Synthesis, characterization and properties of a water soluble, naphthyl monoimide based unnatural amino acid and its binding studies with DNA

M.Sc. Thesis

by

Ankan Biswas

DISCIPLINE OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY INDORE
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Synthesis, characterization and properties of a water soluble, naphthyl monoimide based unnatural amino acid and its binding studies with DNA

A THESIS

Submitted in partial fulfillment of the

requirements for the award of the degree

of

Master in Science

by

ANKAN BISWAS

INDIAN INSTITUTE OF TECHNOLOGY INDORE

JUNE, 2015

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Synthesis, characterization and properties of a water soluble, naphthyl monoimide based unnatural amino acid and its binding studies with DNA** in the partial fulfilment of the requirements for the award of the degree of **MASTER IN 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 time period from JULY 2014 to JUNE 2015 of M.Sc. under the supervision of Dr. Apurba K. Das, Assistant Professor, Discipline 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.

(ANKAN BISWAS)

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

(Dr. APURBA K. DAS)

Ankan Biswas has successfully given his/her M.Sc. Oral Examination held on **3rd July, 2015**.

Signature of Supervisor of MSc thesis
Date:

Convener DPGC
Date:

Signature of PSPC Member
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Date:

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Ankan Biswas
M.Sc. II year
Discipline of Chemistry
IIT INDORE

Dedicated to My

Family

Abstract

Fluorescence molecules with long emission region and high intensity are very important to detect biomolecules. Deoxyribonucleic acid (DNA) is one of the most important biomolecule in our body. Small molecules binding with DNA can be used as several biological applications, such as treatment of cancer, parasitic diseases and infections. Small organic molecules binding with DNA in water medium and physiological conditions are very rare. Our aim of this project is to synthesize such molecule which can act as fluorophore, can bind or interact with DNA and can also be soluble in water. 1,8-naphthalic based molecules are important class of DNA binder. Another important thing of these types of molecule is they have high cytotoxicity ability. We can make it fluophore compound from this moiety. In this project, we have designed and synthesized a 1,8-naphthalic monoimide based unnatural amino acid; which is effectively binds with calf-thymus DNA (ct-DNA) at pH 7.4.

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Acronyms

Abbreviations used for compounds, substituents, reagents, etc. are largely in accordance with the recommendations of the IUPAC. Additional abbreviations used in this thesis are listed below.

CD	Circular Dichroism
$CDCl_3$	Chloroform-d
DMSO-d_6	Dimethyl Sulfoxide-d_6
d	Doublet
DCM	Dichloromethane
DMF	Dimethyl Formamide
EtOH	Ethanol
EtOAc	Ethyl Acetate
ESI-MS	Electrospray Ionization Mass Spectrometry
HCl	Hydrochloric Acid
MeOH	Methanol
NaOH	Sodium Hydroxide
NMR	Nuclear Magnetic Resonance
pH	The negative logarithm of hydrogen-ion activity ($-\log_{10}[H_3O^+]$)
s	Singlet
t	Triplet
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV-Vis	UV-Visible Spectroscopy

Nomenclatures

θ	Angle
λ	Wavelength
α	Alfa
β	Beta
Å	Angstrom
nm	Nanometer
δ	delta
µm	Micrometer
µM	Micro molar

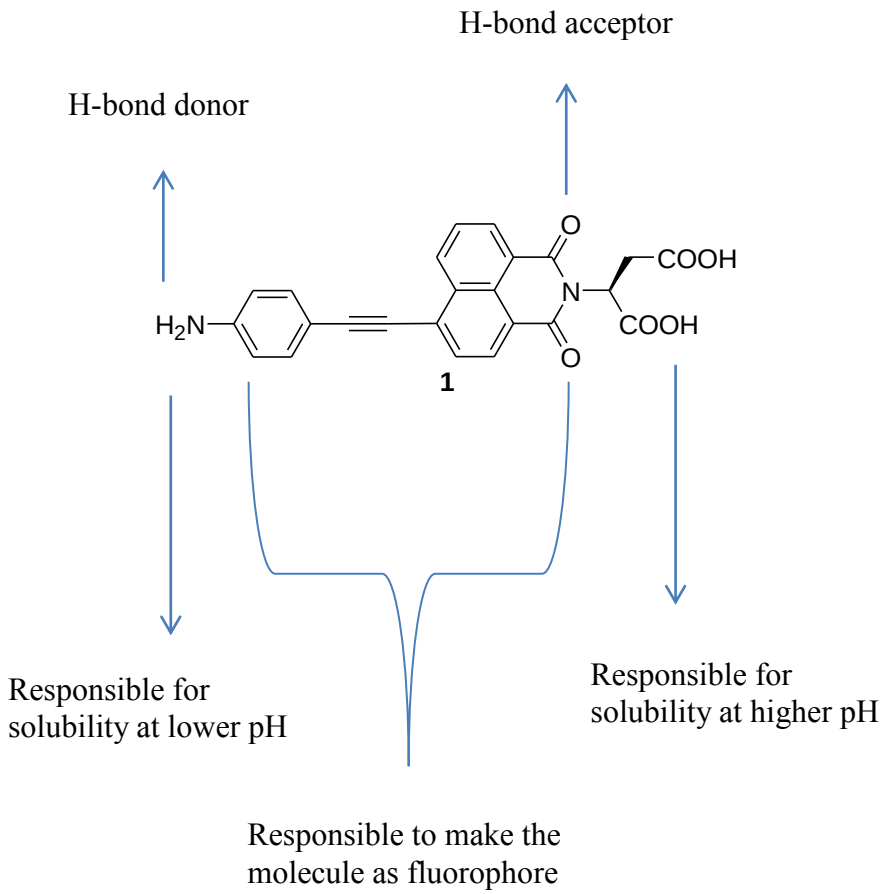
Chapter 1:

Introduction and Reaction Scheme:

1.1 Introduction:

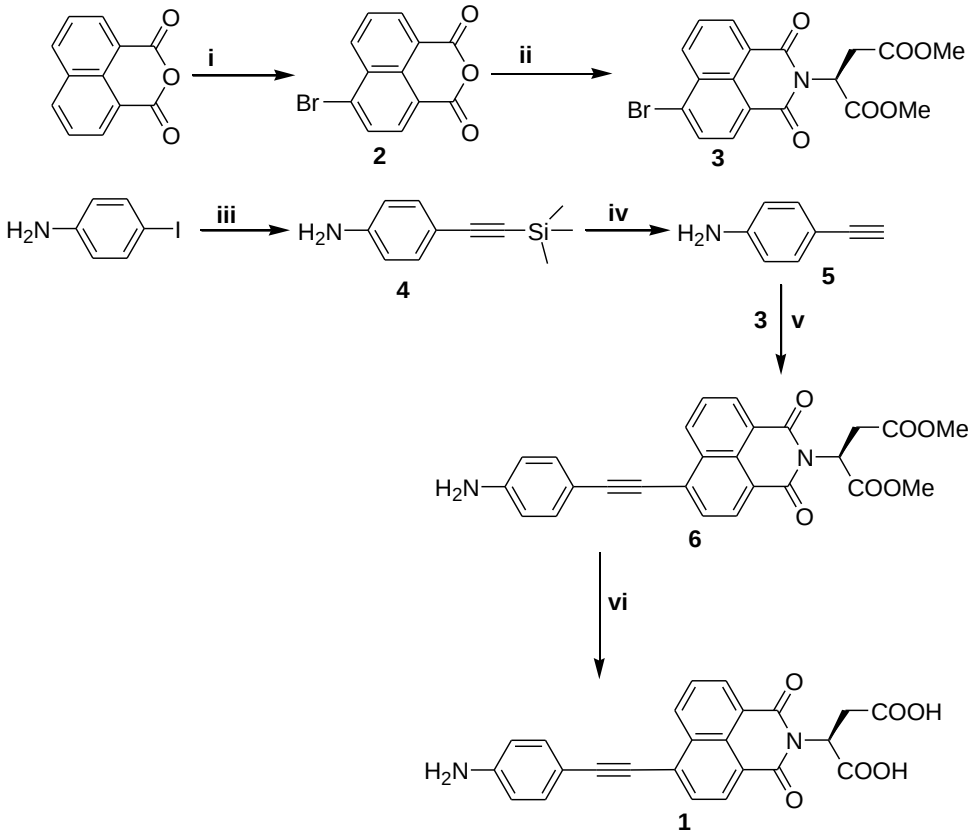
Fluorescent molecules are very sensitive to detect and for the probing of structure, dynamics and interactions of biomolecules.[1-3] Therefore, design and synthesis of such molecules are very important to know biological process and interbiomolecular interactions. Fluorophores having low emission intensity, short wavelength emission region, quenching properties are unsuitable for practical use. Therefore, highly intensive and long wavelength emissive flourophores compounds are very useful for the detection and sensing of biomolecules.[4-6] Deoxyribonucleic acid (DNA) is one of the most important biomolecules. It controls the heredity of life by its base sequences and plays very important roles in many biological processes like transcription, cell division, mutagenesis, *etc*.[7-9] Small molecules which are able to target and bind with DNA, have important applications in the treatment of several diseases such as cancer, viral infections or parasitic diseases. Therefore, small molecule binding with DNA are very active area of interest in research.[10,11] 1,8-naphthalimide based compounds are an important class of DNA intercalators, which are also used as drugs in antitumor therapy and thus have high cytotoxic activity against a variety of tumor cells.[12-15] Biological studies showed that, these naphthalimide based intercalating reagents are capable of targeting DNA without DNA damaging and mutation, which reduces side effects and the risk of developing new secondary cancers.[16] Inspiring from these various important applications of naphthalimide based compounds, we have selected 1,8-napthalic anhydride as our starting material and by brominating of this we have prepared 4-bromo-1,8-naphthalic anhydride. We can substituted bromine by any conjugated donor group for long range conjugation and to achieve highly fluorophores probe. Bromo group of 4-bromo-1,8-naphthalic anhydride was substituted by 4-ethynyl-phenylamine by Sonogashira coupling reaction. To make it anhydride to imide, we have used

dimethyl ester of L-aspartic acid. Amine group of dimethyl ester of L-aspartic acid was attacked at anhydride to form imide and finally we have hydrolyzed the methyl ester groups to acid groups. Here we have synthesized a new type of unnatural amino acid, in which free amine group from 4-ethynyl-phenylamine is in N-terminal position and two carboxylic acid groups from L-aspartic acid are in C-terminal position. In this compound free amine group acts as donor and imide acts as acceptor. from L-aspartic acid are in C-terminal position. In this compound free amine group acts as donor and imide acts as acceptor.



The advantage of this molecule is, it is soluble in water at higher pH, forming carboxylic acid salt, as well as at lower pH by formation of ammonium salt by protonation of free amine and also act as fluorophore because of long range conjugation and donor-acceptor internal charge transfer (ICT) at excited state between phenyl and naphthyl ring.[17-18]

1.2. Reaction Scheme:



Reagents: **i.** 1) Br_2, KOH, 24h, 60 ^oC 2) H_2SO_4, 1h, 60 ^oC, **ii.** dimethyl ester of L-aspartic Acid, EtOH, 24h, 80 ^oC, **iii.** $Pd(PPh_3)_2Cl_2$, CuI, TMSA, Et_3N, 24h, r.t., **iv.** DCM:MeOH (1:3), K_2CO_3, 6h, r.t., **v.** $Pd(PPh_3)_2Cl_2$, CuI, THF, Et_3N,24h, 60 ^oC, **vi**.1) 0.5 (N) NaOH, THF:MeOH (1:1) 2) 1 (N) HCl.

Chapter 2:

Results and discussion:

2.1. Synthesis of compounds:

In this project, our desire compound, 2-[6-(4-amino-phenylethynyl)-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl]-succinic acid (**1**) was synthesized according to described reaction scheme. Starting from commercially available 1,8-naphthalic anhydride, which was treated with liquid bromine in aqueous KOH medium followed by acidification to synthesis 4-bromo-1,8-naphthalic anhydride (**2**) according to reported method.[19] Treating **2** with dimethyl ester of L-aspartic acid in ethanol, intermediate **3** was synthesized. Compound **4** was synthesized by Sonogashira coupling reaction between 4-iodoaniline and TMSA and after removal of trimethyl silyl group from **4** by K_2CO_3, terminal alkyne **5** was obtained; which was carried out according to literature method.[20] Compound **6** was synthesized by Sonogshira coupling reaction between **5** and **3**. After base catalyzed hydrolysis of **6**, desire compound **1** was synthesized.

2.2. UV-Vis and Fluorescence spectra of compound 1 at different pH:

After synthesize, UV-Vis spectra of compound **1** were studied at different pH to observe the variation in absorption maxima peak and intensity at different pH. Absorption peak observed at 247 nm and 355 nm may be due to π-π^* transition and at 415 nm probably for n-π^* transition from free amine lone pair electron to ring (**Figure 1**). When pH was varied minor change in peak position (absorption wavelength) of compound **1** was observed. But increasing pH, the absorption intensity was slightly increases (**Figure 1**).

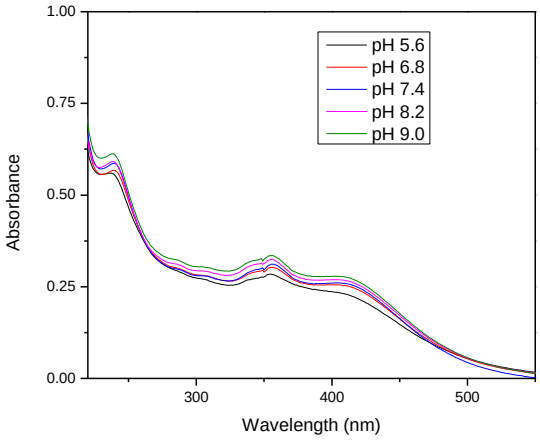


Figure 1: UV-Vis spectra of compound **1** at different pH

After UV-Vis spectroscopic investigation, fluorescence spectra of compound **1** were studied at different pH, to study photophysical properties of the compound. The compound was excited at 355 nm and 415 nm. From fluorescence graph, it was found that emission intensity was maxima at 7.4-8.2 pH range. When pH was lowered to 5.8 there was a huge decrease in intensity. The reason of the decreasing in fluorescence emission intensity may be due to the protonation of free amine of **1** at lower pH. The lone pair of electron of amine group is not available due to protonation and sharp charge transfer from donating amine group to accepting imide moiety is hampered. When the compound was excited at 415 nm, there was a blue shift at pH 5.8, but when it was excited at 355 nm, slight red shift was observed at the same pH in fluorescence spectra.

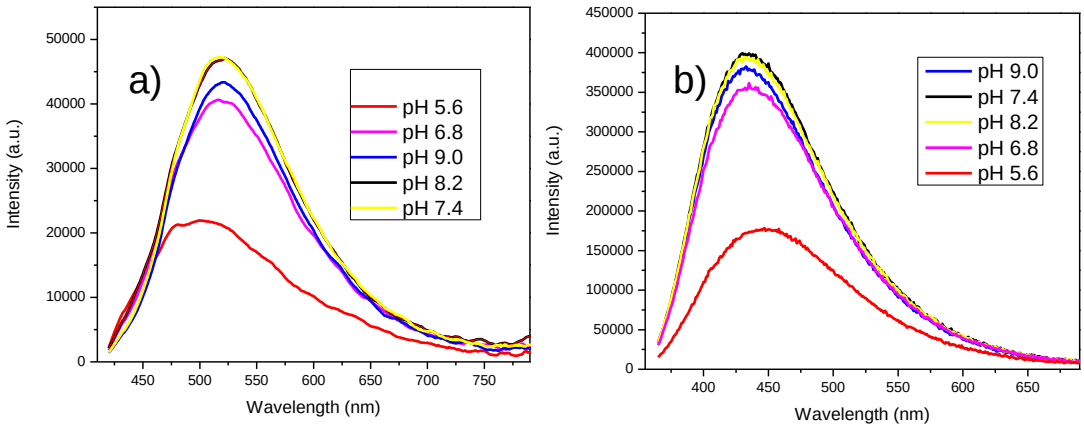


Figure 2: Fluorescence spectrum of **1** at different pH, **a)** λ_{ex}=415 nm, **b)** λ_{ex}=355 nm.

2.3. Binding study of compound 1 with DNA: UV-Vis and Fluorescence study:

As our desire compound contains imide group as H-bond acceptor and free ammine or acid groups as H-bond donor, it was expected that, the compound could be bind with DNA by electrostatic interactions or H-bonding with aromatic base pair of DNA. For the DNA binding study of probe **1**, UV-Vis spectra (**Figure 3a**) and emission spectra (**Figure 3b**) at pH 7.4 (5 mM phosphate buffer solutions of **1** (concentration = 30 µm)) with successive increasing DNA concentration (0 µm to 67.63 µm) was investigated.

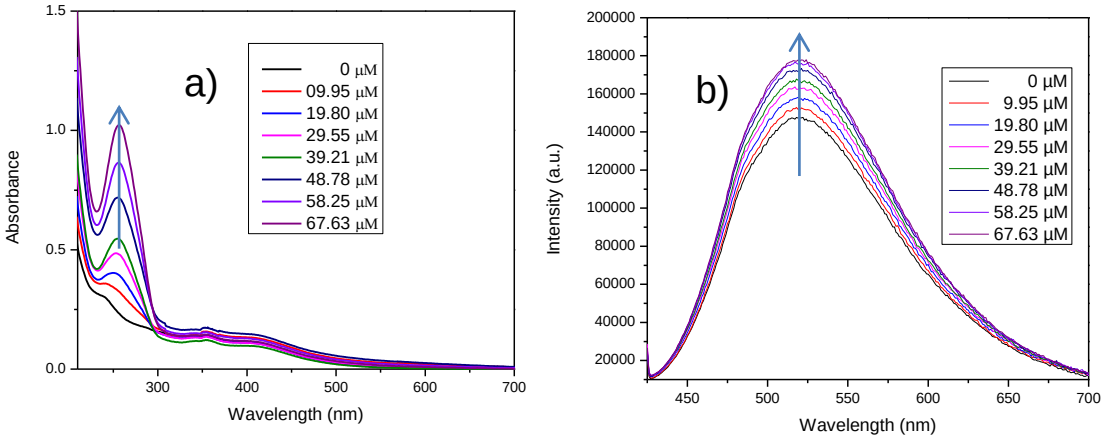


Figure 3: UV-Vis and fluorescence spectra of compound **1** in prescence of ct-DNA at pH 7.4. **a)** UV-Vis spectra of compound **1** with increasing DNA concentration**.** [compound **1**] = 30 µM and [ct-DNA] = 9.95, 19.80, 29.55, 39.21, 48.78, 58.25 and 67.63 µM. **b)** Fluorescence spectra of compound **1** with increasing DNA concentration (λ_{ex} = 415 nm). [compound **1**] = 30 µM and [ct-DNA] = 9.95, 19.80, 29.55, 39.21, 48.78, 58.25 and 67.63 µM.

In UV-Vis spectra, with increasing ct-DNA concentration, increasing absorbance intensity at 260 nm was observed, which is normal absorbance wavelength of double stand DNA and there was no deviation in absorbance wavelength and intensity from pure compound **1** after the addition of DNA solution.

With increasing non-fluorophore DNA concentration in solution of compound **1** (concentration = 30 µM), fluorescence intensity was increases gradually. These indicate that there is some charge transfer enhancement of **1** in presence of DNA. This was happenning may be due to H-bonding or electrostatic interaction between DNA and compound **1.**

2.4. Benesi-Hildebrand plot to Evaluate Binding Constant:

The association constant (K) of the compound **1** with ct-DNA was determined by a Benesi-Hildebrand plot using the following equation 1,

$1/(I-I_0) = 1/(I_\alpha-I_0) + 1/(I_\alpha-I_0)K[ct\text{-}DNA]$……. 1

Where **I_0**, **I** and **I_α** are emission intensities of compound **1** in absence of ct-DNA, in an intermidiate concentration of ct-DNA and an infinity concentration of ct-DNA respectively. The binding constant (K) was determind from the slope of **$1/(I - I_0)$** vs.**1/[ct-DNA]** plot in equation 1. K was found 18.55 M^{-1}.

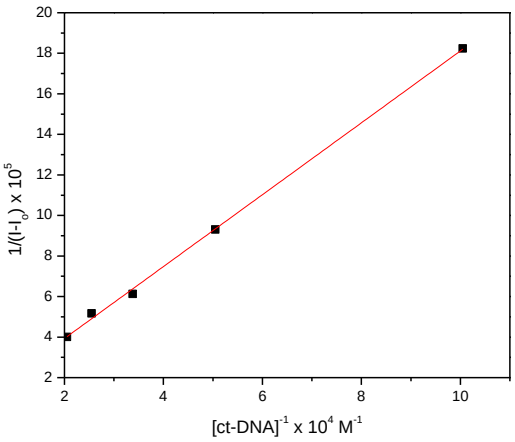


Figure 4: Benesi- Hildebrand plot of compound **1** in presence of increasing ct-DNA concentration. [compound **1**] = 30 µM and [ct-DNA] = 9.95, 19.80, 29.55, 39.21 and 48.78 µM.

Value of ΔG was also calculated by using equation **2**. **ΔG = -RTlnK…….. 2.**

Value of ΔG was found -1.73 Kcal mol^{-1}, R = 1.987 cal mol^{-1} T^{-1}, T = 298K.

2.5. CD spectra of ct-DNA with increasing probe concentration:

CD spectra of ct-DNA was investigated with increasing probe concentration at phosphate buffer medium (5 µM, pH 7.4) to study the effect of helical structure of DNA in presence of compound **1**. CD spectra showed that there is no change in helical structure of DNA after addition of compound **1**.

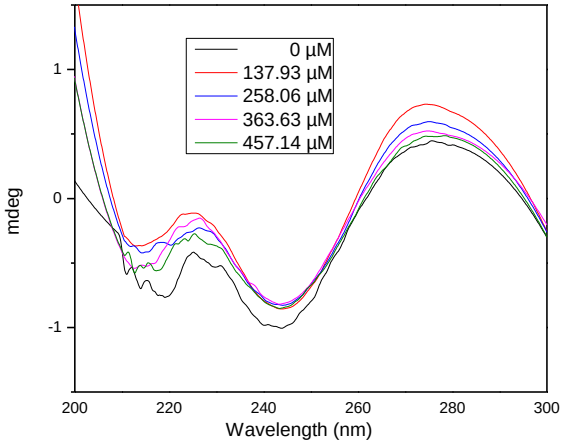


Figure 5: CD spectra of DNA solution in presence of compound **1**. [ct-DNA] = 148.14 µM, [compound] = 0, 137.93, 258.06, 363.63 and 457.14 µM.

Chapter 3

Experimental sections:

3.1. Synthesis and characterizations of compounds:

3.1.1. General method:

All the chemicals and reagents were obtained commercially. All NMR spectra were recorded at 400 MHz Bruker Advance III 400 NMR. Compounds concentrations were in the range of 1-10 mmol in $(CD_3)_2SO$ and $CDCl_3$. Mass spectra were recorded on Bruker micrOTOF-Q II by positive mode electrospray ionizations. All the solvents, which were used in the reactions and for column chromatography were properly dried and distilled. All the synthesized products were dried under high vacuum pump before sample characterizations (^{1}H NMR, ESI-MS, HRMS). Milli-Q water was used for reaction purposes and for photophysical studies.

3.1.2. Synthesis of 4-bromo-1,8-naphthalic anhydride (2):

To a 250 mL round bottom flask, 1,8-naphthalic anhydride (3.96g, 20 mmol) was added into aqueous KOH solution (5.6 g, 24 mL H_2O) and stirred with a magnetic stirrer at 60 ºC for 10 min. After complete dissolution, the reaction mixture was cooled at room temperature and cooled to 0 ºC. Then (2.06 mL, 80 mmol) Br_2 was added into the reaction mixture by dropping funnel for 1 h with vigorous stirring at this cooling condition. After the addition of Br_2, the flask was fitted with a condenser and was refluxed for 24h at 60 ºC. After cooling down at room temperature, it was neutralized by 4 (N) H_2SO_4 and again refluxed for 1h at 60 ºC. After cooling down at room temperature the product was collected by suction filtration and was washed with cold water, methanol and di-ethyl ether and collected as amorphous white solid. Yield = 4.432 g (15.94 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.70 (dd, 2H, J = 7.04, 8.28 Hz), 8.46 (d, 1H, J = 7.8 Hz) 8.13 (d, 1H, J = 7.8 Hz), 7.94 (t, 1H, J = 7.9 Hz) ppm. HRMS (ESI) m/z calculated for $C_{12}H_5BrO_3$: 300.9299 $(M+Na)^+$; Found: 300.9299 $(M+Na)^+$.

3.1.3. Synthesis of 2-(6-bromo-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-succinic acid dimethyl ester (3):

To a 250 mL round bottom flask (1.118 g, 4.035 mmol) of compound **2** was added and dissolved with 30 mL of ethanol. After that, (0.780 g, 4.845 mmol) of dimethyl ester of L-Aspartic acid was added into the reaction mixture and stirred with a magnetic stirrer. The flask was fitted with a condenser and refluxed at 80 ºC for overnight. The reaction was monitored by TLC. After completion of reaction; reaction mixture was extracted by ethyl acetate and washed with 1 M HCl (30 mL x 3). The organic layer was collected; dried over dry Na_2SO_4 and collected as white solid. Yield = 1.45 g, (3.46 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.68 (d, 1H, *J* = 7.28 Hz), 8.62 (d, 1H, *J* = 8.52 Hz), 8.44 (d, 1H, *J* = 7.76 Hz), 8.07 (d, 1H, *J* = 7.80 Hz), 7.87 (t, 1H, *J* = 7.60 Hz), 6.25 (t, 1H, *J* = 6.76 Hz), 3.73 (s, 3H), 3.67 (s, 3H), 3.54 (dd, 1H, *J* = 6.8 Hz, 6.52 Hz), 2.98 (dd, 1H, *J* = 7.28, 7.24) ppm. ESI-MS m/z Calculated for $C_{18}H_{14}BrNO_6$: 441.9902 $(M+Na)^+$; Found: 441.9870 $(M+Na)^+$.

3.1.4. Synthesis of 4-trimethylsilanylethynyl-phenylamine (4):

2.19 g (10 mmol) of 4-iodoaniline was dissolved in 25 mL of tri-ethylamine in a dry, 250 mL two necked round bottom flask. The flask was capped by septum and argon gas was purged into the solution for 10 min by syringe. After that, CuI (10 mol % of alkyne) and $Pd(PPh_3)_2Cl_2$ (5 mol % of alkyne) were added followed by addition of TMSA. The reaction mixture was stirred for 24h under argon atmosphere at r.t. Reaction was monitored by TLC. The reaction mixture was filtered and filtrate part was extracted with ethyl acetate and washed with NH_4Cl, brine solution (3 x 50 mL). Ethyl acetate was evaporated to get solid, which was purified by column chromatography (100 mesh silica gel, 1% ethyl acetate in hexane as eluent) to get desire product as white solid. Yield 1.81 g (9.6 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27 (d, 2H, *J* = 7.52 Hz), 6.57 (d, 2H, *J* = 7.52 Hz), 0.22 (s, 9H) ppm. ESI-MS m/z Calculated for $C_{11}H_{15}NSi$: 190.1052 $(M+H)^+$; Found: 190.3127 $(M+H)^+$.

3.1.5. Synthesis of 4-ethynyl-phenylamine (5):

To a solution of 1 g (5.29 mmol) of compound **4** in 3:1 MeOH-DCM, 2.07 g (15 mmol) K_2CO_3 was added. The reaction mixture was stirred in a magnetic stirrer under argon atmosphere for 5h. The reaction was monitored by TLC. After completion of the progress of the reaction, reaction mixture was extracted with ethyl acetate and washed with brine (3 x 50 mL) and dried over Na_2SO_4. Ethyl acetate was evaporated to obtain desire product as white solid. Yield = 0.610 g (5.21 mmol, 98.5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (d, 2H, *J* = 7.52 Hz), 6.60 (d, 2H, *J* = 7.52 Hz), 3.82 (s, 2H), 2.96 (s, 1H) ppm. ESI-MS m/z Calculated for C_8H_7N: 235.1234 $(2M+H)^+$; Found: 235.3540 $(2M+H)^+$.

3.1.6. Synthesis of 2-[6-(4-amino-phenylethynyl)-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl]-succinic acid dimethyl ester (6):

251.4 mg (0.60 mmol) of compound **3** was dissolved in 5 mL THF and 5 mL of Et_3N in a 100 mL, round bottom flask. The flask was capped by septum and argon gas was purged into the solution for 10 min by syringe. After that, CuI (5 mol% of **5**) and $Pd(PPh_3)_2Cl_2$ (5 mol% of **5**) were added followed by addition of 77.25 mg (0.66 mmol) of **5**. The reaction mixture was refluxed for overnight under argon atmosphere at 70 ºC. The reaction was monitored by TLC and after completion; reaction mixture was extracted with ethyl acetate and washed with brine, dried over dry Na_2SO_4. Ethyl acetate was evaporated to get solid crude, which was purified by column chromatography (100 mesh silica gel, 30% ethyl acetate in hexane as eluent) to yield compound **6**. Yield = 213.5 mg (0.46 mmol, 78.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.78 (d, 1H, *J* = 8.28 Hz), 8.64 (d, 1H, *J* = 7.28 Hz), 8.55 (d, 1H, *J* = 7.52 Hz), 7.90 (d, 1H, *J* = 8.0 Hz), 7.83 (t, 1H), 7.48 (d, 2H, *J* = 7.28 Hz) 6.70 (d, 2H, *J* = 7.28), 6.27 (t, 1H, *J* = 6.52) 4.01 (s, 2H), 3.74 (s, 3H), 3.68 (s, 3H) 3.56 (dd, 1H, *J* = 6.56 Hz, 6.76 Hz), 2.96 (dd, 1H, *J* = 6.52 Hz, 6.56 Hz) . HRMS (ESI) m/z calculated for $C_{26}H_{20}N_2O_6$: 479.1214 $(M+Na)^+$, Found: 479.1215 $(M+Na)^+$

3.1.7 Synthesis of 2-[6-(4-amino-phenylethynyl)-1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl]-succinic acid (1):

200 mg (0.44 mmol) of compound **6** was dissolved in 1:1 methanol-THF. After that, 2 mL of 1 (N) aqueous NaOH solution was added into the reaction mixture. The reaction mixture was stirred for overnight. After completion of reaction, reaction mixture was dissolved in 50 mL water and washed with ether (3 x 50 mL). Aqueous part was collected and neutralized by 1 (N) HCl. After getting slightly acidic pH, the desired product was extracted by ethyl acetate (3 x 50 mL) from red turbid aqueous part. Ethyl acetate was dried over dry Na_2SO_4 and evaporated to get desired product as red solid. Yield = 179.3 mg (0.42 mmol, 95.2 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.82 (d, 1H, *J* = 9.92 Hz), 8.58 (d, 1H, *J* = 7.04 Hz), 7.98 (t, 1H, *J* = 8.0 Hz), 7.47 (d, 2H, *J* = 7.52 Hz), 6.64 (d, 2H, *J* = 7.56 Hz), 5.89 (s, 2H) ppm. HRMS (ESI) m/z calculated for $C_{24}H_{16}N_2O_6$: 451.0901 $(M+Na)^+$, Found: 451.0902 $(M+Na)^+$.

3.2. UV-Visible and Fluorescence Study of compound 1 at different pH:

UV-visible absorption spectra were recorded using a Varian Cary 100 Bio spectrophotometer. Fluorescence spectra were studied by Horiba Fluoromax-4 spectrofluorometer. The cell (length = 1 cm, capacity = 2 mL) was used for this study at 298 K. The concentration of probe was 30 µm. Na_2HPO_4 and $NaH_2PO_4.H_2O$ were used for preparation of phosphate buffer solutions. All the UV-Visible studies were carried out in 5 mM phosphate buffer of pH 7.4 solution at 298 K. All the experiments were carried out by freshly prepared sample solution.

3.3. Studies on the interaction of compound 1 with ct-DNA:

3.3.1 Preparation of ct-DNA solution:

Calf thymus DNA (ct-DNA) was purchased from Sigma Aldrich (USA) and used without further purification. The supplied ct-DNA was dissolved in 5 mM sodium

phosphate buffer solution of pH 7.4 and reconstituted overnight at 2 – 8 ºC to dissolve all the material and then the solution was collected through a 0.45 µm syringe filter. The concentration of ct-DNA was determined by measuring the absorbance at 260 nm (A_{260}) and using the formula, concentration of ct-DNA (µg/mL) = A_{260} x 50 µg/ml x Dilution factor, where A_{260} = absorbance of the DNA solution at 260 nm and 50 µg/mL is the concentration of double stand DNA when A_{260} = 1. Thus, from the above formula we determined the concentration of our stock ct-DNA solution as 1.37 mg/mL. The molar concentration of ct-DNA was determined from UV-visible spectra using molar absorption coefficient (ε) of 6600 $L.mol^{-1}$ cm^{-1} at 260 nm, which was found to be 4436.7 µM. The purity of ct-DNA was checked by measuring the ratio of absorbance at 260 nm to 280 nm (A_{260}/A_{280}) which was found 1.80 – 1.85 i.e. the ct-DNA is sufficiently free of protein.[21] From that stock solution sub stock of 2000 µM ct-DNA was prepared.

3.3.2. UV-Visible and fluorescence study of compound 1 in presence of ct-DNA at pH 7.4:

5 mM sodium phosphate buffer solution of pH 7.4 was prepared. Compound **1** was dissolved in this solution keeping concentration 30 µM. Sub stock 2000 µM ct-DNA solution was added 10 µL per time in cubed containing 2 mL of 30 µM probe solution. In this process, concentration of ct-DNA solution was increased 0 µm to 67.63 µm in UV-Vis and fluorescence study.

3.3.3. CD spectra study of ct-DNA with increasing probe concentration:

CD spectra of ct-DNA were investigated with increasing probe concentration at phosphate buffer medium (5 µM, pH 7.4). Initial concentration of ct-DNA was 148.14 µM. In this ct-DNA solution, 2000 µM probe solution was added 20 µL per time, increasing concentration from 0 to 457.14 µM.

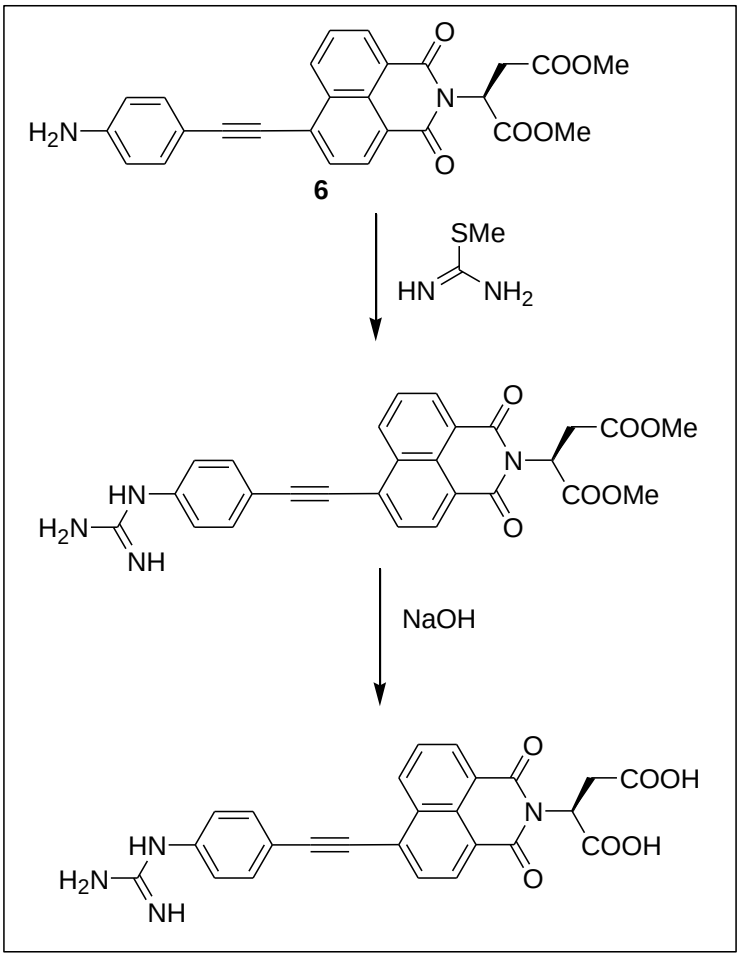
Chapter 4:

Conclusion and Scope of future work:

We have synthesized and characterized one water soluble, fluorogenic and unnatural amino acid. Further we have done photo-physical studies and also DNA binding study of this compound which was carried out by UV-Vis and fluorescence spectroscopy at pH 7.4. It was observed that the unnatural amino acid binds with ct-DNA. Fluorescence intensity increases with successive increase of non-fluorophore DNA concentration.

In future we want to carry out dye displacement study of the unnatural amino acid with ct-DNA and also want to synthesis one more compound. In this regard we want to incorporate guanidine moiety in place of free amine.

The proposal synthetic scheme is given below:



Appendix A:

^{1}H NMR and Mass spectrum of compounds:

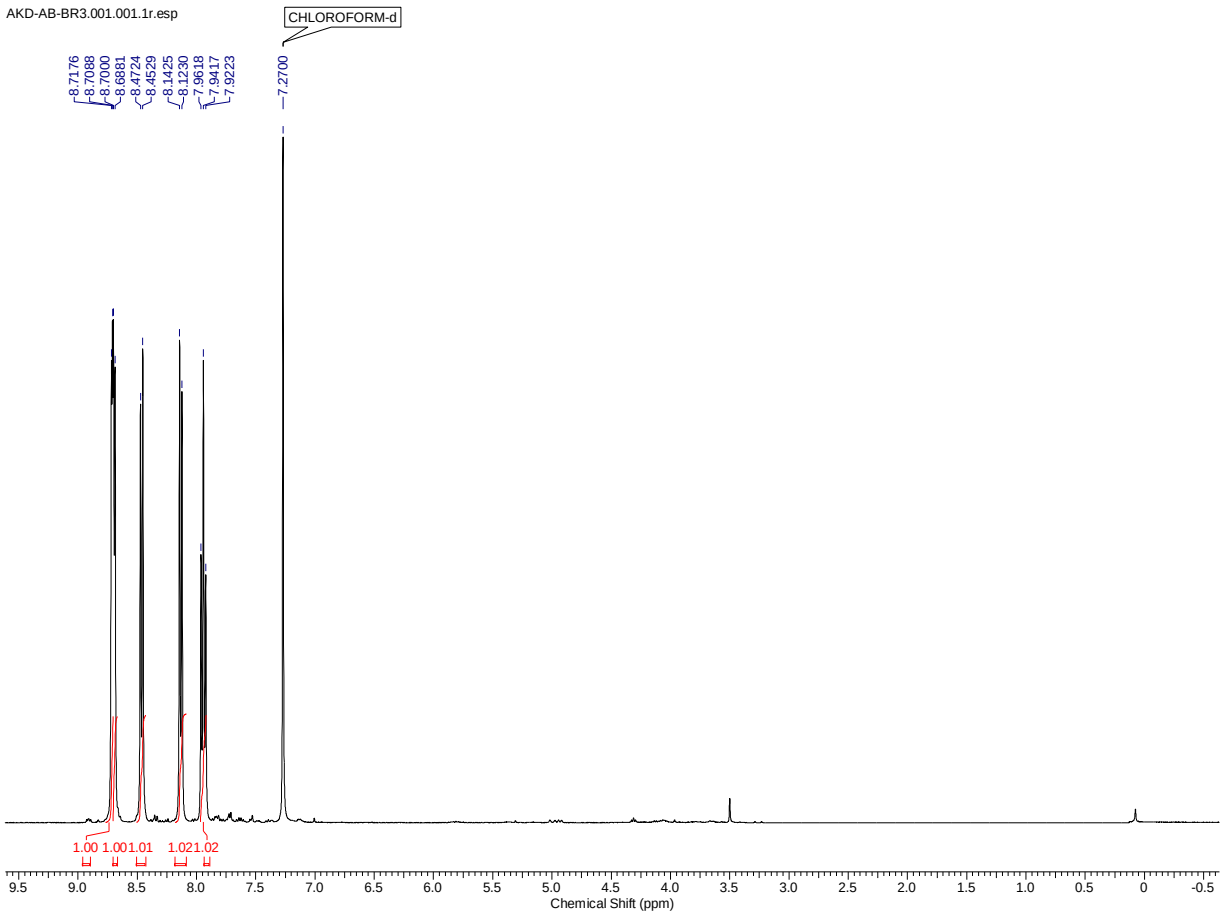


Figure 6: 400 MHz ^{1}H NMR spectrum of compound **2** in $CDCl_3$

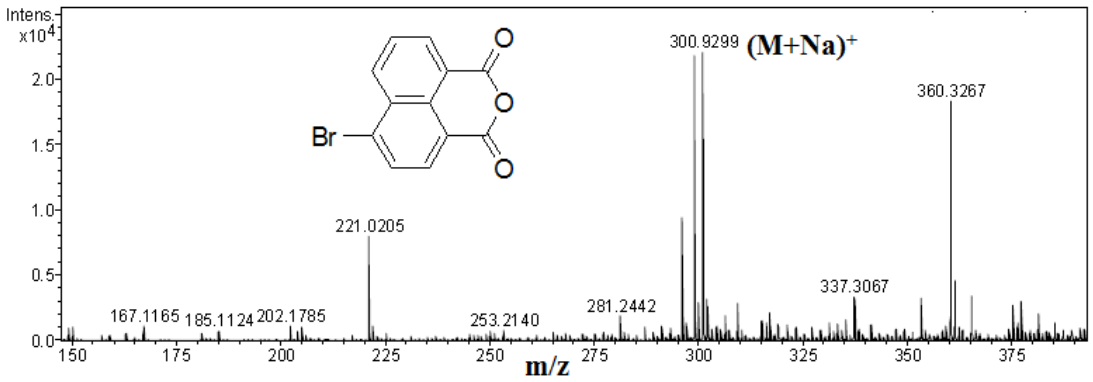


Figure 7: HRMS(ESI) spectrum of compound **2**

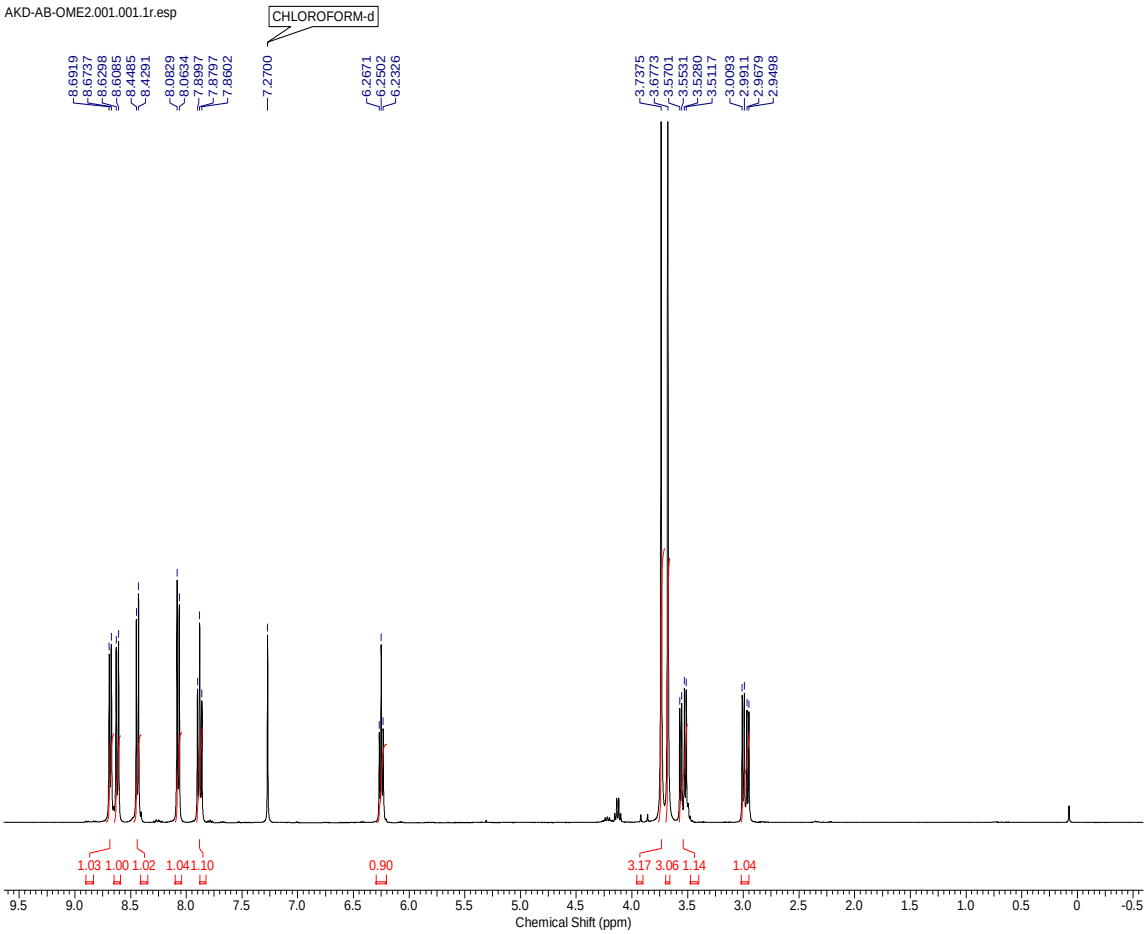


Figure 8: 400 MHz ^{1}H NMR spectrum of compound **3** in $CDCl_3$

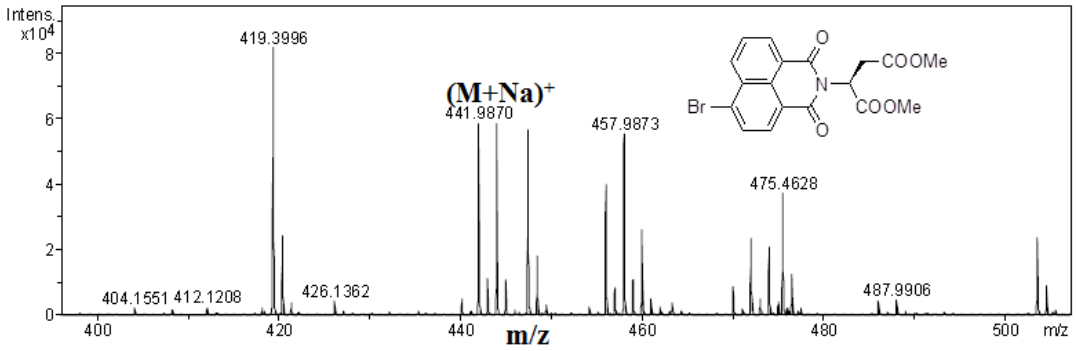


Figure 9: ESI-MS spectrum of compound **3**

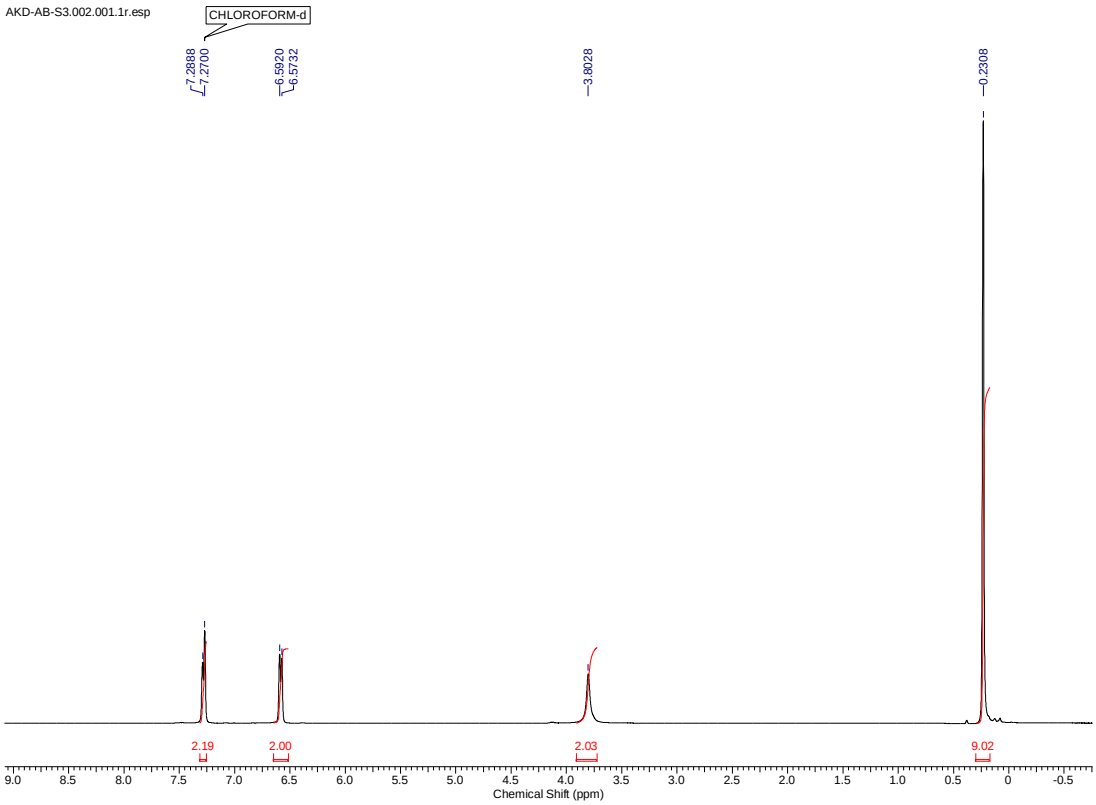


Figure 10: 400 MHz ^{1}H NMR spectrum of compound **4** in $CDCl_3$

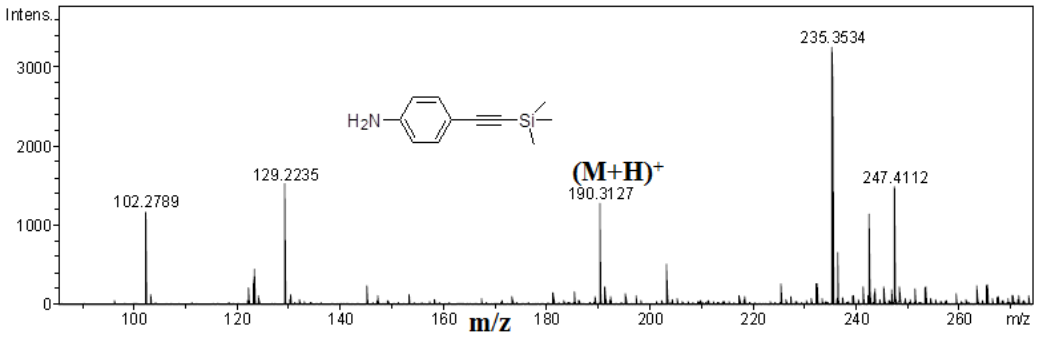


Figure 11: ESI-MS spectrum of compound **4**

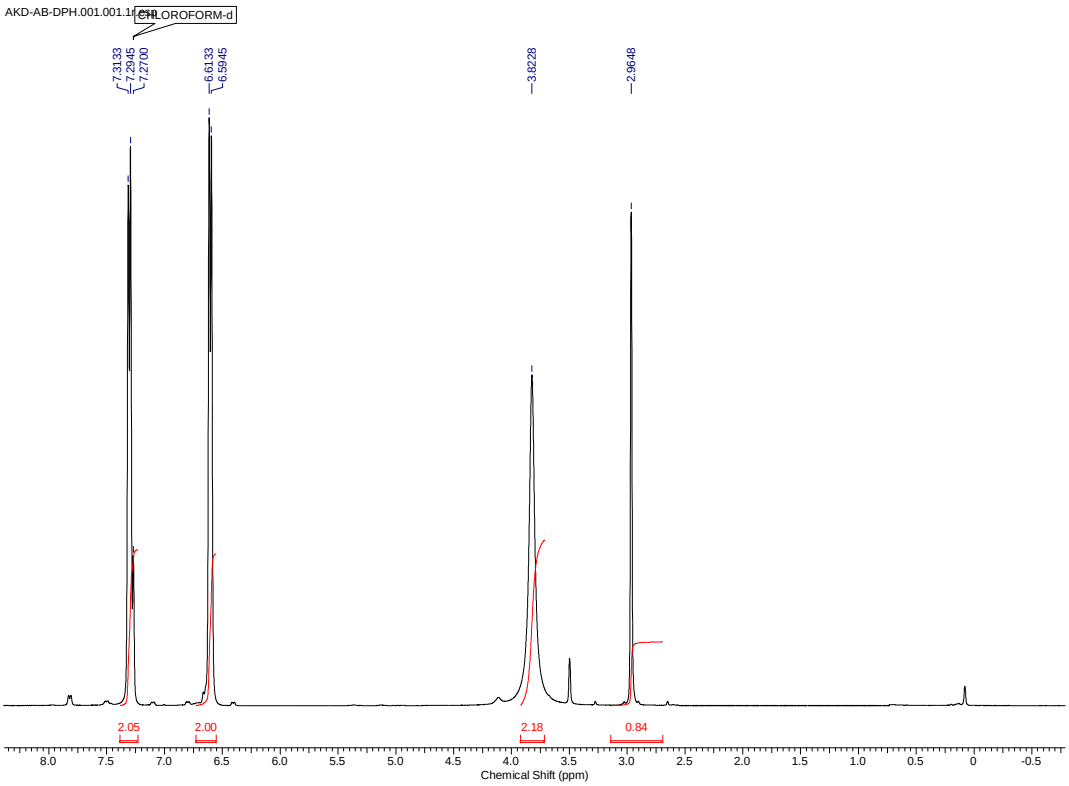


Figure 12: 400 MHz ^{1}H NMR spectrum of compound **5** in $CDCl_3$

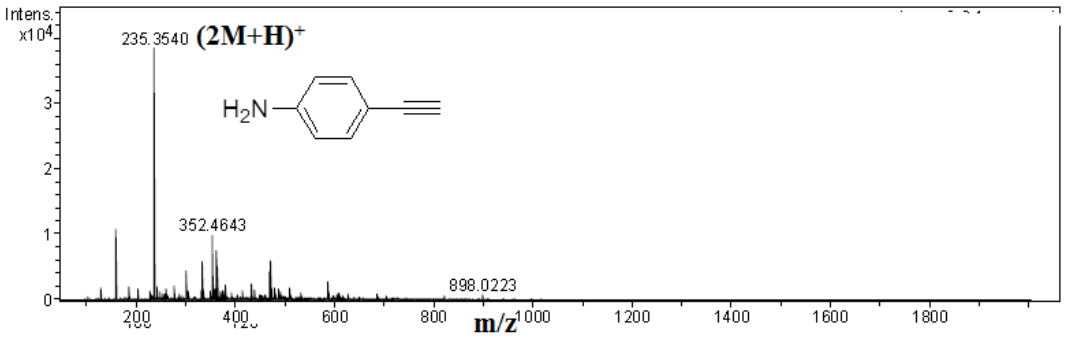


Figure 13: ESI-MS spectrum of compound **5**

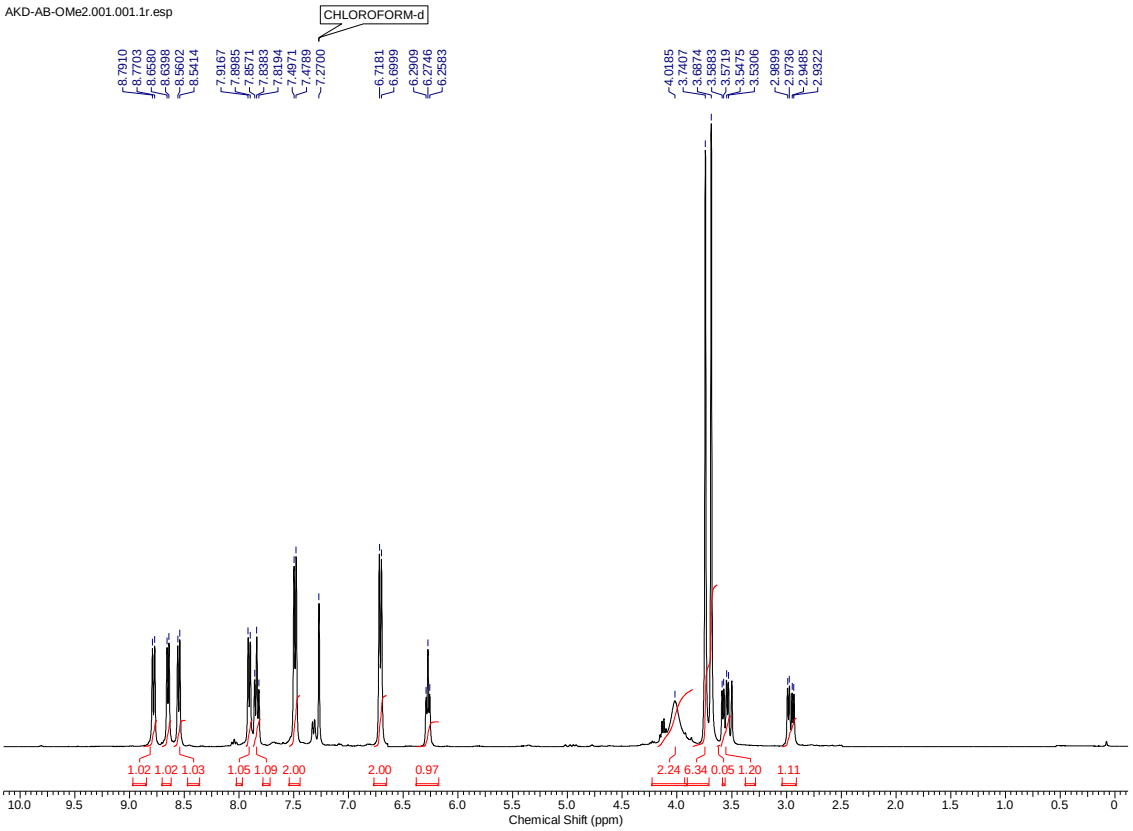


Figure 14: 400 MHz ^{1}H NMR spectrum of compound **6** in $CDCl_3$

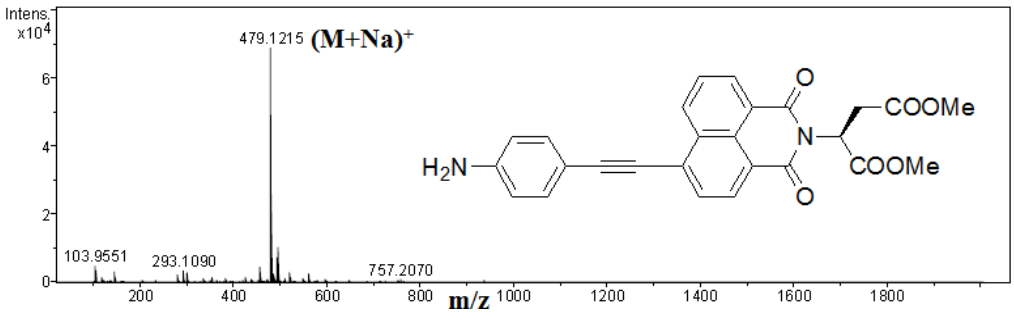


Figure 15: HRMS(ESI) spectrum of compound **6**

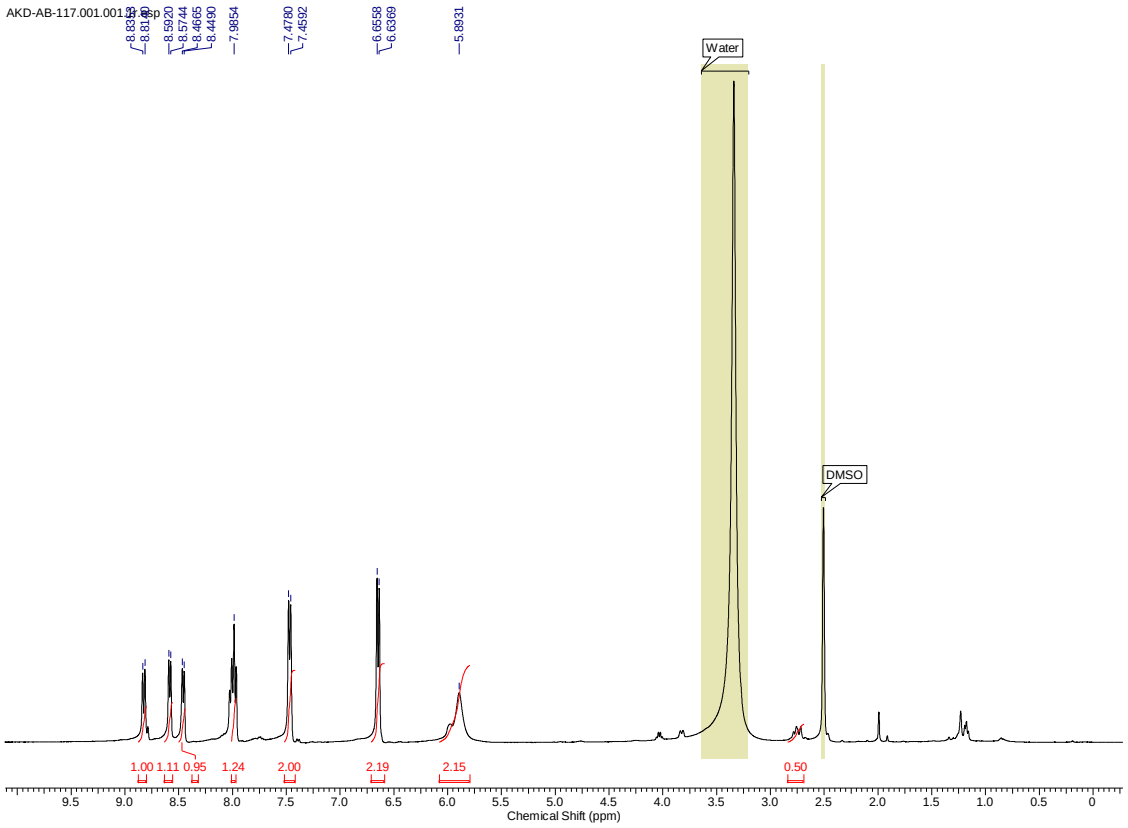


Figure 16: 400 MHz ^{1}H NMR spectrum of compound **1** in DMSO-d_6

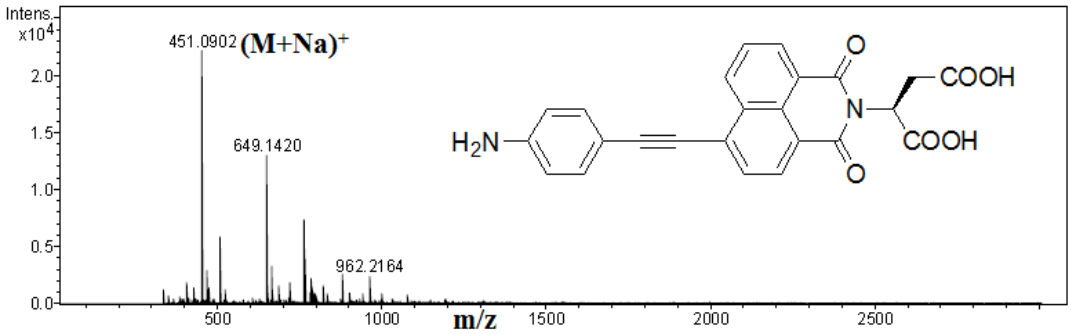


Figure 17: HRMS(ESI) spectrum of compound **1**

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