TOWARDS THE SYNTHESIS OF DIMERS FROM 9,10-DIHYDROANTHRACENE 9,10- α, ß SUCCINIC ANHYDRIDE/ACID AND THEIR FLUORESENCE PROPERTIES

A
thesis submitted
in partial fulfillment of requirement for the
Degree of Master of Science in Chemistry

Submitted by	**Supervisor**
Shilpa Narang	Dr. Manmohan Chhibber
Reg. No : 300802019	Assistant Professor

School of Chemistry and Biochemistry
Thapar University
Patiala 147001
July 2010

DEDICATED TO MY PARENTS

Acknowledgement

It is said that life is a carnival of experiences and a journey with various goals. So in my journey where I experienced this project I want to thank the supreme almighty for his presence in my soul and in my mind.

I take this opportunity to thank my guide Dr. Manmohan Chhibber, Assistant Proffessor, School of Chemistry and Biochemistry without whose presence the whole project just would have been a dream. I am extremely indebted to him for the scientific attitude and utmost patience he has installed in me which will definitely stand in all future endeavour and it was because of him that I was able to learn so much in this short period.

My sincere thanks to Dr. Susheel Mittal, Head, School of Chemistry and Biochemistry for his guidance and suggestions. I express my regards to all faculty members of School of Chemistry and Biochemistry for their help and moral support during my stay .

I express my gratitude to my golden friends Shalini , Karamjeet , Jasneet, Rumit whose support and friendship was like a cold shower in the heat of work and pressure .

I am highly obliged to Chander sir and other laboratory staff who were very helpful in every possible way.

I thank my lab mates Shalini, Charanjeet and Jasneet to cooperate me in the laboratory . It was like a family with them. A vote of thanks goes to PhD. Scholars Miss Ramandeep Kaur for her suggestions and help.

Last but not least I owe my thesis to my parents and my sister Neha and my brother Harshit Narang who are the building pillar of my life .

Shilpa

Dated: July 15, 2010.

SHILPA NARANG

Candidate's Declaration

I hereby declare that the work being presented in the dissertation entitled "Towards the synthesis of dimmers from 9,10dihydroanthracene-9,10-α,ßsuccinic anhydride/acid and their fluorogenic properties." , in partial fulfillment of the requirement for the award of degree of Masters of Science (Chemistry) done at School of Chemistry and Biochemistry (SCBC), Thapar University , Patiala, is my own work. The said work is carried out during the period January 2010 to May 2010, under the supervision of Dr. Manmohan Chhibber, Assistant Proffessor , School of Chemistry and Biochemistry , Thapar University, Patiala. I have not submitted the matter embodied in this dissertation for the award of any other degree.

Patiala

Date: 15 July 2010

Shilpa

Shilpa Narang

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

Dr. Manmohan Chhibber

Project Supervisor,

Assistant Professor (SCBC),

Thapar University

Susheelmittal 15/7/X

Dr. Susheel Mittal

Head,SCBC

Thapar University

Certificate

This is to Certify that the project entitled "Towards the synthesis of dimers of "9, 10 dihydroanthracene 9,10-α,ß-succinic anhydride/acid and their fluorescence properties. " , being submitted by Miss SHILPA NARANG in partial fulfillment of the requirement for the award of degree of Master of Science (Chemistry), Thapar University , Patiala, is a bonafide work carried out under the supervision of Dr. Manmohan Chhibber(Assistant Proffessor) and that no part of this project has been submitted for the award of any other degree.

Dr. Manmohan Chhibber

Assistant Proffessor

Project Supervisor,

Thapar University

Susheel Mittal 15/7/X

Head,

Dr. Susheel Mittal

School of Chemistry and Biochemistry,

Thapar University

20/7

Dean , Academic Affairs,

(Dr. R.k. Sharma)

Thapar University

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Introduction:

The florescent compounds are the ones that show emission of electromagnetic radiation, especially of visible light, stimulated in a substance by the absorption of incident radiation. The emission persists only as long as the stimulating radiation is continued. George Gabriel Stokes named the phenomenon florescence in 1852 [1].The name was derived from the mineral fluorite, Calcium difluoride (CaF_2), which contain traces of divalent europium, that serves as the fluorescent activator to emit blue light. [2]

Fluorescence has become an important investigational tool in many areas of analytical, biochemical and medical research due to its extremely high sensitivity and selectivity. It is an essential investigational technique allowing detailed, real-time observation of the structure and dynamics of intact biological systems with extremely high resolution. In pharmaceutical industry it has almost completely replaced radiochemical labeling.

The fluorophore, that is the probe that shows fluorocence, may be covalently or noncovalent linked to a sample to be analyzed, producing the respective conjugates or complexes. Different molecules that show fluorescence from short to very long wavelengths are used as probes. Among organic fluorophores oxobenzopyrans, naphthofurans, oligothiophenes, 4,7-phenanthroline-5,6-diones, benzooxadiazoles, dansyl chloride, naphthalene 2,3-dicarboxaldehyde, and 6-propionyl-2-(dimethylamino)naphthalene are used that show fluorescence from the near-ultraviolet to approximately 500 nm. Other organic fluorescent molecules that emit between 500 nm and the near-infrared (ca. 900 nm) are fluoresceins (including biarsenical dyes), rhodamines, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacenes (BODIPY dyes), squaraines, and cyanines .[3]

Anthracene, since it's discovery has found various chemical applications in electroluminicent devices and fluoroscent probes because of its highly fluorescent nature. A number of fluorescent probes with an anthracene unit have been reported by various research groups and found useful applications in different fields. For example, Stack et al [4] have synthesized fluorescent probe specific for catechols. Malakhov et al[.5] synthesized 1-(phenylethynyl)- pyrene and 9,10-bis(phenylethynyl)anthracene fluorescent dyes useful for DNA labeling. Swamy et al.[6] have synthesized anthracene derivative containing two boronic acid groups at 1,8-position and these derivatives are found to be sorbitol-selective fluorescence sensors. Recently , Kotha et. al. reported the synthesis and structural properties of a functionalized anthracene derivative, which they proposed potentially useful fluorescent probe for proteins.[7]

Present work relates to synthesis of some anthracene derivatives and observation of their fluorescence spectra.

Review of Literature

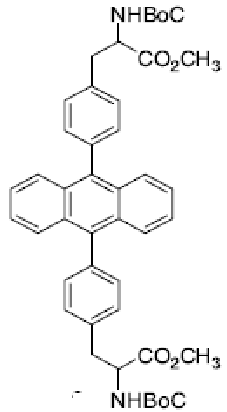
Anthracene has been subjected to a good deal of experimental and theoretical investigation both for practical purposes and for academic reasons. One of the main reasons for its extensive study is its florogenic properties due to which it can be tagged to many molecules and biomolecular. The florogenic properties of anthracene skelton are retains in most of its derivatives and has been studied quite extensively.

Anthracene-based sensors encompass all areas of molecular recognition; for example, anion sensors[8] glucose sensors[8, 9] and metal-organic switches.[10] Anthracene is exploited due to its relative abundance (i.e., low cost) and depth of synthetic manipulation. Anthracene also possesses favorable photophysical properties, such as strong absorption and high quantum yield. Limitations include photoinduced cycloaddition reactions and formation of peroxides in the presence of oxygen.[11]

Following paragraphs represent a brief literature survey of such organic molecules. Tryptophan has a multi-exponential decay of fluorescence, making the situation complicated even for single-tryptophan proteins. Moreover, tryptophan absorbs ultraviolet light, which is scattered to a significant extent in heterogeneous media. So, it is desirable to synthesize fluorescent probes with amino acid moieties, which have single exponential fluorescence decays and preferably, absorb light of longer wavelengths, particularly in the range of emission of tryptophan, so that they can be used as energy acceptors from tryptophan. A number of unusual amino acids have been incorporated into peptides and the photophysical properties of the corresponding peptides have been studied. Kotha et. al[7] have synthesized an amino acid that quenches tryptopan fluorescence significantly, presumably by FRET while its own fluorescence is not quenched by tryptophan. However, iodide ion quenches the fluorescence of the fluorophore **(Figure-1)**. Therefore, this highly fluorescent bis-armed amino acid derivative can be used to monitor unfolding of proteins.

Macrocyclic ditopic anthracenyl receptors namly 1,8-Bis(azacrown)anthracenes **(Scheme-1)** were synthesized that displayed chelation-enhanced fluorescence effects(CHEF) upon the addition of linear dications $^{+}NH_3(CH_2)_nNH^{+}{}_3$. The effect, which is dependent on the chain

Figure-1

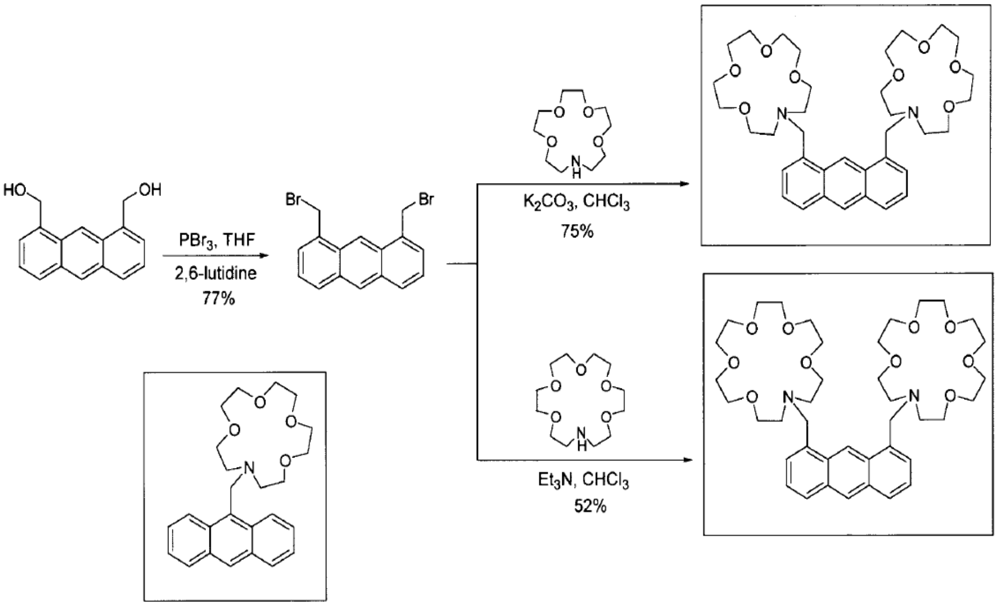


Macrocyclic ditopic anthracenyl receptors namly 1,8-Bis(azacrown)anthracenes **(Scheme-1)** were synthesized that displayed chelation-enhanced fluorescence effects(CHEF) upon the addition of linear dications $^{+}NH_3(CH_2)_nNH^{+}{}_3$. The effect, which is dependent on the chain length, was at a maximum for propylene diammonium cation. The synthesis of the compounds was carried out starting with 1,8-bis(hydroxymethyl)anthracene, which was then transformed to 1,8-bis(bromomethyl)anthracene. Final compound was synthesized by adding 1,8-bis(bromomethyl)anthracene to a mixture of 1-aza-15- crown-5, mild base in solvent at room temperature **(Scheme-1).**

There are several other studies where anthracene has been coupled to various metal ion receptors to monitor quenching of its fluorescence. Ghosh et. al[12]. synthesized anthracene coupled adenines **(Figure-2A and B)** and established their Cu ion recogonegition properties based on quenching of fluorescence. Unob et. al. synthesized anthracene-bascd fluorescent calixl4]arene **(Figure-2C)** and calix[4]arenc **(Figure-2d)** that could chelate to transition metal cations such as zinc and nickel.

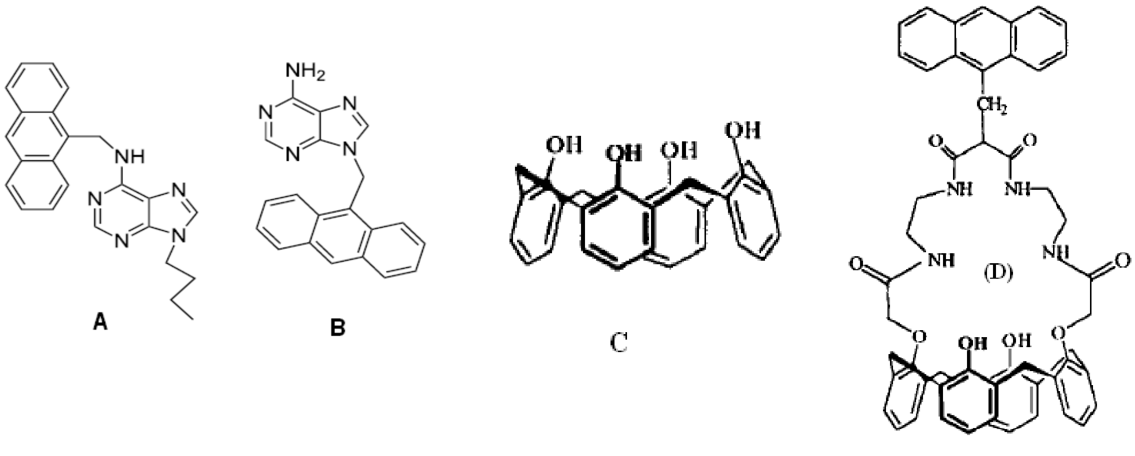
Most of the work cited above refers to compounds where anthracene unit as whole was used for the fluorescence properties. Following references discuss examples where the anthracene unit has been modified without hampering its photoemission properties.

Scheme1: Schematic representation of synthesis of 1,8-bis(bromomethyl)anthracene



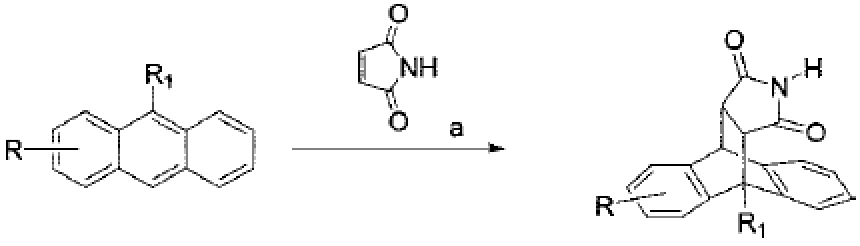
Bova et. al.[13] synthesized a series of rigid anthracene derivatives via Diels Alder cycloaddition of anthracene and maleimide **(Scheme-2)**. The substitution on the anthracene ring was varied as shown **(Table 1)** below. The aim of their study was to observe the blocking of L-Type Ca^{2+} channels (LTCCs) that regulate Ca^{2+} influx in a number of cell types. LTCCs play a key role in the regulation of vascular smooth muscle contraction, and are widely used in the therapy of

Figure- 2: Simple anthracene coupled receptors



hypertension. These compounds bind preferentially to inactivated LTCCs, directly interacting with the pore forming subunit of the channel.

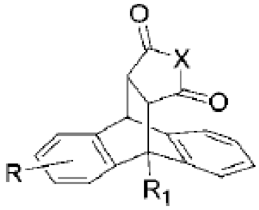
Scheme-2



1-10

Reagents: a) Xylene, refluxing 4-16h

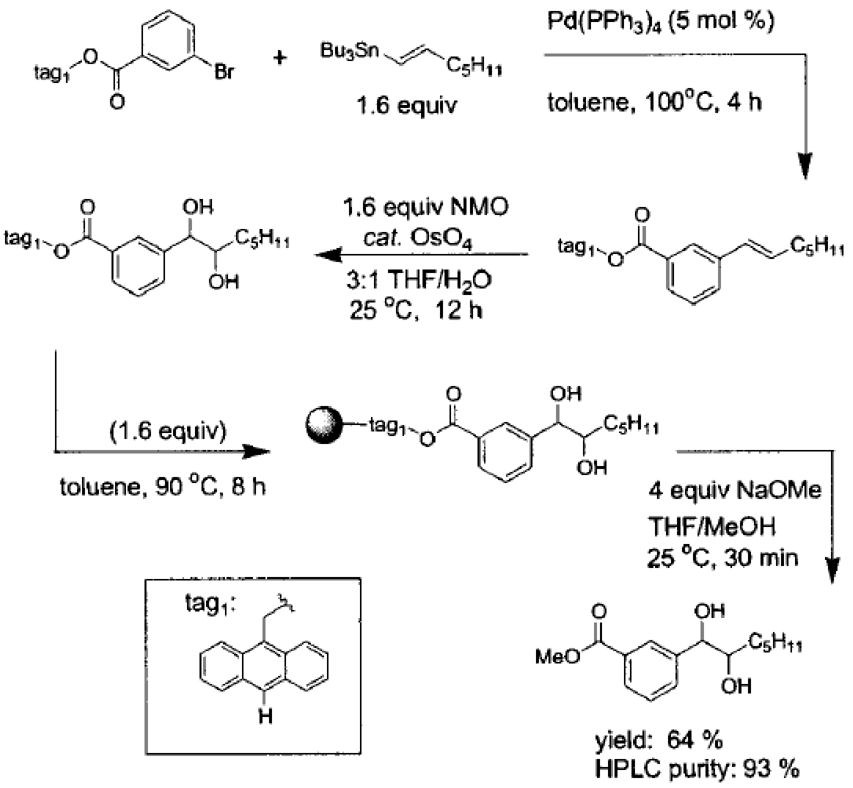
Table- 1: Structures of the studied compounds



compd	R	R_1	X
1	H	H	NH
2	4-CH_3	H	NH
3	4-Cl	H	NH
4	3-Cl	H	NH
5	H	CHO	NH
6	H	CH_2OH	NH
7	H	Cl	NH
8	H	CH_3	NH
9	H	NO_2	NH
10	H	CN	NH
11	H	H	$NCH_2{-}{\equiv}$

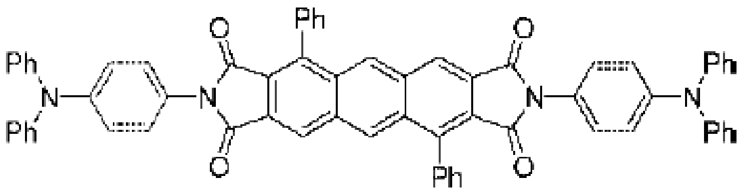
The fluorescent property of anthracene was used for parallel synthesis of benzyl esters by Wang et. al[14]**.** Maleimides, a dienophiles, was bound to resin and used for sequestration of anthracene-tagged compounds. Use of 9-anthrylmethyl ester tagged substrates was demonstrated in a multistep synthesis sequence. **(Scheme-3)**

Scheme-3: Multistep synthesis using a 9-Anthrylmethyl Ester



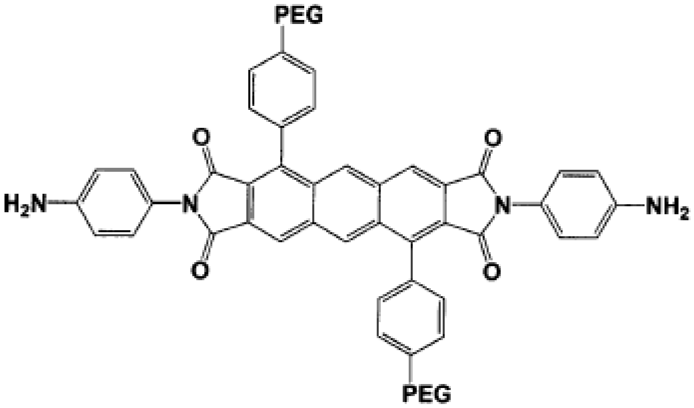
Anthracene-tagged benzoate was reacted in a Stille coupling with (*E*)-tributyl-1-heptenyl stannane to afford long chain aromatic compound bound to resin. After concentration, the crude product was dihydroxylated to afford diol which was incubated with excess maleimide resin to provide resin-captured diol. After resin washing, the product was cleaved to benzoate in good yields.

Figure-3



Tyson et. al [15,16] have synthesized anthracene diimide derivatives that show potential use as a fluorescent sensor for optical based chemical sensing properties[15] (**Figure-3)** pH and nitroaromatics and as an indicator in thermochromic materials[16] **(Figure-4)**.

Figure-4



One of the very common reactions of anthracene is their Diels-Alder product which results in 9,10-dihydroanthracene-9,10- α,ß-succinic anhydride and acid. Although a lot derivates of these compounds have been reported, we have not come across the dimmers of these molecules which can act as ion chelators. Present work has been undertaken with an objective to synthesize the dimmers of anthracene based molecules and study their fluorescent properties.

MATERIALS AND METHODS:

A. *Source of reagents and analytical facilities*

Anthracene, maleic acid, maleic anhydride, phosphorus tricloride and xylene were obtained from M/S SD Fine Chemical Limited, Mumbai. All the chemicals and solvents were used as received without any further purification. ^{1}H NMR spectra were recorded on 400MHz FT-NMR cryo magnet spectrometer(bruker), from Sophisticated Analytical Instrumentation Facility (SAIF) at Punjab University, Chandigarh. Fluorescense and Ultraviolet absorption studies were carried out on Perkin Elmer LS55 Fluorescense spectrophotometer and Specord 205 ultraviolet spectrophotometer respectively at School of Chemistry and Biochemistry, Thapar University, Patiala.

B. *Experimental*

B1. Synthesis of 9,10-dihydroanthracene-9,10-α,βsuccinic anhydride (1): Anthracene (3.6g, 0.018 mol) was taken in a solution of xylene (25 ml) and maleic anhydride (1.96g,0.02 mol) added. The reaction mixture was allowed to reflux for 2 hr after which it was cooled and the precipitates collected under vacuum filtration. Washing was given with cold methanol to obtain product (3.83g, Yield 69.0 %) as white solid having melting range 260-264 ^{0}C.

B2. Synthesis of 9,10-dihydroanthracene-9,10-α,βsuccinic acid (2): Anthracene (3.6g, 0.018 mol) was taken in a solution of xylene (25 ml) and maleic acid (2.13g,0.183 mol) added. The reaction mixture was allowed to reflux for 2 hr after which it was cooled and the precipitates collected under vacuum filtration. Washing was given with cold methanol to obtain product (3.89g, Yield 68 %) as white solid.

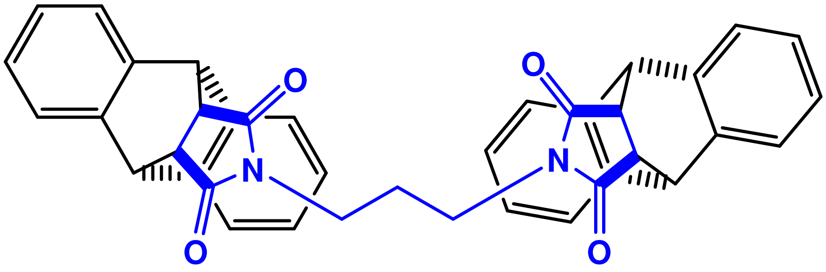
B3. Attempted Synthesis of dimer with ethylenediamine for 9, 10-dihydroanthracene-9, 10-α, β, succinic anhydride (3): Compound (1) (1.5g, 0.0026 mol) was taken in methanol (7 ml) and ethylenediamine (0.153g, 0.00225 mol) was added. The reaction mixture was refluxed for two hours and methanol evaporated. Thin layer chromatography (TLC) of the product gave three spots and desired product was separated using column chromatography using chloroform: methanol (90:10) solvent system as white crystalline solid. Characterization of the product using ^{1}H NMR gave peaks that showed presence of 9, 10-dihydroanthracene-9, 10-α, β succinicacid.

B3. Dimer of 9,10-dihydroanthracene-9,10-α,β,succinic anhydride with 1,3-diaminopropane (4) : Compound (1) (1.5g, 0.0026 mol) was taken in methanol (7 ml) and 1,3-diaminopropane (0.1954g,0.0026 mol) was added. The reaction mixture was refluxed for two hours and methanol evaporated. Thin layer chromatography (TLC) of the product gave three spots and desired product was separated using column chromatography using chloroform: methanol (90:10) solvent system as white crystalline solid. Characterization of the product using ^{1}H NMR gave peaks that were compatible with the proposed structure.

B4. Attempted Synthesis of dimer with 1,3-diaminopropane for 9, 10-dihydroanthracene-9, 10-α, β, succinic acid (5): Phosphorous trichloride (1.18g, 0.0085 mol) was added to a soild sample of compound (2) on water bath and allowed to react till the liberation of HCl from top of condenser stopped.

In another round bottom flask was taken 1,3-diamine (0.392g, 0.0065mol) and K_2CO_3 (0.65g, 0.0046 mol) in acetone (10ml) and water (10ml) mixture in ice bath. After libration of HCl had stopped from the above mixture dichloromethane was added and the reaction mixture added drop wise over a period of 15 minutes. The reaction was left overnight for stirring and work up was done after evaporating the solvent. The organic compound was extracted using methylenechloride and solvent evaporated to get white solid. However, ^{1}H NMR of the compound showed it to be a starting material only.

B5. Fluorescence spectra of the synthesized compounds: Each of the compounds synthesized by above procedure were dissolved in methylene chloride. The UV-Vis and Fluorecence were observed at two concentrations (0.0001mol & 0.00005mol). Prior to fluorescence measurement of the compound the absorption for the solvent was made zero.

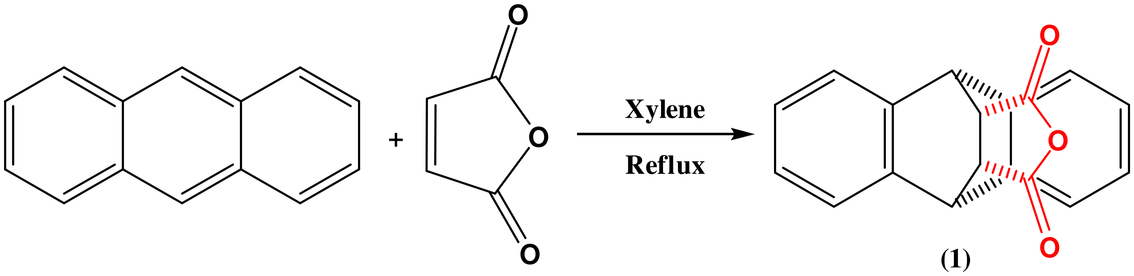


Results and discussion

a) Synthesis

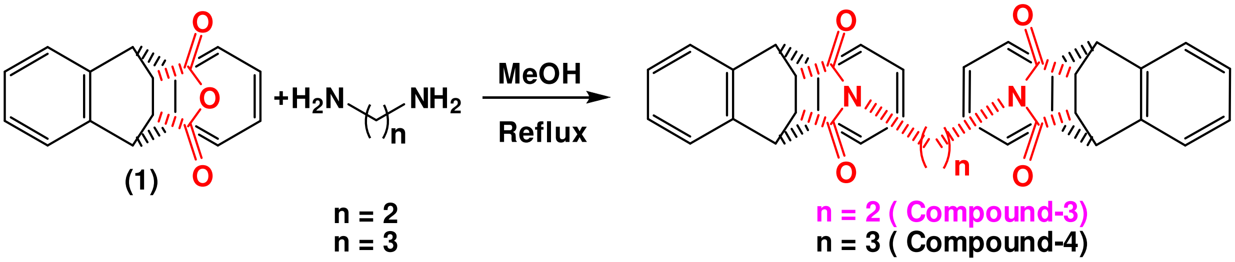
The Diels Alder reaction between anthracene and maleic anhydride was carried out in xylene . **(Scheme-4)** The reaction mixture after refluxing for two hours gave product, identified by TLC, as 9, 10-dihydroanthracene-9, 10-α, β-succinic anhydride **(1)**. In this reaction anthracene served as the diene and maleic anhydride was the dienophile. The crude product obtained after filtration was purified by washing with chilled methanol to remove any starting materials. The white solid was recrystallized by dissolving in methanol. Melting point of the product observed was matching with the authentic sample.

Scheme-4



The compound **(1)** synthesized above was subjected to amidation with 1,2-diamino ethane and 1,3-diamino propane respectively in two separate reactions using methanol as solvent. **(Scheme-5)** The reactant amine in this reaction also acts as base to attack the carbonyl carbon of the anhydride there by making an amide bond. The resulting intermediate again undergoes dehydration to form a lactone. A mixture of products were formed in case of 1,3-diamine as confirmed by TLC. The products were separated using column chromatography with chloroform methanol as the solvent system. The compound obtained **(Compound-4)** was

Scheme-5

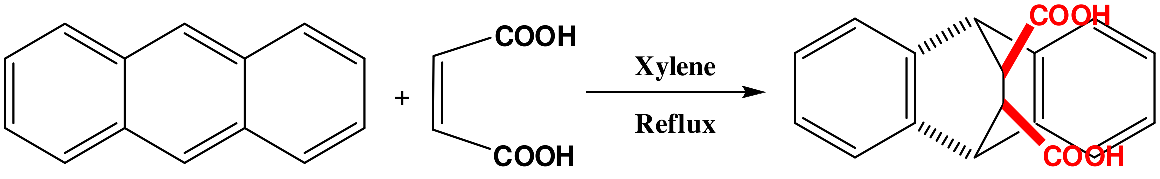


identified as dimer of 9, 10-dihydroanthracene-9, 10-α, β-succinic anhydride **(4)**.The ^{1}H NMR spectra of the compound shows multiplet at 7.3 ppm due to aromatic protons. The multiplet at 3.06-3.15 represented CH_2 groups attached to two N of the dimer. The middle methylene group of the dimer was represented by another multiplet at 2.0 ppm.

The reaction attempted with 1,2-diamino ethane for the synthesis of compound **(3)** showed the presence of starting compound **(1)**. Perhaps the steric hindrance offered by the phenyl groups of the anthracene ring was responsible non occurrence of this reaction. The purified compound showed very clear spectra of the starting material **(1)** with two doublets at 3.5 and 4.5 ppm respectively.

In an attempt to synthesize a cavity based dimer of anthracene, we attempted the synthesis initial bicyclic compound namely of 9,10-dihydroanthracene-9,10α,β succinic acid **(3)** using similar conditions as reported for compound **(1)** above **(Scheme-6)**. The dinophile malic anhydride was replaced with malic acid and refluxed in xylene in the presence of anthracene.

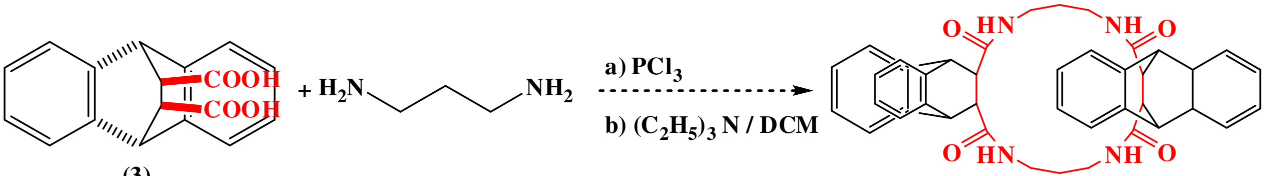
Scheme-6



(3)

An attempt was made to synthesize the cyclic dimer of compound **(3)** by converting it into acid chloride with phosphorous trichloride and then reacting it with 1,3-diamino propane in the presence of triethyl amine and methylene chloride **(Scheme-6)**. Work up of the reaction mixture and evaporation solvent gave starting diacid as the only product instead of anticipated product **(5)**.

Scheme- 6



(3)

(5)

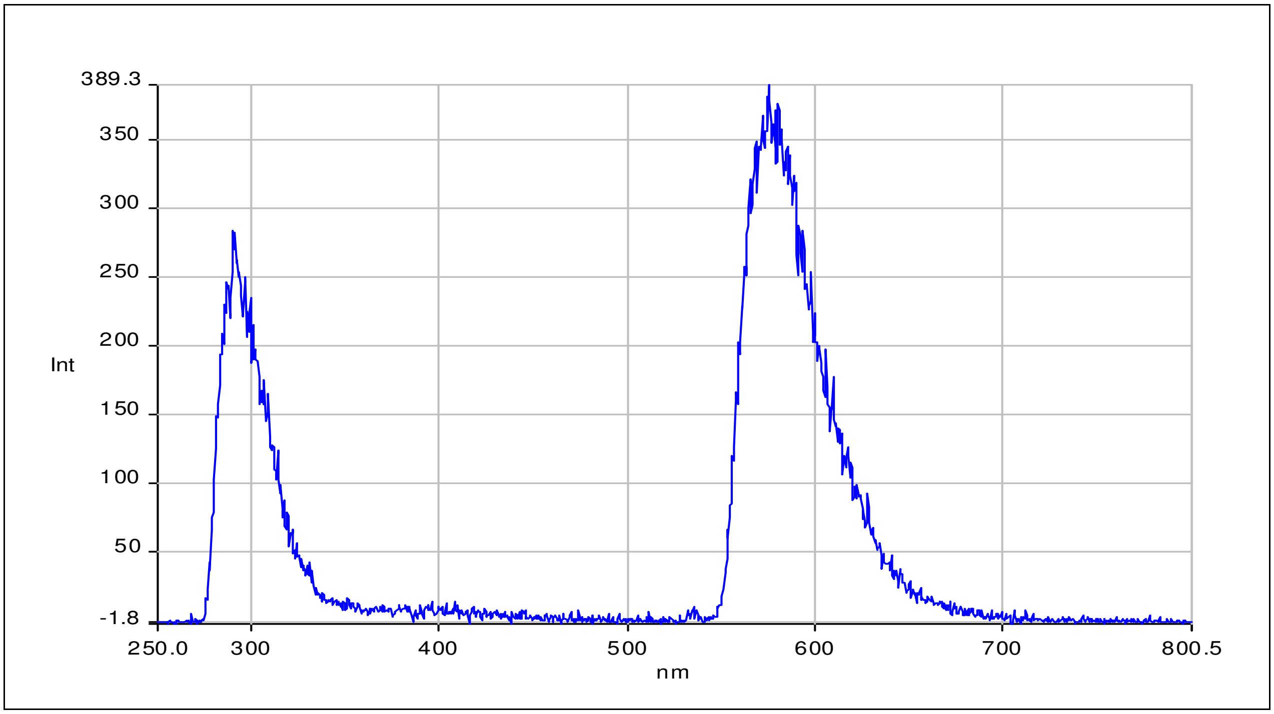
The product in this case was again a mixture of a number of products. However, separation and purification of the product gave only starting material as evident from the ^{1}H NMR peaks obtained at 3.5 and 4.7 ppm respectively for only aliphatic protons in the molecule. It is proposed here that perhaps the compound gets hydrolysed getting adsorbed on silica surface. Above preposition is also supported by the reports that prove hydrolysis of peptide bonds in the presence of metal ions and neutral water[R]. However in later case the rate of hydrolysis is very slow.

b) Fluorescence study of synthesized compounds

All the synthesized compounds were subjected to fluoresence studies at concentrations 1x 10-3 and 0.5 x 10-3 M concentrations using methylene chloride as solvent. To ensure complete dissolution of compounds in the solvent they were kept in ultra sound bath prior to study. After neutralizing the fluorescence due to solvent, the compounds were subjected to the desired study.

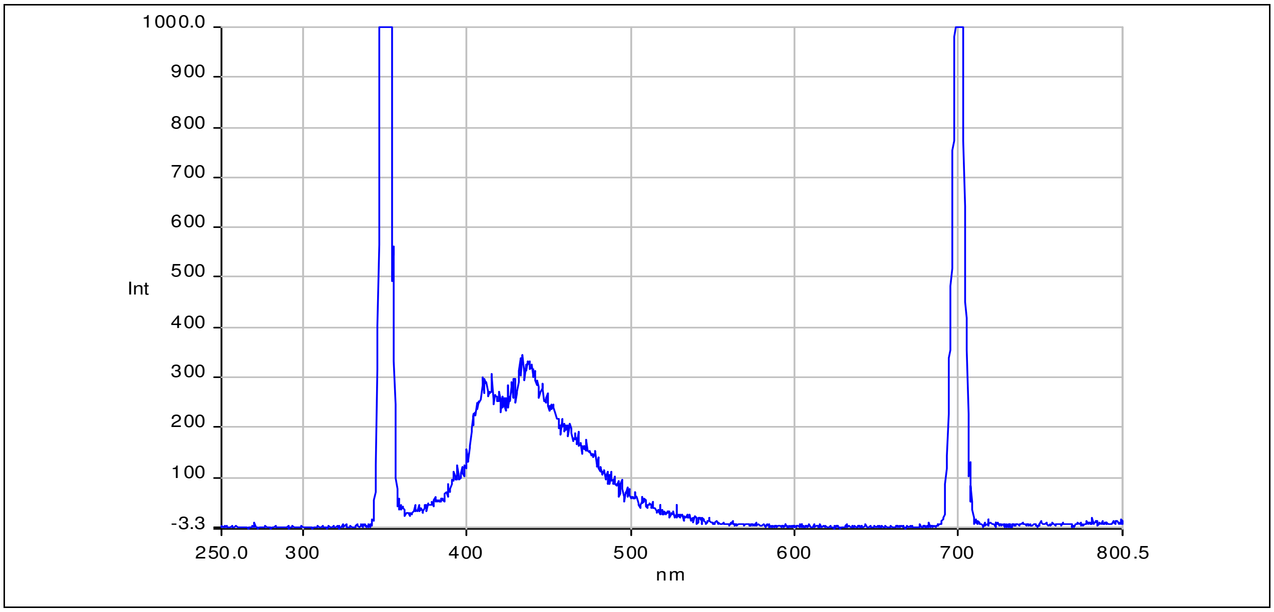
Both Diels-alder products compound **(1)** and **(2)** gave similar spectra where excitation peak and emission peaks were observed at 280 and 570 nm respectively **(Figure- 5)**. In case of

Figure-5: Fluorescence spectra of Compounds (1)



compound **(4)** decreased intensity the fluorescence was observed with excitation and emission spectra overlapping with each other , the maxima for which was observed at 410 and 440 nm respectively **(Figure-6)**. The quenching of fluorescence in case of compound **(4)** may be attributed to the absence of a pair of electron in amide bond formation as compared to malic anhydride derivate, where due to two lone pair of electrons on oxygen intense emission and excitation peaks are observed.

Figure-6: Fluorescence spectra of Compounds (4)



All the other compounds obtained, subjected to fluorescence studies gave a spectra similar to that of compound **(1)** and **(2)** thereby confirming the presence of starting material. Perhaps an alternative synthetic strategy needs to be worked for successful synthesis of compounds attempted in this study.

Conclusion

An attempt was made to synthesize the dimmers of Diels- Alder reaction from anthracene and malic anhydride. From the targeted three compounds that were attempted synthesis of only one was successful. The studies with the synthesized compound showed quenching of fluorescence due to non availability of free electron pairs.

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