PHOTOCHEMICAL REACTION AND CHARACTERISATION OF SYNTHETIC PEPTIDE-FUNCTIONALIZED MOLECULES

M.Sc. Thesis

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A THESIS

Submitted in partial fulfillment
of the requirements for the
award of the degree
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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Photochemical reaction and characterization of synthetic peptide functionalized molecules** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DEPARTMENT OF CHEMISTRY, Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the time period from **July 2020** to **June 2021** under the supervision of **Dr. Apurba K. Das**, Associate 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.

Sweta Agarwal

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

04.06.2021 **Dr. Apurba K. Das**

SWETA has successfully given his/her M.Sc. Oral Examination held on 08.06.2021.

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Date: 04/06/2021

Date:

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ABSTRACT

Organic conjugated polymers have various applications in diverse subjects. Among them PDAs are studied most widely because of their various structures and applications. In this study I have started with 6-aminocaproic acid as a precursor which then has been coupled with 4-pentynoic acid. The coupled product then undergoes homo-coupling to form 1,3-diacetylene monomer. The monomer formed then has been polymerized under UV light (254 nm) and the color changed from white to blue. Each step has been monitored by Thin Layer Chromatography (TLC).All synthesized compounds have been purified by Column Chromatography and characterized by mass spectrometry and nuclear magnetic resonance (NMR) techniques.

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NOMENCLATURE

nm	Nanometer
δ	Delta
mmol	Milimol
μM	Micro molar
mL	Milliliter
mg	Milligram
g	Gram
λ	Lambda

ACRONYMS

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

$CDCl_3$	Chloroform-*d*
DMSO-*d*6	Dimethyl Sulfoxide-*d*6
DMF	N,N'-Dimethylformamide
HCl	Hydrochloric acid
Na_2CO_3	Sodium Carbonate
aq.	Aqueous
d	Doublets
ESI-MS	Electron Ionization Mass Spectrometry
NaOH	Sodium Hydroxide
MeOH	Methanol
NMR	Nuclear Magnetic Resonance
s	Singlet
min.	Minute
$SOCl_2$	Thionylchloride
TLC	Thin Layer Chromatography
t	Triplet
UV-Vis	Ultra violet-visible
m	Multiplet
HOBt	N-Hydroxybenzotriazole
Na_2SO_4	Sodium sulphate

EDC.HCl	1-(3-dimethylaminopropyl)-ethylcarbodiimidehydrochl-oride
DMAP	4-dimethylaminopyridine
RB Flask	Round bottomflask
DCM	Dichloromethane
EtOAc	Ethyl acetate

Chapter1

1.1Introduction

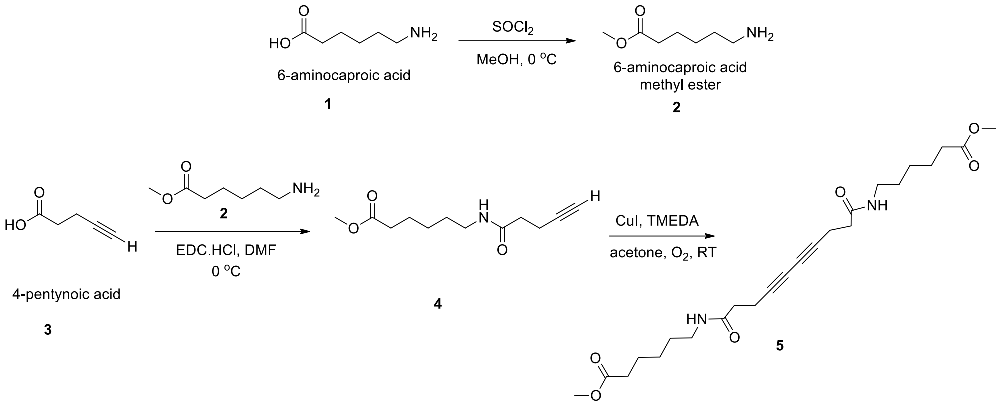
Now-a-days, researchers are mostly attracted by metal-free organic polymers for detection and solving environmental problems.[1]Due to increase in the population size, there is a tremendous increase in the greenhouse gases in the atmosphere which eventually increases the earth's temperature.[2-3] Among the greenhouse gases, CO_2 is the main concern. As CO_2 is inert in nature, it becomes very difficult to capture it. Therefore, capturing of CO_2 via chemisorption is quite difficult and even it leaves a side product, so capturing by physisorption is widely accepted. Different porous materials like Metal Organic Framework (MOF)s, Covalent Organic Framework(COF)s and Covalent Organic Polymer (COP)scan be utilized for capturing of gases.[5-7] Due to their hydrothermal stability, covalent organic polymers (COPs) are the most efficient. COPs are also widely used in energy storage, drug delivery, gas absorption. Poly-diacetylene is one of the most interested π- conjugated systems and polymerization can be achieved under visible light irradiation.[4] Unlike other covalent polymers, no chemical reactions, catalyst or heating is required in the polymerization of diacetylene which ensures its purity.[8] Poly-diacetylene basedpolymers(PDA) has been selected for the purpose due to their unique chromatic and emissive property as biosensing material and detection. PDAs are usually formed by the 1,4 addition of diacetylene under UV light.[9-10]Diacetylene derivative have been selected because they can self-assemble through non covalent interactions like hydrogen-bonding, van der waals interactions and π-π interactions. Generally, diacetylene undergoes topochemical polymerization only when properly oriented. [11-12]Polydiacetylene (PDAs) are formed by photo light without chemicals initiators and catalyst. COPs are also used as a photocatalyst for dye degradation.

Earlier semiconductors like TiO_2, ZnO has been used for this purpose but due to their toxicity and expensiveness, now COPs are used as photocatalysts for photodecomposition of organic dyes under UV light radiation.[13-17] Upon UV irradiation permanent porosity has been developed in the PDAs.

PDAs are π-conjugated polymers that show fluorescent transformations and optical property. Due to alternating ene-yne backbone in the structure they show different color upon irradiation with UV light. PDAs have a dual signal output, therefore can be used as sensors.[18-21] PDAs are attracted by many scientists due to its colorometric and florescent transition. Upon Exposing to different stimuli like solvent (solvochromism), temperature (thermochromism), pH, mechanical stress (mechanochromism) and molecular binding events (affinochromism/biochromism), PDAs result in color transformation along withthechange in their fluorescence. Color changes occur mainly due to interaction of side groups with themselves and also with the external stimuli. It has generally been known that the blue color transformation is due the change of the planar into non-planar structure.[22-25] As PDAs crystals are insulators, so this property can be used to exploit their applications like capacitive based sensors of vapor substances. PDAs due to their π-conjugation system can be used as photocatalyst.[26-28]

In the current work, we have synthesized theunnatural amino acid conjugated 1,3-diacetylene based monomer.The unnatural amino acid, 6-aminocaproic acid has been used and first coupled with 4-pentynoic acid. The coupled product has been further polymerized under UV light (254 nm) and the polymerization is confirmed by the color transformation from white to blue.All synthesized compounds have been purified by Column Chromatography and characterized by mass spectrometry and nuclear magnetic resonance (NMR) techniques.

1.2 Reaction Scheme



Scheme 1: Overall synthetic scheme of 1,3-diacetylene base monomer **5**.

Chapter 2

2.1 Experimental methods

2.1.1 Materials

Chemicals including 4-pentynoic acid, 6-aminocaproic acid, copper chloride (I) and *N*, *N*, *N*, *N*-tetramethylethylenediamine (TMEDA) were bought from Alfa Aesar India. N, N- dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimidehydrochloride (EDC.HCl), 1-hydroxybenzotriazole (HOBt) and thionyl chloride were purchased by SRL India Pvt. Ltd. Solvents such as DCM, EtOAc, hexane were purchased from Merck India Pvt. Ltd and distilled before use by following procedures of reportedliterature.

2.1.2 General Characterization

Mass spectrometry (ESI-MS) was performed on a Bruker instrument *via* electron spray ionization on positive-mode. All NMR spectroscopy was recorded out on a 400 MHz Bruker AVANCE 400 NMR.The deuterated solvents like DMSO-d_6 and $CDCl_3$ have been used for NMR analysis.($CHCl_3$: δ 7.26, DMSO: δ =2.50 For ^{1}H NMR; and $CHCl_3$: δ 77.00, DMSO:
δ 39.50 for ^{13}C NMR). Alloftheinvolvedreactionswere constantly monitored by employing thin-layer chromatography technique (TLC).

Chapter 3

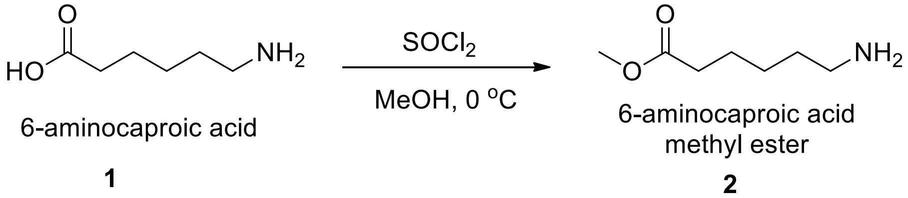
3.Synthesis of diacetylene-based monomers

3.1 Synthesis of compound2

In a 100 mL R.B. flask, 6-aminocaproic acid (2 g, 15.24 mmol) was dissolved in methanol (40mL) and kept for stirring for 15 min at 0 ºC then thionylchloride ($SOCl_2$) (76.2 mmol, 5.52 mL) was carefully added dropwisethrough the walls of the RB flask and covered the R.B. with a calcium chloride guard tube. There action mixture was kept for stirring for overnight at 30ºC. The left over solvent was evaporated at room temperature by rotary evaporation. Obtained crude compound was washed two times with diethylether.The desired product was obtained as white powder. Yield- 90%

^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.14 (s, 1H), 3.57 (s, 1H), 2.96 - 2.61 (m, 1H), 2.29 (dd, *J* = 9.2, 5.4 Hz, 1H), 1.54 (m,), 1.30 (dd, *J* = 14.3, 7.1 Hz, 1H).

ESI-MS: m/z calculated for $C_7H_{15}NO_2[M+H]^+$: 146.1176, found 146.1190



Scheme2: Synthesis of compound **2**

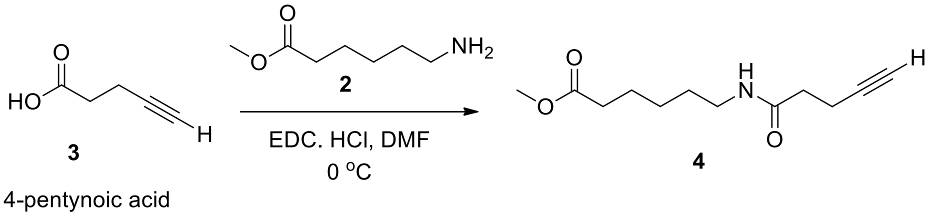
3.2Synthesis of 4

In a 100 mL R.B. flask, 4-pentynoic acid (5.09 mmol, 0.5 g) was dissolved indry DMF (5 mL). The reaction mixture was stirred for 15 min in ice-cold condition, followed by addition of EDC.HCl(5.60 mmol, 1.074 g). After 5 min, HOBt (5.096 mmol, 0.688 g) was added. The mixture was stirred for 30 min, followed by the addition of 6-aminocaproic ester. The reaction mixture was keptfor stirring for 12 h. The resultant mixture was diluted by ethyl acetate. The organic part was then washed with 1NHCl (3×15mL), Na_2CO_3 (3×15mL) and brine (3 × 15 mL). After that, the collected organic parts were dried over Na_2SO_4 andthesolventwasevaporatedusingrotavapor. The crude product was purified using column chromatography by using hexane- ethylacetate (6:4) as an eluent.White crystalline powder was obtained as pure product **4**. Yield-88%

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.74 (s, 1H), 3.65 (s, 3H), 3.26 (dd, *J* = 13.1, 6.9 Hz, 2H), 2.51 (m,), 2.37 (t, *J* = 7.2 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 2.00 (t, *J* = 2.6 Hz, 1H), 1.63 (m), 1.51 (dd, *J* = 14.9, 7.3 Hz, 2H), 1.39 – 1.30 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.07 (s), 170.89 (s), 83.06 (s), 69.34 (s), 51.54 (s), 39.30 (s), 35.43 (s), 33.84 (s), 29.21 (s), 26.31 (s), 24.43 (s), 14.97 (s).

ESI-MS: m/z calculated for $C_{12}H_{19}NO_3[M+Na]^+$:248.1257, found 248.1233

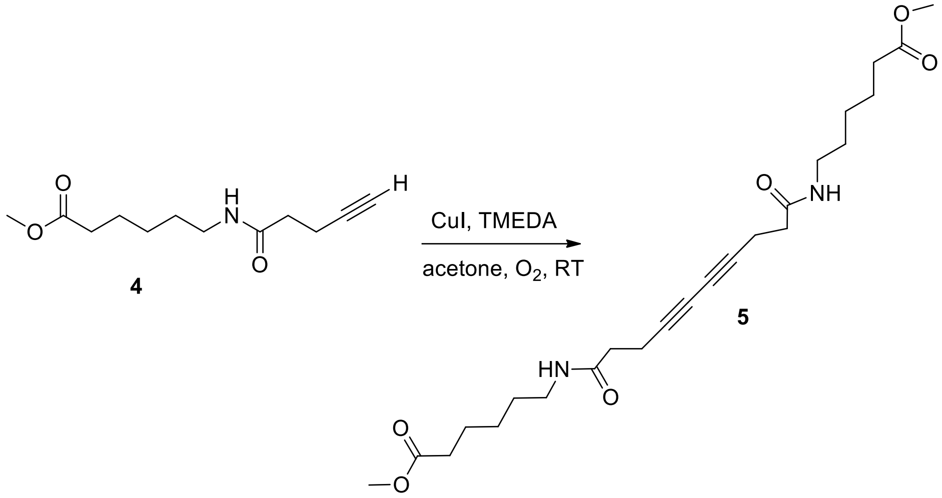


Scheme 3: Synthesis of compound **4**

3.3 Synthesis of 5

In a 100 mL R.B. flask, dry acetone (10 mL) was taken followed by the addition of CuCl (6.70 mmol, 0.556 g) and TMEDA (3.107 mmol, 0.475mL). The reaction mixture was kept for stirring for 30 min followed by the addition of compound **4** (1.55 mmol, 0.350 g). After that, the reaction mixture was connected with O_2balloon. The reaction mixture was then stirred for 48 h. After that, the remaining solvent was evaporated using rotary evaporation to obtain the crude product. The obtained product was purified by using column chromatography using hexane-ethyl acetate (3:7) as eluent to get the compound**5**. The compound**5** was obtained as white powder. Yield- 60%

ESI-MS: m/z calculated for $C_{24}H_{36}N_2O_6$ $[M+K]^+$: 487.2205, found 487.2328



Scheme 4: Synthesis of compound **5**

3.4 Polymerization

The photopolymerization of the monomer **5** were carried out under the UVlightirradiation.Inthispolymerization,white powderedmonomers(50mg)weretaken on a glass slide and then it the glass slide was placed in a UV chamber for 15 min. The chamber was fitted with 6 UV tubes (12W each) which provided UV light of wavelength 254 nm.

Chapter 4

Resultsand Discussion

The biomolecules functionalized diacetylene based symmetrical monomer was synthesized and well characterized by ^{1}H and ^{13}C NMR spectroscopy and ESI-MS spectrometry.

4.1 Polymerization analysis

The self-assembled and orderly arranged diacetylene units undergo topochemical photopolymerization. The two obligatory factors for undergoing photopolymerization are (i) the angle of 45between two diyne axes and (ii) the distance between two adjacent diyne axes should be 4.9 Å (Figure 2a). The peptide functionalized and self-assembled diacetylene monomers underwent photopolymerization through 1,4-addition to form conjugated ene-yne alternated polymer when treated with UV-Vis light.The color of the obtained product changed from white to blue upon irradiation by UV light for 15 min that confirms the polymerization.

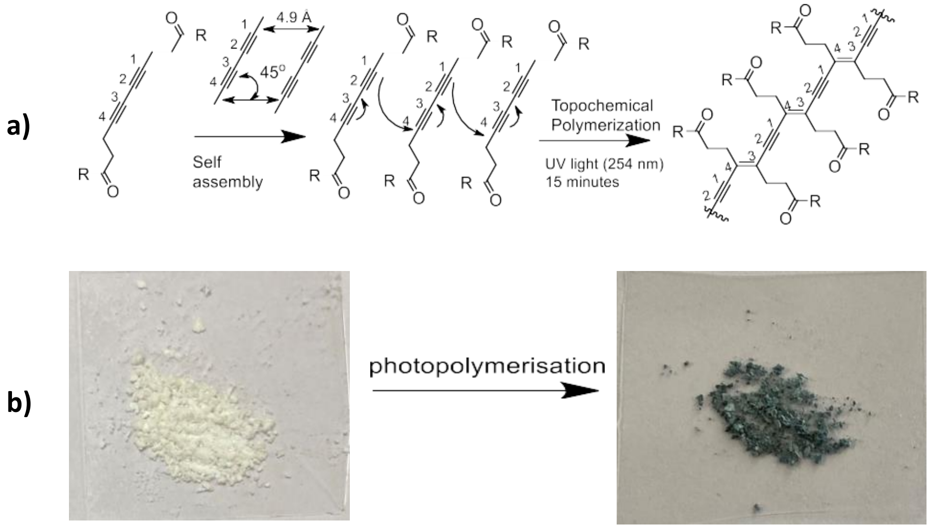


Figure 1.(a) Schematic mechanism of photopolymerization, (b) solid state photopolymerization of compound **5**

4.2Characterization

4.2.1 Mass Spectra

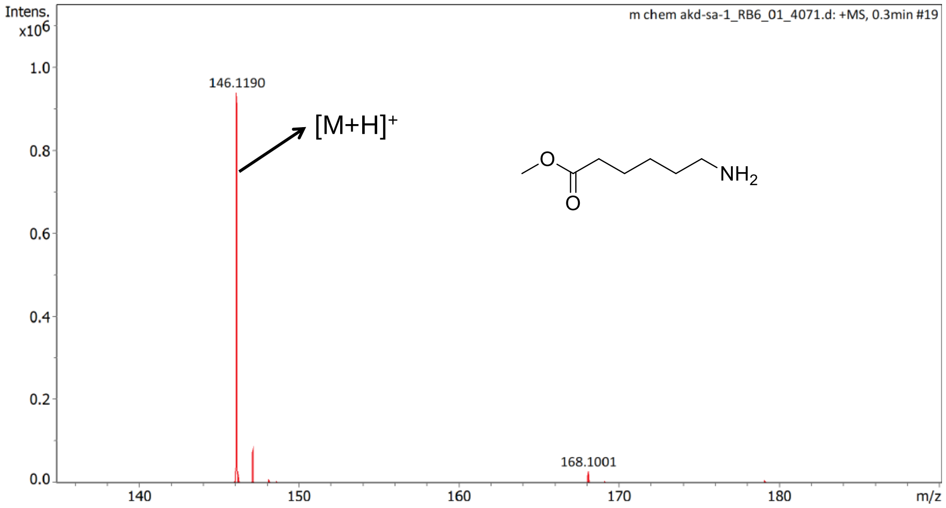


Figure 2: Mass spectrum of compound (**2**)$C_7H_{15}NO_2$ $[M+H]^+$: 146.1176, found 146.1190

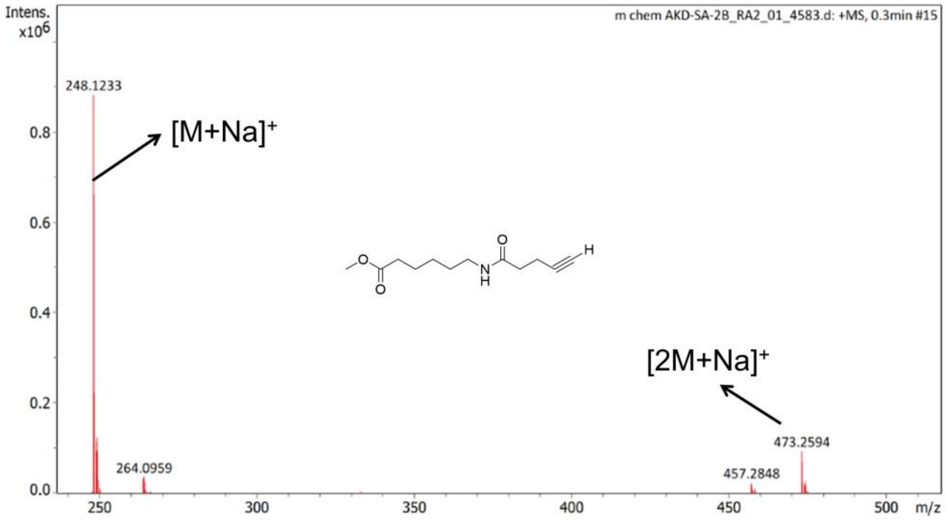


Figure 3: Mass spectrum of compound (**4**)calculated for $C_{12}H_{19}NO_3$ $[M+Na]^+$:248.1257, found 248.1233

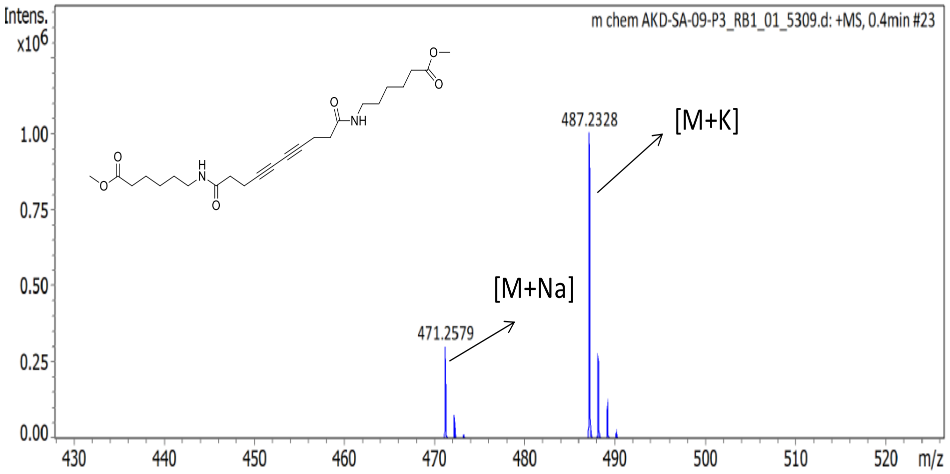


Figure 4:Mass spectrum of compound (**5**)calculated for $C_{24}H_{36}N_2O_6$ $[M+K]^+$: 487.2205, found 487.2328

4.2.2 ^{1}H and ^{13}C NMR spectral data

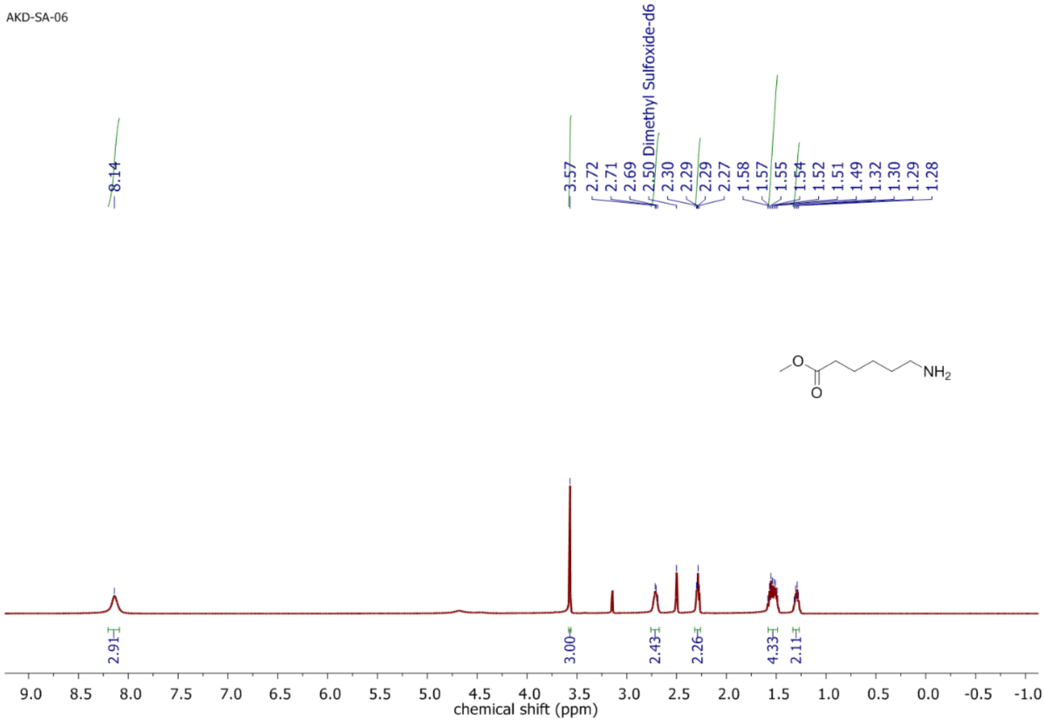


Figure 5: ^{1}H NMR (400MHz, DMSO-*d6*) spectrum of compound (**2**)

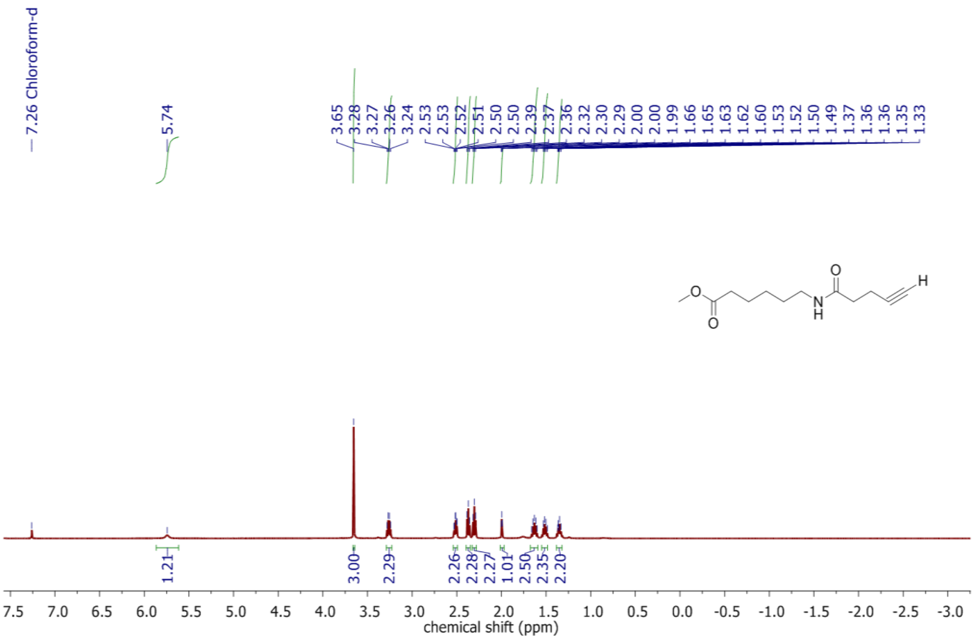


Figure 6: ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound (**4**)

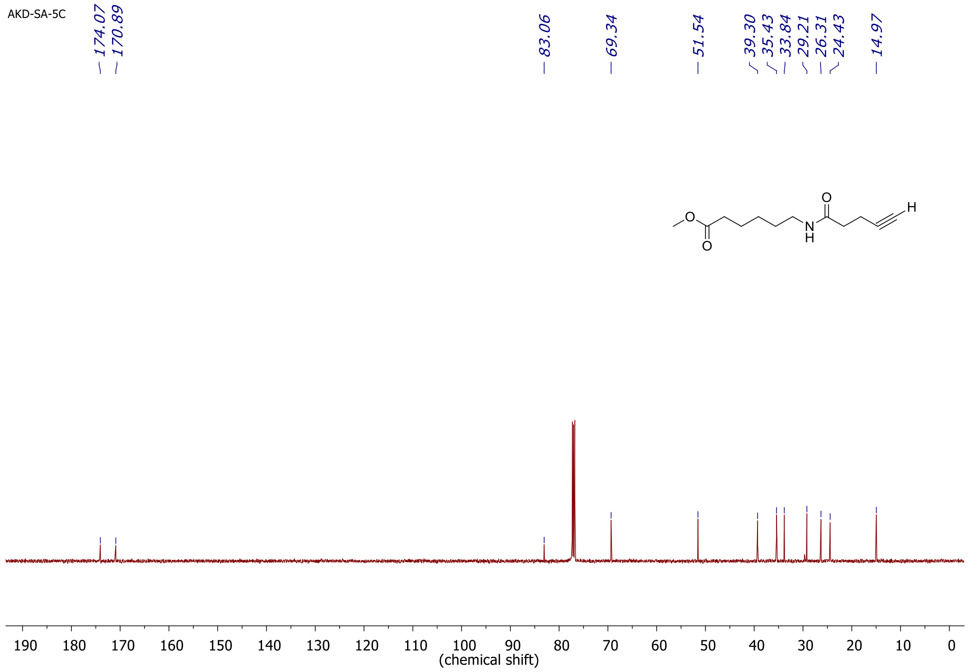


Figure 7: ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound (**4**)

Chapter 5

5.1 Conclusion

Ihave successfully synthesized and characterizedpolydiacetylene based peptide functionalized conjugated compound, 6-aminocaproic acid functionalized polydiacetylene. The monomer underwent photopolymerization when irradiated with UV-light. The white coloured compound transformed into blue color upon irradiation for 15 minand the formation of conjugated polydiacetylene covalent organic polymer took place.All the compounds were successfully synthesized and purified over column chromatography. NMR, and mass spectrometry were used for further characterization of these purified compounds

5.2 Future Plans

Ihave successfully synthesized the unnatural amino acid conjugated 1,3-diacetylene based monomer. Polymerization was confirmed by color change from white to blue upon UV irradiation for 15 min. Our future plan focuses on the application part of the synthesized polydiacetylene. The compound synthesized can be further checked for photocatalytic property of degrading pollutant dye whenirradiatedwithUV-Vislight and also carbon-dioxide capture.

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