen and some other 1,2-benzoselenazol-3-one have shown promising biological activity in antibacterial, antioxidant, and anticancer studies.[3]

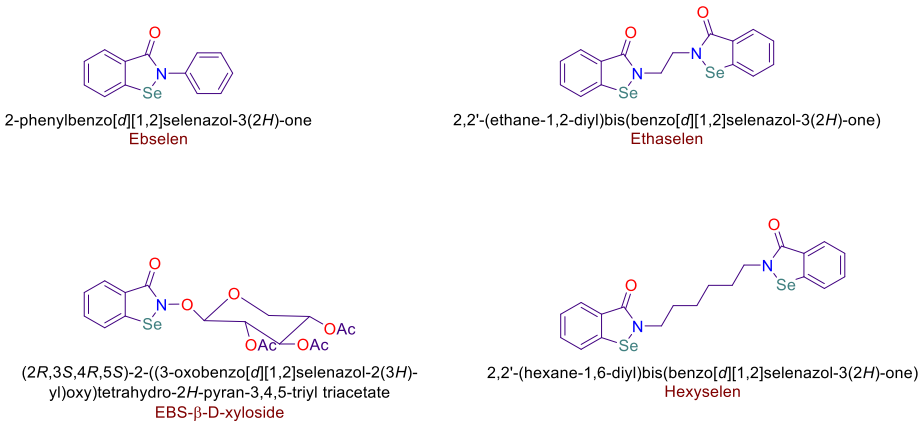

Fig. 1: 1,2-Benzoselenazol-3-one derivatives.

1,2-Benzoselenazol-3-one contains a five-membered heterocyclic ring, which contains a Se-N bond. Ebselen, a 1,2-benzoselenazol-3-one, was first synthesized by a group of researchers in 1924.[1] Since then, ebselen has been subjected to numerous clinical trials and has shown anti-proliferation activity through ROS production. Ebselen showed minimal toxicity too. Ebselen has also been subjected to various studies in which

it inhibited covid disease. In a certain xenograft model of a human pancreatic tumor in mice, regular oral consumption of Ebselen decreased tumor size by 58%.[1] Besides that, Ebselen has shown promising biological activity as an antimicrobial, antibacterial, antiviral, and anti-inflammatory agent.

Ebselen is also known to show similar activity shown by glutathione peroxidase. Glutathione peroxidase enzyme catalyses the reduction of free H_2O_2 into H_2O.[4] Hence, it protects cells from oxidative damage caused by free radicals. Therefore, due to Ebselen's well-documented anti-oxidant property and its ability to mimic glutathione peroxidase(GPx), scientists are intrigued by its biological properties.

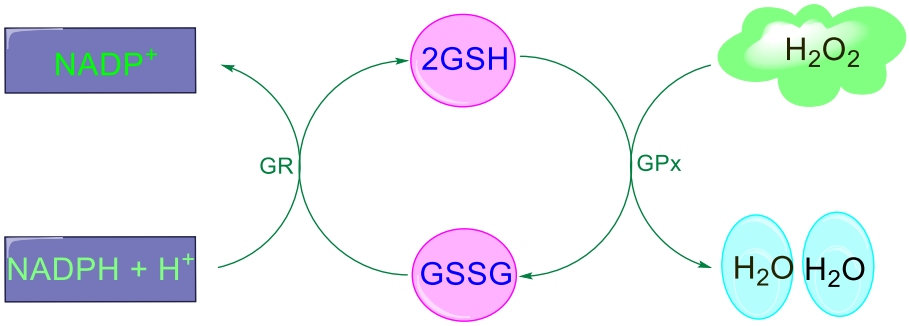

Fig. 2: Hydrogen peroxide is converted into two molecules of water, while two molecules of GSH are dimerizing into GSSG in a reaction catalysed by glutathione peroxidase(GPx).[11] GSSG further forms two molecules of GSH and this reaction is catalysed by glutathione reductase.

It is currently being tested for treating several diseases like arthritis and cancer[10]; Because of its low toxicity, researchers are pretty optimistic regarding ebselen's future applications. A similar compound ethaselen when subjected to Anti-cancer studies demonstrated a significant decrease in the growth of cancer cells in various cancer cell models. EBS-β-D-xyloside and hexyselen also inhibited cancer growth in various in-vitro cancer cell models.[3]

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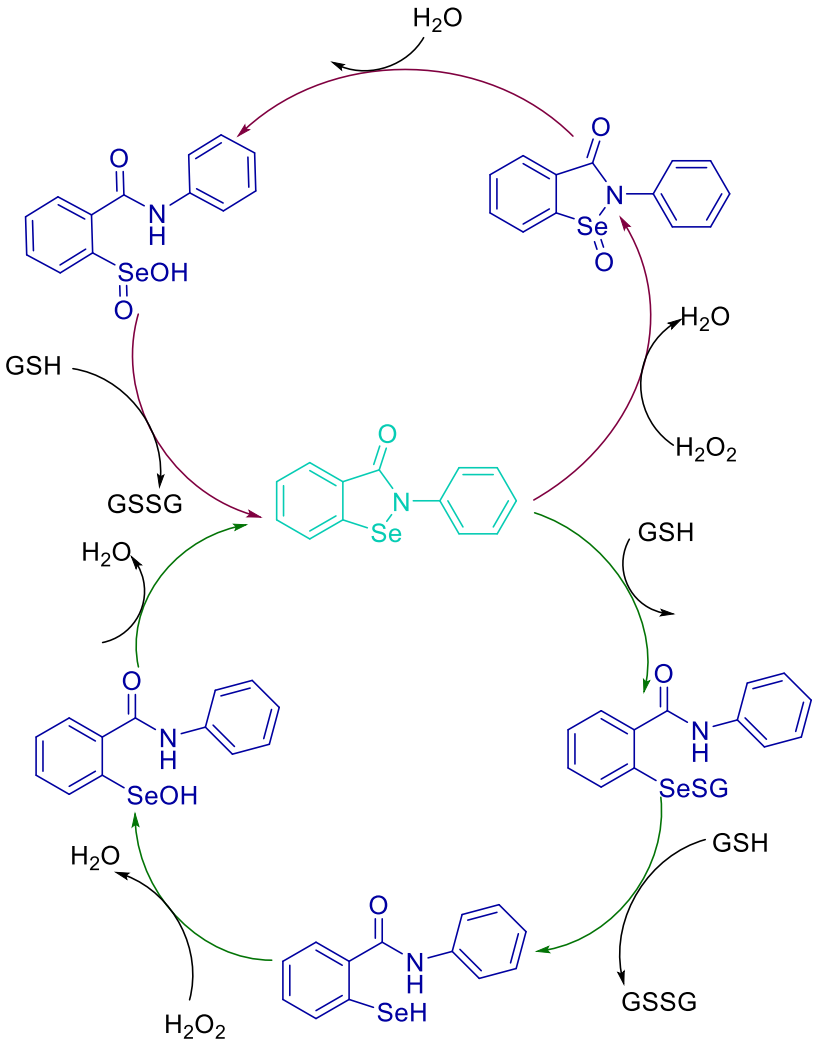
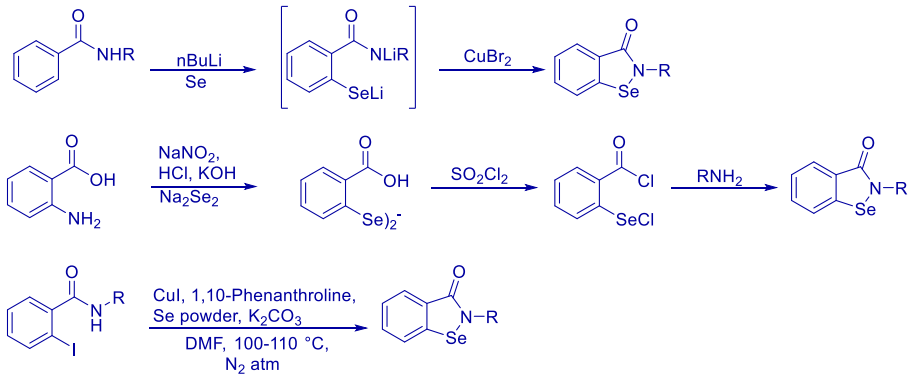


Fig. 3: Mechanism similar to GPx demonstrated by ebselen, where H_2O_2 is getting converted to H_2O and at the same time glutathione(GSH) is getting reduced to its dimer form.[4]

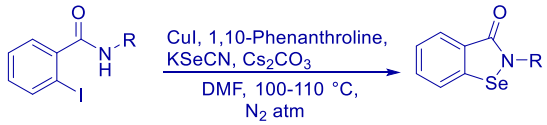
Chapter 2

2.1 Previous Work

Due to 1,2-benzoselenazol-3-one's promising biological and chemical effects, they have gained the attention of scientists worldwide. As a result, various scientists have devised several methods to synthesize these 1,2-benzoselenazol-3-one using a variety of reagents at their disposal, as mentioned in the following schemes.[10]



Our synthesis involves basic coupling, methyl-esterification and hydrolysis reactions, which provides a facile route to synthesize a dipeptide-based 1,2-benzoselenazol-3-one derivative. The cyclization reaction used to establish a heterocyclic ring containing selenium and nitrogen atom utilized reagents such as CuI, KSeCN, 1,10-Phenanthroline and Cs_2CO_3. This chemical route turned out to be better than another conventional method of synthesizing ebselen-like 1,2-benzoselenazol-3-one. Our goal is to test dipeptide-based 1,2-benzoselenazol-3-one derivative for potential medicinal properties like antitumor, antibacterial and antioxidant etc.



Chapter 3

3.1 Experimental section

Materials required

The solvents and reagents were purchased from commercially available sources. Alfa Aesar, Sigma Aldrich-India, Merck, Spectro-chem, and TCI are some available sources.

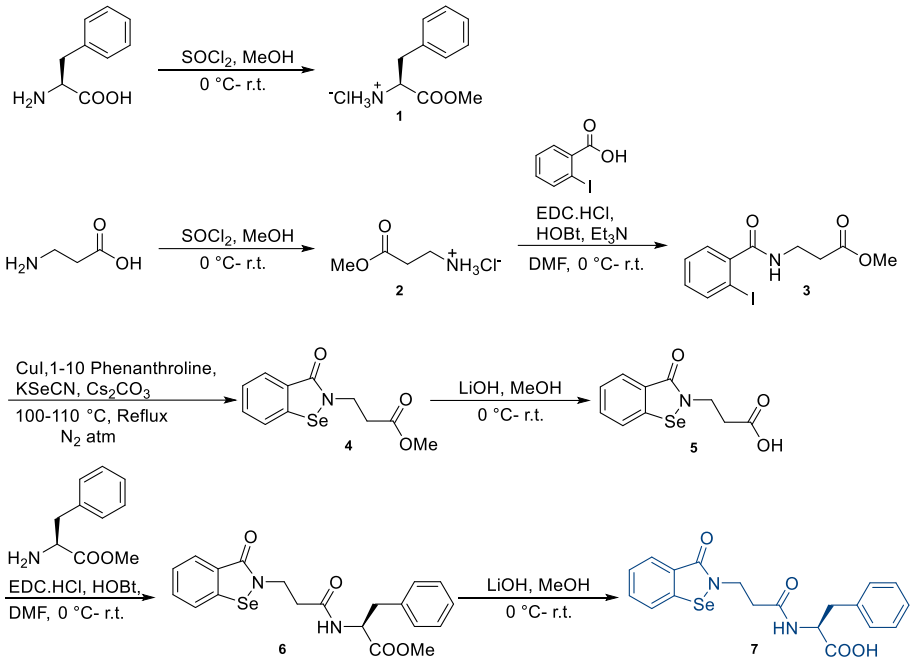
L-Phenylalanine, β-alanine, diethyl ether, EDC.HCl, HOBt, Et_3N, 2-iodobenzoic acid, and lithium hydroxide pellets were purchased from the Sisco Research Laboratory. Thionyl chloride was purchased from Spectro-chem. KSeCN was purchased from Alpha Aesar. DMF and methanol were purchased from Merck, ethyl acetate was purchased from FINAR limited, and petroleum ether was purchased from Advent. After completion of the reaction, silica (230-400 mesh) was used in the flash column to purify the compound with ethyl acetate and petroleum ether as mobile phase. For moisture-sensitive reactions, the dry solvents were used in N_2 gas.

General

TLC monitored the course of reactions. NMR spectra were recorded on Bruker Avance (500 MHz) instrument at 25 °C. Mass spectra were set down on the Bruker instrument by using ESI positive mode. The NMR spectra of all substrates were analysed using MestReNova software. The NMR samples were prepared in DMSO-d_6 and $CDCl_3$. Solvent. The chemical shift was expressed in the form of ppm (δ) relative to surplus solvents protons ($CHCl_3$: δ= 7.26, DMSO: δ= 2.50 For ^1H NMR; and $CHCl_3$: δ 77.00, DMSO: δ= 39.50 for ^{13}C NMR).

Chapter 4

4.1 Reaction Scheme

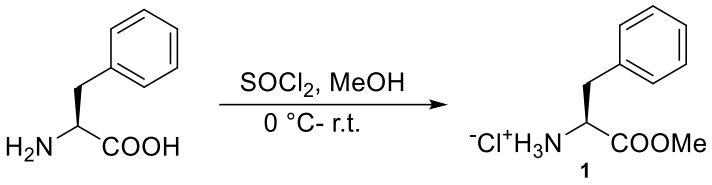


In the first step of the scheme, L-phenylalanine was converted to phenylalanine methyl ester (**1**), followed by the conversion of β-alanine to β-alanine's methyl ester (**2**) using $SOCl_2$ in alcohol. In the next step, the coupling reaction using EDC.HCl, HOBt, and Et_3N between methyl 3-aminopropanoate hydrochloride and 2-iodobenzoic acid yielded methyl 3-(2-iodobenzamido)propanoate(**3**). After that, methyl 3-(2-iodobenzamido)propanoate was converted to methyl 3-(3-oxobenzo[d][1,2]selenazol-2(3*H*)-yl)propanoate (**4**) by refluxing in the presence of CuI, 1-10 Phenanthroline, KSeCN, Cs_2CO_3 at 100 to 110 ºC. In the following step **4** was hydrolysed to 3-(3-oxobenzo[d][1,2]selenazol-2(3*H*)-yl)propanoic acid (**5**) using 1N LiOH. In the next step, **5** and methyl L-phenylalaninate were coupled together using EDC.HCl and HOBt which yielded methyl (3-(3-oxobenzo[d][1,2]selenazol-2(3*H*)-yl)propanoyl)-L-phenylalaninate (**6**)

which in last step was hydrolysed to (3-(3-oxobenzo[d][1,2]selenazol-2(*3H*)-yl)propanoyl)-L-phenylalanine (**7**).

4.2 Synthesis of compounds

Synthesis of compound **1**



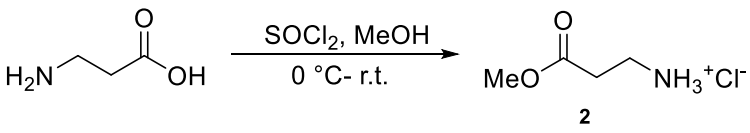
In a 250 mL R.B. flask, MeOH (30 mL) was taken and kept to stir under ice-cold conditions. After cooling for 5 min, L-phenylalanine(1 g, 6 mmol) was added to it. Then $SOCl_2$ (1 mL, 1.2 mmol) was poured, covered with guard tube, and stirred for 12 h. Then resulting solution was dried in the Rota. The solid residue was solubilized in H_2O; further, its pH was maintained at 9 by adding 1N NaOH dropwise. The solution was extracted in EtOAc and evaporated in rota vapor, producing a pure L-phenylalanine's methyl ester.

Yield = 91% (0.98 g, 5.4 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.77 (s, 1H, H of OH), 7.35-7.24 (m, 5H, H of Phe), 4.2 (s, $C^{\alpha}H$ of F), 3.65 (s, 3H, H of OCH_3), 3.23-3.09 (m, 2H, $C^{\beta}H$ of F).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.9, 133.5, 129.6, 129.1, 127.9, 54.2, 53.1, 36.1.

ESI-MS m/z: $[M+H]^+$ calc for $C_{10}H_{13}NO_2$ = 180.1019; detected = 180.1053.

Synthesis of compound **2**

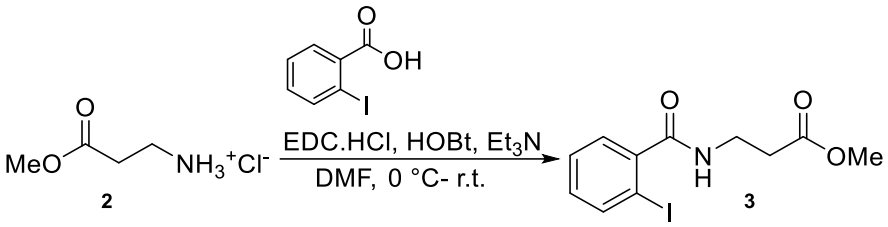


In a 250 Ml RB flask, MeOH (30 mL) was taken and kept to stir under ice-cold conditions. After cooling for 5 min, β-alanine (3 g, 33.6 mmol) was added to it. Then $SOCl_2$ (4.9 mL, 67.3 mmol) was poured in RB, covered with guard tube, and allowed to react overnight. After that solution was evaporated in rota. The white solid obtained was further used in another reaction.

Yield = 81% (3.8 g, 21.5 mmol).

ESI-MS m/s: $[M+H]^+$ calc for $C_4H_9NO_2$ = 104.0706; detected = 104.0721.

Synthesis of compound **3**



2 3

In a 250 mL R.B. flask, 2-iodobenzoic acid (1.19 g, 4.8 mmol) was dissolved in DMF in ice-cold condition. After cooling for 15 min, EDC.HCl (1.1 g, 5.7 mmol) and Et_3N (0.35 mL, 2.4 mmol) were added. 15 minutes later, HOBt (0.77 g, 5.7 mmol) was added to the R.B. while stirring. After 20 min, methyl 3-aminopropanoate hydrochloride (1 g, 7.1 mmol) was added along with Et_3N (1 ml, 7.1 mmol) to R.B. and stirred overnight.

After 12 h, contents in R.B. were diluted with EtOAc and given 1N HCl, 1N NaOH and brine solution wash, each 3x10 mL. The organic part was dried by Na_2SO_4 and evaporated in Rota. A yellow-coloured solid of **3** (1 g, 3 mmol) was obtained.

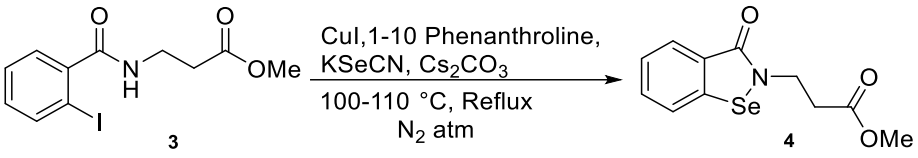
Yield = 63% (1 g, 3 mmol). ^{1}H NMR. (500 MHz, $CDCl_3$): δ = 7.86-7.84 (d, 1H, J = 7.9Hz, H of Ibx), 7.37-7.36 (d, 2H, J = 4.2, H of Ibx), 7.11-

7.07 (m, 1H, H of Ibx), 6.36 (s, 1H, H of NH), 3.74-3.71 (m, 5H, $C^{\beta}H$ of β-Ala, H of OCH_3), 2.72-2.70 (t, 2H, J = 5.95, $C^{\alpha}H$ of β-Ala).

^{13}C N.M.R. (125 MHz, $CDCl_3$): δ = 173.0, 169.4, 142.0, 139.8, 131.1, 128.2, 128.1, 92.3, 51.9, 35.3, 33.6.

ESI-MS m/z: $[M+Na]^+$ calcd. for $C_{11}H_{12}INO_3$ = 355.9754; detected = 355.9740.

Synthesis of compound **4**



In 100 ml, two-neck R.B. CuI (0.5 g, 1.6 mmol) and 1,10-phenanthroline (0.475 g, 2.6 mmol) were added and dissolved in DMF and stirred for 10 min in an inert environment. After that, KSeCN (0.38 g, 2.6 mmol) was taken to the R.B. and stirred for 30 min. After that, methyl 3-(2-iodobenzamido)propanoate (0.5 g, 1.5 mmol) was added to RB and left for stirring for 30 min. At last, Cs_2CO_3 (1.3 g, 2.5 mmol) was taken in R.B. and refluxed for 3 h in N_2 atm.

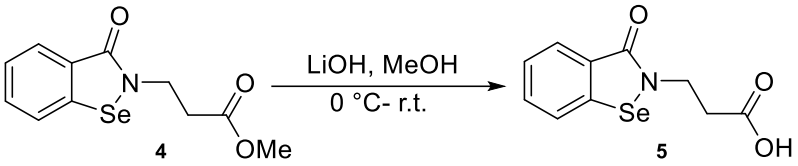
Reaction upon completion was quenched with cold brine (stirred for 30 min). EtOAc was used to extract the compound from the solution obtained and dried in rotavapor. The yellow-coloured liquid obtained was Separated using Flash-chromatography (40% EtOAc/Hex) yielding **4**.

Yield = 43% (0.187 g, 0.65 mmol). ^1H NMR (500 MHz, $CDCl_3$): δ = 8.05-8.03 (d, 1H, J = 10Hz, H of Ibx), 7.62-7.57 (q, 2H, J = 10, H of Ibx), 7.43-7.40 (t, 1H, J = 10Hz, H of Ibx), 4.15-4.13 (t, 2H, J = 5Hz, $C^{\beta}H$ of β-Ala), 3.73 (s, 1H, H of OCH_3), 2.80-2.77 (t, 2H, J = 5Hz, $C^{\alpha}H$ of β-Ala).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.1, 138.4, 132.1, 128.7, 126.8, 126.2, 123.8, 52.0, 40.6, 34.4.

ESI-MS m/z: $[M+Na]^+$ calcd. for $C_{11}H_{11}NO_3Se$ = 307.9985; detected = 307.9797.

Synthesis of compound **5**



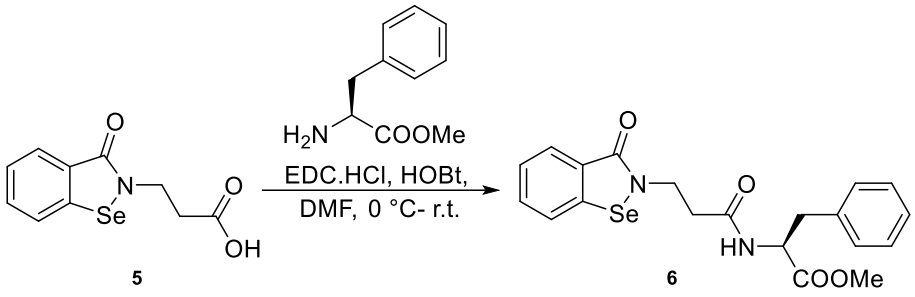
In a 250 ml R.B. flask **4** (0.15 g, 0.526 mmol) was solubilized in methanol at ice-cold condition while stirring. After that, 1N LiOH (1 mL) was poured to the solution dropwise at regular intervals; TLC was used to observe the course of reaction and upon completion solution in R.B. was concentrated in rotavapor. The residue obtained was Solubilized in distilled H_2O, followed by acid neutralization by 1N HCl, and at last compound was extracted with EtOAc and evaporated in rotavapor, yielding a white solid of **5**.

Yield = 93% (0.135 g, 0.49 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 12.43 (s, H of OH), 8.04-8.02 (d, 1H, J = 10Hz, H of Ibx), 7.82-7.80 (d, 1H, J = 10, H of Ibx), 7.62-7.59 (t, 1H, J = 10Hz, H of Ibx), 7.43-7.40 (t, 1H, J = 10Hz, H of Ibx), 3.94-3.91 (t, 2H, J = 10Hz, $C^{\beta}H$ of β-Ala), 2.62-2.59 (t, 2H, J = 10Hz, $C^{\alpha}H$ of β-Ala).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2, 166.8, 140.0, 131.9, 128.1, 127.7,126.2, 126.1, 35.

ESI-MS m/z: $[M+H]^+$ calcd for $C_{10}H_9NO_3Se$ = 271.9821; detected = 272.0042.

Synthesis of compound **6**



In a 250 mL RB flask, **5** (0.135 g, 0.5 mmol) was solubilized in DMF under ice-cold condition and left to stir for 5 minutes. After that, EDC.HCl (0.12 g, 0.65 mmol) was added in the R.B. flask while stirring. After 15 min HOBt (0.88 g, 0.65 mmol) was added to the R.B. and left to stir. After 20 minutes, L-phenylalanine's methyl ester (0.18 g, 1 mmol) was taken in the R.B. and stirred overnight. The R.B. flask was covered with aluminium foil.

After 12 h, contents in R.B. were solubilized in EtOAc and given 1N HCl, 1N NaOH and brine wash each 3x10 mL. The organic part was dried with Na_2SO_4 and evaporated in Rota. A white solid of **6** was obtained. The white solid was further separated using Flash-chromatography. (60%, EtOAc/Hex).

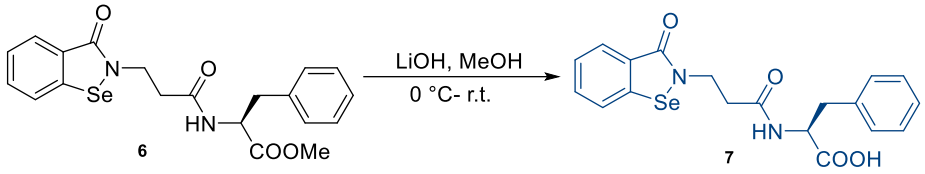
Yield = 69%, (0.15 g, 0.347 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.04-8.03 (d, 1H, J = 7.85Hz, H of Ibx), 7.62-7.57 (q, 2H, J = 7.85, H of Ibx), 7.43-7.40 (t, 1H, J = 6.4Hz, H of Ibx), 7.14-7.11 (m, 3H, H of F), 7.00-6.99 (m, 2H, H of F), 6.25-6.23 (d, 1H, J = 5.5, H of NH), 4.92-4.88 (m, 1H, $C^{\alpha}H$ of F), 4.20-3.67 (m, 2H, $C^{\beta}H$ of β-Ala), 3.67 (s,1H, H of OCH_3), 3.16-2.63 (m, 2H, $C^{\beta}H$ of F), 2.65-2.63 (t, 2H, J = 5Hz, $C^{\alpha}H$ of β-Ala).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.7, 169.9, 167.5, 138.9, 135.6, 132.0, 129.1, 128.6, 128.5, 127.0, 126.8, 126.1, 123.9, 53.3, 52.3, 40.8, 37.8, 36.2.

ESI-MS m/z: $[M+Na]^+$ calcd for $C_{20}H_{20}N_2O_4Se$ = 455.0482; detected = 455.0519.

Melting Point = 146 ºC

Synthesis of compound **7**



In a 250 ml R.B. flask methyl **6** (0.15 g, 0.347 mmol) was solubilized in methanol in ice-cold condition while stirring and after that, 1N LiOH (1mL) was taken in the solution dropwise at regular intervals. The reaction was observed with TLC and upon completion, the solution was dried in rotavapor. The residue obtained was dissolved in distilled H_2O, followed by acid neutralization with 1N HCl and ether wash. At last, the compound was extracted with ethyl acetate and evaporated in rotavapor, yielding a brown solid of **7**.

Yield = 89.3% (0.13 g, 0.31 mmol). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 12.73 (s, 1H, H of OH), 8.34-8.32 (d, 1H, J = 7.7Hz, H of NH), 8.02-8.01 (d, 1H, J = 7.85, H of Ibx), 7.80-7.79 (d, 1H, J = 7.5Hz, H of Ibx), 7.61-7.58 (t, 1H, J = 7.15, H of Ibx), 7.42-7.39 (t,1H, J = 7.2Hz, H of Ibx), 7.26-7.13 (m, 5H, H of F), 4.49-4.45 (m, 1H, $C^{\alpha}H$ of F), 3.89- 3.81 (m, 2H, $C^{\beta}H$ of β-Ala), 3.06-3.02(m, 1H, $C^{\alpha}H$ of F), 2.89-2.84(m, 1H,$C^{\alpha}H$ of f).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.3, 170.4, 166.8, 140.2, 137.9, 131.8, 129.5, 128.5, 128.2, 127.6, 126.8, 126.2, 126.0, 53.9, 37.2, 35.9, 21.5.

ESI-MS m/z: $[M+Na]^+$ calcd for $C_{19}H_{18}N_2O_4Se$ = 419.0506; detected = 419.0635.

Melting Point = 138 ºC

Chapter 5

5.1 Characterization

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

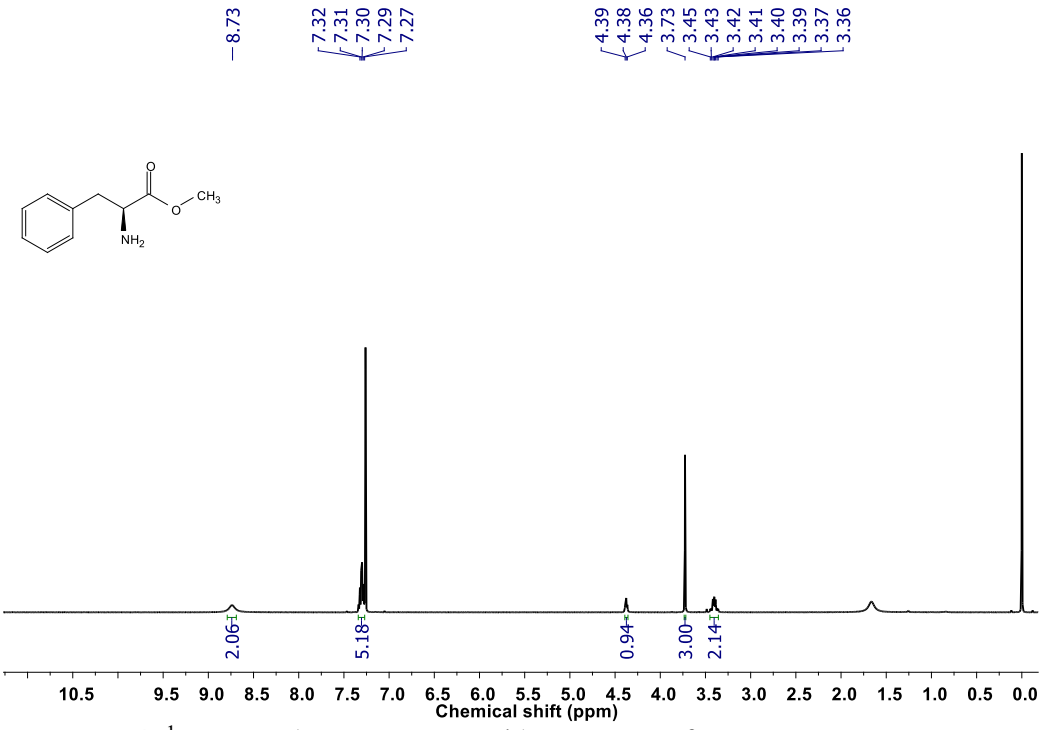


Fig. 4: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **1.**

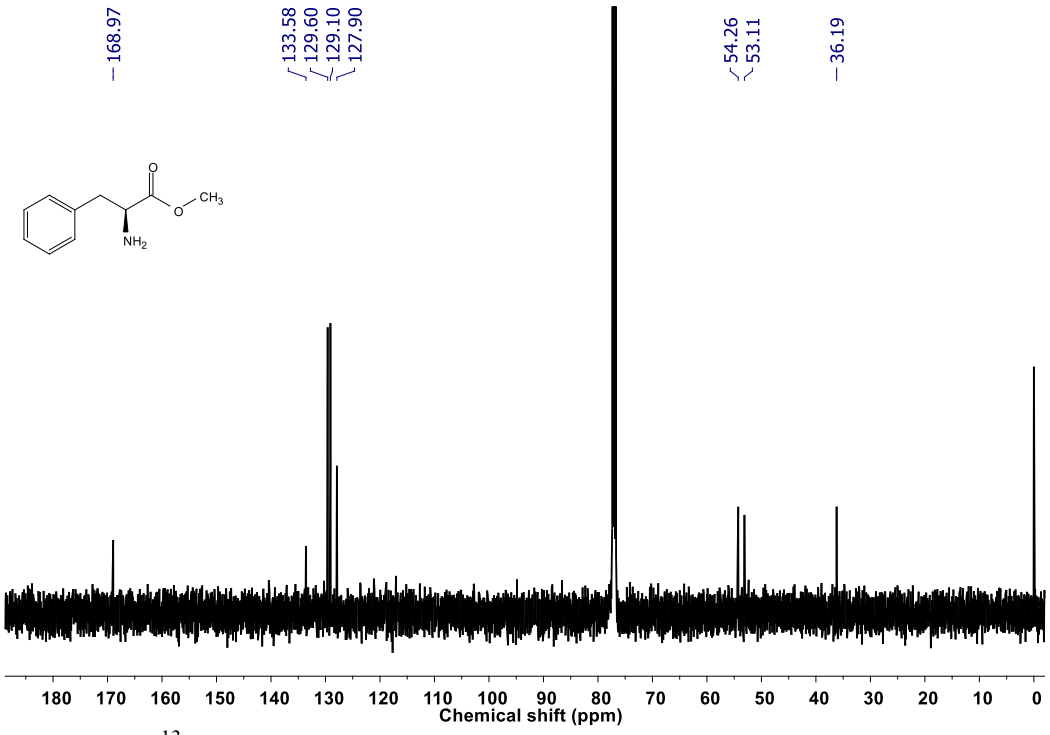


Fig. 5: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **1.**

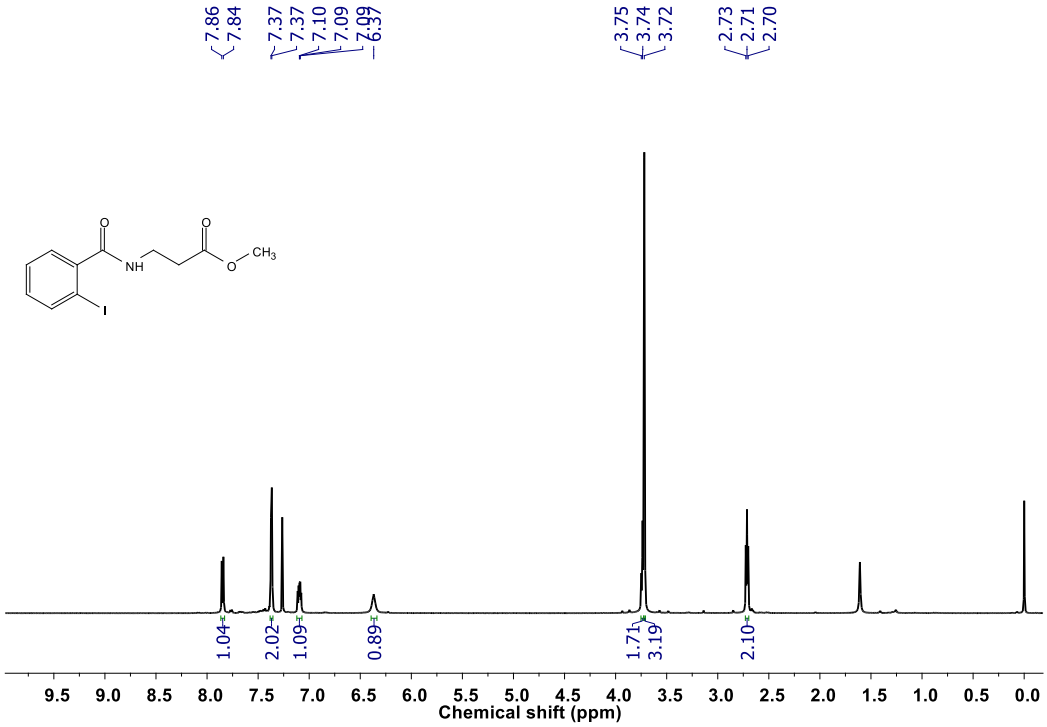


Fig. 6: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3.**

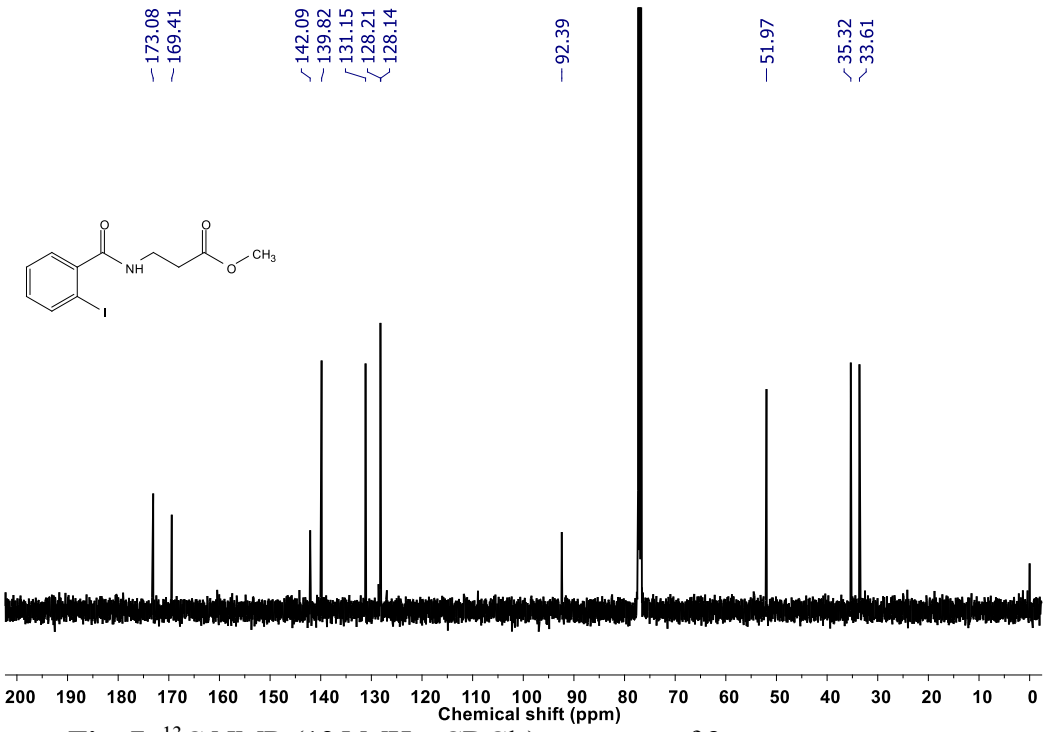


Fig. 7: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **3.**

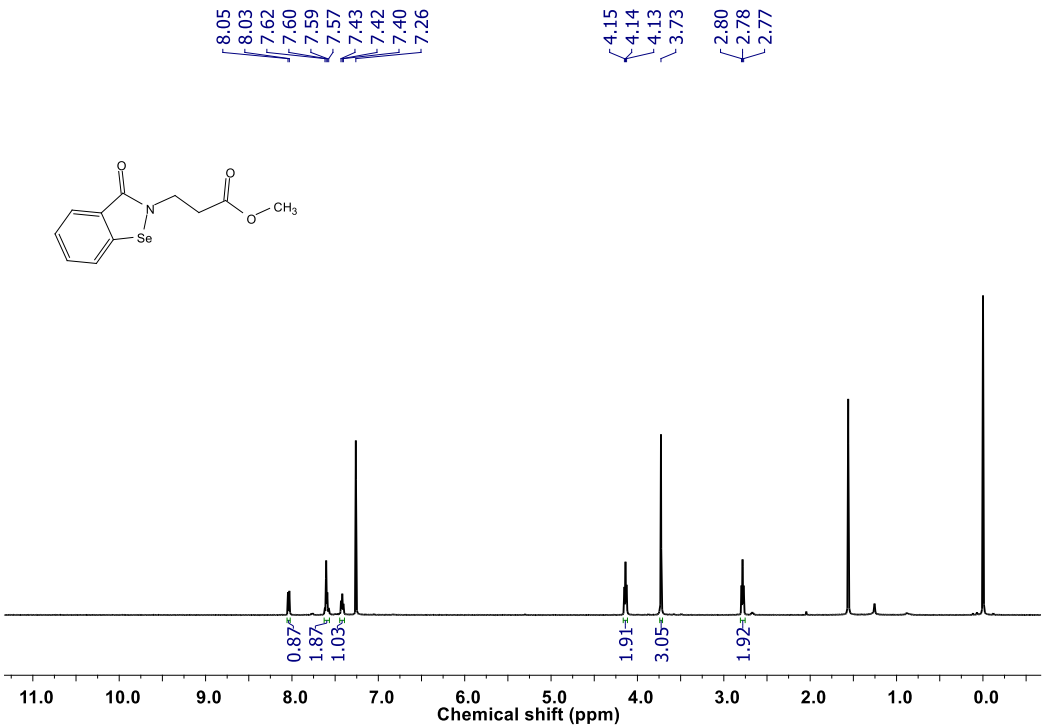


Fig. 8: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **4.**

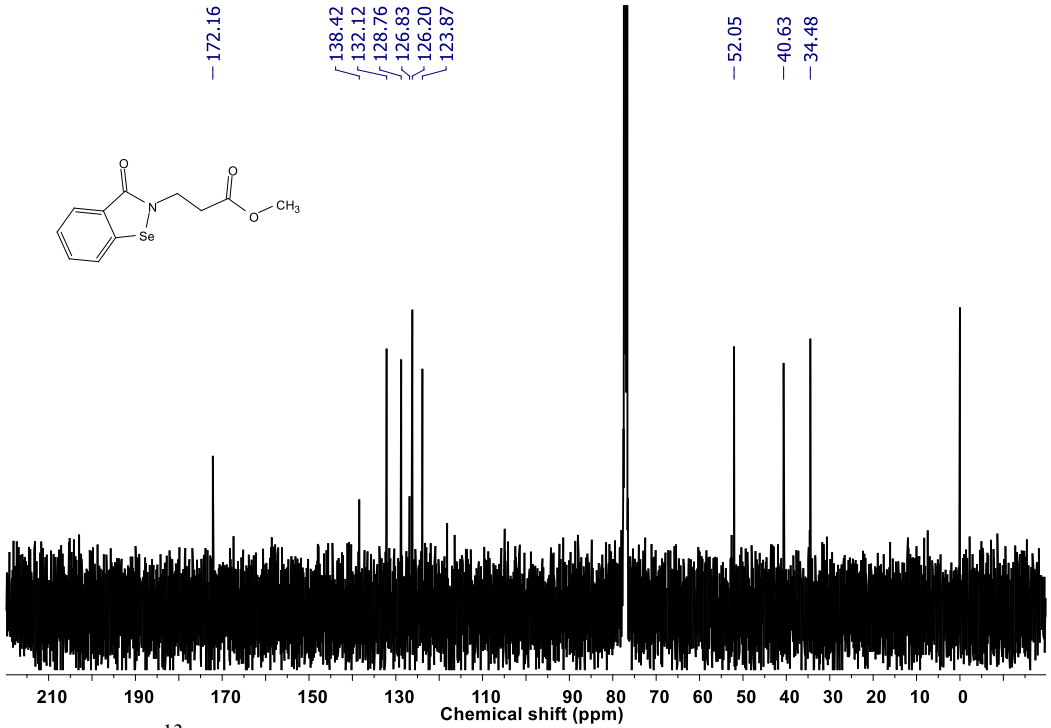


Fig. 9: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **4.**

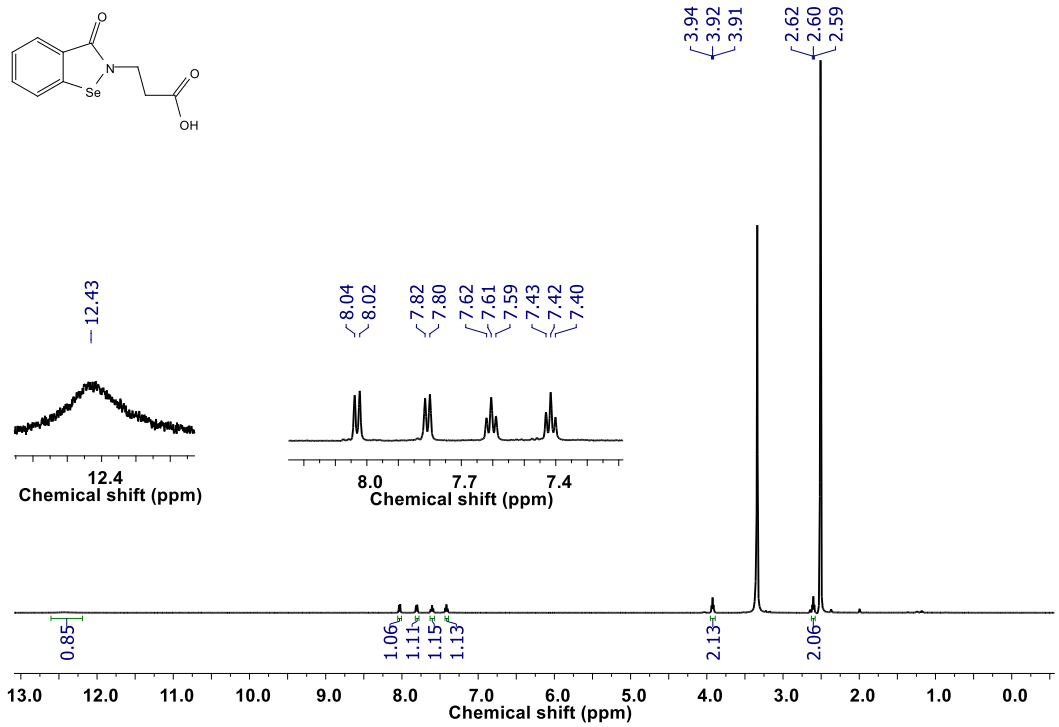


Fig. 10: ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **5.**

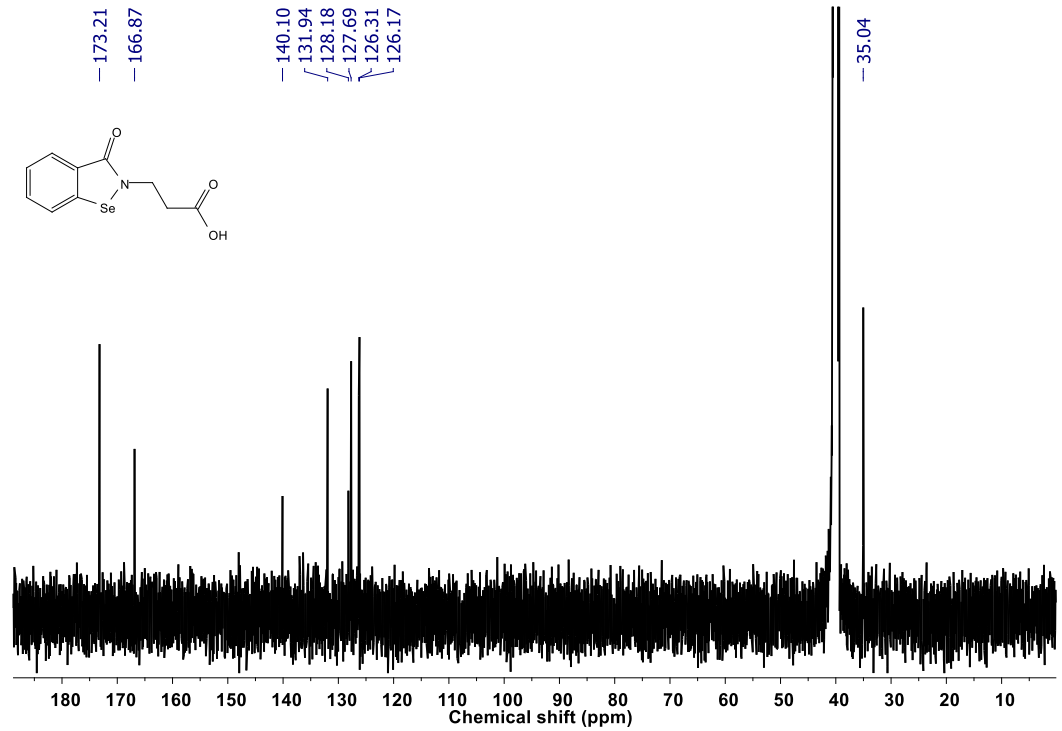


Fig. 11: ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of **5.**

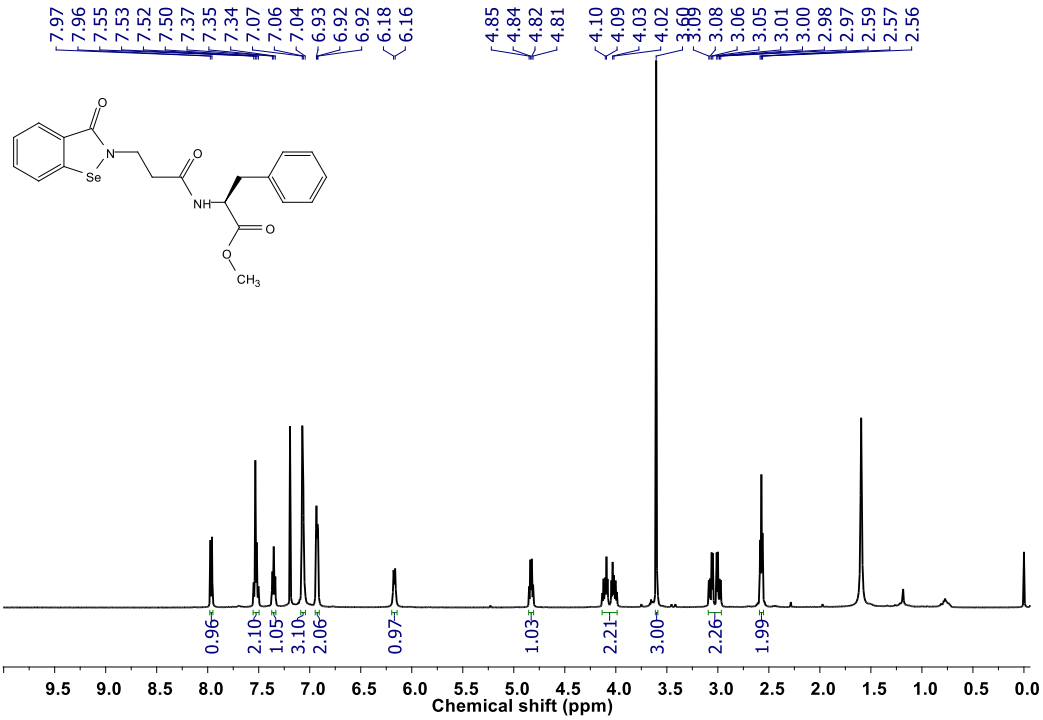


Fig. 12: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **6.**

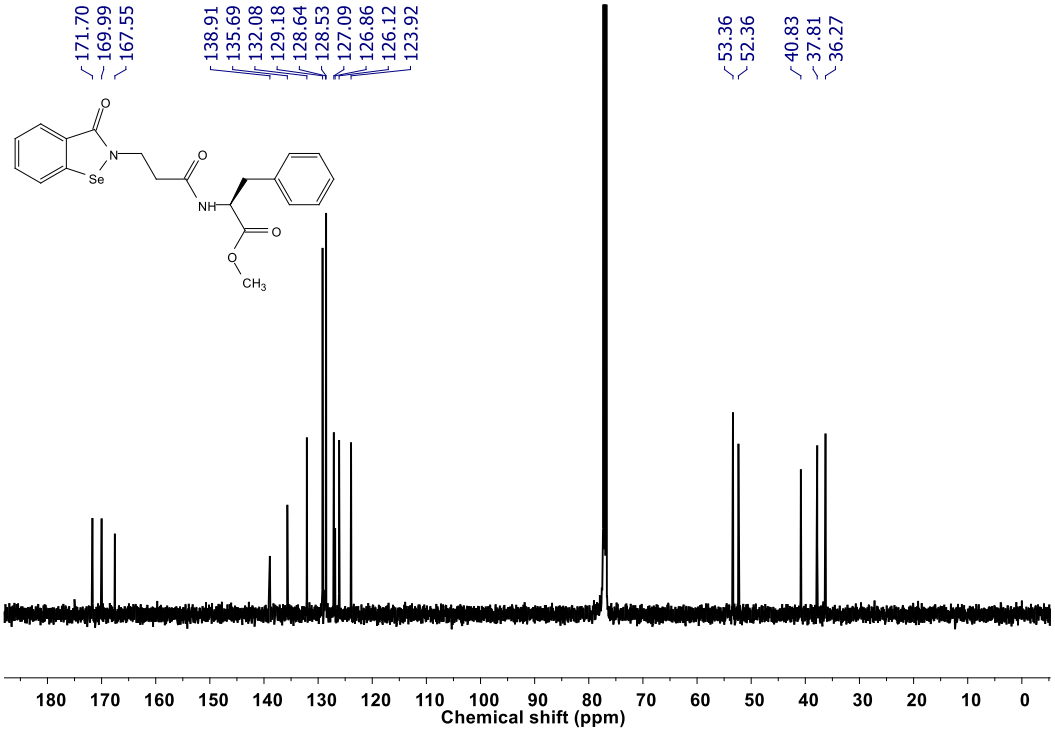


Fig. 13: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **6.**

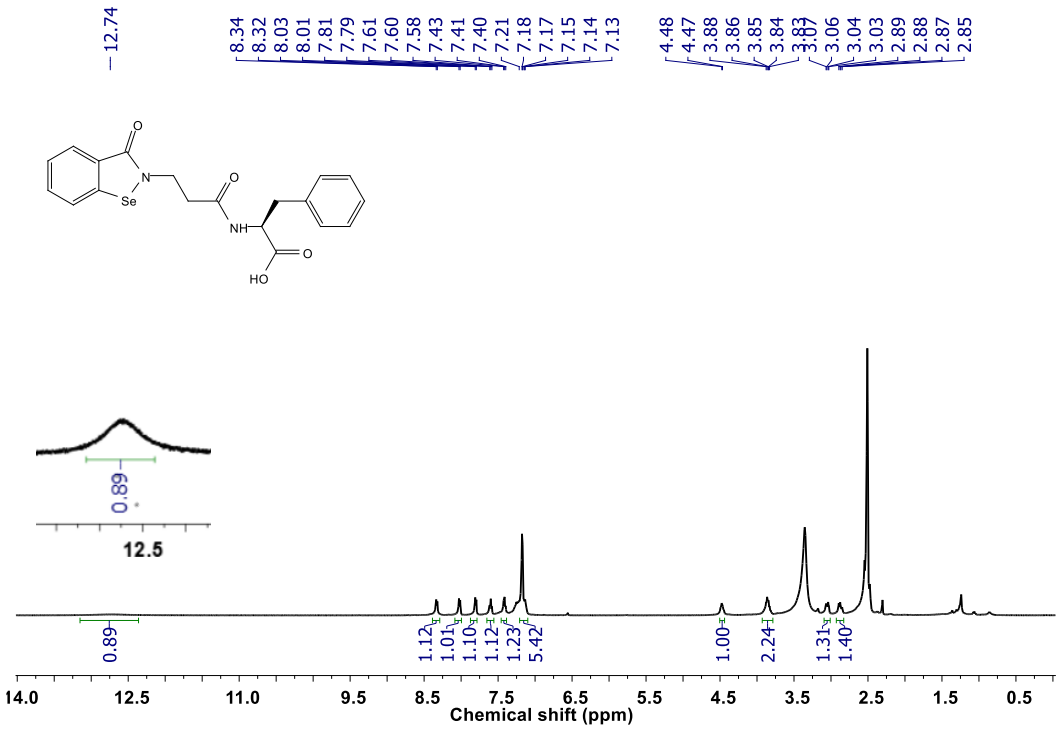


Fig. 14: ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **7.**

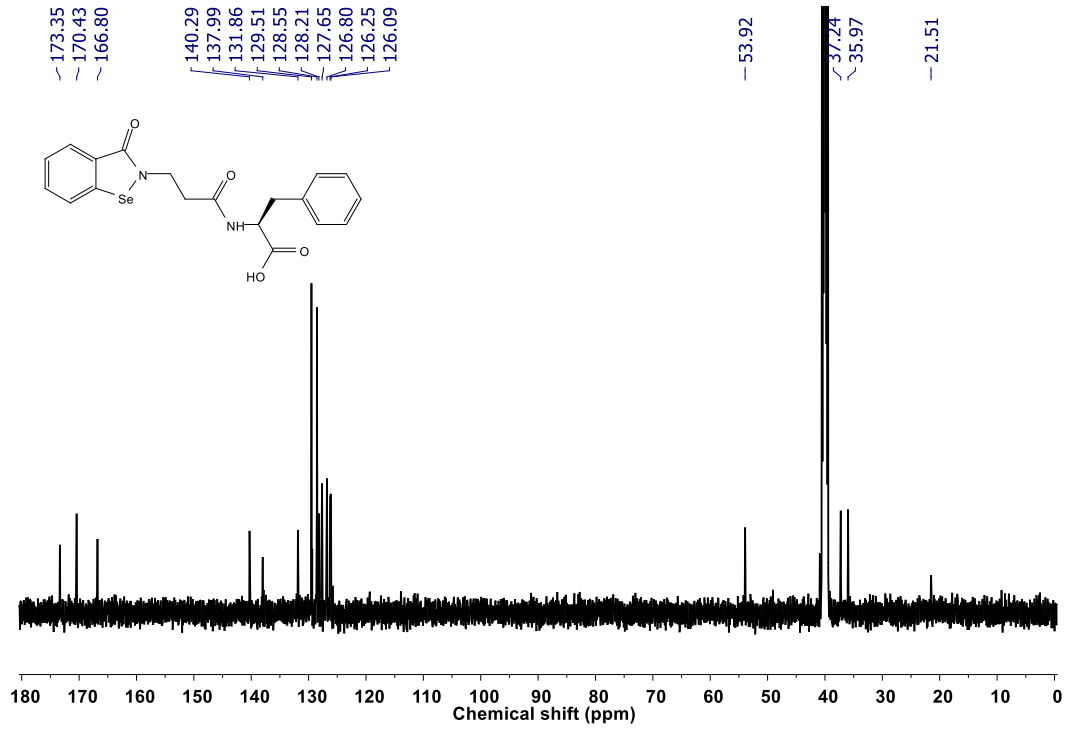


Fig. 15: ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of **7.**

Mass Spectra

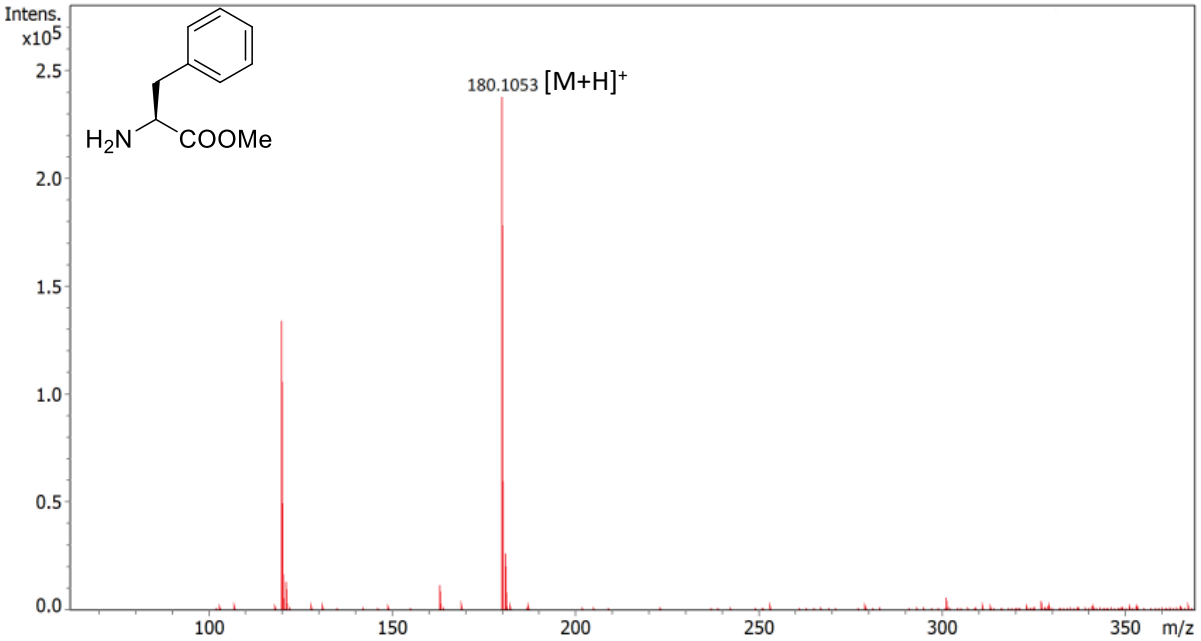


Fig. 16: Mass spectrum of compound **1.**

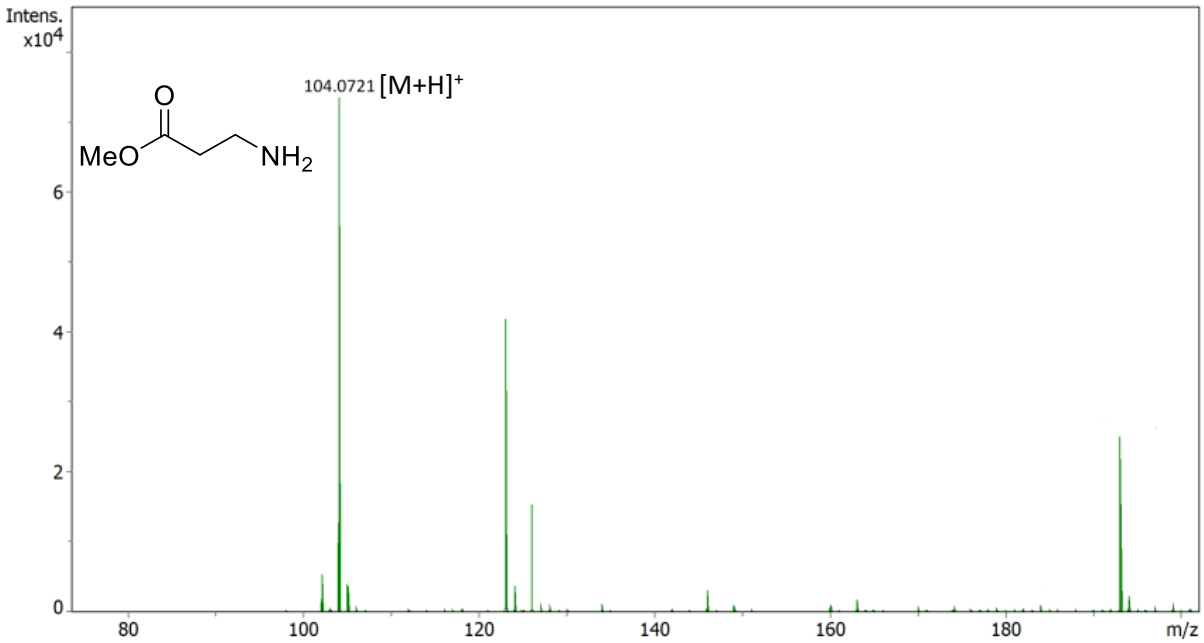


Fig. 17: Mass spectrum of compound **2**.

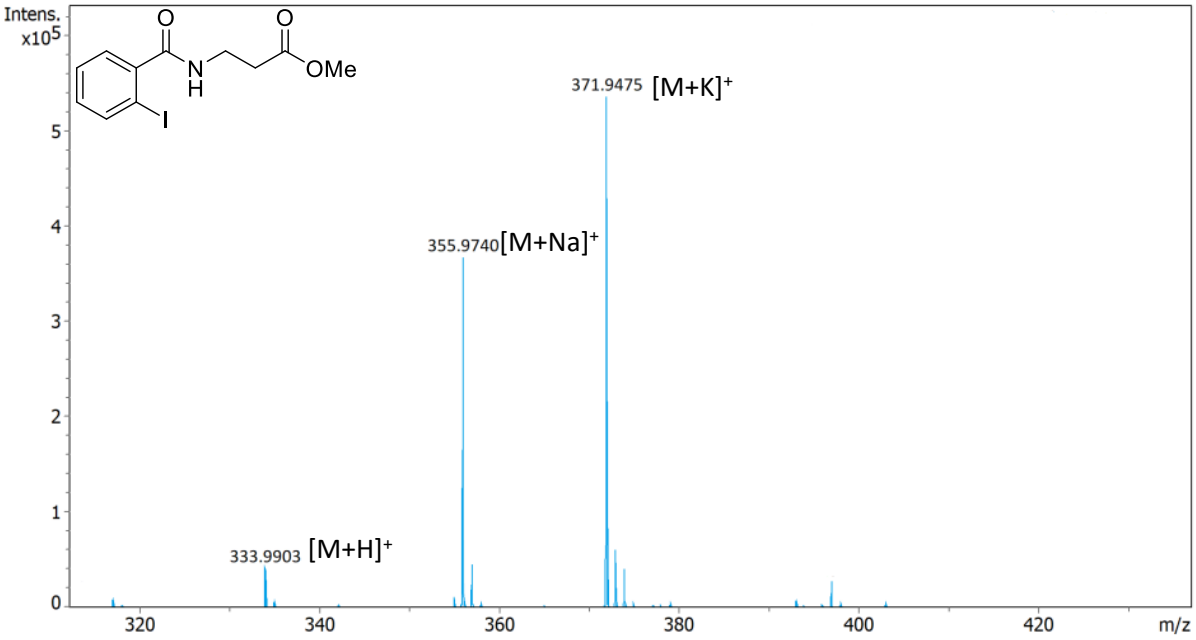


Fig. 18: Mass spectrum of compound **3**.

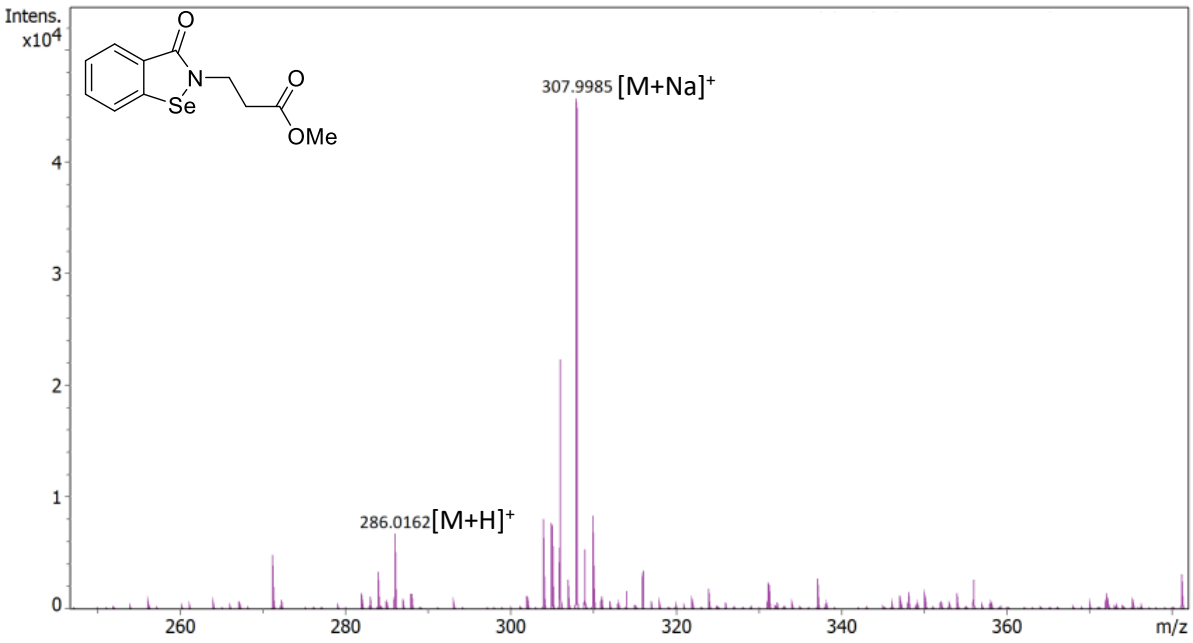


Fig. 19: Mass spectrum of compound **4**.

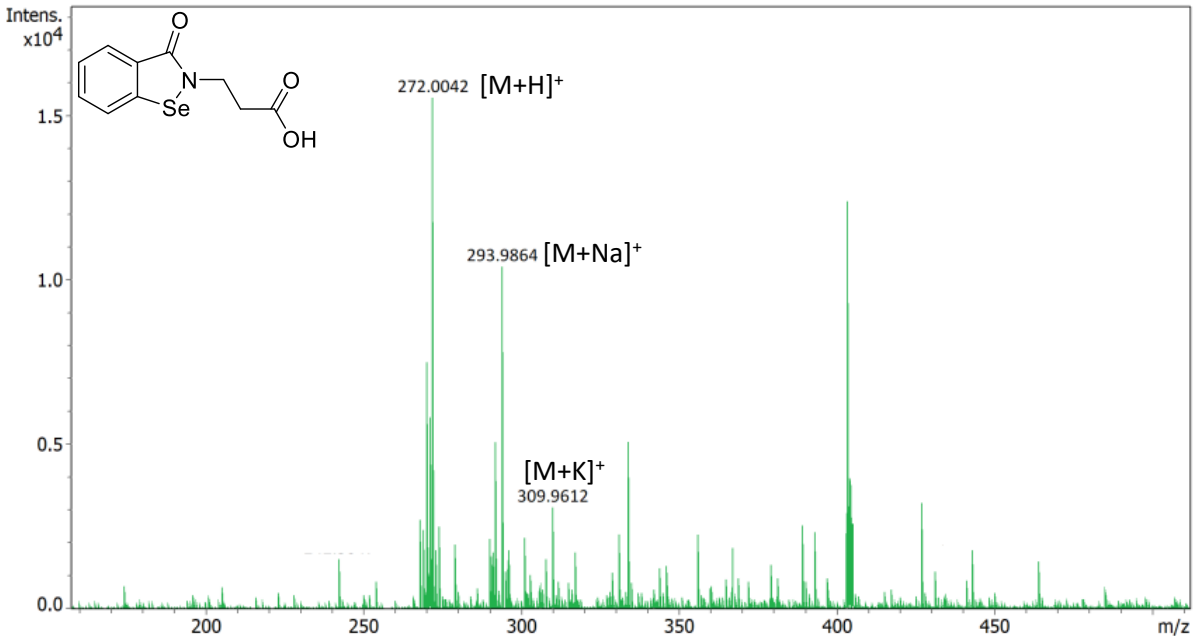


Fig. 20: Mass spectrum of compound **5**.

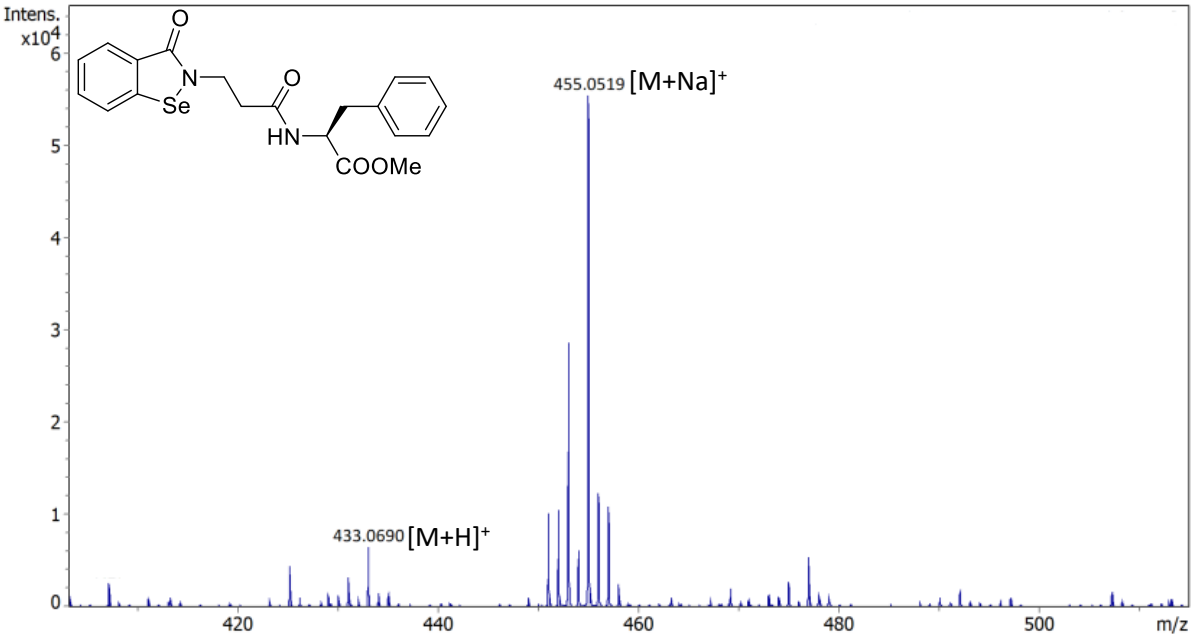


Fig. 21: Mass spectrum of compound **6**.

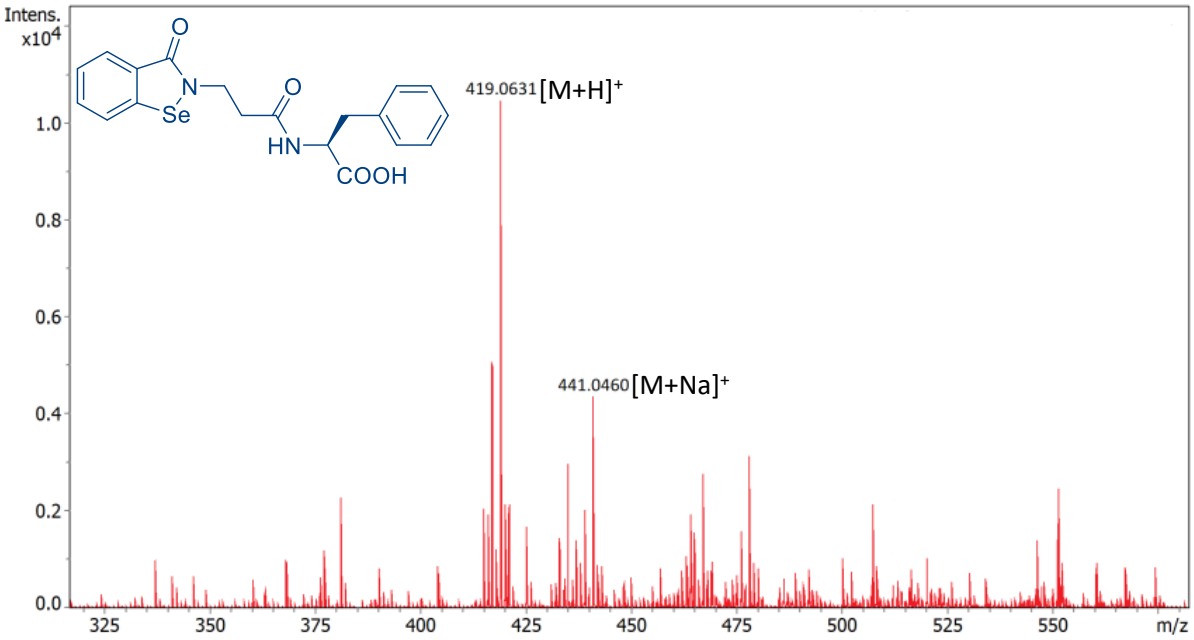


Fig. 22: Mass spectrum of compound **7**.

Chapter 6

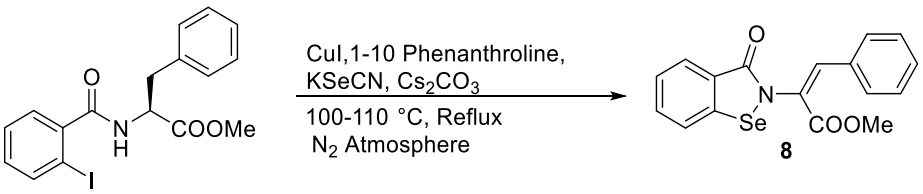
6.1 Results and Discussion

β-alanine's methyl ester was coupled with 2-iodobenzoic acid using EDC.HCl, HOBt mediated coupling reaction, yielding methyl 3-(2-iodobenzamido)propanoate (**3**).

(**4**), which is a β-alanine based 1,2-benzoselenazol-3-one derivative, was synthesized using CuI, 1,10-phenanthroline, KSeCN and Cs_2CO_3 mediated cyclization of (**3**).

(**4**) was then hydrolysed and coupled with L-phenylalanine's methyl ester using EDC.HCl, HOBt mediated coupling reaction (**6**) which was further hydrolysed to (**7**). All the substrates were analysed by ^{1}H,^{13}C NMR, and Mass spectrometry.

Initially the cyclization reaction was done with different substrate which was methyl (2-iodobenzoyl)phenylalaninate but an undesired product was formed with double bond between α and β carbon. That is why we replaced our substrate with methyl 3-(2-iodobenzamido)propanoate.



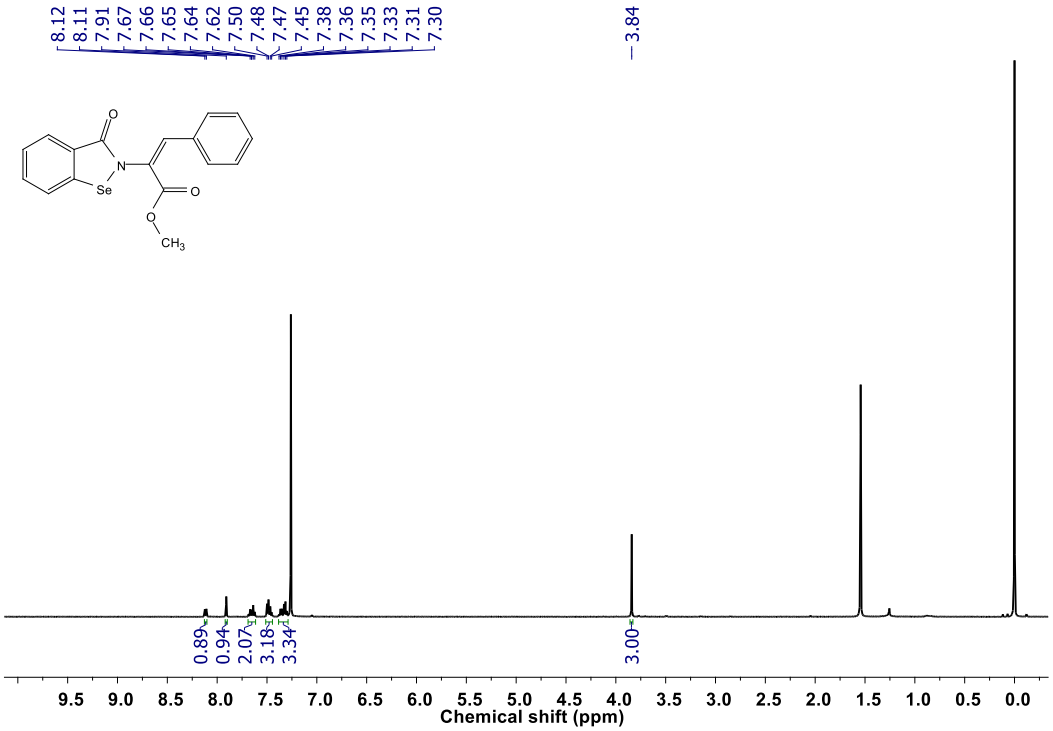


Fig 23: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **8**.

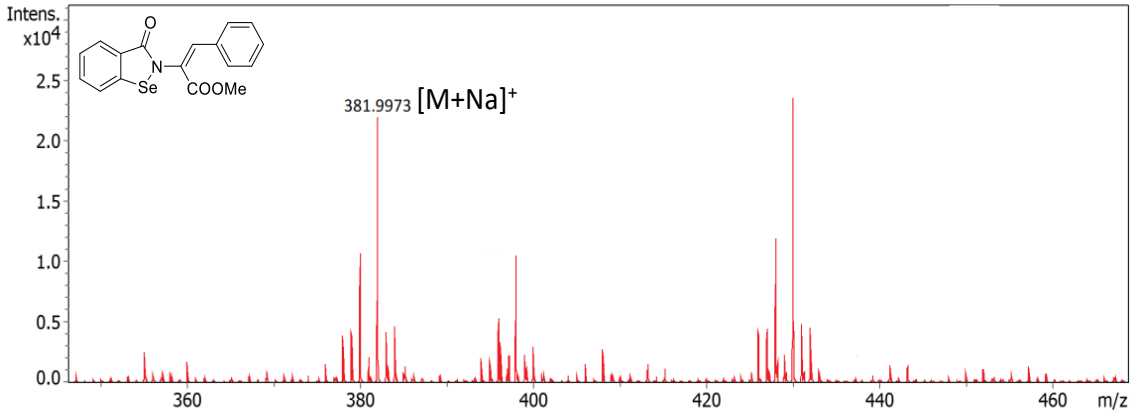
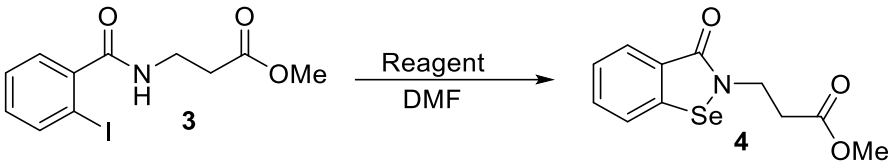


Fig. 24: Mass spectrum of **6**: ESI-MS m/z: $[M+Na]^+$ calcd. for $C_{17}H_{13}NO_3Se$ =381.9954; detected = 381.9973.

Optimization table



S. No.	Substrate	Reagent	Condition	Time(h)	Product	Yield %
1	3	CuI,1,10-phenanthroline,Se-powder, K_2CO_3	100-110 ºC, N_2 atm	12	NA	0
2	3	CuI,1,10-phenanthroline,KSeCN, Cs_2CO_3	100-110 ºC, N_2 atm	1.5	4	23
3	3	CuI,1,10-phenanthroline,KSeCN, Cs_2CO_3	100-110 ºC, N_2 atm	3	4	43
4	3	CuI,1,10-phenanthroline,KSeCN, Cs_2CO_3	100-110 ºC, N_2 atm	6	4	10

Fig. **25**: The above table is showing the optimization of cyclization reaction involved in reaction scheme.

It is clear from the optimization table that KSeCN mediated cyclization reaction produced better results when refluxing was done for 3 hours.

6.2 Conclusion

1,2-benzoselenazol-3-one derivatives are employed in various fields of chemistry, whether it be sensing, drug delivery, etc., and because of that reason, in this project, we aimed at synthesizing a peptide-based ebselen derivative that could be studied further for its properties, and we were successful at synthesizing a 1,2-benzoselenazol-3-one derivative. Since ebselen is also known for its excellent antioxidant and antibacterial properties therefore, (**7**) can also be subjected to various biological analysis.

6.3 Future Plan

Current work demonstrated the synthesis of peptide-based Ebselen derivatives (**6**) and (**7**). Since these 1,2-benzoselenazol-3-one derivatives are already known to present a wide range of biological activities therefore, these compounds will be subjected to specific biological analyses such as cell viability tests. Current and future results will encourage scientists to focus on these organoselenium compounds and their application in various scientific fields.

6.4 References

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