REGIOSELECTIVE BROMINATION OF NAPHTHALENETETRACARBOXYLIC DIANHYDRIDE AND SYNTHESIZING ITS DERIVATIVES FOR DNA BINDING STUDIES

A thesis submitted

in partial fulfilment for the degree of

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(Chemical Science)

by

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April 2014

Dedicated to

beloved Bhagawaan

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CERTIFICATE

I hereby certify that the work described in this thesis entitled "Regioselective bromination of naphthalenetetracarboxylic dianhydride and synthesizing its derivatives for DNA binding studies" has been carried out by **Ms. Y. V. Suseela** under my supervision at the Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India and that it has not been submitted elsewhere for the award of any degree or diploma.

Dr. T. Govindaraju

(Research supervisor)

DECLARATION

I hereby declare that the matter embodied in this thesis "Regioselective bromination of naphthalenetetracarboxylic dianhydride and synthesizing its derivatives for DNA binding studies" is the result of investigations carried out by me under the supervision of Dr. T. Govindaraju at the New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India and that it has not been submitted elsewhere for the award of any degree or diploma. In keeping with the general practice in reporting the scientific observations, due acknowledgement has been made whenever the work described is based on the findings of other investigators. Any omission that might have occurred due to oversight or error in judgement is regretted.

Suseela Y.V.

(Y. V. Suseela)

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PREFACE

The thesis entitled "**Regioselective bromination of naphthalenetetracarboxylic dianhydride and synthesizing its derivatives for DNA binding studies**" is divided into three chapters as follows

Chapter 1: Introduction

Brief overview of chemical structure, photophysical properties and synthesis of naphthalene diimides (NDIs) and their applications in various fields. Introduction about DNA binding small molecules and various characterization techniques.

Chapter 2 : Effective brominating reagents for naphthalenetetracarboxylic dianhydride and preparation of imide- and core-substituted naphthalene dimides.

Describes an elegant regioselective bromination of NDA using dibromohydantoin (DBH) and tribromoisocyanuric acid (TBCA) as new reagents and functionalization of obtained brominated NDAs. Discussion includes photophysical properties of imide- and core-substituted NDIs and characterization of all compounds through various spectroscopic techniques.

Chapter 3 : Synthesis of NDI derivatives for DNA binding studies.

Describes the synthesis of NDI derivatives with benzimidazole moiety as a side chain of varying lengths. The effect of these NDI derivatives on DNA groove binding was studied using DAPI displacement technique.

Table of Contents

Chapter 1

Introduction

1.1. Rylene diimides

Functional π-conjugated organic systems have drawn considerable attention owing to their numerous applications in wide areas ranging from electronics to biomedicine.[1] Rylene tetracaboxylic diimides shown in Figure 1.1 are class of robust polycyclic aromatic molecules with excellent thermal and oxidative stability, high electron affinities, high electron mobilities and therefore, promising candidates for a variety of organic electronics applications.[1] Interest in rylene diimides such as those based on benzene and naphthalene stems, arrived from early observations of electron transfer behavior and the ability to tune molecular electronic properties by well-established organic chemistry protocols, either through variation of substituents on the imide nitrogen atoms or on the rylene skeleton.[2] The three simplest rylene diimde systems pyromellitic diimides, 1,4,5,8-naphthalenetetracarboxylic diimide (NDI), perylene-3,4,9,10-tetracarboxylic diimide (PDI) are shown in Figure 1.1. The most intensively studied systems are NDIs and PDIs. Among all rylene diimides, NDIs show significant implications in biomedicine, organic electronics and materials science due to their remarkable electronic, spectroscopic and self-assembly properties.[3,4]

1.1.1. Naphthalene diimides (NDIs)

NDIs (also known as naphthalene carbodiimides) have attracted much attention due to their propensity to form n-type semiconductor materials.[5] NDIs have shown extensive applications in materials chemistry including the formation of organized structures (films, nanotubes etc.,) that could pave the way for constructing electronic devices such as field effect transistors,[6] sensors,[7] energy and electron transfer,[8-11] host guest chemistry including foldamers,[12,13] and ligand-gated ion channels.[14] NDIs can act as supreme components for the creation of self-assembly systems (e.g. Catenanes, rotaxanes and barrels) and materials.[15,16] The desirable electronic, spectroscopic and enhanced solubility properties lead to the preference for NDIs over pyromellitic diimides and perylene diimides. In general, NDIs can assist better fabrication properties than PDI dyes (Figure 1.1). NDI has also extended its applications in biological and medical fields highlighting their versatility.[17]

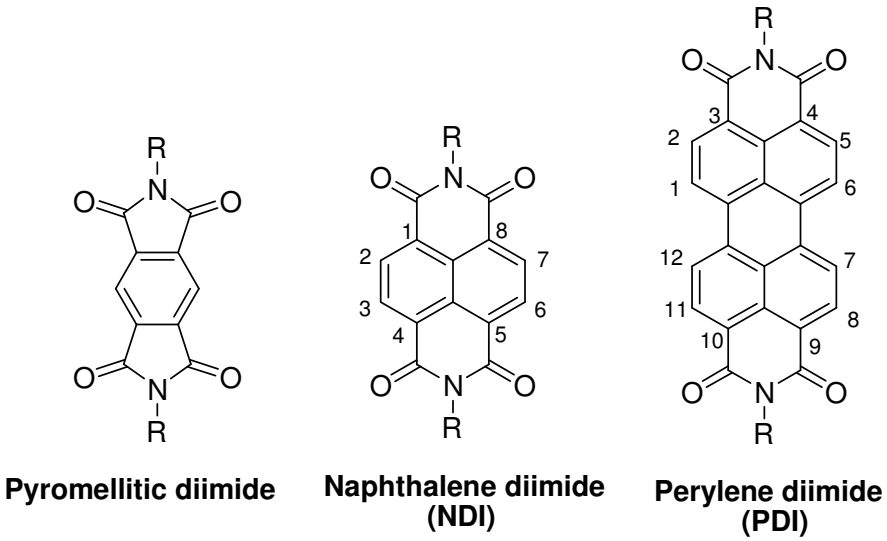
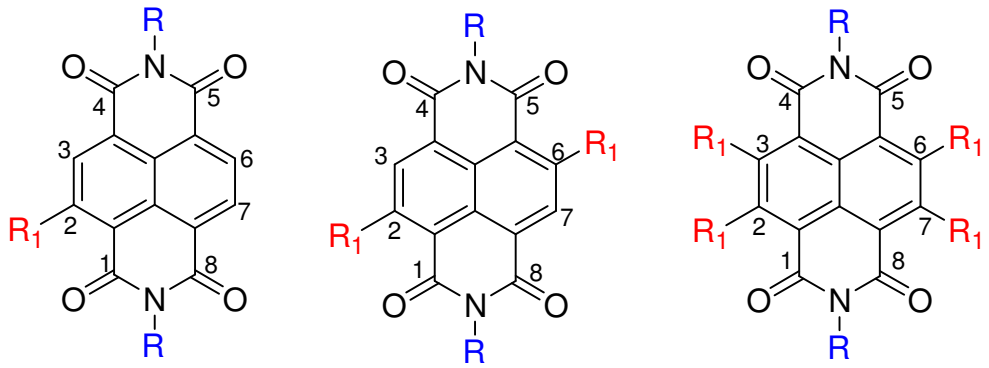


Figure 1.1 Chemical structures of some of the rylene diimide molecules

1.1.2. Core functionalized NDIs

Functionalized NDIs can be prepared from naphthalenetetracarboxylic dianhydride (NDA) which are primarily modified into NDIs through imide substitution and followed by core substitution of naphthalene core. Functionalization through diimide nitrogens has minimal influence on the optical and electronic properties, although they can be used to control solubility, aggregation, and intermolecular packing in the solid state, whereas the naphthalene core substitution in NDIs significantly alter their electrical, optical, and redox properties.[18-20] Core substituted NDIs (cNDIs) have found popularity in the latter half of the 20th century due to their remarkable properties. In the early 1930's, the pioneering work of vollman *et.al.,*[21] involves NDIs bearing aryl amino core substituents, which however lacked remarkable optical properties. It might be the reason why cNDIs were not studied deeply until recent developments by the Würthner and Matile groups,[22,23] and later by various other groups who developed better synthetic protocols.[24] cNDIs are those containing functionality at positions 2, 3, 6, 7 on the naphthalene core, as shown in Figure 1.2.[25,26] At present, there are many ways to prepare core-substituted NDIs, especially with alkyl amino- and alkoxy-core substitution resulting in desirable optical properties such as bright coloring and rainbow fluorescence, both

of which can be tuned over a wide wavelength range by varying the electron donating ability of the core substituents.[27,28]



R - alkyl or aryl groups
R1 - NHR_2, OR_2, or SR_2

Figure 1.2 General structures of cNDIs

Functionalization of NDIs directly on the naphthalene core at 2- or 2, 6-positions via C–C linkages open up new strategies for solar cell applications.[24] In this regard, many groups have devoted their efforts towards core-substitution of NDIs with electron withdrawing and/or electron donating moieties such as aryl,[29-31] thiophene,[32] and cyano[29,33] utilizing Suzuki coupling reactions.[34] The nature of molecular structure determines the supramolecular assembly of cNDIs. The high π-acidity is ideal for their ability to form face-to-face π-stacks.[35,36] Mostly aromatic rings are π-basic, with electron clouds above and below the aromatic plane. An electron deficient aromatic plane produces a negative quadrupole moment Q_{zz} as shown in Figure 1.3. The computed quadrupole moment Q_{zz} for unsubstituted NDIs is -18.6 B (Buckinghams) whereas introduction of two cyano acceptors in the core of cNDIs produces with exceptional π-acidity Q_{zz} of -39.2 B. The elusive tetracyano cNDI possess the highest predicted Q_{zz} of -55.5 B.[24]

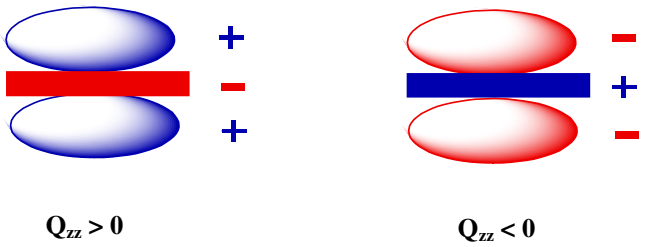


Figure 1.3 Schematic view of π-acidic (left) and π-basic (right) aromatic rings between their electron-poor (blue) and electron-rich (red) π-clouds.

1.1.3. Photophysical properties

The combination of electron donating substituents in the core with electron withdrawing imides in NDIs gives versatile push–pull chromophores, whereas electron acceptors produce exceptionally π-acidic systems. The HOMO-LUMO transition of the parent NDI is polarized along the long molecular axis (i.e. between the two imide groups), whereas it is polarized perpendicular to this axis for the core-substituted derivatives.[22] This aspect arises from a 'new' HOMO orbital that is created from the interaction of electron donating 2,6-substituents in the naphthalene core. Therefore, the UV/Visible absorption spectra of cNDIs can be understood based on two allowed optical transitions, one at 350 nm that is characteristic for all NDIs and a new long-wavelength charge transfer transition that is polarized perpendicular to the transition polarized along the long molecular axis and whose spectral position is highly dependent on the 2- and 6-electron donor substituents as shown in Figure 1.4. Varying electron donor substituents on the naphthalene core of NDI has resulted in wide visible range spectral absorption (Figure 1.4).

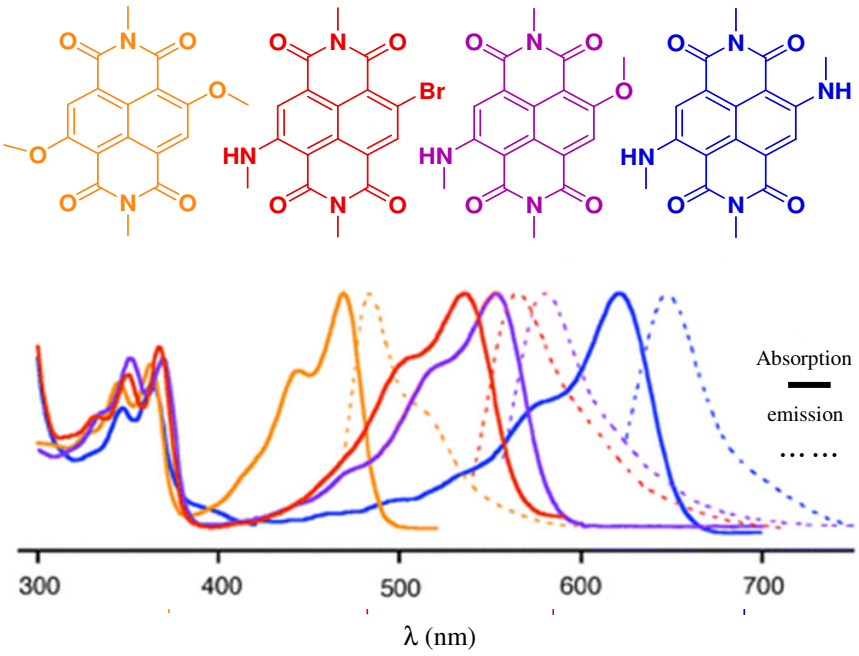
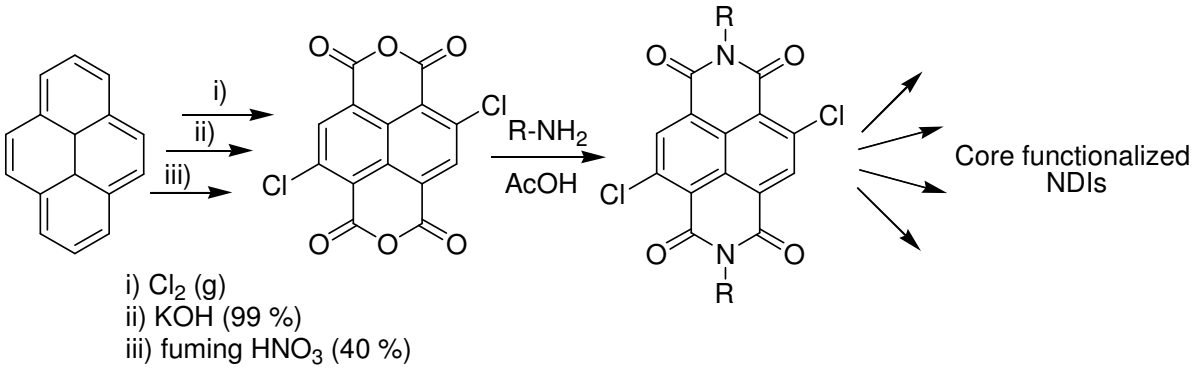


Figure 1.4 Absorption (solid line) and emission spectra (dashed line) of cNDIs.[23]

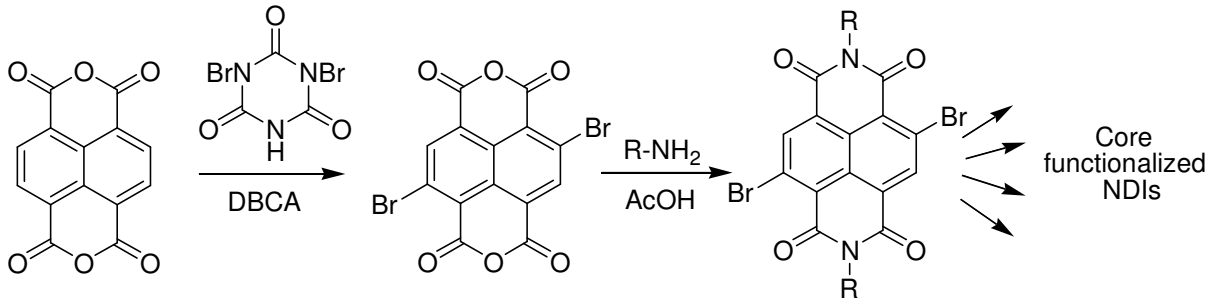
1.1.4. Synthetic approach

The classical synthetic approach to cNDIs involve oxidation of pyrene as shown in Scheme 1.1, developed by vollman *et.al.*[21,23] This synthetic protocol includes use of strong acids,



Scheme 1.1 The classical synthetic route to cNDIs

strong bases and chlorine gas. The resulting dianhydride with two chlorides in the core is then converted into diimide by reacting with amines in presence of AcOH to prevent core substitution. From these diimide intermediates, cNDIs are accessible by nucleophilic aromatic substitution. The above conventional synthetic method is not suitable to prepare cNDIs due to harsh reaction conditions required for the initial pyrene oxidaton. Recently, alternating synthetic routes to cNDIs have been developed to avoid harsh reagents like chlorine gas in large amounts as shown in Scheme 1.2.[25-28] In this method, the commercially available NDA is brominated with dibromoisocyanuric acid (DBCA) leading to a mixture of brominated NDAs, which are directly converted into diimides by condensing with amines under acidic conditions. Although the DBCA method of preparing cNDIs is a facile route, still there is a need for improving



Scheme 1.2 An alternative synthetic approach to cNDIs.

bromination efficiency and simple workup procedure to achieve large scale preparation of cNDIs. Hence, the present work illustrates the development of efficient and cost effective brominating reagents for regioselective bromination of NDA under mild conditions which will be discussed in detail in Chapter 2. The synthesized bromo-derivatives of NDA act as starting precursors for further core functionalization to obtain cNDIs.

1.1.5. Applications

Research on core-substituted NDIs has experienced rapid growth due to advances in their synthesis and corresponding optical properties. cNDIs could be used to prepare novel

structures for applications in organic electronics, bio-supramolecular chemistry, biomedicine and materials science.[37,38] The extended conjugation achieved in cNDIs has gained applications in organic solar cells.[39] cNDIs have provided an intellectual grounding in molecular recognition, particularly in DNA intercalation,[40] acid/base pH responses,[41-43] anion sensing, and for the assembly of complex molecular arrays based on conjugated molecules. cNDI derivatives have also been used as potent G-quadruplex ligands for intercalation, targeting cancer cells.[44,45] The ability of achieving electron transport and absorption in the entire visible wavelength range make cNDIs ideal for the construction of artificial photosystems.[46] These wide range applications signifies the contribution of core-substituted NDI systems towards development of many scientific disciplines.

1.2. DNA recognition

Deoxyribonucleic acid (DNA) is an important biomacromolecule that encodes genetic information responsible for functioning and development of most of the living organisms. The double helical structure of DNA was first discovered by James Watson and Francis Crick in 1953 using X-ray diffraction studies.[47] DNA is very long, thread like double stranded polymer, made up of linear array of monomers called nucleotides. Nucleotides have three primary components viz. nitrogenous base, a ribose carbon sugar and a phosphate group as shown in Figure 1.5. The nitrogenous bases found in nucleotides are monocyclic pyrimidines and bicyclic purines. Purines are adenine (A) and guanine (G) whereas pyrimidines are cytosine (C) and thymine (T). The two common base pairs that exists are A=T and G≡C by means of Watson and Crick hydrogen bonding as shown in Figure 1.5. The right handed double helix has two polynucleotide chains wound antiparallel to each other and linked by Watson-Crick (A·T and G·C) base pairs that stack on one another. The sequence of these bases determines the biological information in a strand of DNA. The DNA double helix has two grooves of unequal width due to the way the base pairs stack and the sugar-phospahte backbones twist. These grooves are called major and minor grooves (Figure 1.5). In addition to the common B-DNA conformation that exist under cellular conditions, there are two main conformations *i.e.,* A-DNA and Z-DNA, which are left handed form. A number of non-B DNA structures have been discovered since past 4 decades such as G-Quadruplex, i-motif,

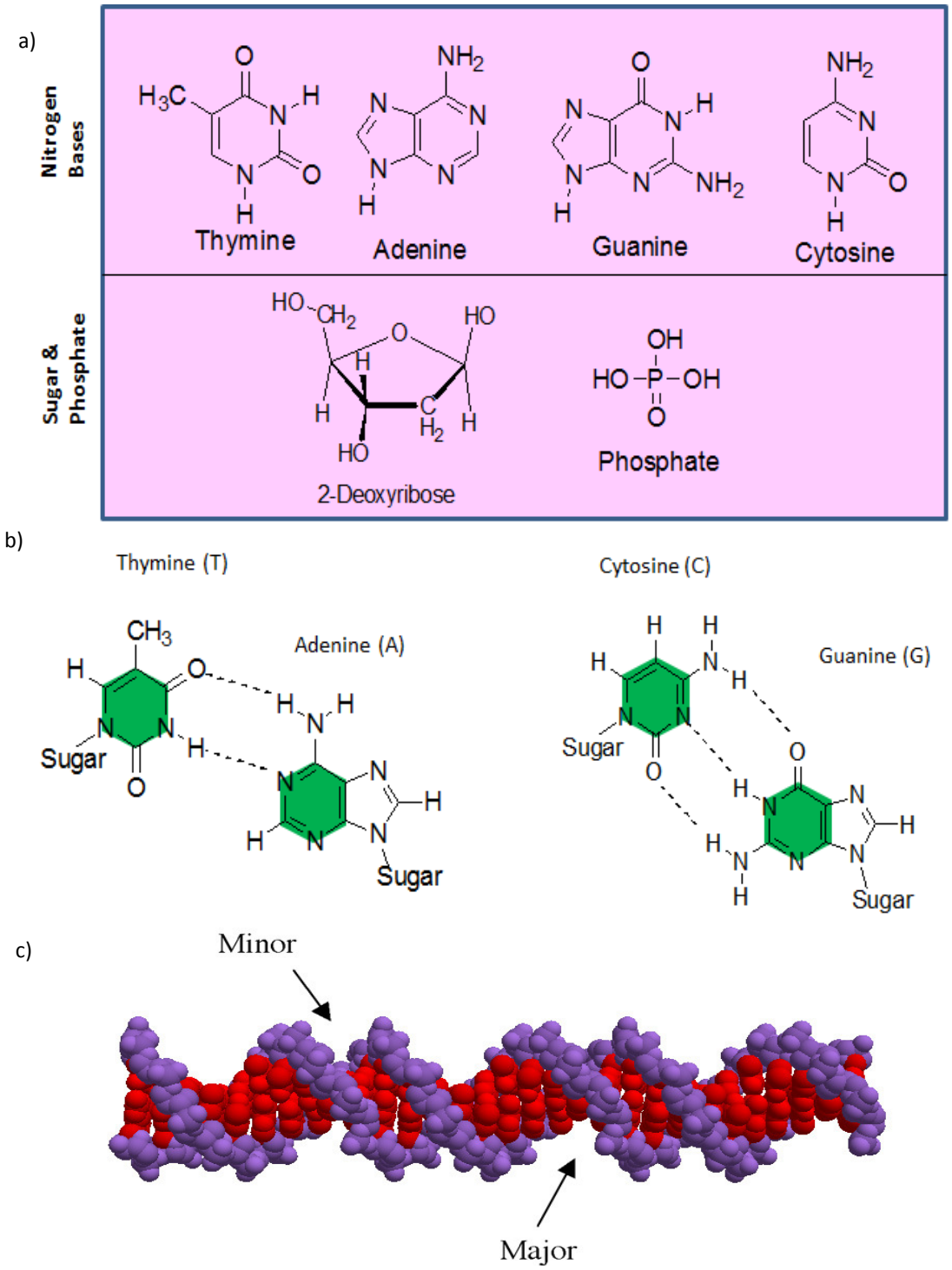


Figure 1.5 a) Components of DNA b) A=T, G≡C base pairs c) DNA double helix structure with minor and major grooves.

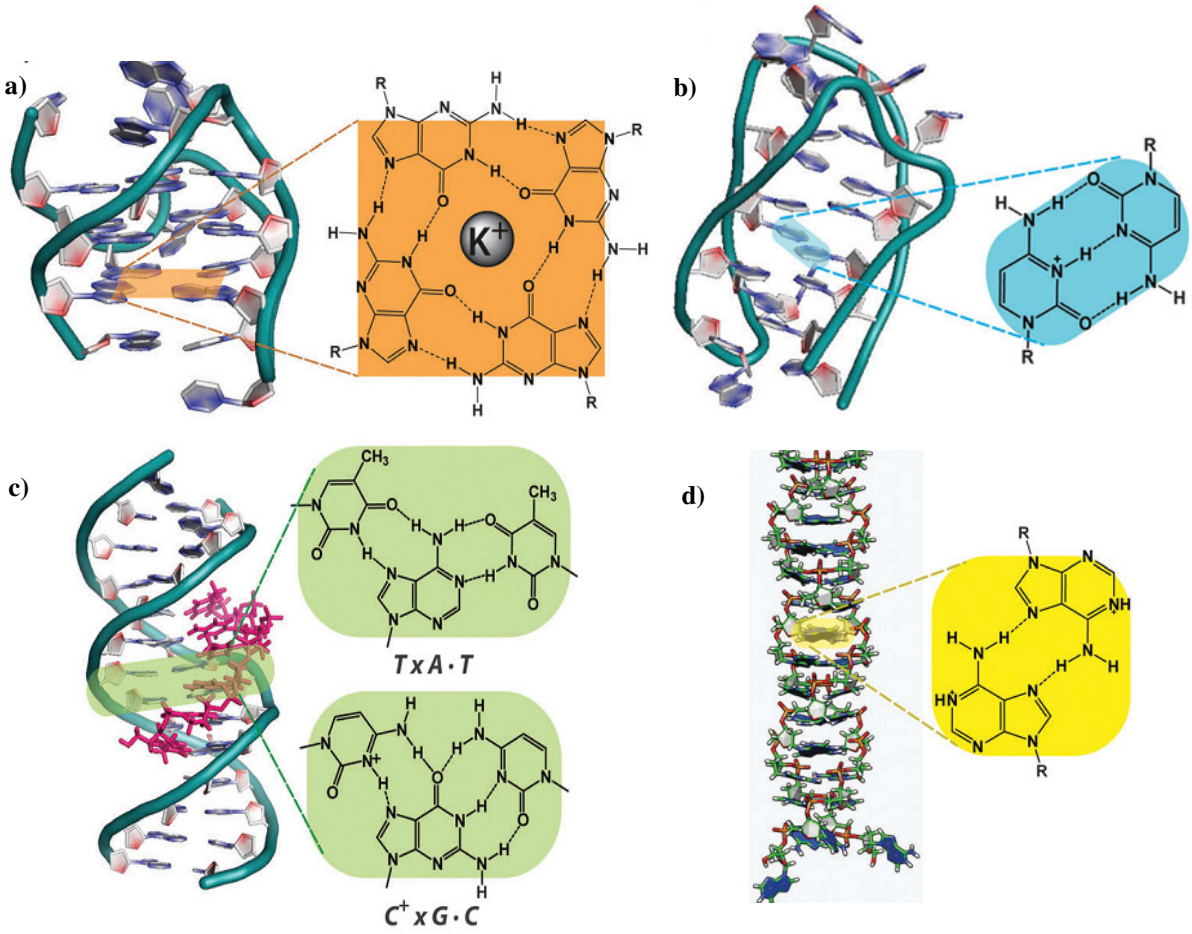


Figure 1.6 Schematic view of non-B DNA structures and their molecular structures a) G-quadruplex and G-tetrads composed of four guanine bases (orange) b) i-motif (i-tetraplex) and hemiprotonated C:C+ base pair (cyan) c) parallel triplex consists of T × A·T and C+ × G·C triads (light green). Triplex forming oligonucleotide (TFO), bound at the major groove of the DNA duplex, is colored in pink d) A-motif and A:A base pair (yellow).[48]

triplex, poly (dA) duplex (A-motif), hairpin etc., as shown in Figure 1.6.[49,50] The term "non-B DNA structures" refers to all DNA conformations other than the conventional right-handed Watson-Crick (double helix) structure. These are formed mainly from repetitive DNA sequences that have potential to fold under certain conditions. In order to form these structures, DNA strands make unusual base pairing such as Hoogsteen base pairs that are

different from Watson-Crick base pairs. For instance, in the case of G-quadruplex, i-motif, triplex and A-motif, the H-bonding between G-G, C-C, G-G-C and A-A respectively is known to be involving Hoogsteen hydrogen bonding that play an important role in stabilizing various conformations. Many research groups have studied conformational dynamics of non B-DNAs as they are regarded as a fascinating material for the nanotechnology[48,51] apart from their biological importance and role in biomedicine.[52-54]

1.2.1. DNA as therapeutic target

DNA is a self-organized chemical code. The right arrangement of the nucleobases provides chemical instructions in the form of genes. Approximately 30,000-40,000 genes are found in human.[55] The obligation of understanding the complex relationship between DNA (gene expression) and the well-being is of particular importance to molecular biology and human health. The unconventional gene expression, either in the form of harmful products or abnormal regulation, is responsible for a variety of deadly diseases[56] for instance cancer, which is of genetic origin. With increasing cases of cancer in recent years, synthesis of new and selective molecules which have antitumor (antiproliferative) activity has been the most important objective of medicinal chemistry. Therefore, designing molecules, to regulate and study the process of gene expression and organization is of prime interest.[57] Hence, small molecular ligands that are able to bind with DNA are pivotal for the development of diagnostic and therapeutic agents targeting DNA. Till date, approximately 130 different kind of antitumor drugs have been approved by food and drug administration (FDA) for the use in treatment of cancer. Some of the earliest compounds discovered to act on DNA were the Sulfur mustards, but their high toxicity provoked renewed interest to a search for less toxic and more efficient compounds.[58] Drugs used in chemotherapy treatment interact with DNA through different mechanisms. In 1960s, some compounds with cytotoxic activity were discovered to act as anticancer agents, although their mechanism of action was unknown. Interestingly, after Leonard Lerman's proposal in 1961, the occurrence of non-covalent interactions suggested an intercalative process.[59] Small molecule–DNA interactions are classified into two major categories, intercalation and groove binding among other modes (Figure 1.7). The binder preferences depends on the structure of molecules interacting with

DNA and the structure of DNA. Small differences in the structure of an interacting molecule may affect the binding mode and stability of the molecule/DNA complex.

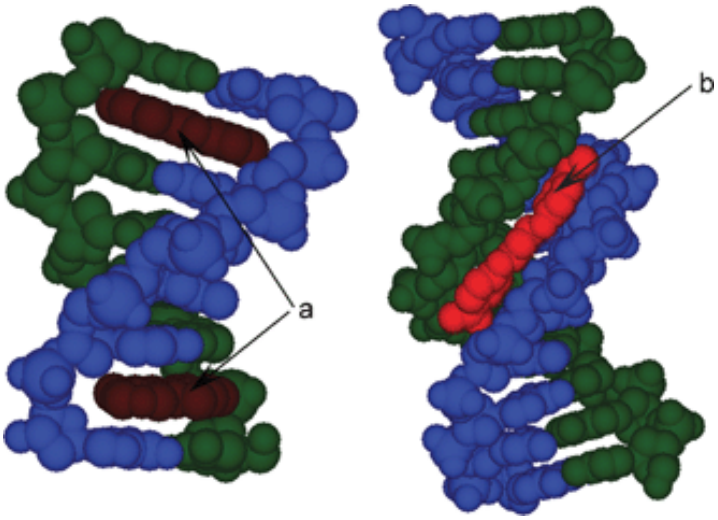


Figure 1.7 Schematic representation of intercalation and groove binding.[60]

1.2.2. Intercalating agents

DNA intercalators are of great importance in treating tumours. Intercalation involves the insertion of a planar molecule between DNA base pairs, which results in decrease in the DNA helical twist, stiffening and lengthening of DNA. The driving forces for this binding mode are dipole-dipole interactions and π-stacking with aromatic nucleobases. These favorable contributions result in association constants of 10^5 to 10^{11} M^{-1}.[61] Intercalation has a significant influence on the DNA structure, because the DNA has to unwind or open up partially so that the intercalator fits between two base pairs. It has been observed that structural changes of DNA upon intercalation leads to limited access to the neighbouring binding pocket for steric reasons. Since the discovery of the DNA intercalation process by Lerman, thousands of organic, inorganic octahedral [particularly ruthenium(II) and rhodium(III)] and square-planar [particularly platinum(II)] compounds have been developed as potential anticancer agents.[62] Variety of organic intercalators which are highly potent and effective drugs as well as certain carcinogenic compounds includes anthracenes, acridines, anthraquinones, phenazines, quinolones, phenanthridines, phenantrolines, pyrene derivatives etc., have been reported.[63]

There are two modes for intercalation of small molecules, viz., classical intercalation and threading intercalation modes. Classical intercalators shown in Figure 1.8 are those without any bulky

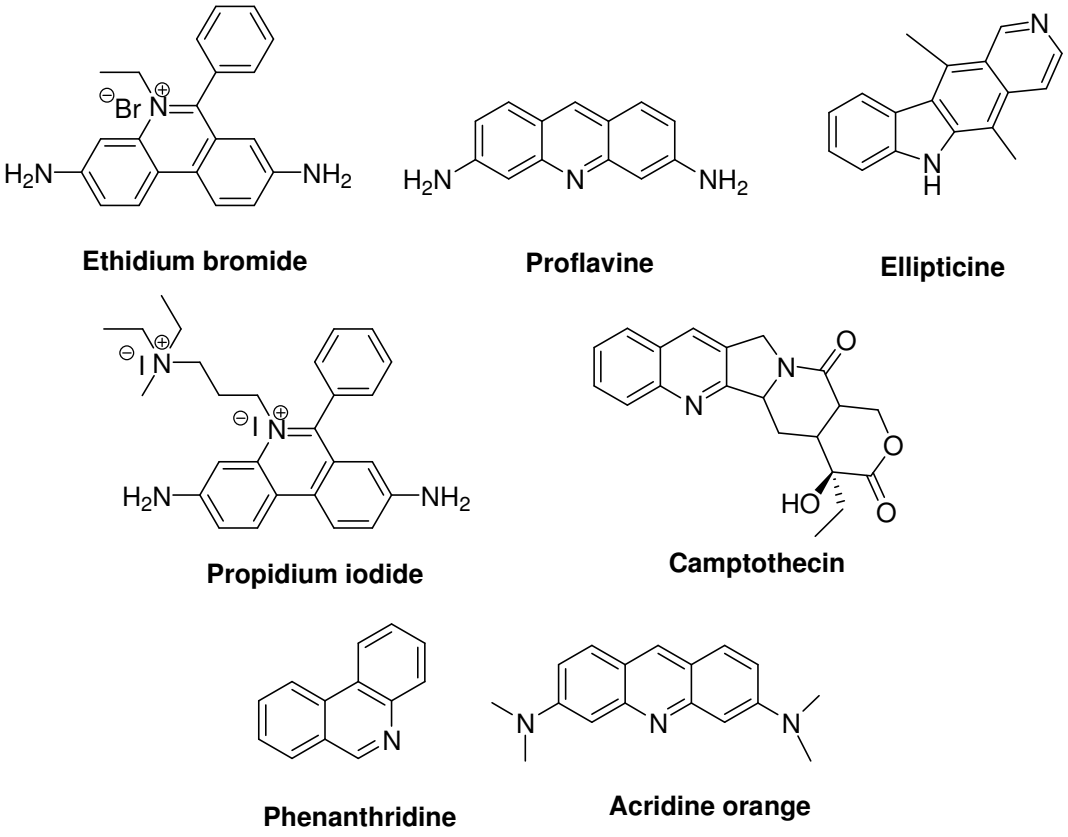
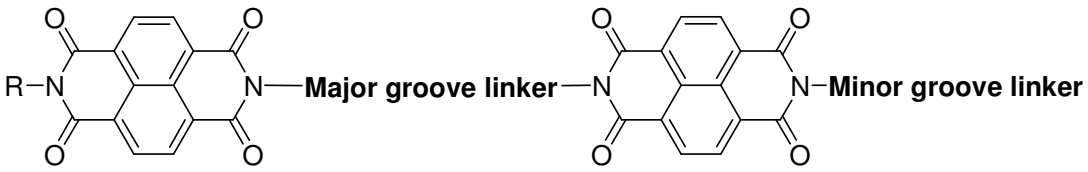


Figure 1.8 Chemical structures of classical intercalators.

substituents or side chains that can presumably intercalate without any binding intercalation in the minor or major grooves, whereas threading intercalators carry bulky substituents next to intercalation moiety that can interact with minor or major grooves of DNA. Since peptides exhibit good pharmacokinetic and biological properties there is an increasing interest in the synthesis of intercalators containing peptide chain which can bring additional element of sequence specificity. Higher affinity is typically displayed by ligands that contain multi-intercalator scaffolds connected by peptide linkers. In view of this, Iverson group has been

involved in developing bis-intercalators and poly-intercalators to target duplex DNA. They developed poly-intercalation system in which intercalating naphthalene diimide (NDI) units are connected by flexible peptide linkers that alternate between the minor and major grooves of DNA when bound as shown in Figure 1.9.[64] They have reported longest sequence specific tetra- and hexa-intercalators with extremely slow dissociation rates and long half-life.[65]



Major groove linker - Gly_3Lys / adipic acid
Minor groove linker - β-Ala_3Lys

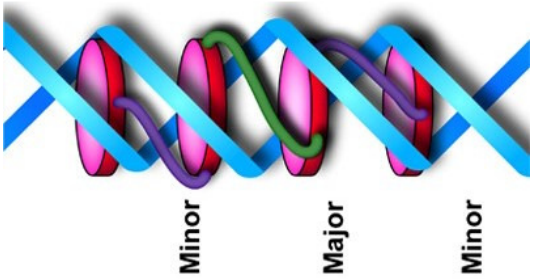


Figure 1.9 Poly-intercalator with NDI (intercalating moiety) and linkers (for minor and major groove binding).[65]

1.2.3. Groove binding agents

Canonical B-form of DNA is characterized by a wide (12 A^o) and shallow major groove and a narrow (4-6 A^o) and deep minor groove as shown in Figure 1.5c.[66] The edges of the base pairs present different hydrogen bond donor and acceptor combinations to the major and minor grooves in the DNA double helix. The minor groove is particularly rich environment for recognition by small molecules. Some of the well-known groove binders are shown in Figure 1.10. Netropsin and distamycin A[67] are natural products possessing amido groups and N-methyl pyrrole rings, that interact with A·T rich regions of DNA in the minor groove through hydrogen bonding and hydrophobic interactions. Groove binders are typically positively

charged 'crescent'-shaped linked aromatic rings. Many groove binders preferentially bind A·T-rich duplexes, because grooves which contain G·C base pairs, are sterically hindered by the guanine amino functionality at C-2. Dervan and co-workers[68] developed minor groove binding distamycin derivatives using hairpin polyamides that can recognize DNA sequence specifically with high affinities. DNA recognition depends on specific contacts of backbone amide hydrogens with edges of DNA bases. Hoechst 33258 and DAPI (Figure 1.10) are often used as fluorescent stains, that binds to minor groove of A·T rich sequences.

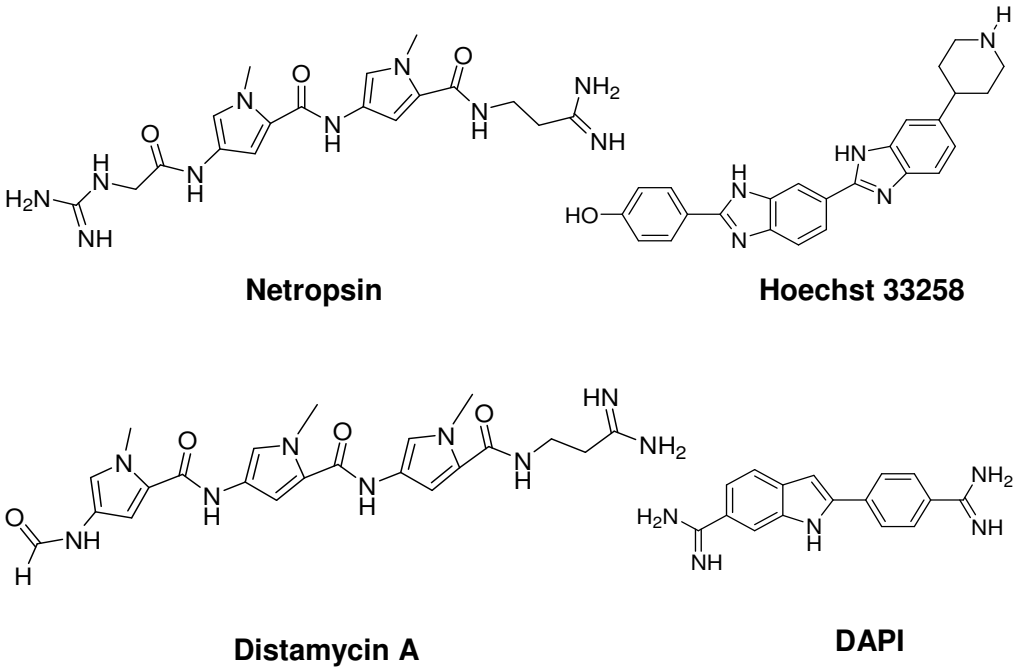


Figure 1.10 Chemical structures of DNA groove binders.

1.2.4. Characterization techniques

The binding mode of small molecule-DNA is important to investigate the properties and discover of new DNA binding agents. The main experimental task is differentiation between intercalation, groove binding, and any other binding mode. A battery of *in vitro* assays readily distinguishes between DNA intercalation and groove binding. Simple methods like thermal

denaturation of DNA in the absence and presence of small molecules, changes in UV absorption spectra and fluorescence properties are extensively used for determining the small molecule-DNA interactions but these techniques are usually not sufficient to give us clear results about binding mode. More accurate binding mode can be obtained from advanced experimental techniques like NMR spectroscopy and X-ray diffraction studies.

1.2.4.1. Thermal denaturation studies

UV-absorption is a sensitive and convenient way to monitor the melting behaviour or denaturation of DNA. The temperature at which half the DNA has transfered into a single stranded DNAs is known as the melting point, T_m. Absorption of UV light can be used to measure the extent of denaturation. Measurements are made at a wavelength of 260 nm, which is close to the absorbance maximum for nucleobases. Single stranded DNA absorbs 12% to 40% more light than double stranded DNA at 260 nm. A plot of the change in absorbance of a DNA solution versus temperature is called a melting curve (Figure 1.11). The sigmoidal shape of the melting curve indicates that denaturation is a cooperative process. The T_m gives information about stability of DNA in presence and absence of ligand. Due to different stability of the DNA-ligand complex the sigmoidal curve can be shifted to higher or lower temperatures compared to that obtained with the DNA alone.[69]

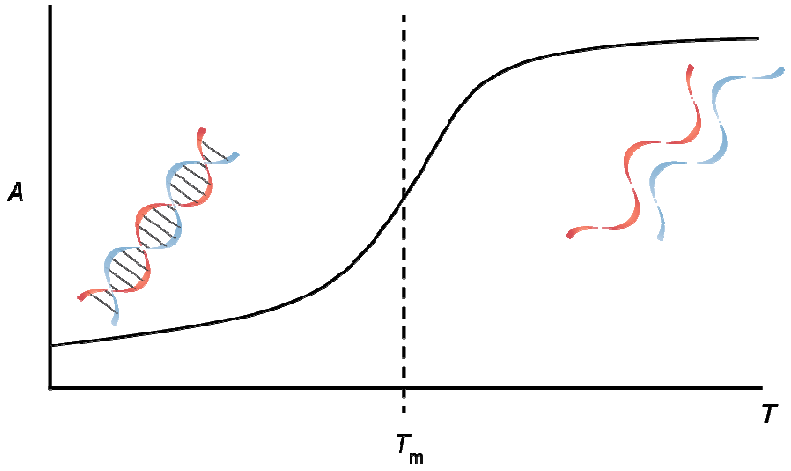


Figure 1.11 Thermal denaturation (melting) curve (plot of absorbance versus temperature) of DNA duplex.

1.2.4.2. UV/Visible spectroscopy

In a complex with DNA, the ligand molecule is positioned in an environment which is different from that of the uncomplexed molecule in solution. Molecules especially solvatochromic compounds such as organic dyes, usually have different absorption properties in complexed and uncomplexed forms. Thus, on addition of DNA to a solution of intercalator or groove binder, a shift in the absorption maximium to longer wavelengths (bathochromic shift or red shift) and a decrease of the absorbance (hyperchromicity) occurs. The data from spectrophotometric titrations can also be used to determine the association constant between the dye and DNA.

1.2.4.3. Fluorescent dye displacement technique

This technique used to establish DNA binding affinity, binding stoichiometry and sequence selectivity.[70] It involves the displacement of intercalator or a groove binder. The classical intercalator ethidium bromide and groove binders DAPI or Hoechst are commonly used for fluorescent dye displacement studies. Ethidium bromide, DAPI and Hoechst are known to show intense fluorescence on association with DNA due to significant suppression of the conformational flexibility and shielding of the dye from solvent molecules. Addition of ligand to intercaltor or groove binder bound DNA solution results in decrease in fluorescence due to displacement of bound intercalator or groove binder. The percentage of decrease in fluorescence is directly related to the extent of binding and affinity of the ligand to DNA under study. Other frequently used methods are viscosity measurements, isothermal titration calorimetry (ITC) and circular dichroism (CD).[71-74] Viscosity measurements can distinguish intercalation from groove binding in some cases.[71] In relative viscosity measurements, a comparative study with standard intercalators and groove binders like Ethidium bromide (Figure 1.8) and Hoechst 33258 (Figure 1.10) respectively are performed, intercalation is clearly distinguished from groove binding.[72] Groove binding typically results in only subtle changes in DNA structure. In contrast, intercalation, in which a planar moiety is inserted between adjacent base pairs, results in a substantial change in DNA structure like unwinding and lengthening, leads to increase in viscosity measurements. Isothermal titration calorimetry

(ITC) is a sensitive technique which can be used to obtain precise thermodynamic data. This technique allows the determination of various binding parameters such as dissociation constant (K_d), enthalpy (ΔH), entropy (ΔS), and the stoichiometry of binding using the standard equation below.[73]

$$\Delta G_{bind} = -RT \ln K_D; \Delta G = \Delta H - T\Delta S$$

CD is not usually observed for achiral ligand molecules. However, when ligand form complexes with DNA they are placed within a chiral environment and give an induced CD (ICD) signal.[74] The appearance of an ICD signal confirms the ligand-DNA interaction and might provide further information about the position of a ligand in its complex with DNA, because the intensity and the phase of the ICD signal depends on the position and orientation of the chromphore relative to the DNA bases. Hence, the small molecule-DNA interactions can be conveniently monitored by employing above discussed techniques and these methods provide useful data for accessing the binding strength and binding mode.

1.3. Present work

The thesis work is mainly focused on developing efficient and cost effective reagents for bromination of naphthalenetetracarboxylic dianhydride (NDA), which will be discussed in Chapter 2. These bromo derivatives serves as precursors for preparing core-functionalized naphthalene diimides (cNDIs). Studies involve characterization and photophysical properties of bromo derivatives of NDIs and cNDIs. Synthesis of NDI derivatives with benzimidazole moiety as side chain for DNA binding studies and their characterization will be discussed in Chapter 3.

1.4. References

1. Zhan, X.; Facchetti, A.; Barlow, S.; Marks, T. J.; Ratner, M. A.; Wasielewski, M. R.; Marder, S. R., Hybrid Supramolecular naphthalene diimide-thiophene structures and their application in polymer electronics. *Adv. Mater.* **2011***, 23*, 268–284.
2. Chen, L.; Li, C.; Mullen, K., Beyond perylene diimides: synthesis, assembly and function of higher rylene chromophores. *J. Mater. Chem. C*. **2014**, *2*, 1938.
3. Bhosale, S.V.; Jani, C. H.; Langford, S., Chemistry of naphthalene diimides. *J. Chem. Soc. Rev*. **2008**, *37*, 331 – 342.
4. Avinash, M. B.; Govindaraju, T., Amino acid derivatized arylenediimides: a versatile modular approach for functional molecular materials. *Adv. Mater.* **2012**, *24*, 3905–3922
5. Katz, H. E.; Lovinger, A. J.; Kloc, C.; Siegrist, T.; Li, W.; Lin, Y, -Y.; Dodabalapur, A., A soluble and air-stable organic semiconductor with high electron mobility. *Nature* **2000,** *404*, 478–481.
6. Brochsztain, S.; Rodrigues, M. A.; Demets, G. L. F.; Politi, M. J., Stabilization of naphthalene-1,8:4,5-dicarboximide radicals in zirconium phosphonate solid materials and thin films. *J. Mater. Chem.* **2002**, *12*, 1250–1255.
7. Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S. J.; J. Yoon., Pyrophosphate-selective fluorescent chemosensor at physiological pH: formation of a unique excimer upon addition of pyrophosphate. *J. Am. Chem. Soc*. **2007**, *129*, 3828–3829.
8. Gosztola, D.; Niemczyk, M. P.; Wasielewski, M. R., Picosecond molecular switch based on bidirectional inhibition of photoinduced electron transfer using photogenerated electric Fields. *J. Am. Chem. Soc*., **1998**, *120*, 5118–5119.
9. Heinen, U.; Berthold, T.; Kothe, G.; Stavitski, E.; Galili, T.; Levanon, H.; Wiederrecht,G.; Wasielewski, M. R. High time resolution Q-band EPR study of sequential electron transfer in a triad oriented in a liquid crystal. *J. Phys. Chem. A.* **2002**, *106*, 1933–1937.

10. Osuka, A.; Yoneshima, R.; Shiratori, H.; Okada, T.; Taniguchi, S.; Mataga, N., Electron transfer in a hydrogen-bonded assembly consisting of porphyrin–diimide. *Chem. Commun*. **1998**, 1567–1568.
11. Ghiggino, K. P.; Hutchison, J. A.; Langford, S. J.; Latter, M. J.; Lee, M. A. -P.; Takezaki, M., A simple dyad exhibiting microsecond charge separation in non-polar solvents. *Aust. J. Chem*. **2006**, *59*, 179–185.
12. Blacker, A. J.; Jazwinski, J.; Lehn, J. -M.; Cesario, M.; Guilhem, J.; Pascard, C., Cyclo-bisintercalands: synthesis and structure of an intercalative inclusion complex, and anion binding properties. *Tetrahedron. Lett*. **1987**, *28*, 6057–6060.
13. Reczek, J. J.; Iverson, B. L., Using aromatic donor acceptor interactions to affect macromolecular assembly. *Macromolecules* **2006**, *39*, 5601–5603.
14. Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S., Ligand-gated synthetic ion channels. *Chem.– Eur. J*. **2005**, *11*, 6525–6532.
15. Fallon, G. D.; Lee, M. A. -P.; Langford, S. J.; Nichols, P. J., Unusual solid-state behavior in a neutral [2] catenane bearing a hydrolyzable component. *Org. Lett*. **2004**, *6*, 655–658.
16. Hansen, J. G.; Feeder, N.; Hailton, D. G.; Gunter, M. J.; Becher, J.; Sanders, J. K. M., Macrocyclization and molecular interlocking via mitsunobu alkylation: highlighting the role of C–H···O interactions in templating. *Org. Lett*. **2000**, *2*, 449–452.
17. Vicic, D. A.; Odom, D. T.; Nunez, M. E.; Gianolio, D. A.; McLaughlin, L. W.; Barton, J. K., Naphthalene- and perylene- based linkers for the stabilization of hairpin triplexes. *J. Am. Chem. Soc*. **2000**, *122*, 8603–8611.
18. Jones, B. A.; Facchetti, A.; Marks, T. J.; Wasielewski, M. R., Cyanonaphthalene diimide semiconductors for air-stable, flexible, and optically transparent n-channel field-effect transistors. *Chem. Mater*. **2007**, *19*, 2703 – 2705.
19. Hu, Y.; Gao, X.; Di, C.-A.; Yang, X.; Zhang, F.; Liu, Y.; Li, H.; Zhu, D., Core-expanded naphthalene diimides fused with sulfur heterocycles and end-capped with electron-withdrawing groups for air-stable solution-processed n-channel organic thin film transistors. *Chem. Mater*. **2011**, *23*, 1204 – 1215.
20. Oh, J. H.; Suraru, S. -L.; Lee, W. -Y.; Konemann, M.; Hoffken, H. W.; Roger, C.; Schmidt, R.; Chung, Y.; Chen, W. -C.; Wurthner, F.; Bao, Z., High-performance air-

stable n-type organic transistors based on core-chlorinated naphthalene tetracarboxylic diimides. *Adv. Funct. Mater*. **2010**, *20*, 2148 – 2156.

21. Vollmann, H.; Becker, H.; Corell, M.; Streeck, H.; Liebigs., Pyrene and its derivatives. *Ann. Chem*. **1937**, *531*, 1–159.
22. Würthner, F.; Ahmed, S.; Thalacker, C.; Debaerdemaeker, T., Core-substituted naphthalene bisimides: new fluorophors with tunable emission wavelength for FRET studies. *Chem.–Eur. J*. **2002**, *8*, 4742-4750.
23. Sakai, N.; Mareda, J.; Vauthey, E.; Matile, S., Core-substituted naphthalenediimides. *Chem. Commun*. **2010**, *46*, 4225-4237.
24. Würthner, F.; Stolte, M., Naphthalene and perylene diimides for organic transistors. *Chem. Commun*. **2011***, 47*, 5109-5115.
25. Thalacker, C.; Miura, A.; De Feyter, S.; De Schryver, F. C.; Würthner, F., Hydrogen bond directed self-assembly of core-substituted naphthalene bisimides with melamines in solution and at the graphite interface. *Org. Biomol. Chem*. **2005**, *3*, 414-422.
26. Thalacker, C.; Röger, C.; Würthner, F., Synthesis and optical and redox properties of core-substituted naphthalene diimide dyes. *J. Org. Chem*. **2006**, *71*, 8098-8105.
27. Kishore, R. S. K.; Ravikumar, V.; Bernardinelli, G.; Sakai, N.; Matile, S., Rapid and mild synthesis of functionalized naphthalenediimides. *J. Org. Chem*. **2008***, 73*, 738-740.
28. Jones, B. A.; Facchetti, A.; Wasielewski, M. R.; Marks, T. J, Tuning orbital energetics in arylene diimide semiconductors. materials design for ambient stability of n-type charge transport. *J. Am. Chem. Soc*. **2007**, *129*, 15259-15278.
29. Chopin, S.; Chaignon, F.; Blart, E.; Odobel, F., Syntheses and properties of core-substituted naphthalene bisimides with aryl ethynyl or cyano groups. *J. Mater. Chem.* **2007**, *17*, 4139-4146.
30. Kruger, H.; Janietz, S.; Sainova, D.; Dobreva, D.; Koch, N.; Vollmer, A., Hybrid supramolecular naphthalene diimide-thiophene structures and their application in polymer electronics. *Adv. Funct. Mater***. 2007**, *17*, 3715-3723.

31. Suraru, -L.; Würthner, F., Core-tetrasubstituted naphthalene diimides by stille cross-coupling reactions and characterization of their optical and redox properties. *Synthesis* **2009**, *11*, 1841.
32. Guo, X.; Watson, D., Conjugated polymers from naphthalene bisimide. *Org. Lett.* 2008, *10*, 5333-5336.
33. Jones, B. A.; Facchetti, A.; Marks, T. J.; Wasielewski, M. R., Core-expanded naphthalene diimides fused with sulfur heterocycles and end-capped with electron-withdrawing groups for air-stable solution-processed n-channel organic thin film transistors. *Chem. Mater.* **2007**, *19*, 2703-2705.
34. Bhosale, V.; Kalyankar, M. B.; Bhosale, S. V.; Langford, S. J.; Reidc, E. F.; Hogan, C. F., The synthesis of novel core-substituted naphthalene diimides via Suzuki cross-coupling and their properties. *New J. Chem.* **2009**, *33*, 2409-2413.
35. Mareda, J.; Matile, S., Anion–π slides for transmembrane transport. *Chem.–Eur. J.* **2009**, *15*, 28–37
36. Kishore, S. K.; Kel, O.; Banerji, N.; Emery, D.; Bollot, G.; Mareda, J.; Gomez-Casado, A.; Jonkheijm, P.; Huskens, J.; Maroni, P.; Borkovec, M.; Vauthey, E.; Sakai, N.; Matile, S., Ordered and oriented supramolecular n/p-heterojunction surface architectures: completion of the primary color collection. *J. Am. Chem. Soc.* **2009**, *131*, 11106–11116.
37. Bhosale, S. V.; Jani, C. H.; Lalander, C. H.; Langford, S. J., Solvophobic control of core-substituted naphthalene diimide nanostructures. *Chem.Commun.* **2010**, *46*, 973-975.
38. Bhosale, S. V.; Jani, C. H.; Lalander, C. H.; Langford, S. J.; Nerush, I.; Shapter, J. G.; Villamainac, D.; Vauthey, E., Supramolecular construction of vesicles based on core-substituted naphthalene diimide appended with triethyleneglycol motifs. *Chem. Commun.* **2011**, *47*, 8226-8228.
39. Bhosale, S. V.; Bhosale, S. V.; Bhargava, S. K., Recent progress of core-substituted naphthalenediimides: highlights from 2010. *Org. Biomol. Chem.* **2012**, *10*, 6455–6468.

40. Collie, G. W.; Promontorio, R.; Hampel, S. M.; Micco, M.; Neidle, S.; Parkinson, G. N., Structural basis for telomeric G-quadruplex targeting by naphthalene diimide ligands. *J. Am. Chem. Soc*. **2012**, *134*, 2723-2731.
41. Zhou, C.; Li, Y.; Zhao, Y.; Zhang, J.; Yang, W.; Li, Y., An unusual addition reaction for constructing a novel pH-controlled fluorescence switch. *Org. Lett*. **2011**, *13*, 292-295.
42. Cox, R. P.; Higginbotham, H. F.; Graystone, B. A.; Sandanayake, S.; Langford, S. J.; Bell, T. D. M., A new fluorescent H^+ sensor based on core-Substituted naphthalene diimide. *Chem. Phys. Lett*. **2012**, *521*, 59-63.
43. Fin, A.; Petkova, I.; Doval, D. A.; Sakai, N.; Vauthey, E.; Matile, S., Naphthalene- and perylenediimides with hydroquinones, catechols, boronic esters and imines in the core. *Org. Biomol. Chem*. **2011**, *9*, 8246-8252.
44. Parkinson, G. N., Cuenca, F., Neidle, S., Topology conservation and loop flexibility in quadruplex–drug recognition: crystal structures of inter- and intramolecular telomeric DNA quadruplex–drug complexes. *J. Mol. Biol.,* **2008**, *381*, 1145-1156.
45. Cuenca, F.; Greciano, O.; Gunaratnam, M.; Haider, S.; Munnur, D.; Nanjunda, R.; Wilson, W.; Neidle, S., Tri- and tetra-substituted naphthalene diimides as potent G-quadruplex ligands. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 1668-1673.
46. Bhosale, R.; Misek, J.; Sakai, N.; Matile, S., Supramolecular n/p-heterojunction photosystems with oriented multicolored antiparallel redox gradients (OMARG-SHJs). *Chem. Soc. Rev*. **2010**, *39*, 138–149.
47. Watson, J. D.; Crick, F. H., Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* **1953**, *171*, 737–738.
48. Choi, J.; Majima, T., Conformational changes of non-B DNA. *Chem. Soc. Rev*. **2011**, *40*, 5893–5909.
49. Zhao, J.; Bacolla, A.; Wang, G.; Vasquez, K. M., Non-B DNA structure-induced genetic instability and evolution. *Cell. Mol. Life Sci*. **2010**, *67*, 43–62.
50. Wang, G.; Vasquez, K. M., Non-B DNA structure-induced genetic instability. *Mutat. Res*. **2006**, *598*, 103–119.

51. Chakraborty, S.; Sharma, S.; Maiti, P. K.; Krishnan, Y., The poly dA helix: a new structural motif for high performance DNA-based molecular switches. *Nucleic Acids Res*. **2009**, *37*, 2810–2817
52. Wang, G.; Vasquez, K. M., Non-B DNA structure-induced genetic instability. *Mutat. Res,* **2006**, *598*, 103–119.
53. Bacolla; Wells, R. D., Non-B DNA conformations as determinants of mutagenesis and human disease. *Mol. Carcinog.* **2009**, *48*, 273–285.
54. Zhao, J.; Bacolla, A.; Wang, G.; Vasquez, K. M., Non-B DNA structure-induced genetic instability and evolution. *Cell. Mol. Life Sci.* **2010**, *67*, 43–62.
55. Venter, J. C. *et al*., The sequence of the human genome. *Science* **2001**, *291*, 1304.
56. Tjian, *R.*, Molecular machines that control genes. *Sci. Am*. **1995**, *2*, 54
57. Gottesfeld, J. M.; Neely, L.; Trauger, J. W.; Baird, E. E.; Dervan, P. B., Regulation of gene expression by small molecules. 1997, *387*, 202-205.
58. Goodman, L. S.; Wintrobe, M. M.; Damescheck, W.; Goodman, M. J.; Gilman, A.; McLennan, M. T., Nitrogen mustard therapy-use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. *J. Am. Med. Assoc*. **1946**, *132*, 126-132.
59. Lerman, L. S., Structural considerations in the interaction of DNA and acridines. *J. Mol. Biol*., **1961**, *3*, 18-30.
60. Liu, X. D.; Diao, H. Y.; Nishi, H. N.; Applied chemistry of natural DNA *Chem. Soc. Rev*. **2008**, *37*, 2745-2757.
61. Jonathan BC., Energetics of drug–DNA interactions. *Biopolymers* **1997**, *44*, 201-215.
62. Liu, H.-K.; Sadler, P.J., Metal complexes as DNA intercalators. *Acc. Chem. Res*. **2011**, *44*, 349-359.
63. Rescifina, A.; Zagni, C.; Varrica, M. G.; Pistarà, V.; Corsaro. A., Recent advances in small organic molecules as DNA intercalating agents: Synthesis, activity, and modeling. *Eur. J. Med. Chem*. **2014**, *74,* 95-115.
64. Holman, G. G.; Zewail-Foote, M.; Smith, A. R.; Johnson, K. A.; Iverson, B. L., A sequence-specific threading tetra-intercalator with an extremely slow dissociation rate constant. *Nat. chem.* **2011**, *3,* 875-881.

65. Smith, A. R.; Iverson, B., Threading polyintercalators with extremely slow dissociation rates and extended DNA binding sites. *J. Am. Chem. Soc*. **2013**, *135*, 12783−12789.
66. Saenger, W., Principles of nucleic acid structure. *Springer-Verlag*: New York, **1984.**
67. Bailly, C.; Chaires J. B., Sequence-specific DNA minor groove binders. design and synthesis of netropsin and distamycin analogues. *Bioconjugate Chem.* **1998**, *9*, 513-538.
68. Dervan, P. B., Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* **2001***, 9,* 2215–2235.
69. Martinez, R.; Chacon-Garcia, L., The search of DNA-intercalators as antitumoral drugs: what it worked and what did not work. *Curr Med Chem.* **2005**, *12*, 127-151.
70. Zaitsev, E. N.; Kowalczykowski, S. C., Binding of double-stranded DNA by Escherichia coli RecA protein monitored by a fluorescent dye displacement assay. *Nuc. Acids Res.* **1998**, *26*, 650–654.
71. Macgregor, R. B.; Clegg, R. M.; Jovin, T. M., Viscosity dependence of ethidium DNA intercalation kinetics, *Biochemistry* **1987**, *26*, 4008-4016.
72. Rentzeperis, D.; Medero, M.; Marky, L.A., Thermodynamic investigation of the association of ethidium, propidium and bis-ethidium to DNA hairpins. *Bioorg. Med. Chem*. **1995**, *3*, 751-759.
73. Ren J.; Jenkins TC.; Chaires J B., Energetics of DNA intercalation reactions. *Biochemistry* **2000**, *39*, 8439-8447.
74. Norden, B.; Kurucsev, T., Analysing DNA complexes by circular and linear dichroism*. J. Mol. Recognit.* **1994**, *7,* 141–156.

Chapter 2

Effective brominating reagents for naphthalenetetracarboxylic dianhydride and preparation of imide- and core-substituted naphthalene diimides

2.1. Introduction

1,4,5,8-Naphthalenediimides (NDIs) (also known as naphthalene carbodiimides) have drawn much attention due to their tendency to form n-type semiconductor materials among aromatic molecules that have enormous applications, particularly in the design of conducting materials.[1] NDIs are neutral, planar, chemically robust and most notably they are electron deficient compounds that are capable of undergoing self-organization. They have found wide use in supramolecular chemistry, artificial photosystems, photo detectors, optical switches, DNA intercalation and bioimaging.[1] The propensity of NDIs to form excimers initiated the development of NDI-based fluorescent chemosensors.[2a] Functionalization through the diimide nitrogen's or via core substitution (substitution on the naphthalene core) produces analogues whose absorption and emission properties are variable. Imide-substituted NDIs are prepared by the simple condensation of 1,4,5,8-naphthalenetetracarboxylic dianhydride (NDA) with suitable amine groups. The imide (*N*, *N'*-) substituents of NDIs have minimal influence on the optical and electronic properties, although they can be used to control solubility, aggregation, and intermolecular packing in the solid state. The core substitution of the NDIs can be used to tune the molecular electronic properties. Core substituted NDIs (cNDIs) are rapidly emerging as a class of their own in an attractive strategy to create highly colorful, conducting and functional materials[2b] with different photophysical properties than their core unsubstituted counterparts. These cNDIs have extensive applications in single molecule fluorescence techniques.[3] The traditional synthetic approach for functionalized NDIs has been discussed in Chapter 1 which has many drawbacks. The preferred starting precursors for accessing core functionalized NDIs are the corresponding bromo derivatives of NDAs. The availability of precursor materials in large scale accelerates the ongoing research activities on core substituted NDIs. This demands the development of reagents for the efficient and economical preparation of brominated derivatives of NDAs. Various bromination methods known till date in the literature for the bromination of NDA involve (i) molecular bromine as a brominating reagent in the presence of catalytic amount of iodine either in oleum or mixture of conc. H_2SO_4 and oleum as a solvent,[4-6] (ii) use of sodium bromide (NaBr) as a brominating agent in oleum[3] and (iii) dibromoisocyanuric acid (DBI) in oleum as a source of bromine.[7-12] However all these literature methods suffer from many drawbacks like molecular bromine involved reactions produce low yields and handling bromine is a difficult task due to its toxicity and

high vapour pressure. Moreover, hazardous and corrosive nature of molecular bromine makes it difficult to handle as a brominating reagent under milder reaction conditions. Furthermore, prolonged reaction time and high reaction temperatures were needed to carry out the reaction (i.e., the tetra-bromo NDA was synthesized at 140 ^{o}C).[4] The NaBr method required harsh reaction conditions and a special equipment to carry out the reaction.[6] DBI is highly expensive, not readily available in large quantities, reactions were performed at high temperature (130 ^{o}C)[8] and gave mixture of brominated products.[10] Most importantly, after the bromination, a convenient procedure for the isolation and purification of brominated NDAs is not available and instead compounds were purified and characterized by converting them into the corresponding core-substituted NDIs. These drawbacks further reiterate the need for developing efficient brominating reagents for the rapid and regioselective bromination of NDA to preferred bromo-products in high yield and purity.

2.2. Objective of the work

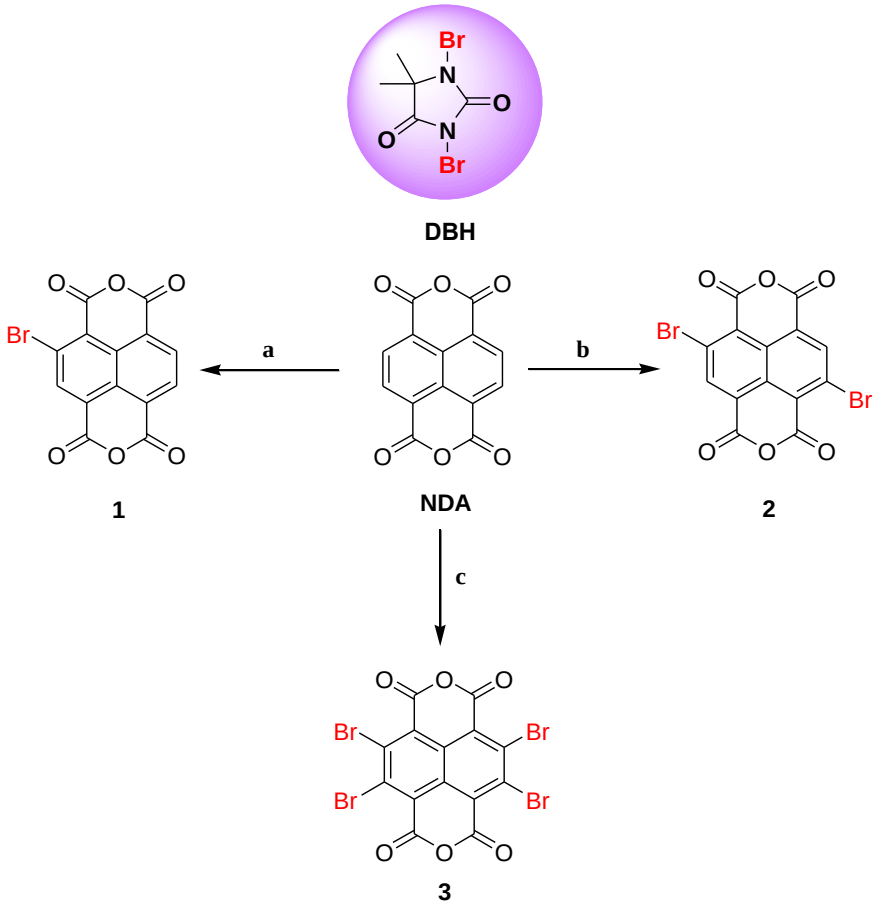
The main aim of the work is to develop simple, economical, practical and industrially viable reagent for the preparation of mono-, di-, and tetra-brominated NDAs in high yield and purity under relatively milder reaction conditions**.** These bromo-NDAs subsequently serve as direct precursors for the synthesis of core-functionalized NDIs. We chose to exploit commercially available and inexpensive 5,5-dimethyl-1,3-dibromo hydantoin (DBH, $45.7/500 g; source: www.sigmaaldrich.com) and less toxic as well as easily synthesizable tribromo isocyanuric acid (TBCA) as new reagents for the bromination of NDA.

2.3. Bromination of NDA using dibromohydantoin (DBH)

DBH is a stable and readily available solid material that is widely used as a disinfectant in drinking water purification, recreational water treatment, as a bleaching agent in pulp and paper mills, and for treating industrial/commercial water cooling systems. Surprisingly, DBH has been used as brominating reagent only in two instances to brominate functionalized alkenes[13] and has not been exploited for other substrates, including bromination of NDA.

2.3.1. Synthesis of bromo NDAs

The mono-, di-, and tetrabromo-NDAs were prepared as shown in Scheme 2.1.



Scheme 2.1 Reagents and conditions a) DBH (0.55 equiv.), Conc. H_2SO_4, rt, 12 h, 70%; b) DBH (1.5 equiv.), Conc. H_2SO_4, 50 ^oC, 10 h, 82%; c) DBH (3.0 equiv.), Conc. H_2SO_4, 4 h at rt then 12 h at 80 ^oC, 96%.

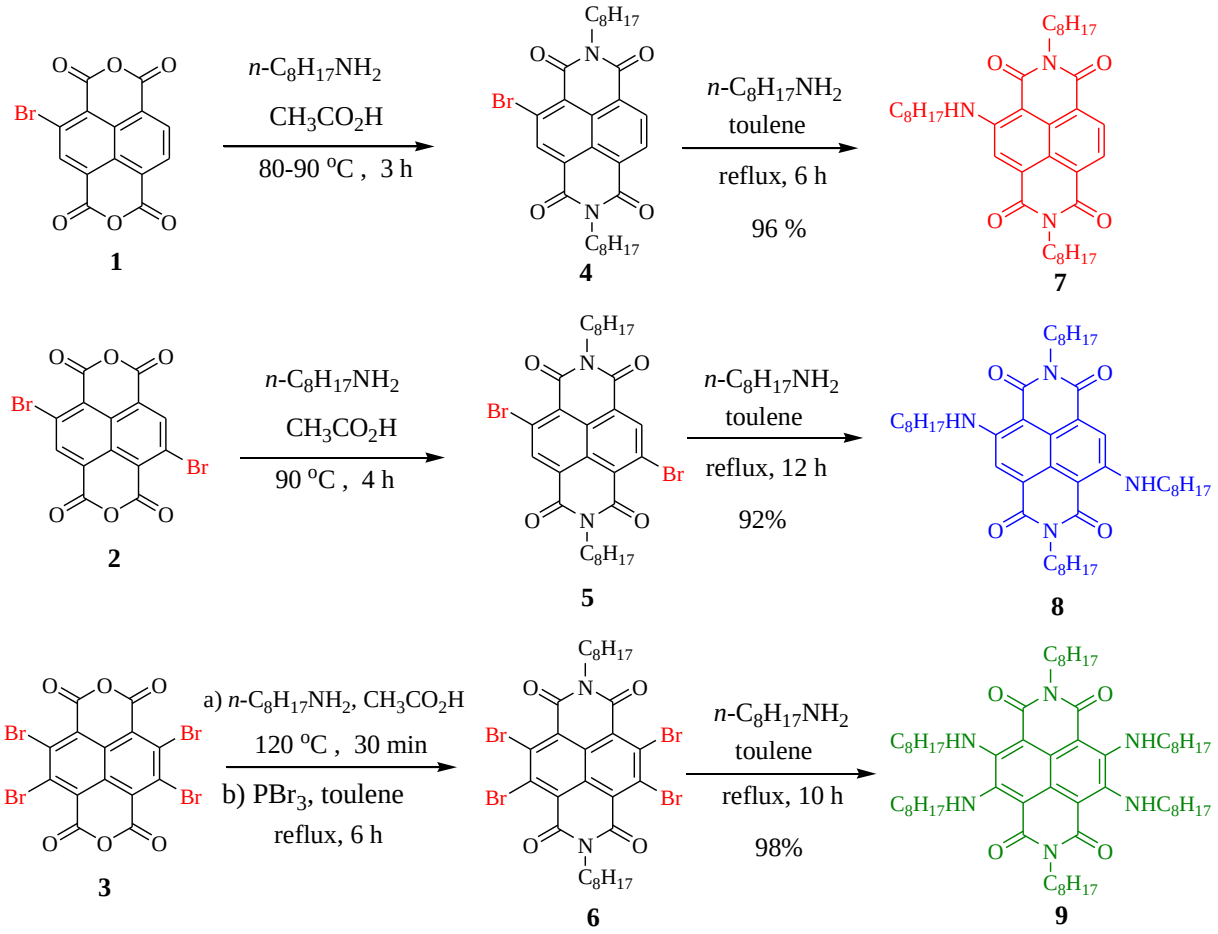
The NDA was treated with DBH (0.55 equiv.) in 98 % H_2SO_4 at room temperature for 12 h in a stoppered round-bottomed flask (100 mL). The reaction afforded crude product consisting of mainly 2-bromo NDA (**1**), a trace of 2, 6-dibromo-NDA (**2**), and unreacted starting material. The crude product was recrystallized from *N*,*N*-dimethylformamide (DMF) to obtain **1** in 70 % yield (Scheme 2.1). The reaction of NDA and DBH (1.5 equiv.) in 98 % H_2SO_4 at 50 ^oC for 10 h gave crude product **2** along with small amount of partially ring-opened side

product. The partially-ring opened side product was selectively recrystallized from DMF solution. The product was then precipitated from the supernatant by adding water to obtain pure **2** in 82 % yield. Tetrabromination of NDA was carried out in 98 % sulfuric acid with three equivalents of DBH at room temperature for 4 h at 80 ^{o}C for 12 h to give 2,3,6,7-tetrabromo-NDA (**3**) in 96% yield. The ^{1}H NMR spectrum ([D6] DMSO) of **3** did not have any proton signals, which indicated the absence of mono- and dibrominated compounds in the product. Furthermore, the identity of all bromo compounds **1**, **2** and **3** was confirmed by converting into corresponding imide- and core- substituted products **4-9** as discussed in the following sections.

2.3.2. Core functionalization of bromo-NDAs 1–3 in two step synthesis

As already discussed, imide-substituted NDIs play a major role in controlling solubility, aggregation, and intermolecular packing in the solid state, whereas core substitutions are particularly important in modulating electronic, optical, and redox properties of NDIs.[14,15] The selective *N,N'*-imidation of bromo-NDAs **1**–**3** is outlined in Scheme 2.2. The *N,N'*- bis(*n*-octyl)-2-bromo-NDI (**4**) was synthesized by treating **1** with three equivalents of *n*-octylamine (*n*-$C_8H_{17}NH_2$) in acetic acid at 80–90 ^{o}C for 3 h. After completion of the reaction, the reaction mixture was filtered and washed with methanol to afford the pure product **4** in 80 % yield without requiring any further purification by column chromatography. Similarly, *N,N'*-bis(*n*-octyl)-2,6-dibromo NDI (**5**) was synthesized in 75 % yield by treating **2** with three equivalents of *n*-octylamine in acetic acid at 90 ^{o}C for 4 h. For the imidation of **3**, first we followed the procedure reported by Würthner and co-workers.[16] Imidation of **3** with three equivalents of *n*-octylamine in glacial acetic acid at 130 ^{o}C for 6 h gave the desired *N,N'*-bis(*n*-octyl)-tetrabromo-NDI (**6**) in >10 % yield, along with ring opened 2,3,6,7-tetrabromo-4,8-bis(*n*-octyl carbamoyl) naphthalene-1,5-dicarboxylic acid as a major product and a trace of core-substituted products. To overcome this difficulty in synthesizing **7** exclusively in good yield, we followed the two step synthesis reported by Liu and co-workers.[17] Accordingly, **3** was treated with three equivalents of *n*-octylamine in glacial acetic acid at 120 ^{o}C for 30 min to obtain ring-opened 2,3,6,7-tetrabromo-4,8-bis(*n*-octylcarbamoyl) naphthalene-1,5-

dicarboxylic acid, which was further treated with PBr_3 in toluene at reflux for 6 h to give **6** in 60 % overall yield from **3**.



Scheme 2.2 Two step synthesis for core substitution of bromo-NDAs with *n*-octyl amine.

Initially, the core substitution of bromo-NDIs with *n*-octylamine in DMF at 130 °C gave complex mixture and after purification by column chromatography, afforded the products in less than 30 % yield. To improve the yield and minimize the side reactions, we tried core substitution of bromo-NDIs in toluene as a solvent as shown in Scheme 2.2. We were delighted to find that the core substitution occurred smoothly to afford good-to-excellent yields of the products. The reaction of **4** with 1.25 equivalents of *n*-octylamine in toluene at reflux for 3 h gave core-substituted *N,N'*-bis(*n*-octyl)-2-(*n*-octylamino)-NDI (**7**) in 96 % yield.

Similarly, core substitution of **5** and **6** afforded the corresponding core-substituted *N,N'*-bis(*n*-octyl)-2,6-di(*n*-octylamino)-NDI (**8**) and *N,N'*-bis(*n*-octyl)-2,3,6,7-tetra-(*n*-octylamino)-NDI (**9**) in 92 % and 98 % yields, respectively. In toluene, the reactions were very clean and no side products were observed.

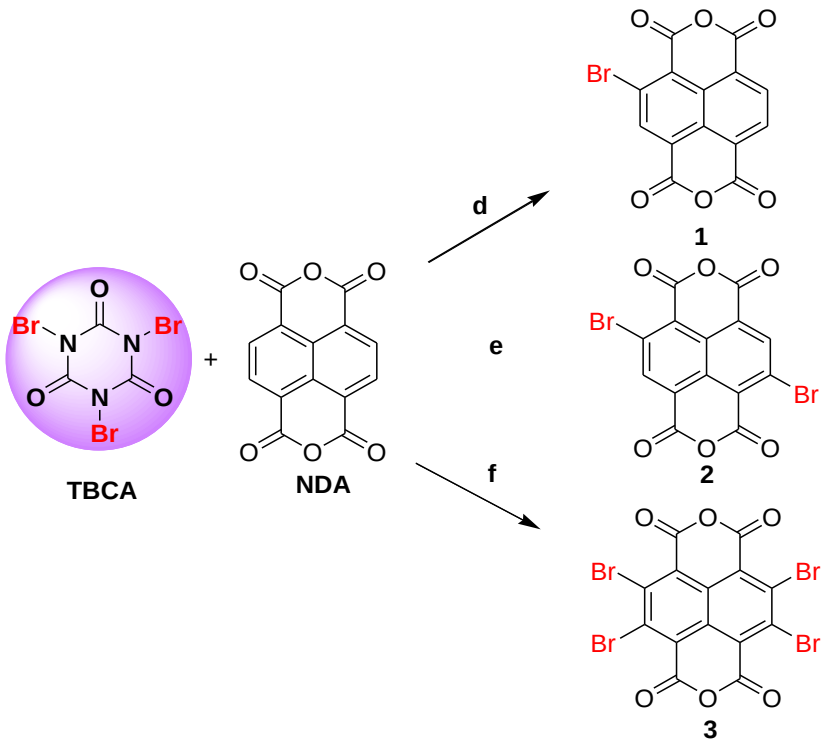
2.4. Bromination of NDA using TBCA

TBCA is a white solid which is less toxic and expected to be a safe brominating reagent that can be easily synthesized in bulk from isocyanuric acid and NaBr in the presence of Oxone.[18] TBCA has been used for the bromination of simple alkenes and aromatic substrates[19] but has not been exploited for bromination of NDA. Thus we performed bromination of NDA using TBCA which was prepared by following the literature procedure[18] and optimized the reaction conditions for regioselective preparation of mono-, di- and tetra-brominated NDA core. Further, it should be noted that TBCA is particularly advantageous because of high atom-economy as it can transfer three Br^+ to one substrate which accelerates the rate of reaction compared to any currently used brominating reagents including dibromoisocyanuric acid (DBI). Easy and straight forward synthesis of TBCA reagent in large quantity makes it an industrially viable reagent for the bromination of NDA while DBI is highly expensive and not readily available in bulk quantity.

2.4.1. Synthesis of mono-, di- and tetra bromo NDAs using TBCA

The mono-, di- and tetra-bromo-NDAs were prepared as shown in Scheme 2.3. The reaction conditions were optimized by varying the equivalents of TBCA and reaction time. NDA was treated with TBCA (0.5 equiv) in 98 % H_2SO_4 at room temperature for 8 h in a stoppered round bottomed flask (100 mL). The reaction afforded crude product consisting of mainly 2-bromo NDA (**1**), trace of dibromo-NDA (**2**) and unreacted starting material. The crude product was recrystallized in dimethylformamide to obtain **1** in 70% yield (Scheme 2.3). Reaction of NDA and 1 equiv of TBCA in 98 % H_2SO_4 at room temperature for 12 h gave 2,6- dibromo-NDA **2** in 92 % yield which was further recrystallized in dimethylformamide to obtain 2,6-dibromo-NDA **2** in 76 % yield. Tetrabromination of NDA was carried out in 98%

sulphuric acid with 2.5 equiv of TBCA at room temperature for 8 h and at 80 ^{o}C for 8 h to give 2,3,6,7-tetrabromo-NDA (**3**) in 98% yield. The product was recrystallized in DMF to obtain pure tetrabromo-NDA **3** in 87 %



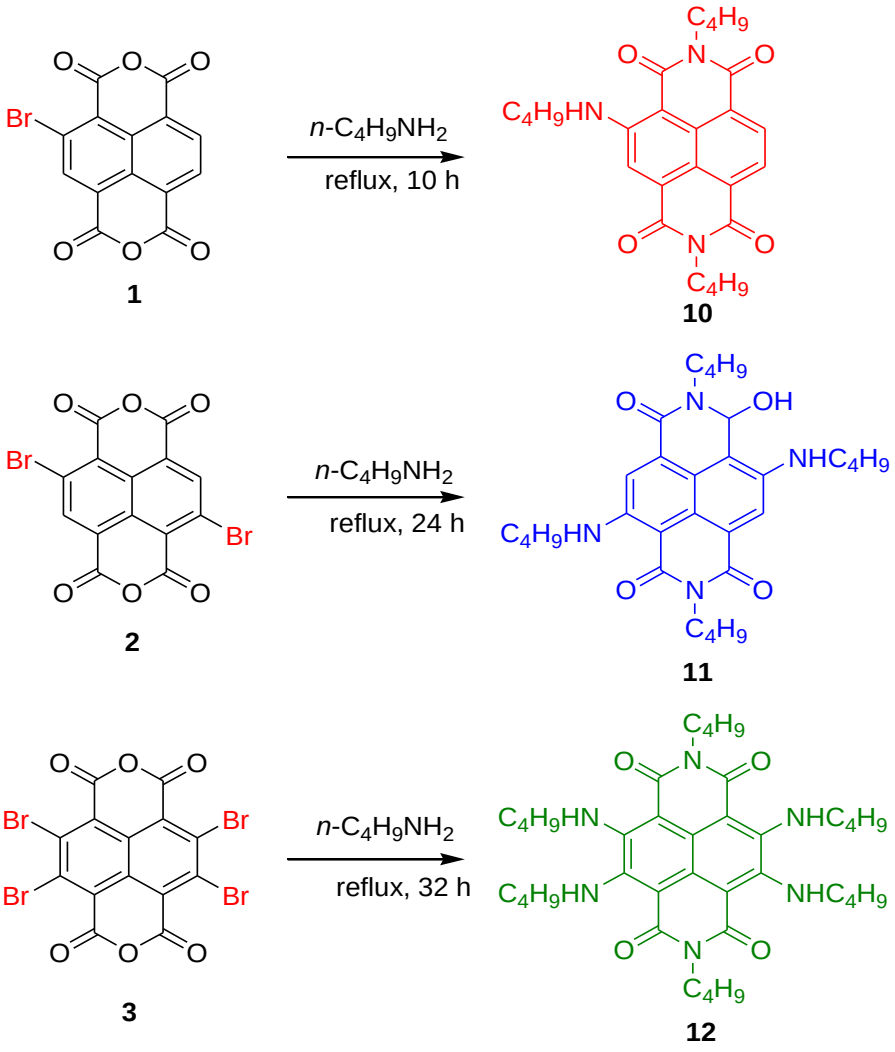
Scheme 2.3 Reagents and conditions d) TBCA (0.5 equiv), Conc. H_2SO_4, rt, 8 h, 70%; e) TBCA (1 equiv), Conc. H_2SO_4, rt, 12 h, 76%; f) TBCA (2.5 equiv), Conc. H_2SO_4, rt, 8 h then at 80 ^{o}C, 8 h, 87%.

yield. The ^{1}H NMR spectrum ([D6] DMSO) of **3** did not show any proton signals, indicating the absence of mono- and di-brominated compounds in the product. The purity and integrity all bromo NDAs was further confirmed by converting into core substituted products as discussed in the following section.

2.4.2. Core substitution of brominated NDAs 1-3 in one step synthesis

The utility of our new brominating reagent TBCA was demonstrated by carrying out the imide and core substitution of brominated NDAs (**1**–**3**) in one step with *n*-butylamine as outlined in Scheme 2.4. Monobromo-NDA **1** in *n*-butylamine as a solvent was refluxed for 10

h to obtain *N,N'*-bis-(*n*-butyl)-2-(*n*-butylamino)-NDI (**10**) in good yield. Similarly, dibromo- and tetra-bromo-NDAs **2** and **3** in *n*-butylamine were refluxed for 24 and 32 h respectively to obtain corresponding imide and core substituted NDIs **11** and **12** in good to moderate yields.



Scheme 2.4 One-step synthesis core substitution of bromo-NDAs with *n*-butyl amine.

2.5. Comparision of dibromohydantoin (DBH) and tribromoisocyanuric acid (TBCA)

As the bromo-NDAs subsequently serve as direct precursors for the synthesis of core-functionalized NDIs, the development of an efficient brominating reagent for the rapid and regioselective bromination of NDA to preferred bromo-products in high yield and purity is

needed. Initially, we exploited commercially available and inexpensive DBH for bromination of NDA as it is readily available stable solid. Moreover this method allows us to avoid the handling of molecular bromine. Furthermore, this method include high conversions, mild reaction conditions, a simple workup, good-to-excellent yields, and high purity through a recrystallization and precipitation technique Table 2.1. However, the DBH method requires relatively prolonged reaction times, fairly high reaction temperature and excess brominating reagent to give bromo NDAs with relatively low yields. These shortcomings provoked us to develop another effective brominating reagent in terms of bromine-atom economy, reaction efficiency and cost effectiveness. For this purpose we chose TBCA as the potential brominating reagent due to its inexpensive synthesis from isocyanuric acid ($22/25 g, www.sigma–aldrich.com) in large quantities. The high atom-economy with respect to transferable bromine atoms compared to other brominating reagents is particularly useful. The bromination reactions of NDA using TBCA were fast, required low equivalents of TBCA and

Table 2.1 Optimized reaction conditions for bromination of NDA using DBH and TBCA.

Reaction conditions	DBH (equivalents)	Reaction time (h)	Reaction Temperature (°C)	Yield (%)
a	0.55	12	RT	70
b	1.5	10	50	82
c	3.0	4+12	RT+80	96
Reaction conditions	**TBCA (equivalents)**	**Reaction time (h)**	**Reaction Temperature (°C)**	**Yield (%)**
d	0.5	8	RT	89
e	1.5	10	50	92
f	3.0	4+12	RT+80	98

performed under milder conditions compared to DBH as shown in Table 2.1. Interestingly mono-, di- and tetra-bromination of NDA core was performed at ambient conditions by varying equiv of TBCA. It should be noted that the reaction time of tetra-bromination of NDA was greatly reduced by heating the reaction mixture towards the end.

2.6. Results and discussion

2.6.1. Photophysical Properties of imide- and core-substituted NDIs

Core substituents are known to have tremendous influence on optical properties of NDIs compared with the corresponding imide substituents. We studied the UV/Visibleabsorption and fluorescence characteristics of imide-substituted NDIs **4**, **5**, and **6** as well as imide and core-substituted NDIs **7**–**9** in chloroform. NDIs with *n*-octyl and *n*-butyl substituents have similar optical properties irrespective of length of the alkyl chain (*n*-butyl or *n*-octyl chain). The absorption spectra of **4**, **5**, and **6** have S_0–S_1 electronic transitions in the range of 300–460 nm (Figure 2.1).

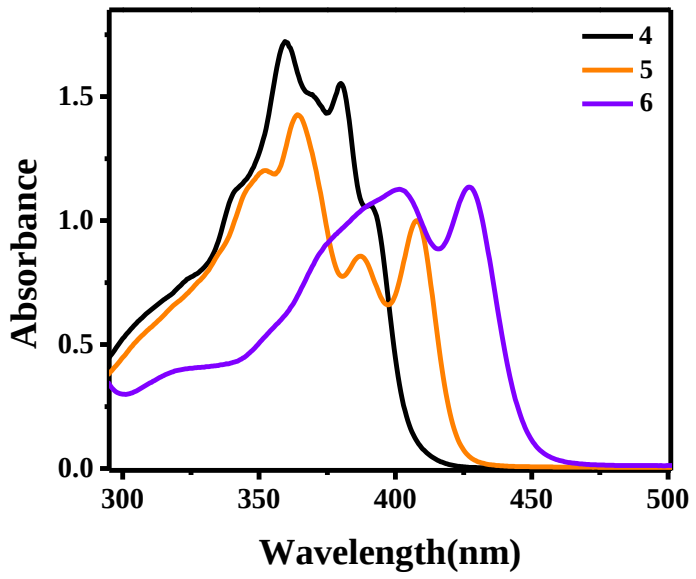


Figure 2.1 UV/Visible absorption spectra of core-unsubstituted bromo-NDIs **4**, **5**, and **6** in chloroform.

The monobromo- and dibromo-NDIs **4** and **5** have vibronic absorption bands (peaks at 340,360, 380, and 390 nm for **4** and 352, 364, 387, and 408 nm for **5**) with absorption maxima at 360 and 364 nm respectively. However, the absorption spectrum of tetrabromo NDI **6** is relatively broad with a remarkable red-shift of 50 nm and prominent absorption maxima at 402 and 427 nm. This pronounced red-shift correlates with number of bromine atoms on the aromatic core of the NDI. The prominent vibronic fine structure of the absorption band indicates the rigid nature of the chromophores and its energy in the order of ca. 1300 cm^{-1} (0.16 eV) corresponds to the skeletal vibrations of the aromatic system.[7]

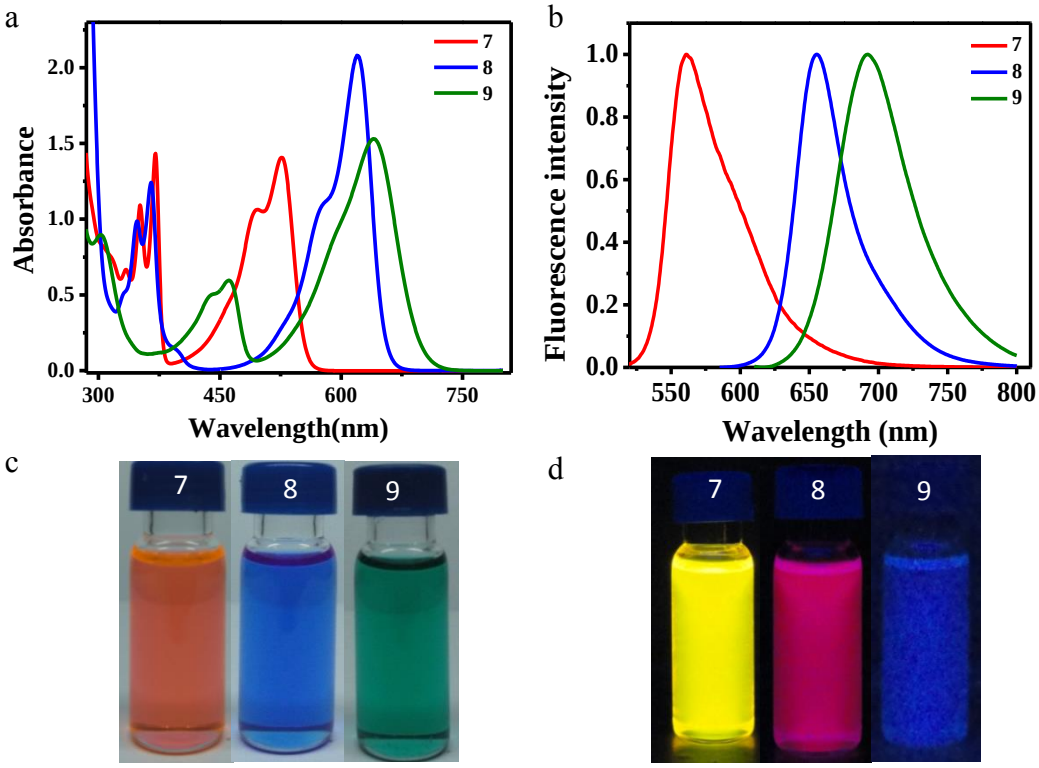


Figure 2.2 a) UV/Visible absorption spectra of core-substituted NDIs **7**–**9** in chloroform b) Normalized Emission spectra of NDIs **7**–**9**; λ_{ex}=497, 577, and 597 nm, respectively. c) Photographs of solutions of NDIs **7**–**9** in chloroform are bright red, blue, and green, respectively, in daylight. d) Photographs of solutions of NDIs **7**–**9** in chloroform fluoresce brightly under in UV light. Excitation wavelength of 365 nm was used.

The introduction of *n*-octyl amino and *n*-butyl substituents at the naphthalene core in **7**–**9** and **10-12** respectively leads to characteristic absorption and emission properties as shown in Figure 2.2. The band I absorption of core-substituted NDIs **7** and **8** is in the region 300–400 nm. Interestingly, for **9** with four *n*-octylamino substituents, band I red–shifted to 461 nm. Furthermore, core substitution caused interesting electronic transitions in the visible region with new absorption bands at 527, 620, and 639 nm for **7**, **8**, and **9** respectively, which cover a wide visible spectral range (Figure 2.2a and Table 2.2). Because of their absorption in the visible region, solutions of **7**, **8**, and **9** in chloroform are bright red, blue, and green, respectively, in daylight (Figure 2.2c). The increase in the number of electron-donating *n*-octylamino substituents at the naphthalene core evoked a bathchromic shift of 93 and 112 nm to the new absorption band of **8** and **9** compared with mono *n*-octylamino substituted **7**. Next we studied the effect of core substitution on the fluorescence emission characteristics of NDIs. The imide substituted bromo-NDIs **4**, **5**, and **6** are not significantly fluorescent. However, solutions of core-substituted NDIs **7**, **8**, and **9** emitted bright fluorescence with emission maxima at 560, 649, and 693 nm respectively (Figure 2.2b and Table 2.2) at excitation wavelengths corresponding to their new absorption bands (497, 577, and 597 nm, respectively, Figure 2.2a and Table 2.2).

Table 2.2 Absorption maximum wavelengths (λ_{max}) and molar absorptivity coefficients (ε) of **1**-**9** in chloroform.

Compound	λ_{max}/nm	ε /M^{-1} cm^{-1}
1	313	15,560
2	314	22,480
3	372	27,480
4	360	34,440
5	364	28,540
6	427	22,700
7	527	28,138
8	620	41,600
9	639	30,600

λ_{max} is absorption maximum wavelengths and ε is molar absorptivity coefficient

The fluorescence colors of solutions of **7**–**9** in chloroform with a common excitation wavelength of 365 nm is shown in Figure 2.2d. Thus, the electrondonating alkylamino substituents transformed the NDIs into valuable fluorophores with relatively good fluorescence quantum yields (Table 2.3) and tunable emission wavelengths from the blue to red spectral region. The quantum yields of compounds **8** and **9** matched with the data reported in literature.[16] The absorbance and fluorescence data of **5**, **6**, **8** and **9** match the reported data in the literature, [7,12]

Table 2.3 Fluorescence parameters of cNDIs **7-9** in chloroform

Compound	λ /nm[a]	λ /nm[b]	φ_f[c]
8	497	560	0.58
9	577	649	0.55[12a]
10	597	693	0.16[12b]

[a] λ is excitation wavelength, [b] λ is emission maximum wavelength, [c] φ_f = Quantum yield.

which further confirms the identity of the products obtained through our DBH and TBCA bromination method. In general our study also showed that the core substitution strongly influences the electronic and optical properties of NDIs.

2.7. Conclusion

We have developed a simple and efficient method for the synthesis of mono-, di-, and tetrabromo-NDAs using DBH and TBCA as brominating reagents.[20, 21] The cost effectiveness of the reagent along with relatively high rate of reaction, the simple procedure, and excellent yields make this a favorable method for the synthesis of mono-, di-, and tetrabromo-derivatives of NDAs. This method of preparation of bromo-NDAs is highly attractive for

further construction of diverse, functionalized NDIs. The significant features of the method include high conversions, mild reaction conditions, a simple workup, good-to-excellent yields, and high purity through a recrystallization and precipitation technique. Core substitution with electron-donating groups tunes the UV/vis absorption and emission properties over a wide visible spectral range compared with minor changes for only imide-substituted NDIs. This method using DBH and TBCA could be easily adopted for the preparation of other brominated derivatives of arylene diimides.

2.8. Experimental Section

Materials and Methods

General: All the reagents were obtained from Sigma–Aldrich and used as received. Column chromatography was performed on silica gel (100–200 mesh). The solvents used for spectroscopic studies were of spectroscopic grade from Sigma–Aldrich. IR spectra were recorded on Bruker (FT)-IR 8400 instrument and absorptions are expressed in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 andusing [D6]DMSO and $CDCl_3$ as solvents. Chemical shifts (δ) are given in parts per million (ppm) with respect to the internal standard tetramethylsilane (TMS), and *J* values are quoted in Hertz. The following abbreviations were used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, brs=broad singlet, dd=doublet of doublet, and dt=doublet of triplet. Elemental analysis was recorded on Thermoscientific Flash 2000 organic elemental analyzer. High resolution mass spectra ware recorded on UHD accurate mass QTOF LC/MS. UV/vis spectra were recorded on a PerkinElmer Model Lambda 900 spectrophotometer and 50mm of the samples were analyzed in quartz cuvette of 1 cm path length. Fluorescence spectra were recorded on PerkinElmer LS 55 Luminescence spectrometer. 50mm of the samples were analyzed in quartz cuvette of 1 cm path length. Fluorescence quantum yields in chloroform were determined by the optically dilute method[22] by using rhodamine 6G as a reference (φ_f (ethanol)=0.95 ± 0.015)[23] and refractive indices of the solvents as published in the CRC handbook.[24]

2-Bromo-1,4,5,8-naphthalenetetracarboxylic dianhydride (1)

In a single-necked round bottom (RB) flask 1,4,5,8-naphthalenetetracarboxylic dianhydride (NDA) (2.68 g, 10 mmol) was slurried in concentrated sulfuric acid (20 mL) at ambient temperature, then the mixture was stirred for 5 min to obtain a solution. DBH (1.57 g, 5.5 mmol) was added in portions over a period of 1 h and the RB flask tightly stoppered to avoid the escape of bromine from the reaction mixture. The resulting brown solution was stirred at room temperature for 12 h. The brown reaction mixture was poured into crushed ice to precipitate the solid. The precipitated solid was filtered, washed with water then with methanol, and finally dried under vacuum to afford 1as pale yellow solid, which was recrystallized from DMF to obtain the pure product as white crystals (2.42 g, 70 %); IR (KBr, cm^{-1}) =1787, 1731, 1192, 1373, 1174, 1149, 1091, 983, 935 cm1; ^{1}H NMR (400 MHz, [D6]DMSO): δ_H ppm 8.71 (s, 1 H), 8.58–8.56 (d, *J* = 7.6 Hz, 1 H), 8.22–8.20 (d, *J* = 7.6 Hz, 1 H); ^{13}C NMR(100 MHz, [D6]DMSO): δ_C ppm 168.1, 160.0, 159.4, 137.4, 131.6, 131.5,130.6, 129.2, 128.3, 125.3, 124.5, 121.8; Elemental analysis: calcd for $C_{14}H_3BrO_6$: C 48.45, H 0.87; found: C 48.47, H 0.85.

2,6-Dibromo-1,4,5,8-naphthalenetetracarboxylic dianhydride (2)

In single-necked RB flask, NDA (2.68 g, 10 mmol) was slurried in concentrated sulfuric acid (25 mL) at ambient temperature, the mixture was stirred at room temperature for 5 min to obtain a solution. DBH (3.57 g, 15 mmol) was added in four portions over a period of 1 h at room temperature. The resulting brown solution was stirred at 50 ^{o}C for 10 h. The mixture was poured into crushed ice to precipitate the solid. The precipitated solid was filtered, washed with water then with methanol, and finally dried under vacuum to afford crude product, which was further purified by crystallization from DMF. During crystallization, the partially ring opened side product preferentially crystallized, leaving the supernatant with pure product **2**, which was further purified by precipitation (3.49 g, 82 %); IR(KBr,cm^{-1})=1787, 1733, 1191, 1172, 1150, 1093, 985,936 cm1; ^{1}H NMR (400 MHz, [D6]DMSO): δ_H ppm 8.78 (s, 2 H); ^{13}C NMR (100 MHz, [D6]DMSO): δ_C ppm 157.9, 156.4, 137.5, 129.4, 127.4,124.2, 123.4; Elemental analysis: calcd for $C_{14}H_2Br_2O_6$: C 39.47, H 0.47; found: C 39.42, H 0.45.

2,3,6,7-Tetrabromo-1,4,5,8-naphthalenetetracarboxylic dianhydride (3)

In single-necked RB flask, NDA (2.68 g, 10 mmol) was slurried in concentrated sulfuric acid (50 mL) at ambient temperature. DBH (8.57 g, 30 mmol) was added in portions and tightly stoppered with glass stopper. The resulting brown solution was stirred at room temperature for 4 h and then the mixture was heated at 80 ^{o}C for 12 h. The mixture was poured into crushed ice to precipitate the solid. The precipitated solid was filtered, washed with water then with methanol, and finally dried under vacuum to obtain **3** as yellow solid (5.6 g, 96 %); IR (KBr, cm^{-1}) =1787, 1733, 1507, 1499, 1421, 1373, 1335, 1188, 1146, 1096, 988, 937, 778,702, 570 cm-^{1}; Elemental analysis: calcd for $C_{14}Br_4O_6$: C 28.80; found: C 28.67.

***N,N'*-Bis(*n*-octyl)-2-bromo-1,4,5,8-naphthalenetetracarboxylic diimide (4)**

A mixture of **1** (867 mg, 2.5 mmol), *n*-octylamine (1.24 mL, 7.5 mmol), and acetic acid (25 mL) was stirred at 90 ^{o}C for 3 h. The mixture was cooled to room temperature, then the precipitate was collected by filtration, washed with methanol, and dried under vacuum to obtain **4** as a pale yellow crystalline solid (1.13 g, 80 %); IR ($CHCl_3$, cm^{-1})=3063, 2954,2919, 2850, 1705, 1656, 1568, 1438, 1371, 1331, 1251, 1238, 1187, 1104, 1076, 780 cm 1; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 8.94 (s, 1 H), 8.83–8.81(d, *J*=8 Hz, 1 H), 8.77–8.75 (d, *J* = 8 Hz, 1 H), 4.22–4.16 (apparent q, *J* = 8 Hz, 4 H, $2CH_2$), 1.78–1.69 (m, 4 H, $2CH_2$), 1.43–1.25(m, 20 H,$10CH_2$), 0.89–0.86 ppm (t, *J* = 6.4 Hz, 6 H, $2CH_3$); ^{13}C NMR (100 MHz,$CDCl_3$): δ_C ppm 162.4, 161.8, 161.7, 138.4, 131.6, 130.7, 128.6, 128.6, 126.8,126.0, 126.0, 125.7, 123.9, 41.5, 41.1, 31.8, 29.3, 29.26, 29.21, 29.1, 28.0,27.9, 27.1, 27.0, 22.6, 14.0 ppm; Elemental analysis: calcd for $C_{30}H_{37}BrN_2O_4$: C 63.27, H 6.55, N 4.92; found: C 63.21, H 6.50, N 4.90; HRMS (APCI):m/z: calcd for $C_{30}H_{37}BrN_2O_4$: 569.2015 $[M+H]^+$; found: 569.1989.

***N,N'*-Bis(*n*-octyl)-2,6-dibromo-1,4,5,8-naphthalenetetracarboxylic diimide (5)**

A mixture of 2,6-dibromo-NDA **2** (1.06 g, 2.5 mmol), *n*-octylamine (1.24 mL, 7.5 mmol), and acetic acid (25 mL) was stirred under nitrogen atmosphere at 90 °C for 4h. The mixture was cooled to room temperature. The precipitate was separated by filtration, washed with methanol and dried under vacuo to obtain *N*,*N*'-bis(*n*-octyl)-2,6-dibromo-NDI **5** as a yellow crystalline solid (1.21g, 75%); IR ($CHCl_3$ cm^{-1}): 3058, 2917, 2848, 2954,1701, 1655, 1561, 1437, 1372, 1315, 1253, 1234, 1218, 1189,1107, 957, 863, 786, 765, 722, 614, 626, 572; ^{1}H-

NMR (400 MHz, $CDCl_3$): δ_H ppm 8.99 (s, 1H, Ar-H), 4.20-4.16 (t, J = 7.6 Hz, 4H, $2CH_2$), 1.77-1.69 (quin, J = 7.6 Hz, 4H, $2CH_2$), 1.42-1.27 (m, 20H, $10CH_2$), 0.89-0.86 (t, J = 6.8 Hz, 6H, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 160.9, 160.8, 139.2, 128.4, 127.8, 125.5, 124.2, 41.7, 31.9, 29.4, 29.3, 28.0, 27.2, 22.7, 14.2. Elemental analysis. Found: C, 55.65, H, 5.63; N, 4.16; Calcd: C, 55.57; H, 5.60; N, 4.32 for $C_{30}H_{36}Br_2N_2O_4$. HRMS (APCI) (*m/z*): calcd for $C_{30}H_{36}Br_2N_2O_4$ $[M + H]^+$, 649.1100, found 649.1069.

_N,N'_-Bis(*n*-octyl)-2,3,6,7-tetrabromo-1,4,5,8-naphthalenetetracarboxylic diimide (6)

2,3,6,7-Tetrabromo-NDA **3** (2.91 g, 5 mmol) and *n*-octylamine (2.48 mL, 15 mmol) in acetic acid (100 mL) were stirred under a nitrogen atmosphere at 120 °C. The reaction was stopped before the color of the reaction mixture changing to red (30 min). The reaction mixture was cooled to room temperature and poured into water, then filtered. The obtained yellow solid was washed with water, after dried under vacuo, the crude product intermediate was used directly for the next reaction without further purification.

A solution of the intermediate and PBr_3 (0.94 mL, 10 mmol) in toluene (50 mL) was refluxed for 6 h under a N_2 atmosphere. The mixture was cooled to room temperature and then poured into water (200 mL). The aqueous phase was extracted with toluene (3 × 50 mL), and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was washed with methanol to obtain *N,N*'-bis(*n*-octyl)-2,3,6,7-tetrabromo-NDI **6** as a yellow crystalline solid (2.4 g, 60%). IR ($CHCl_3$ cm^{-1}): 2956, 2917, 2851, 1713, 1669, 1377, 1288, 1156, 788, 576; ^1H NMR (400 MHz, $CDCl_3$): δ_H ppm 4.22-4.18 (t, J = 7.6 Hz, 4H), 1.79-1.71 (m, 4H), 1.42-1.25 (m, 20H), 0.88-0.86 (t, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δc ppm 159.9, 135.6, 126.7, 125.7, 43.0, 31.9, 29.3, 29.3, 28.1, 27.2, 22.7, 14.2; Elemental analysis. Found: C, 44.01; H, 4.26; N, 3.22; Calcd: C, 44.69; H, 4.25; N, 3.47 for $C_{30}H_{34}Br_4N_2O_4$. HRMS (APCI) (*m/z*): calcd for $C_{30}H_{34}Br_4N_2O_4$ $[M + H]^+$, 806.9289, found 806.9283.

_N,N'_-Bis-(*n*-octyl)-2-(n-octylamino)-1,4,5,8-naphthalenetetracarboxylic diimide (7)

A mixture of **4** (569 mg, 1 mmol) and *n*-octylamine (0.2 mL, 1.25 mmol) in toluene (10 mL) was heated to reflux for 3 h under nitrogen. After completion of the reaction, toluene was

removed on a rotary evaporator and the product was purified by chromatography on a silica gel column(100–200 mesh) eluted with 25 % dichloromethane in *n*-hexane. 7 was obtained as red crystals (570 mg, 96 %); IR ($CHCl_3$, cm^{-1}) =3250, 2957, 2923, 2854, 1707, 1675, 1635, 1587, 1521, 1459, 1323, 1279, 1184, 875, 786 cm1; ^1H NMR (400 MHz, $CDCl_3$): δ_H ppm 10.14–10.12 (t, *J* = 4.8 Hz,1 H, N-H), 8.66–8.64 (d, *J*=8 Hz, 1 H, Ar-H), 8.35–8.33 (d, *J* = 8 Hz, 1 H, Ar-H), 8.21 (s, 1 H, Ar-H), 4.20–4.14 (m, 4 H, $2CH_2$), 3.60–3.55 (q, *J* = 7.2 Hz, 2 H, CH_2), 1.86–1.79 (quin, *J*=7.2 Hz, 2 H, CH_2), 1.76–1.69 (quin, *J* = 7.2 Hz, 4 H, $2CH_2$), 1.47–1.28 (m, 30 H, $15CH_2$), 0.91–0.87 ppm (t, *J* = 6.8 Hz, 9 H, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.4, 163.5, 163.2, 163.1, 152.5, 131.4, 129.7, 128.1, 126.3, 124.5, 123.7, 119.9, 119.5,99.9, 43.5, 41.0, 40.5, 31.97, 31.93, 29.6, 29.5, 29.4, 29.4, 29.34, 29.31, 28.2, 27.3, 27.2, 27.1, 22.7, 14.2; Elemental analysis: calcd for $C_{38}H_{56}N_3O_4$: C 73.87, H 8.97, N 6.80; found: C 73.79, H 8.74, N 6.79; HRMS (APCI):m/z: calcd for $C_{38}H_{56}N_3O_4$: 618.4271 $[M+H]^+$; found: 618.4247.

***N,N′*-Bis-(*n*-octyl)-2,6-di(*n*-octylamino)-1,4,5,8-naphthalenetetracarboxylic diimide (8)**

A mixture of *N,N'*-bis(*n*-octyl)-2,6-bromo-NDI **5** (648 mg, 1 mmol) and *n*-octylamine (0.41 mL, 2.5 mmol) in toluene (10 mL) was refluxed for 12 h under nitrogen. Completion of the reaction was monitored by TLC. After completion, toluene was removed on a rotary evaporator, and the product was purified by column chromatography on silica gel (100-200 mesh) column eluted with 15% dichloromethane in hexane. *N,N′*-bis(*n*-octyl)-2,6-di(*n*-octylamino)-NDI **8** was obtained as dark blue crystals (685 mg, 92%); IR ($CHCl_3$, cm^{-1}): 3283, 2956, 2925, 2854, 1682, 1631, 1587, 1487, 1465, 1318, 1186, 886, 789; ^1H NMR (400 MHz, $CDCl_3$): δ_H ppm 9.33-9.31 (t, *J* = 4.6 Hz, 2H, N-H), 8.10 (s, 2H, Ar-H), 4.17-4.13 (t, *J* = 8 Hz, 4H, $2CH_2$), 3.50-3.45 (q, *J* = 8 Hz, 4H, CH_2), 1.83-1.67 (m, 8H), 1.36-1.28 (m, 40H, $20CH_2$), 0.89-0.86 (t, *J* = 6.6 Hz, 12H, $4CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.3, 163.2, 149.3, 125.9, 121.2, 118.4, 101.9, 53.5, 43.3, 40.6, 31.9, 31.9, 29.6, 29.5, 29.4, 29.4, 29.3, 28.2, 27.4, 27.3, 22.8, 14.2; Elemental analysis. Found: C, 73.59, H, 9.70; N, 7.41; Calcd: C, 74.15; H, 9.74; N, 7.52 for $C_{46}H_{72}N_4O_4$. HRMS (APCI) (*m/z*): calcd for $C_{46}H_{73}N_4O_4$ $[M + H]^+$, 745.5632, found 745.5596

_N,N′_-Bis-(*n*-octyl)-2,3,6,7-tetra(*n*-octylamino)-1,4,5,8-naphthalenetetracarboxylic diimide(9)

A mixture of *N,N′*-bis-(*n*-octyl)-2,3,6,7-tetra(*n*-octylamino)-NDI **6** (806 mg, 1 mmol) and *n*-octylamine (0.83 mL, 5 mmol) in toluene (10 mL) was refluxed for 10 h under nitrogen. Completion of the reaction was monitored by TLC. After completion, toluene was removed on a rotary evaporator and the product was purified by column chromatography on silica gel (100-200 mesh) column eluted with 5% dichloromethane in hexane, *N,N′*-Bis-(*n*-octyl)-2,3,6,7-tetra(*n*-octylamino)-NDI **9** was obtained as dark green crystals (980 mg, 98%). IR ($CHCl_3$, cm^{-1}): 3264, 2955, 2921, 2850, 1643, 1629, 1577, 1520, 1454, 1283, 1180, 1142, 1124, 843, 791, 724; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 9.39 (bs, 4H), 4.21-4.17 (t, J = 7.6 Hz, 4H, $2CH_2$), 3.38-3.35 (apparent t, J = 6.4 Hz, 8H, $4CH_2$), 1.76-1.68 (m, 4H, $2CH_2$), 1.54-1.46 (m, 8H, $4CH_2$), 1.29-1.21 (m, 60H, $30CH_2$), 0.89-0.85 (m, 18H, $6CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.1, 147.1, 117.1, 108.0, 45.2, 40.4, 32.0, 31.9, 31.3, 29.5, 29.4, 29.4, 29.3, 28.2, 27.4, 27.2, 22.8, 22.7, 14.3, 14.2; Elemental analysis. Found: C, 74.22, H, 10.30; N, 8.23; Calcd: C, 74.50; H, 10.69; N, 8.41 for $C_{62}H_{106}N_6O_4$. HRMS (APCI) (*m/z*): calcd. for $C_{62}H_{107}N_6O_4$ $[M + H]^+$, 999.8354, found 999.8328.

General procedure for one-step synthesis of imide and core substituted NDIs (9-10) with *n*-butylamine

The mono-, di- and tetra-brominated NDA compounds **1**–**3** (2 mmol each) in *n*-butylamine were refluxed under nitrogen atmosphere for 10, 24 and 32 h respectively. The reaction progress was monitored by thin layer chromatography (TLC). After completion of the reaction, excess *n*-butylamine was removed on rotary evaporator and the products were purified by column chromatography on silica gel (100–200 mesh) to obtain imide and core functionalized NDIs **10**, **11**, and **12** with overall 60%, 50% and 46% yields respectively.

Compound **10**: IR ($CHCl_3$,cm^{-1}): 3254, 2959, 2926, 2854, 1704, 1673, 1634,1589, 1519, 1460, 1324, 1016, 786; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 10.13 (broad t, 1H, N–H), 8.66–8.64 (d, J= 8 Hz, 1H, Ar-H), 8.35–8.33 (d, J = 8 Hz, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 4.21–4.15 (m, 4H, $2CH_2$), 3.61–3.56 (q, J = 6.8 Hz, 2H, CH_2), 1.86–1.78 (quin, J = 7.2 Hz, 2H, CH_2), 1.75–1.68 (quin, J = 7.2 Hz, 4H, $2CH_2$), 1.49–1.28 (m, 6H, $3CH_2$), 1.04–0.97 (m, 9H, $3CH_3$);

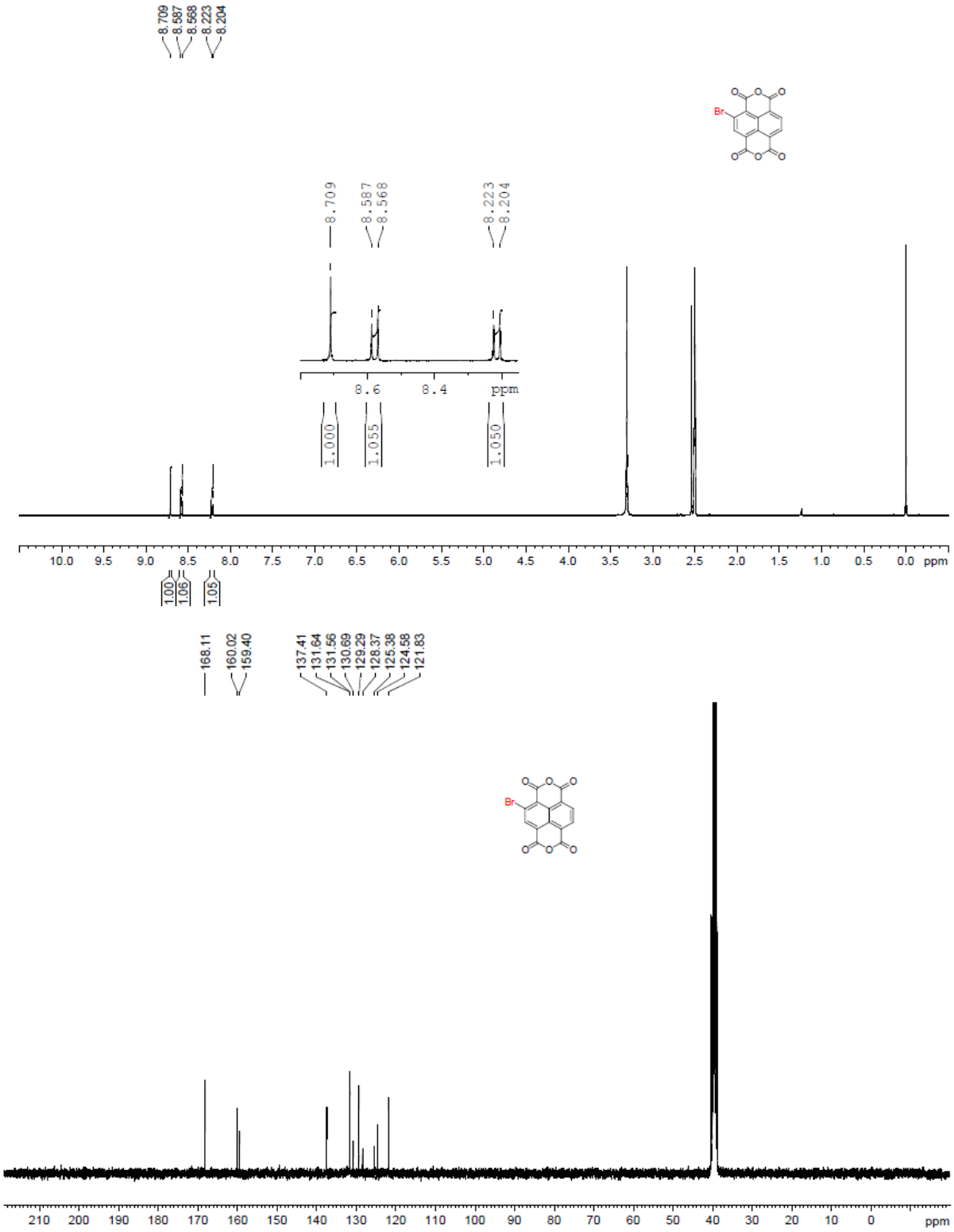
13C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.4, 163.5, 163.24, 163.22, 152.5, 131.4, 129.7, 128.1, 126.3, 124.5, 123.7, 119.9, 119.5, 99.9, 43.1, 40.8, 40.3, 31.6, 30.3, 20.6, 20.4, 20.3, 14.0, 13.95, 13.92; HRMS (APCI) (m/z): calcd for $C_{26}H_{31}N_3O_4$ $[M+H]^+$, 450.2393, found 450.2395.

Compound **11**: IR ($CHCl_3$,cm^{-1}): 3286, 2959, 2926, 2854, 1679, 1632, 1596, 1489, 1465, 1320, 1209, 789; ^{1}H NMR (400 MHz, CDCl3): δ_H ppm 9.33-9.30 (t, *J* = 5.1 Hz, 2H, N–H), 8.10 (s, 2H, Ar-H), 4.18–4.14 (t, *J* = 8 Hz, 4H, $2CH_2$), 3.51–3.46 (q, *J* = 6.8 Hz, 4H, $2CH_2$), 1.83–1.75 (quin, *J* = 7.2 Hz, 4H, $2CH_2$), 1.74–1.66 (m, 4H, $2CH_2$), 1.56–1.41 (m, 8H, $4CH_2$), 1.03–0.97 (m, 12H, $4CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.2, 163.1, 149.2, 125.8, 121.1, 118.3, 101.8, 43.0, 40.3,31.6, 30.3, 29.8, 20.6, 20.4, 14.0, 13.9; HRMS (APCI) (m/z): calcd for $C_{30}H_{40}N_4O_4$ $[M+H]^+$, 521.3128, found 521.3127.

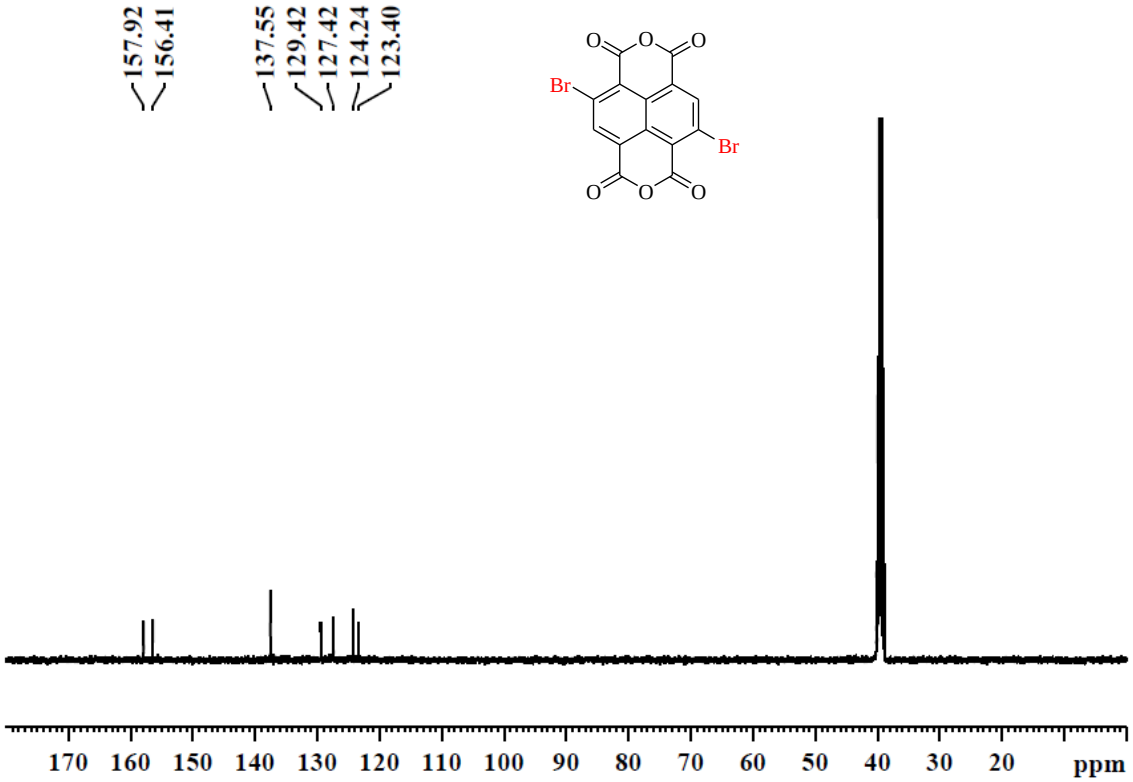
Compound **12**: IR ($CHCl_3$,cm^{-1}): 3274, 2961, 2916, 2850, 1645, 1629, 1577,1462, 1263, 1093, 1026, 798; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 9.38 (broad t, 4H), 4.23–4.19 (t, *J* = 7.6 Hz, 4H, $2CH_2$), 3.41–3.36 (q, *J* = 6.7 Hz, 8H, $4CH_2$), 2.06–2.01 (m, 24H, $12CH_2$), 1.01–0.97 (t, 18H, $6CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 166.1,139.4, 114.2, 108.05, 44.9, 40.2, 33.9, 33.5, 32.0, 29.3, 29.1, 22.8, 14.2,14.1, 14.0; HRMS (APCI) (m/z): calcd. for $C_{38}H_{58}N_6O_4$[M+H]$^+$, 663.4598, found 663.4563.

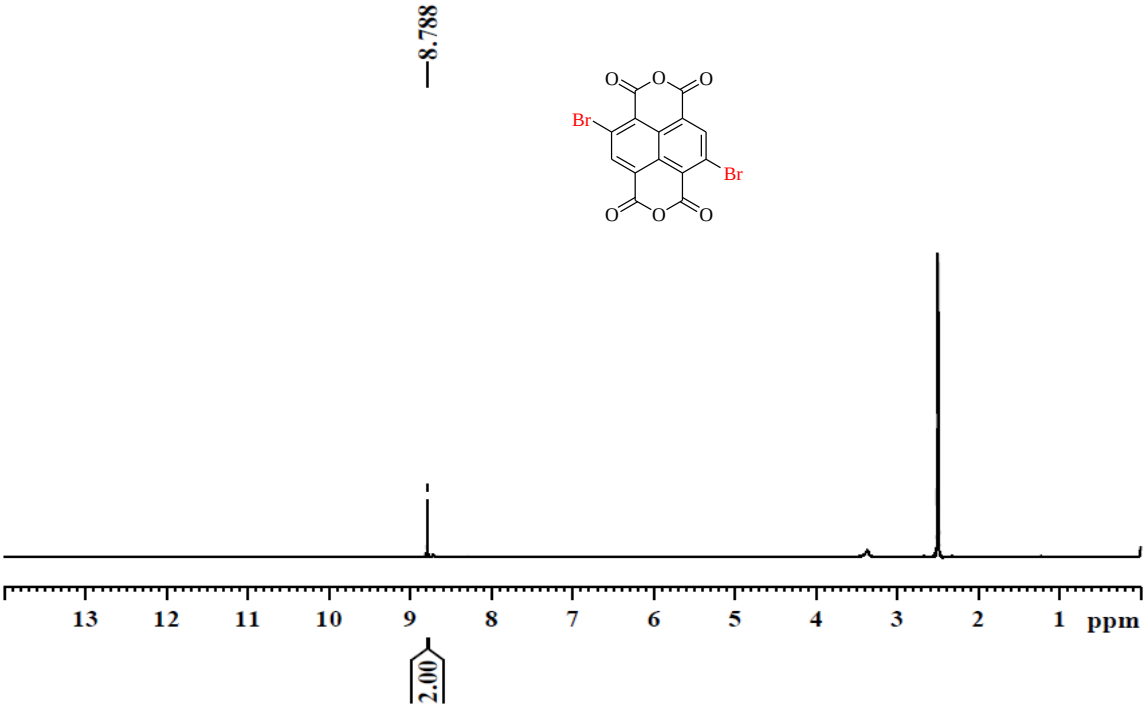
2.9. Appendix

^{1}H and ^{13}C NMR spectrum of compound **1**

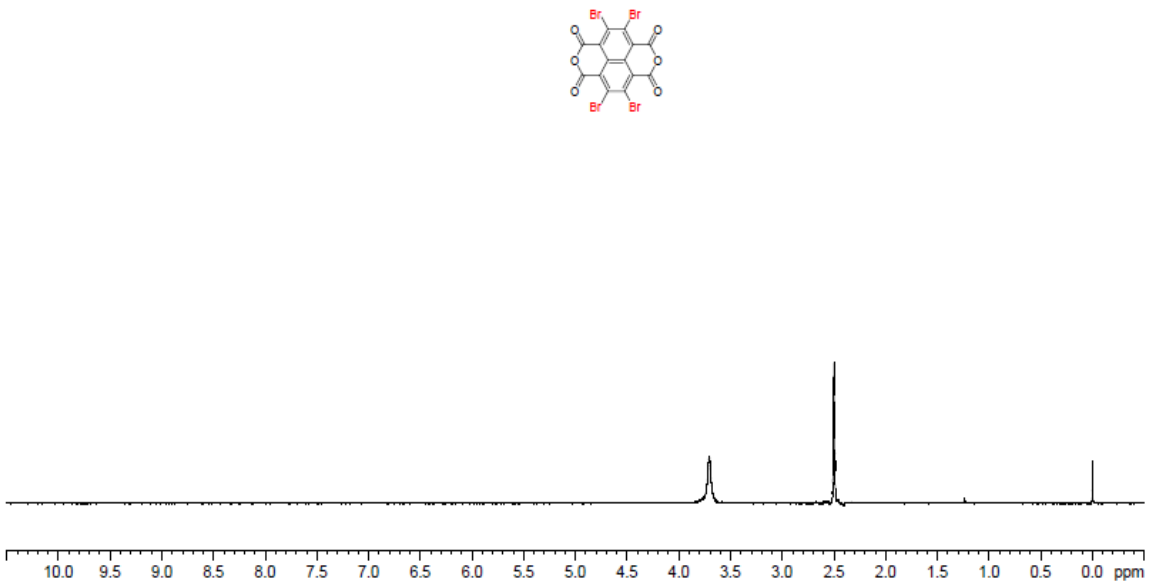


^{1}H and ^{13}C NMR spectrum of compound **2**

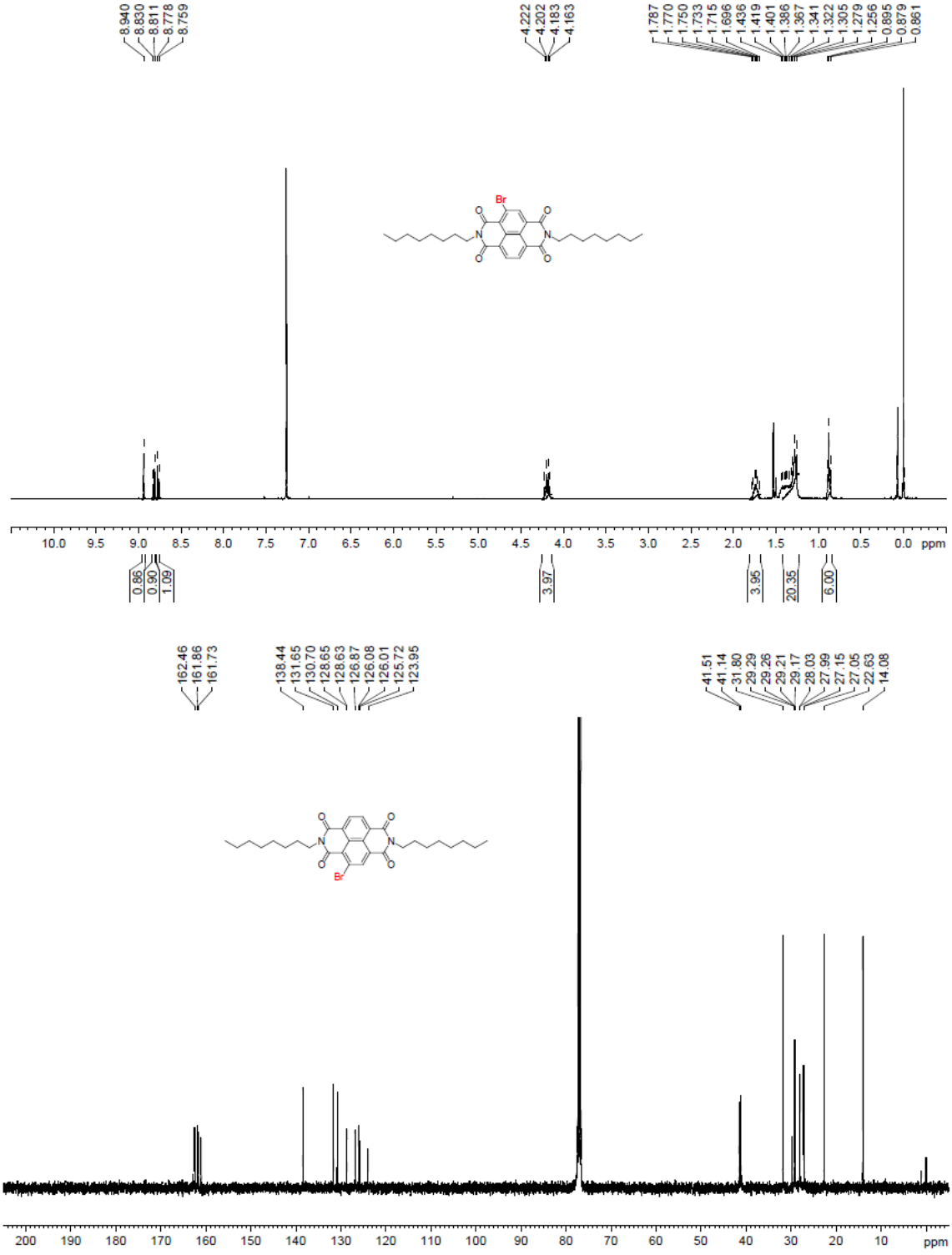




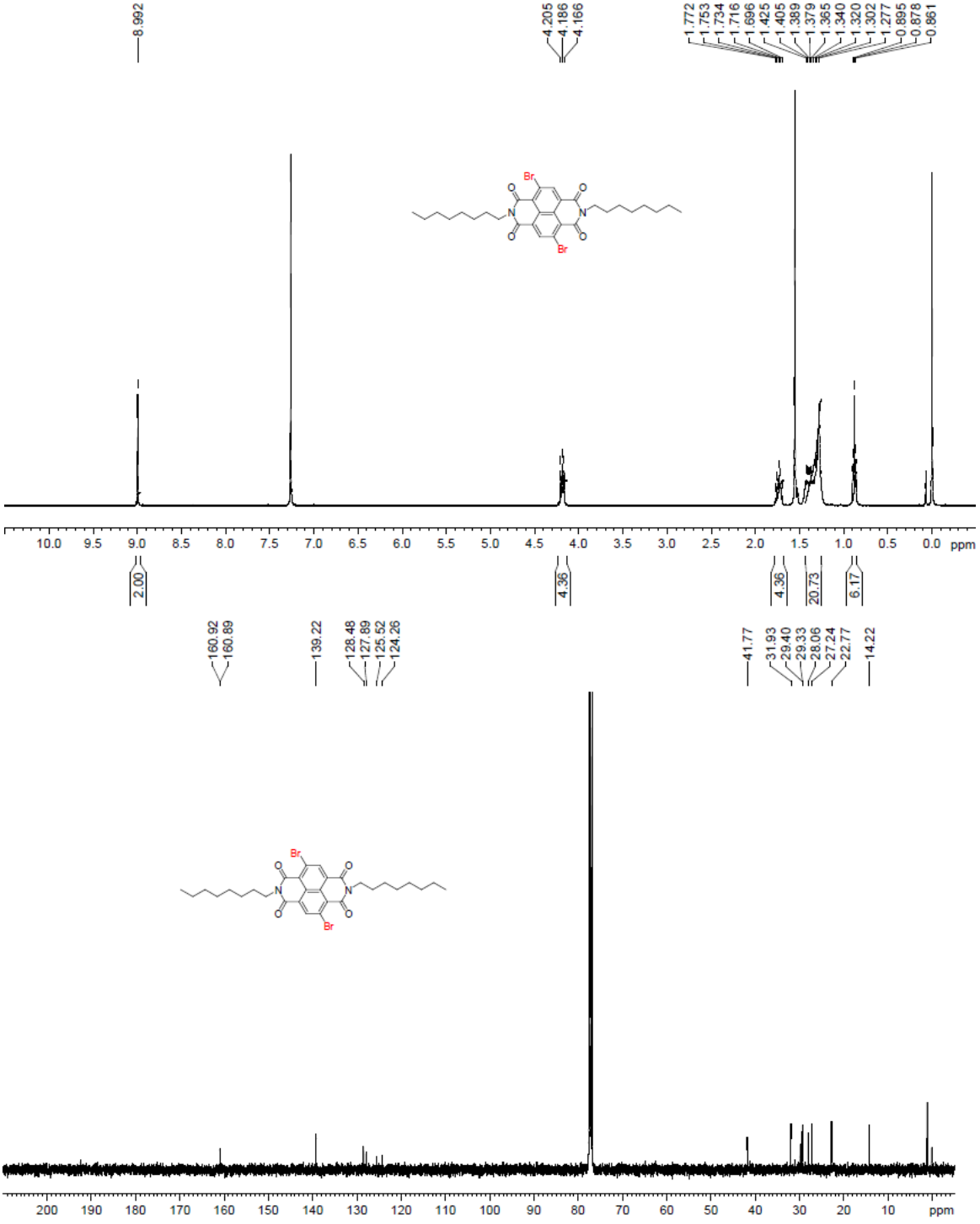
^{1}H NMR spectrum of compound **3** (there are no protons in **3** and hence no signals except for solvent observed in the spectrum)



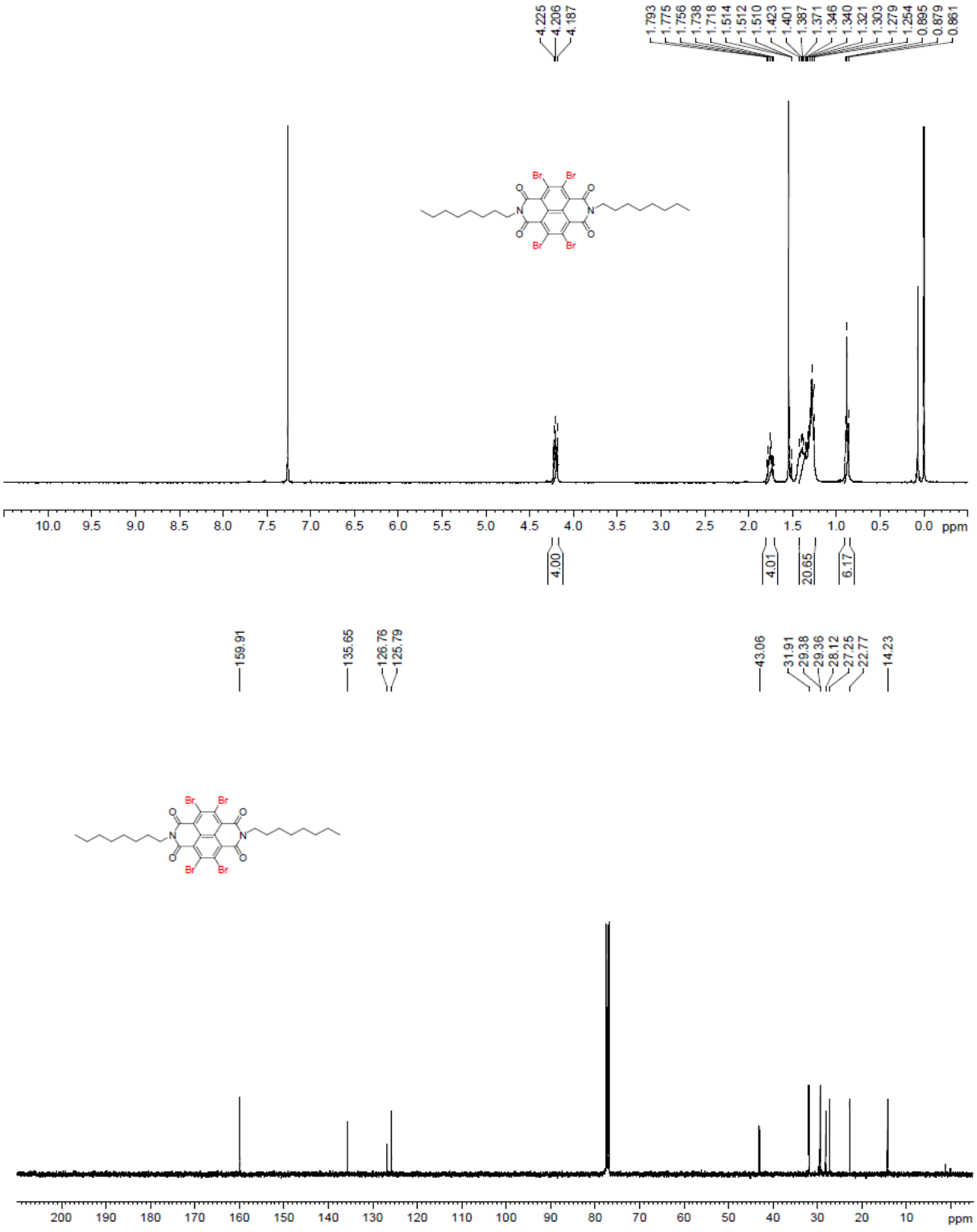
^{1}H and ^{13}C NMR spectrum of compound **4**



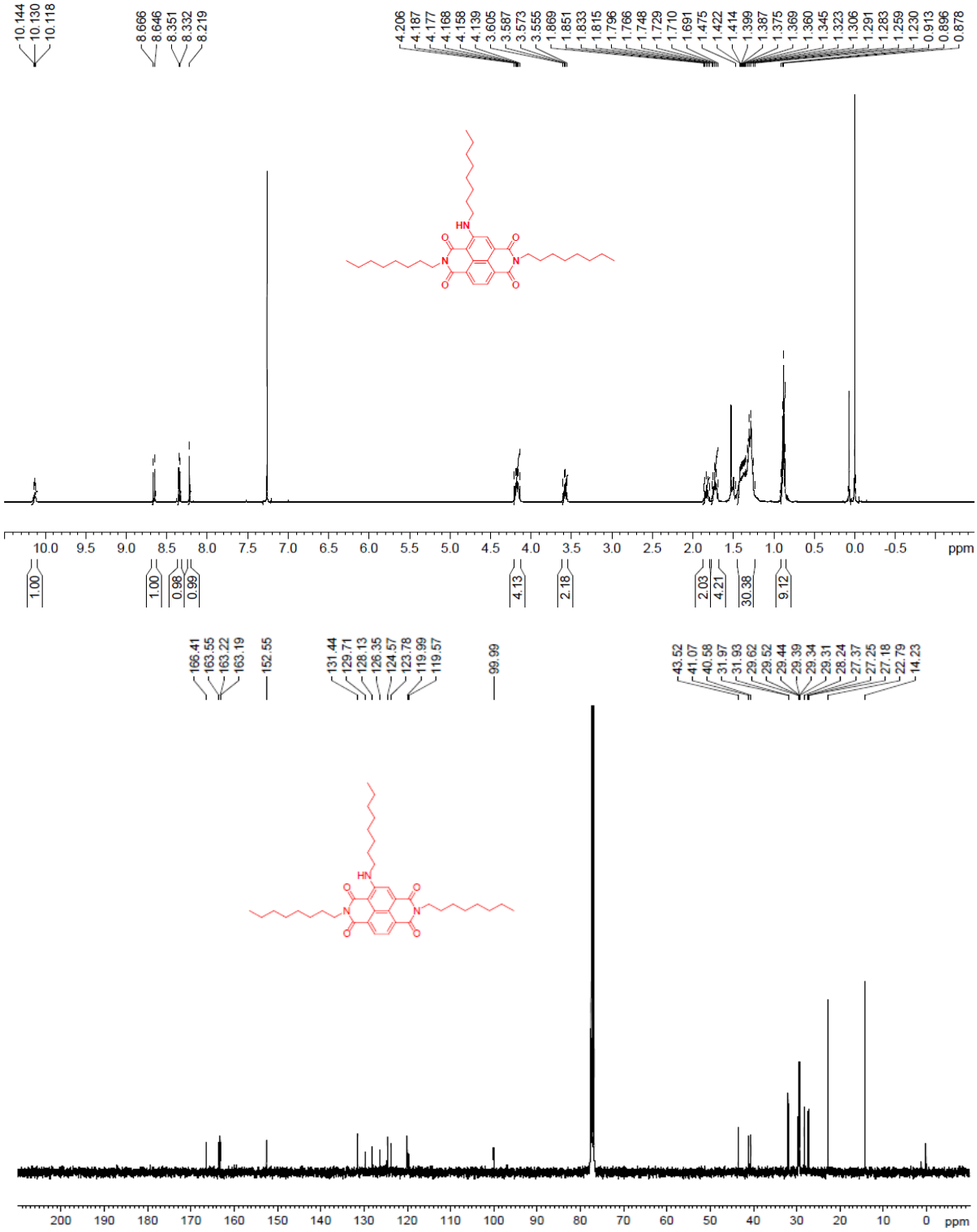
^{1}H and ^{13}C NMR spectrum of compound **5**



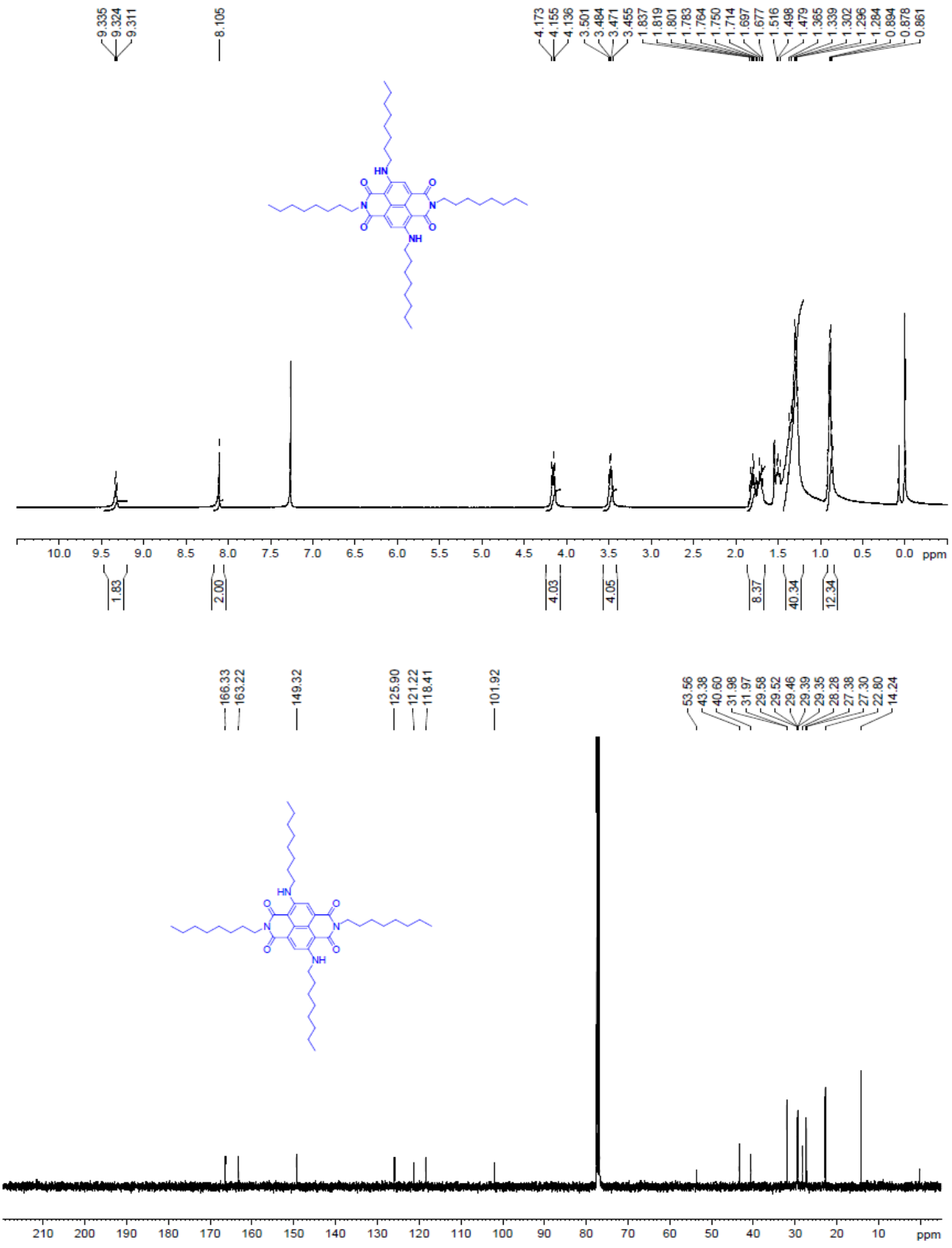
^{1}H and ^{13}C NMR spectrum of compound **6**



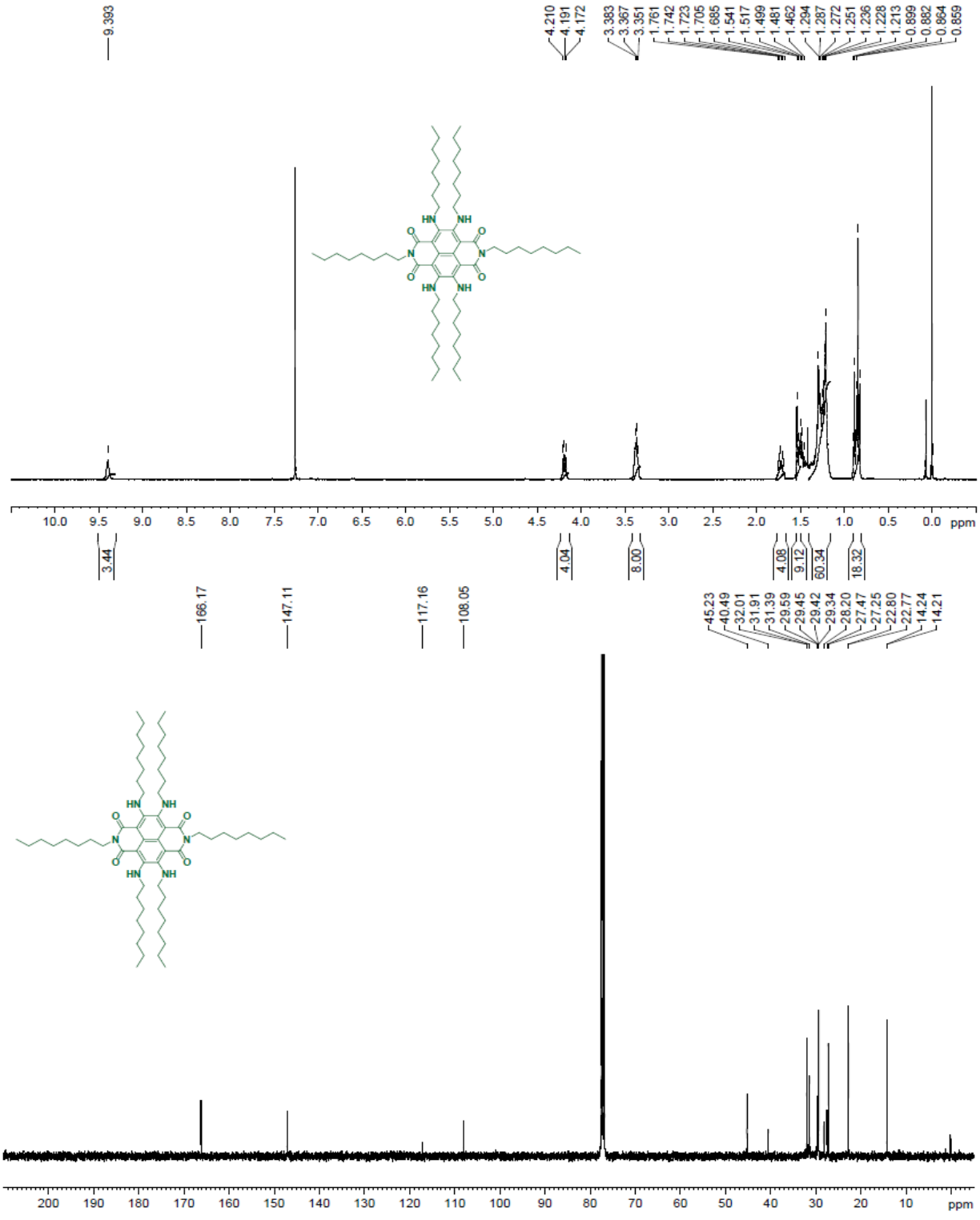
^{1}H and ^{13}C NMR spectrum of compound **7**



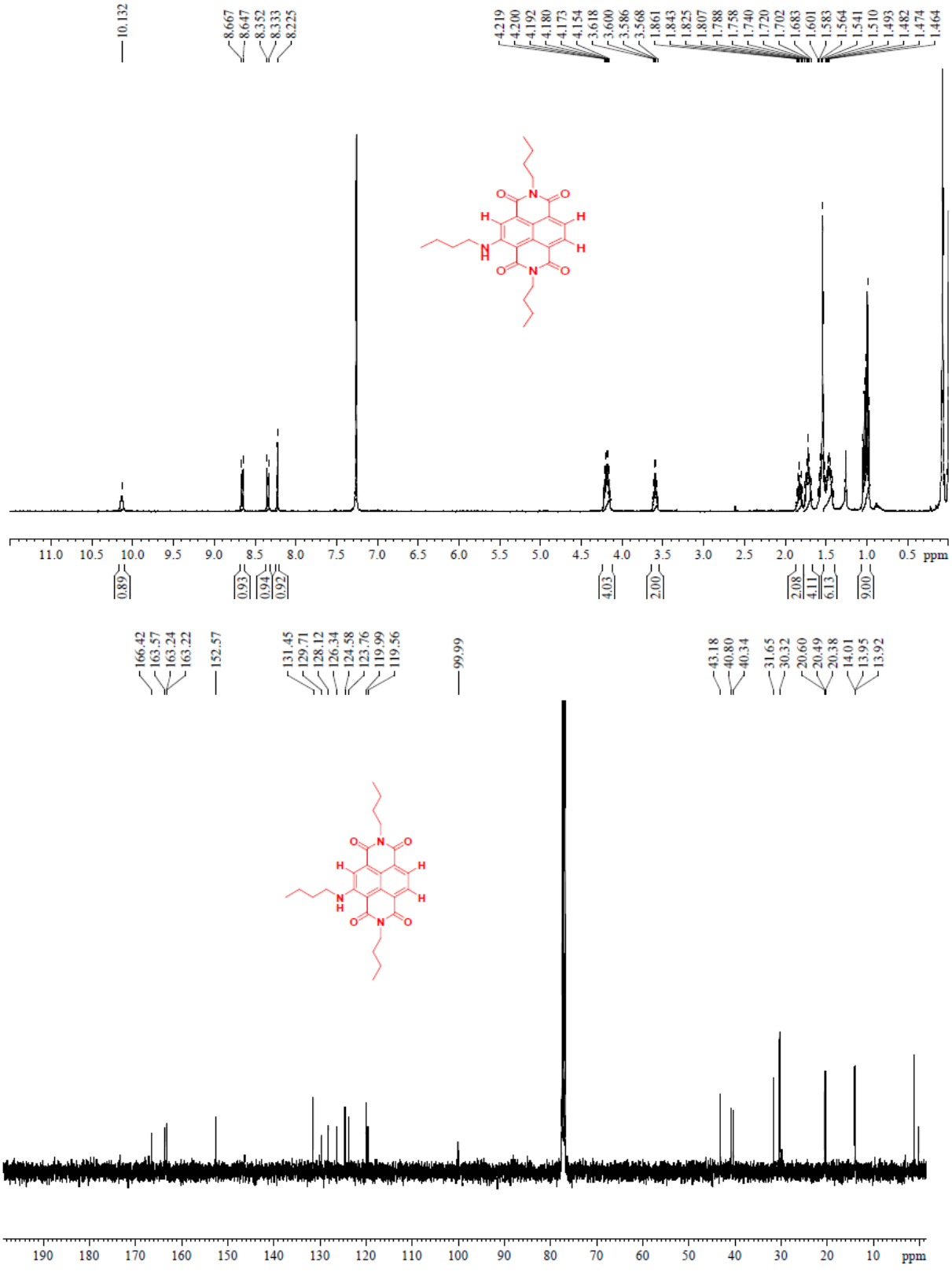
^{1}H and ^{13}C NMR spectrum of compound **8**



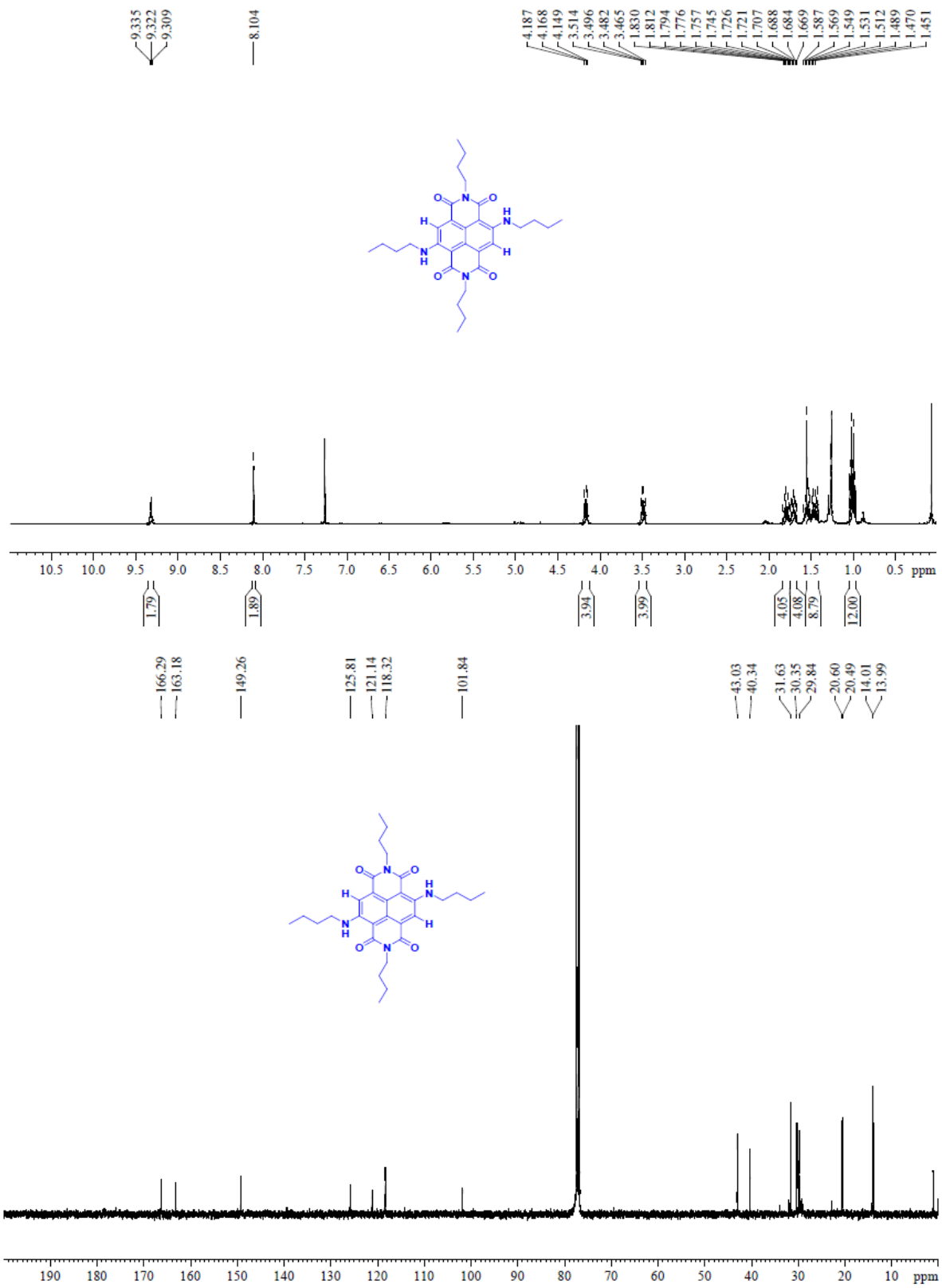
^{1}H and ^{13}C NMR spectrum of compound **9**



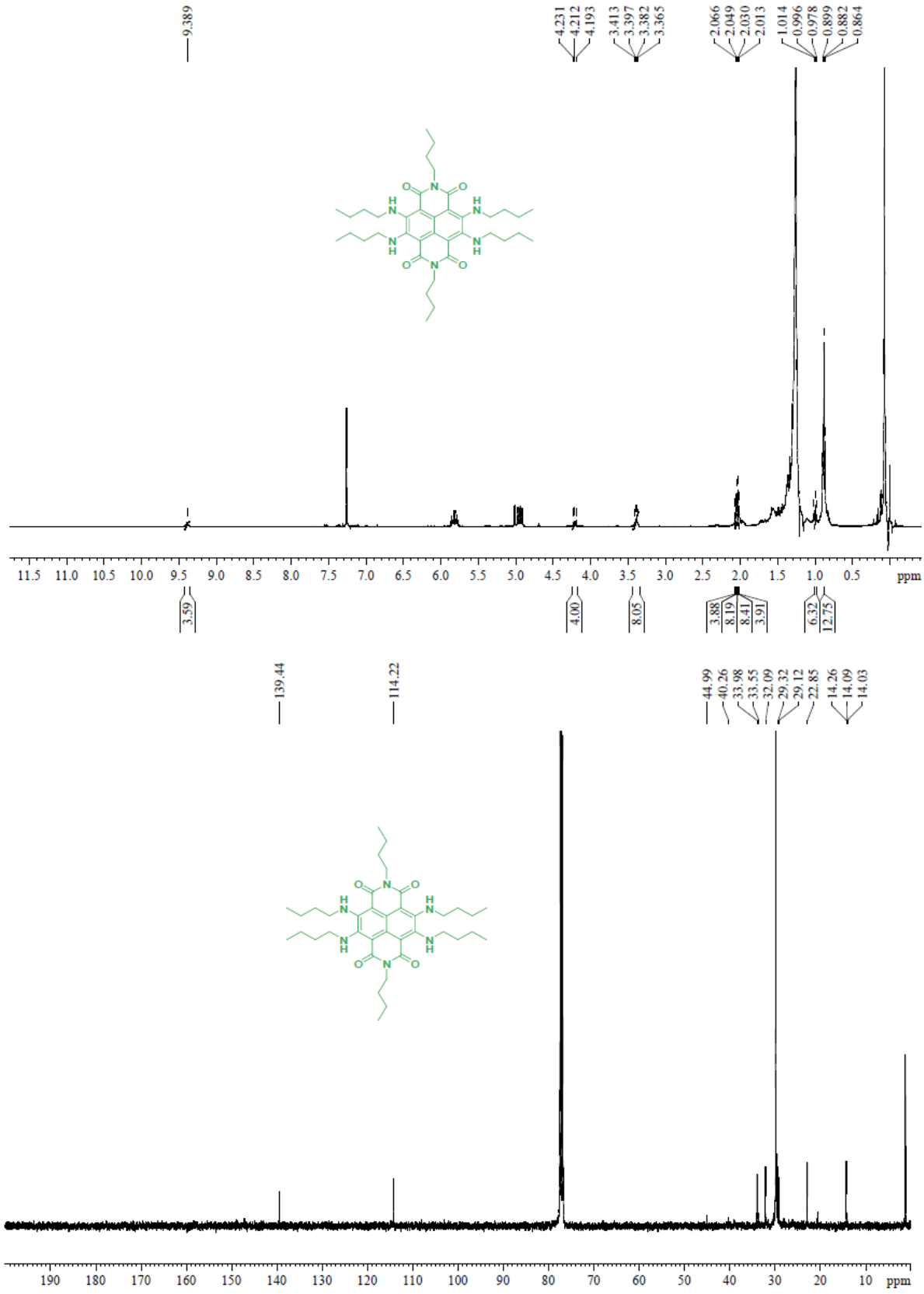
^{1}H and ^{13}C NMR spectrum of compound **10**



^{1}H and ^{13}C NMR spectrum of compound **11**

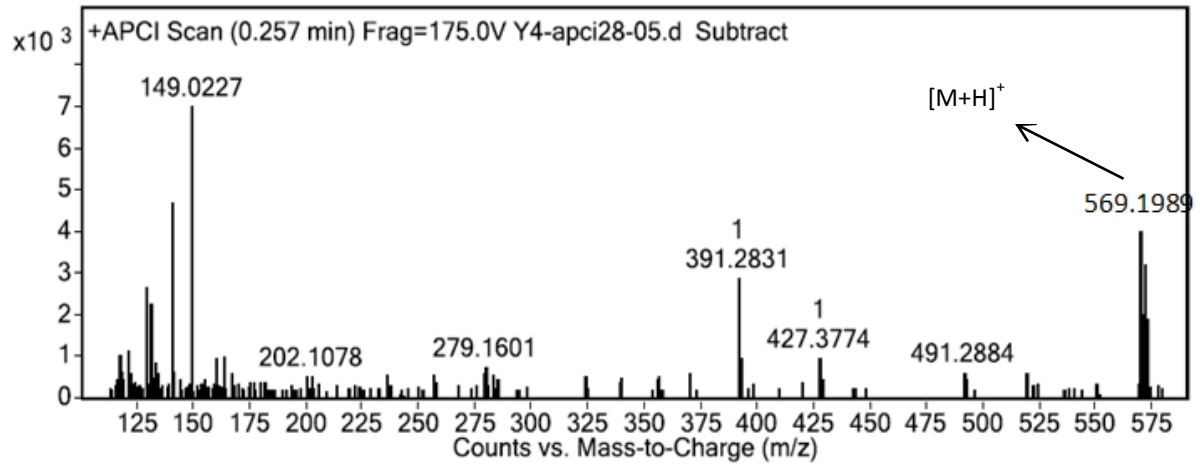


^{1}H and ^{13}C NMR spectrum of compound 12

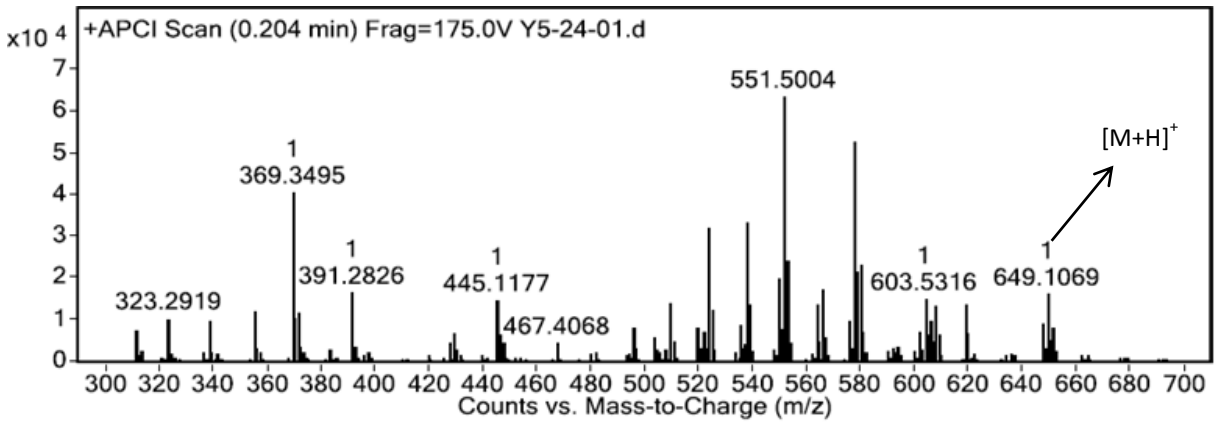


HRMS data

Mass spectrum of compound **4 (Calculated $[M+H]^+$ = 569.2015)**

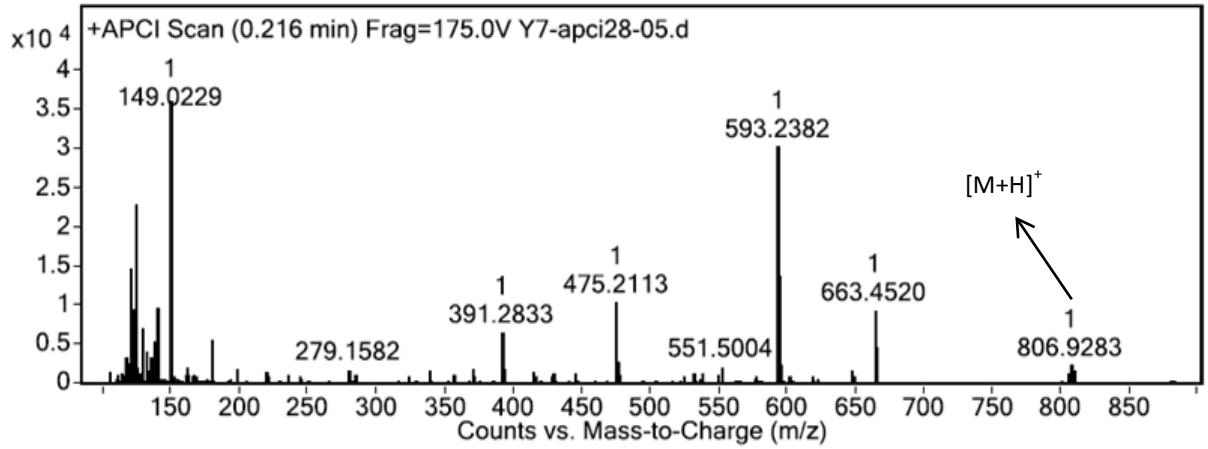


Mass spectrum of compound **5 (Calculated $[M+H]^+$ = 649.1100)**

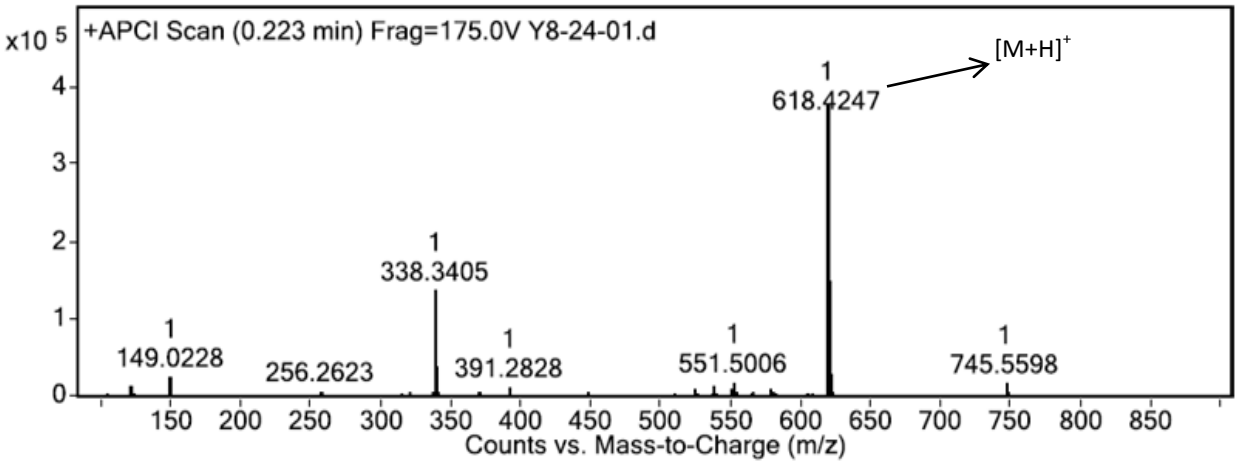


M

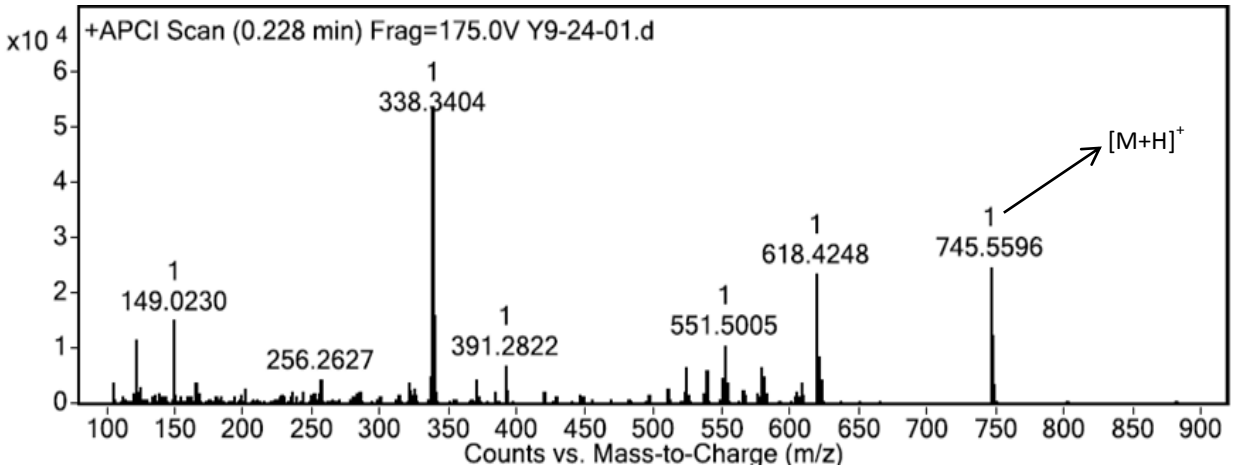
Mass spectrum of compound **6 (Calculated $[M+H]^+$ = 806.9289)**



Mass spectrum of compound **7 (Calculated $[M+H]^+$ = 618.4271)**

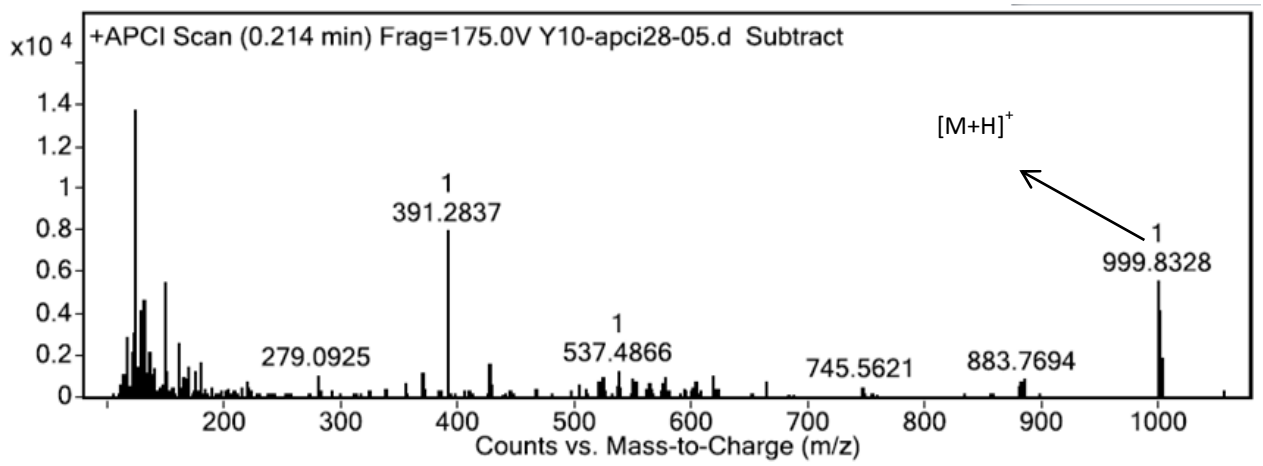


Mass spectrum of compound **8 (Calculated [M+H]$^+$ = 745.5632)**

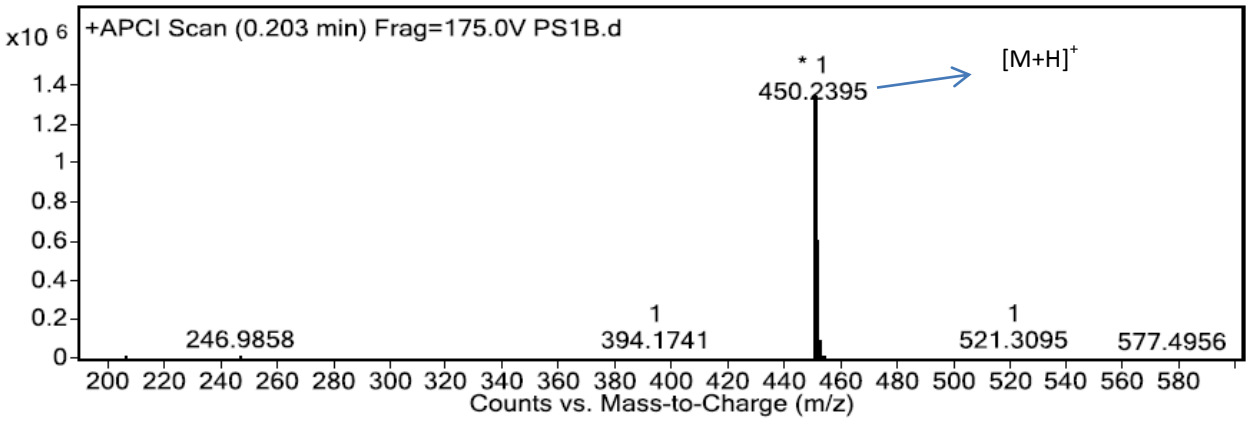


Mass spectrum of compound **9** (**Calculated [M+H]$^+$ = 999.8354**)

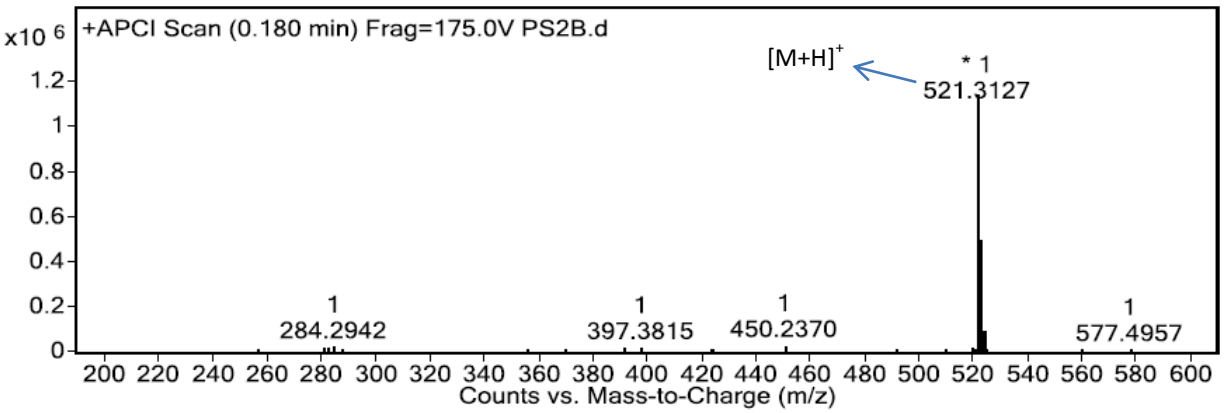
M



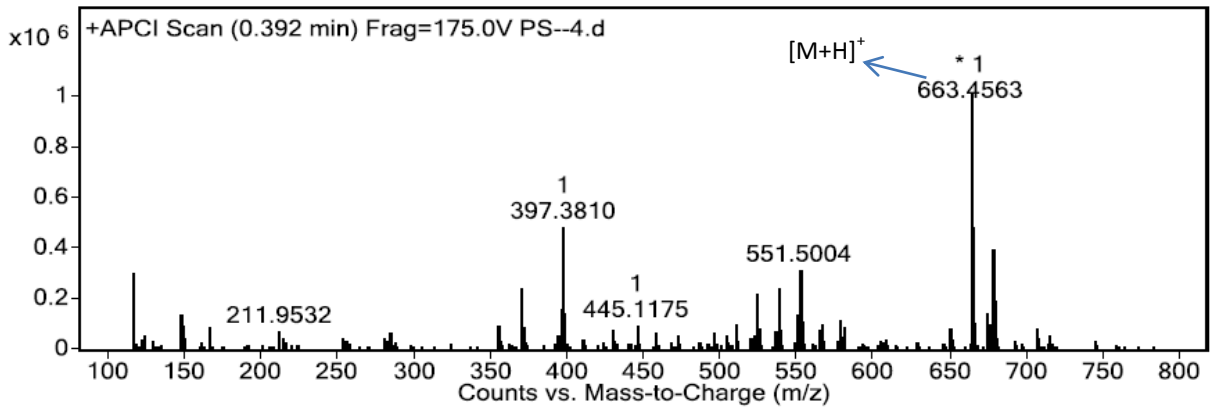
Mass spectrum of compound **10**(**Calculated [M+H]$^+$ = 450.2393**)



Mass spectrum of compound **11** (**Calculated [M+H]$^+$ = 521.3128**)



Mass spectrum of compound **12**(**Calculated [M+H]$^+$ = 663.4598**)



2.10. References

1. Sakai, N.; Mareda, J.; Vauthey, E.; Matile, S., Core-substituted naphthalenediimides. *Chem. Commun*. **2010**, *46*, 4225-4237.
2. a) Lee, H. N.; Xu, Z.; Kim, S. K.; Swamy, K. M. K.; Kim, Y.; Kim, S. J.; J. Yoon., Pyrophosphate-Selective fluorescent chemosensor at physiological pH: formation of a unique excimer upon addition of pyrophosphate. *J. Am. Chem. Soc*. **2007**, *129*, 3828–3829. b) Oh, J. H.; Suraru, S.-L.; Lee, W.-Y.; Kçnemann, M.; Hçffken, H. W.; Rçger, C.; Schmidt, R.; Chung, Y.; Chen, W.- C.; Wurthner, F.; Bao, Z., Rylene and related diimides for organic electronics. *Adv. Funct. Mater*. **2010**, *20*, 2148–2156.
3. Bell, T. D. M.; Yap, S.; Jani, C. H.; Bhosale, S. V.; Hofkens, J.; Schryver, F. C. D.; Langford, S. J.; Ghiggino, K. P., Synthesis and photophysics of core-substituted naphthalene diimides: fluorophores for single molecule applications. *Chem. Asian J.* **2009**, *4*, 1542–1550.
4. Jacquignon, P.; Buu-Hoi, N. P.; Mangane, M., *Bull. Soc. Chim. Fr.* **1964**, *10*, 2517–2523;
5. Gao, X.; Qiu, W.; Yang, X.; Liu, Y.; Wang, Y.; Zhang, H.; Qi, T.; Liu, Y.; Lu, K.; Du, C.; Shuai, Z.; Yui, G.; Zhu, D., First synthesis of 2,3,6,7- tetrabromonaphthalene diimide. *Org. Lett.* **2007**, *9*, 3917–3920.
6. Piyakulawat, P.; Keawprajak, A.; Chindaduang, A.; Hanusch, M.; Asawapirom, U., Synthesis and preliminary characterization of novel naphthalene bisimide based copolymers. *Synth. Metals*. **2009**, *159*, 467–472.
7. Thalacker, C.; Roger, C.; Würthner, F., Synthesis and optical and redox properties of core-substituted naphthalene diimide dyes. *J. Org. Chem.* **2006**, *71*, 8098–8105.
8. Chaignon, F.; Falkenstrom, M.; Karlsson, S.; Blart, E.; Odobel, F.; Hammarstrom, L., Very large acceleration of the photoinduced electron transfer in a Ru(bpy)3–naphthalene bisimide dyad bridged on the naphthyl core. *Chem. Commun.* **2007**, 64–66.
9. Krüger, H.; Janietz, S.; Sainova, D.; Dobreva, D.; Koch, N.; Vollmer., Hybrid supramolecular naphthalene diimide-thiophene structures and their application in polymer electronics. *Adv. Funct. Mater.* **2007**, *17*, 3715–3723.

10. Kishore, R. S. K.; Ravikumar, V.; Bernardinelli, G.; Sakai, N.; Matile, S., Rapid and mild synthesis of functionalized naphthalenediimides. *J. Org. Chem.* **2008**, *73*, 738–740.
11. Doria, F.; Marco di Antonio, M. D.; Michele Benotti, M.; Daniela Verga, D.; Freccero, M., Substituted heterocyclic naphthalene diimides with unexpected acidity. synthesis, properties, and reactivity. *J. Org. Chem.* **2009**, *74*, 8616–8625.
12. Guo, S.; Wu, W.; Guo, H.; Zhao, J., Room-temperature long-lived triplet excited states of naphthalenediimides and their applications as organic triplet photosensitizers for photooxidation and triplet−triplet annihilation upconversions. *J. Org. Chem.* **2012**, *77*, 3933–3943.
13. Hernandez-Torres, G.; Tan, B.; Barbas III, C. F., Organocatalysis as a safe practical method for the stereospecific dibromination of unsaturated compounds. *Org. Lett*. **2012**, *14*, 1858–1861.
14. Jones, B. A.; Facchetti, A.; Marks, T. J.; Wasielewski, M. R., Cyanonaphthalene diimide semiconductors for air-Stable, flexible, and optically transparent n-channel field-effect transistors. *Chem. Mater*. **2007**, *19*, 2703–2705.
15. Hu, Y.; Gao, X.; Di, C.-A.; Yang, X.; Zhang, F.; Liu, Y.; Li, H.; Zhu, D., Core-expanded naphthalene diimides fused with sulfur heterocycles and end-capped with electron-withdrawing groups for air-stable solution-processed n-channel organic thin film transistors. *Chem. Mater*. **2011**, *23*, 1204–1215
16. Roger, C.; Würthner, F. Core-tetrasubstituted naphthalene diimides: synthesis, optical properties, and redox characteristics. *J. Org. Chem*. **2007**, *72*, 8070–8075.
17. Gao, X.; Qiu, W.; Yang, X.; Liu, Y.; Wang, Y.; Zhang, H.; Qi, T.; Liu, Y.; Lu, K.; Du, C.; Shuai, Z.; Yui, G.; Zhu, D., First synthesis of 2,3,6,7-tetrabromonaphthalene diimide. *Org. Lett.* **2007**, *9*, 3917–3920.
18. de Almeida, L. S.; Esteves, P. M.; de Mattos, M. C. S., Tribromoisocyanuric acid: a new reagent for regioselective cobromination of alkenes. *Synlett* **2006**, *10*, 1515–1518.
19. de Almeida, L. S.; Esteves, P. M.; de Mattos, M. C. S., A new regioselective bromination of activated aromatic rings. *Synthesis* **2006**, *2*, 221–223.

20. Sasikumar, M.; Suseela, Y. V.; Govindaraju, T. Dibromohydantoin: a convenient brominating reagent for 1,4,5,8-naphthalenetetracarboxylic dianhydride. *Asian J. Org. Chem.* **2013**, *2*, 779–785.
21. Suseela, Y. V.; Sasikumar, M.; Govindaraju, T. An effective and regioselective bromination of 1,4,5,8-naphthalenetetracarboxylic dianhydride using tribromoisocyanuric acid. *Tetrahedron Lett.* **2013**, *54*, 6314–6318.
22. Lakowicz, J. R.; Principles of fluorescence spectroscopy, 2nd ed., Kluwer/Plenum, New York, **1999**, pp. 52–53.
23. Fischer, M.; Georges, J., Fluorescence quantum yield of rhodamine 6G in ethanol as a function of concentration using thermal lens spectrometry. *Chem. Phys. Lett.* **1996**, *260*, 115–118.
24. Lide, D. R., CRC Handbook of chemistry and physics, 72nd ed., CRC, Boca Raton, **1991**.

Chapter 3

Synthesis of NDI derivatives for DNA binding studies

3.1. Introduction

Deoxyribonucleic acid (DNA) is a well-known carrier for genetic information and is important for functioning of all living organisms. DNA is considered as one of the most promising biological receptors for the development of chemotherapeutic agents.[1] DNA has been identified as a primary target for developing drugs to many diseases. These drugs can change the DNA conformation and inhibit replication or transcription. Since, DNA has been the target for majority of antibiotic and anticancer drugs, study of small molecule and DNA interactions play a key role in pharmacology.[2-5] In the past few years, remarkable advances in the design of sequence-selective DNA binding agents have been achieved.[2,5-13] Recognition of DNA base pairs via specific hydrogen bonding interactions in the minor groove has been achieved by the development of polyamides based inhibitors.[4,6-8] Apart from polyamides various other successful small molecules and oligomers have been successfully developed which includes triple-helix forming oligonucleotides,[9] peptide nucleic acids (PNAs),[10] modified zinc-finger proteins,[11] and small molecules such as minor-groove binders like diamidino-2-phenylindole (DAPI) and bis-benzimidazoles (Hoechst dyes), and several intercalators *e.g.,* ethidium bromide and propidium iodide.[2,12,13] The interaction of small molecules with DNA plays an essential role in many biological processes. Many of the DNA recognizing compounds have allowed chemists to synthesize numerous derivatives in order to understand and modify their sequence specificity and binding affinity. Intercalation is a well-studied mode of small molecule binding to DNA. The extended planar heteroaromatic ring systems are usually regarded as representative DNA intercalators, especially if they possess electron deficiency or charged aromatic cores. Some complex intercalators such as daunomycin having side chains that allows binding in groove through van der waals interaction and hydrogen bonding to the nearby bases gives extra sequence specificity to the intercalating moiety. The binding affinity and DNA sequence recognition of intercalators can be improved by covalently attaching side chains to the intercalating unit. Simple modifications in intercalating compounds can produce derivatives with novel sequence specificity and selectivity. Eventually, threading intercalators have been developed based on naphthalene diimide (NDI) based derivatives.[14-19] NDI unit is a well-studied threading intercalator in which functional groups attached to two imide nitrogens reside in different grooves of DNA.[16,19] The threading intercalation mode may have several advantages

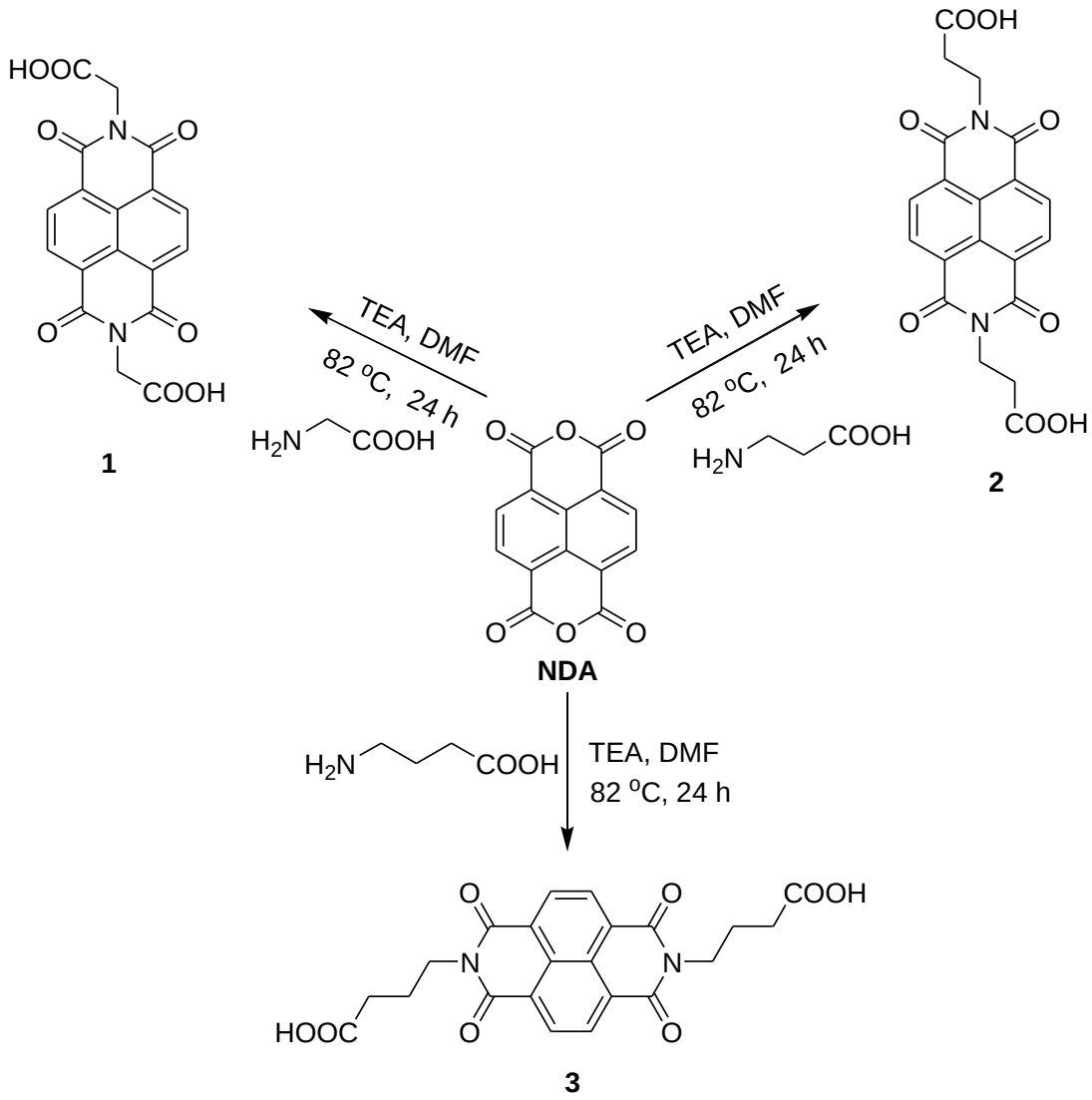
pertaining to biological activity, such as simultaneous blockage of both DNA grooves which may help in effectively regulating gene expression, improved DNA interactions, as well as slow dissociation rates from cellular DNA.[20] NDI derivatives have become major players in this field due to easy synthetic derivatization of the parent naphthalene structure. Wilson *et.al.*[16] investigated kinetic behaviour of symmetric NDI monomers with alky amino substituents attached to both sides which revealed that both association and dissociation rates of the NDIs are significantly lower compared to those of classical intercalators. For the past few years, Iverson and co-workers have developed NDI based bis-intercalators and poly-intercalators with peptide linkers to target duplex DNA with high sequence specificity. It is interesting to note that linker residues binds to both minor and major grooves. The observed binding specificities are explained by proposing possible hydrogen bonds between linkers and base pairs in the groove. Hence, intercalators with linkers on opposite sides represents an effective strategy for targeting specific DNA sequences for the purpose of cancer therapy and antibiotic development.

3.2. Objective of the work

The objective of the work is to synthesize NDI derivatives with benzimidazole moiety as side chains for DNA binding studies. Many classical groove binders like DAPI[21] and Hoechst 33258[22] dyes includes indole and imidazole moieties which have hydrogen bond donor and acceptor sites. These donor and acceptor groups have tendency to involve in hydrogen bonding with base pairs present in minor and major grooves of duplex DNA. NDI derivatives are well known non-specific intercalators for DNA. Therefore, modification of naphthalene dianhydride with benzimidazole attached to imide nitrogens, might result in additional hydrogen binding (amide bond) interactions in the grooves along with an intercalation mode (NDI core). Condensation of NDA with different amino acids of varying chain lengths such as glycine, β-alanine and γ-aminobutyric acid can be used to investigate the contribution of linker flexibility to DNA binding affinity. Hence, NDI derivatives with benzimidazole moiety as a linker of varying length have been synthesized, characterized and used to study DNA binding activity through displacement assays.

3.3. Synthesis of NDI derivatives

NDI derivatives were synthesized in two steps. Initially, naphthalenetetracarboxylic dianhydride (NDA) was condensed with amino acids of varying lengths such as glycine, β-alanine and γ–aminobutyric acid as shown in Scheme 3.1. The NDA was treated with glycine in presence of triethylamine (TEA) base and heated at 82 ^{o}C in DMF for 24 h. The reaction afforded product **1** in 80 % yield.



Scheme 3.1 Synthesis of NDI intermediates **1-3**

Similarly, NDA was treated with β-alanine and γ–aminobutyric acid in TEA base in DMF, heated to 82 ^{o}C for 24 h to yield **2** and **3** in 90 % and 92 % yields respectively. Compound **4** in Figure 3.1 was synthesized according to Scheme 3.1. NDA was treated with 2-(aminomethyl) benzimidazole dihydrochloride in TEA in DMF, heated to 82 ^{o}C for 24 h to yield compound **4** in 90 % yield. Compounds **1**, **2** and **3** act as intermediates with acid group in the side chain for further coupling with benzimidazole moiety.

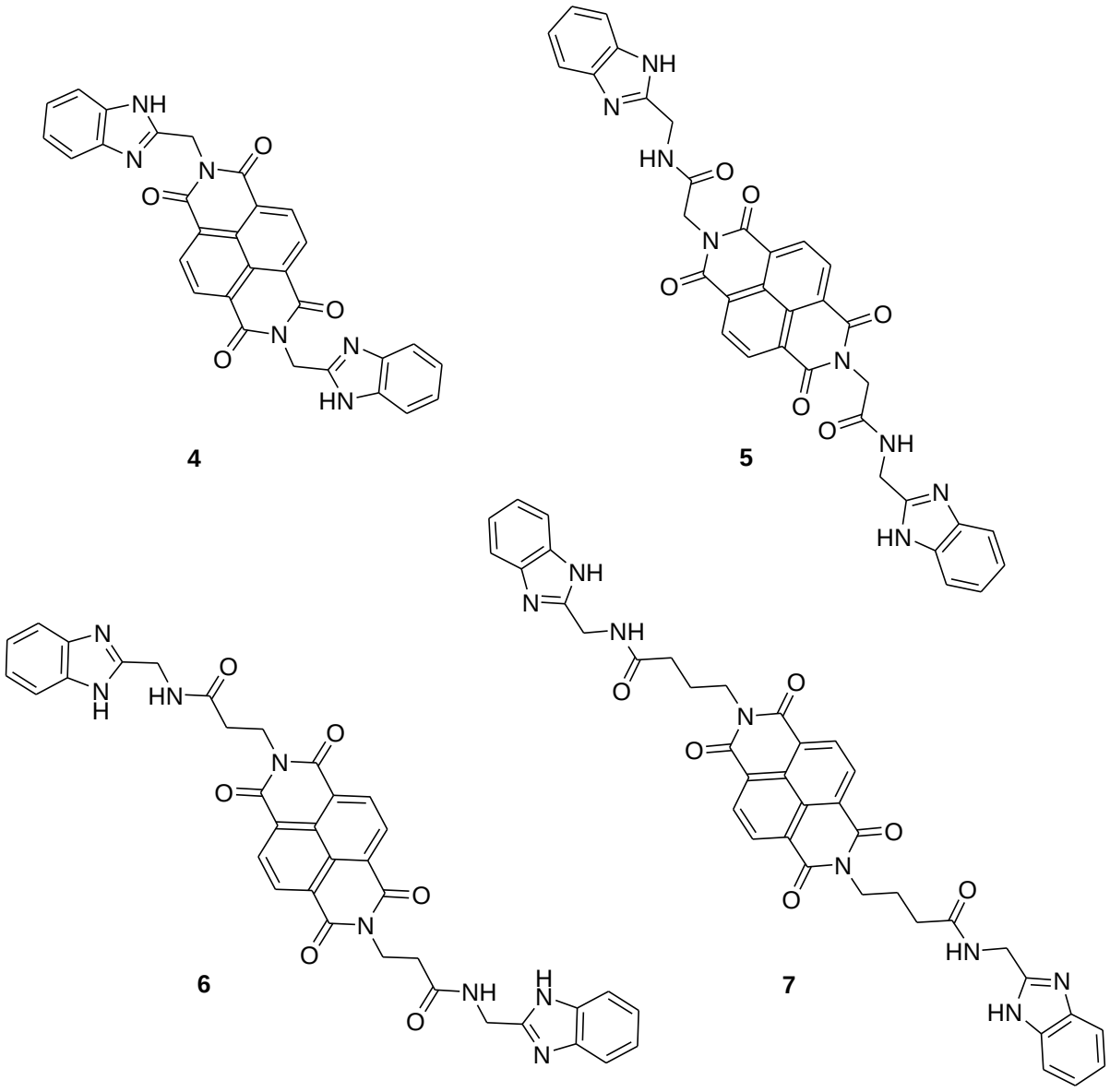


Figure 3.1 Structures of NDI derivatives with benzimidazole side chain.

Further, the intermediate compounds **1**, **2** and **3** were coupled with 2-(aminomethyl) benzimidazole dihydrochloride to yield final NDI derivatives as **5**, **6** and **7** as shown in Figure 3.1. The acid group in the side chain of NDI intermediate **1** is treated with 2-(aminomethyl)benzimidazole dihydrochloride in presence of coupling reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl), hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIPEA) base at room temperature for 12 h which afforded compound **5** in 70 % yield. Similarly, NDI intermediates **2** and **3** were treated with 2-(aminomethyl) benzimidazole dihydrochloride to afford compounds **6** and **7** with 70 % and 76 % yields respectively.

3.4. Results and discussion

3.4.1. Competitive binding of ligands 4-7 with 4',6-diamidino-2-phenylindole (DAPI)

Compounds **4-7** did not show fluorescence in aqueous solution or in organic solvents. Therefore, fluorescence titrations with DNA could not be carried out. However, competitive binding studies using DAPI bound to DNA were carried out. In order to study the binding mode of compounds **4-7** with double standed (ds) DNA, we have performed the competitive binding studies in presence of DAPI. DAPI is a well-known minor groove binder which shows the good fluorescence enhancement upon complexation with DNA. In the presence of minor groove binding ligand (DAPI), new ligand can compete for binding to the DNA, thereby leading to a decrease in the fluorescence intensity of the DAPI−DNA complex. Such competitive binding studies enable the location of the binding site of the new ligand. Compounds **4-7** with increasing concentrations (1-25 μM) were added to DNA which was pretreated with DAPI (10 μM), then decrease in DAPI emission intensity was observed for all compounds as shown in Figure 3.2. However, a pronounced decrease (~50%) in the fluorescence intensity of the DAPI−DNA complex was observed upon the addition of ligand **4** as shown in Figure 3.2. The decrease in fluorescence of DAPI-DNA complex upon addition of **5**, **6**, and **7** was 46 %, 40 % and 33 % respectively. The data in Figure 3.2 shows comparatively high fluorescence quenching for the compound **4** (50 %) than for compounds

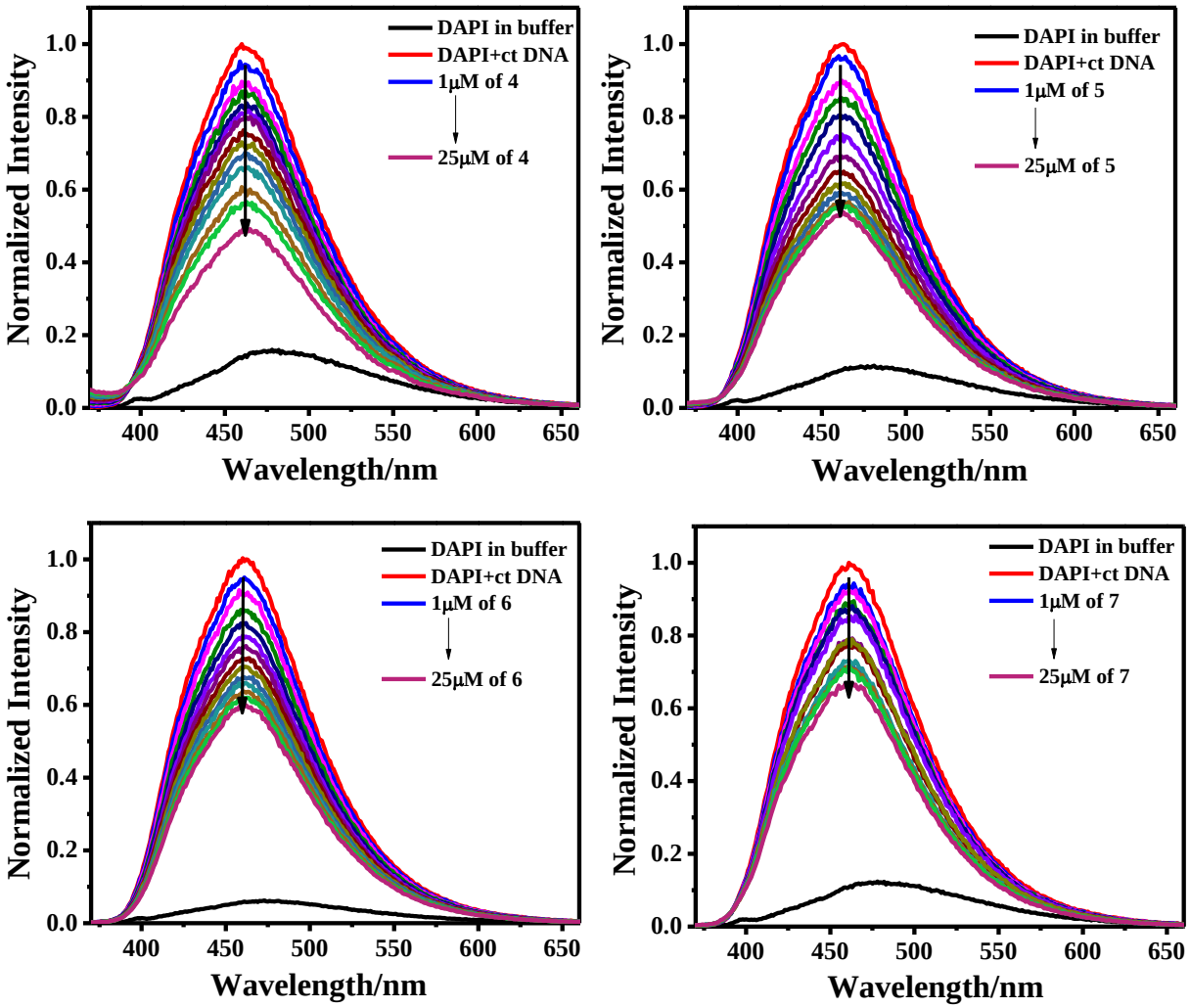


Figure 3.2 Changes in the fluorescence emission spectra of DAPI−DNA upon addition of increasing concentrations of ligands **4**-**7**. [DAPI] = 10 μM, [DNA] = 20 μM, Ligands **4**-**7** =1-25 μM, λ_{ex} = 350 nm.

5, **6**, and **7**, suggesting that NDI derivative with limited flexibity in the side chain or high rigidity binds in the groove efficiently. In general, the quenching of the fluorescence intensity suggested that compounds **4**-**7** have the ability to displace DAPI from minor grooves of the DNA. Therefore, compounds **4**-**7** are more likely to have groove binding in DNA. The basic competitive binding studies with DAPI were performed inorder to prove the binding ability of

NDI derivatives in the grooves of DNA. Further, displacement studies with Hoechst and intercalating ligands are yet to be performed to confirm the selectivity and actual mode of binding of NDI derivatives to DNA.

3.5. Conclusion

We have synthesized NDI derivatives having imidazole moiety in the side chains and characterized. These derivatives were shown to have groove binding affinity in the double helix DNA through preliminary DAPI displacement studies. The imidazole moiety having hydrogen bond donor and acceptor groups have proved to be involved in hydrogen bonding with the base pairs in the groove of DNA. Hence, these NDI derivatives can be further modified to increase the driving force for DNA binding by introducing positive charges in the side chains containing imidazole moiety. Further, we are trying to modify NDI derivatives with ring opened side chains as well as core substitution to improve optical properties and water solubility for DNA binding studies.

3.6. Experimental section

Materials and methods

All the reagents, Calf thymus-DNA (ct-DNA) and DAPI were obtained from Sigma and used as purchased without any further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 and using [D6] DMSO as solvent. Chemical shifts (δ) are given in parts per million (ppm) with respect to the internal standard tetramethylsilane (TMS), and *J* values are quoted in Hertz. The following abbreviations were used s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, broad s=broad singlet.

Fluorescence displacement studies

Fluorescence spectra were recorded on Cary 5000 Eclipse Fluorescence spectrophotometer. Samples were analyzed in quartz cuvette of 1 cm path length. All the solutions for fluorescent

displacement studies were prepared in aqueous 100 mM tris-HCl buffer (pH 7.32) using water from the Millipore system and measurements were performed at 25 °C unless otherwise stated. Stock solutions of ligands were prepared in DMSO (c = 2000 μM). 5 nm excitation and 5 nm emission slit widths were used.

Synthesis procedure

Compound 1 In a single-necked round bottom (RB) flask, 1,4,5,8-naphthalenetetracarboxylic dianhydride (2.68 g, 10 mmol) was slurried in 20 mL DMF at ambient temperature. Glycine (1.87 g, 25 mmol), triethyamine (3.48 mL, 25 mmol) were added and the mixture was stirred at 82 ^{o}C for 12 h. The mixture was cooled to room temperature and added to ice cold water. 2N HCl was added dropwise to reduce the pH to 4 and stirred for 10 min. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **1** as colorless solid (3.0 g, 80 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 13.21 (broad s, 2H, COOH), 8.74 (s, 4H, Ar-H), 4.78 (s, 4H, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 168.9, 162.2, 131.0, 126.2, 125.9, 41.4.

Compound 2 In a single-necked round bottom (RB) flask, 1,4,5,8-naphthalenetetracarboxylic dianhydride (2.68 g, 10 mmol) was slurried in 20 mL DMF at ambient temperature. β-Alanine (2.27 g, 25 mmol), TEA (3.48 mL, 25 mmol) were added and the mixture was stirred at 82 ^{o}C for 12 h. The mixture was cooled to room temperature and added to ice cold water. 2N HCl was added dropwise to reduce the pH to 4 and stirred for 10 min. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **2** as colorless solid (3.69 g, 90 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 12.39 (broad s, 2H, COOH), 8.65 (s, 4H, Ar-H), 4.29-4.25 (t, J = 8 Hz, $2CH_2$), 2.65-2.61 (t, J = 8 Hz, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 172.3, 162.4, 130.3, 126.2, 126.0, 36.0, 31.9.

Compound 3 In a single-necked round bottom (RB) flask, 1,4,5,8-naphthalenetetracarboxylic dianhydride (2.68 g, 10 mmol) was slurried in 20 mL DMF at ambient temperature. γ–Aminobutyric acid (2.57 g, 25 mmol), TEA (3.48 mL, 25 mmol) were added and the mixture was stirred at 82 ^{o}C for 12 h. The mixture was cooled to room temperature and added to ice

cold water. 2N HCl was added dropwise to reduce the pH to 4 and stirred for 10 min. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **3** as colorless solid (4.03 g, 92 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 12.0 (s, 2H, COOH), 8.64 (s, 4H, Ar-H), 4.12-4.09 (t, *J* = 6.8 Hz, $2CH_2$), 2.35-2.32 (t, *J* = 7.2 Hz, $2CH_2$), 1.95-188 (m, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 173.9, 162.6, 130.2, 126.2, 126.1, 31.2, 22.8. ^{13}C DEPT NMR (100 MHz, [D6]DMSO): δ_C ppm 130.0, 39.3, 31.0, 22.5.

Ligand 4 In a single-necked round bottom (RB) flask, 1,4,5,8-naphthalenetetracarboxylic dianhydride (2.68 g, 10 mmol) was slurried in 20 mL DMF at ambient temperature. 2-(Aminomethyl) benzimidazole dihydrochloride (5.50 g, 25 mmol), TEA (3.48 mL, 25 mmol) were added and the mixture was stirred at 82 ^{o}C for 12 h. The mixture was cooled to room temperature and added to ice cold water. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **4** as brown color solid (4.61 g, 90 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 8.78 (s, 4H, Ar-H), 7.72-7.70 (m, 4H, Ar-H), 4.46-4.43 (m, 4H, Ar-H), 5.73 (s, 4H, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 162.7, 149.9, 130.8, 126.6, 126.5, 124.4, 114.2, 37.0.

Ligand 5 In a single-necked round bottom (RB) flask, compound **1** (3.82 g, 10 mmol) was dissolved in 20 mL dry DMF at ambient temperature. The coupling reagents EDC.HCl (5.75 g, 30 mmol), HOBt (4.05 g, 30 mmol) were added and stirred for 5 min for dissolution. 2-(Aminomethyl) benzimidazole dihydrochloride (4.84 g, 22 mmol) and DIPEA (6.84 mL, 40 mmol) were added and stirred at room temperature for 12 h. The mixture was cooled to room temperature and added to water. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **5** as brown color solid (4.48 g, 70 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 12.25 (broad s, 2H, N-H), 8.98-8.95 (t, *J* = 5.2 Hz, 2H, N-H), 8.72(s, 4H, Ar-H), 7.51-7.50 (broad m, 4H, Ar-H), 7.16-7.14 (m, 4H, Ar-H), 4.81 (s, 4H, $2CH_2$), 4.53-4.52 (d, *J* = 5.2 Hz, ,4H, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 170.1, 166.7, 162.5, 151.5, 130.7, 126.3, 42.7, 37.1.

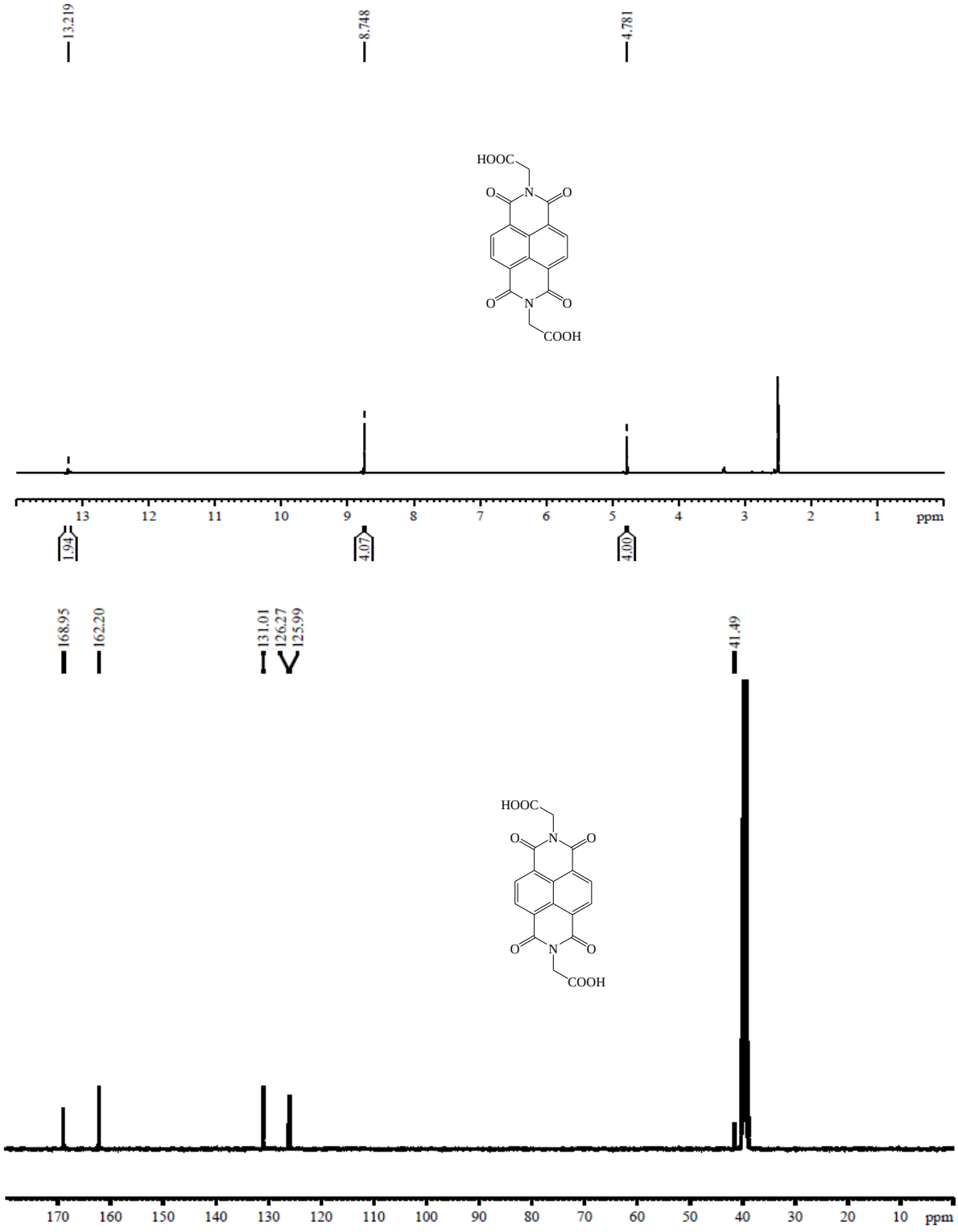
Ligand 6 In a single-necked round bottom (RB) flask, compound **2** (4.10 g, 10 mmol) was dissolved in 20 mL dry DMF at ambient temperature. The coupling reagents EDC.HCl (5.75 g, 30 mmol), HOBt (4.05 g, 30 mmol) were added and stirred for 5 min for dissolution. 2-

(Aminomethyl) benzimidazole dihydrochloride (4.84 g, 22 mmol) and DIPEA (6.84 mL, 40 mmol) were added and stirred at room temperature for 12 h. The mixture was cooled to room temperature and added to water. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **6** as brown color solid (4.67 g, 70 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 12.17 (broad s, 2H, N-H), 8.65-8.63 (broad t, 2H, N-H), 8.61 (s, 4H, Ar-H), 7.45-7.43 (m, 4H, Ar-H), 7.13-7.10 (m, 4H, Ar-H), 4.47-4.45 (d, J = 6 Hz, 4H, $2CH_2$), 4.35-4.31 (t, J = 7.6 Hz, 4H, $2CH_2$), 2.66-2.62 (t, J = 7.6 Hz, 4H, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 170.2, 162.5, 152.0, 130.2, 126.3, 126.1, 121.4, 37.0, 33.2.

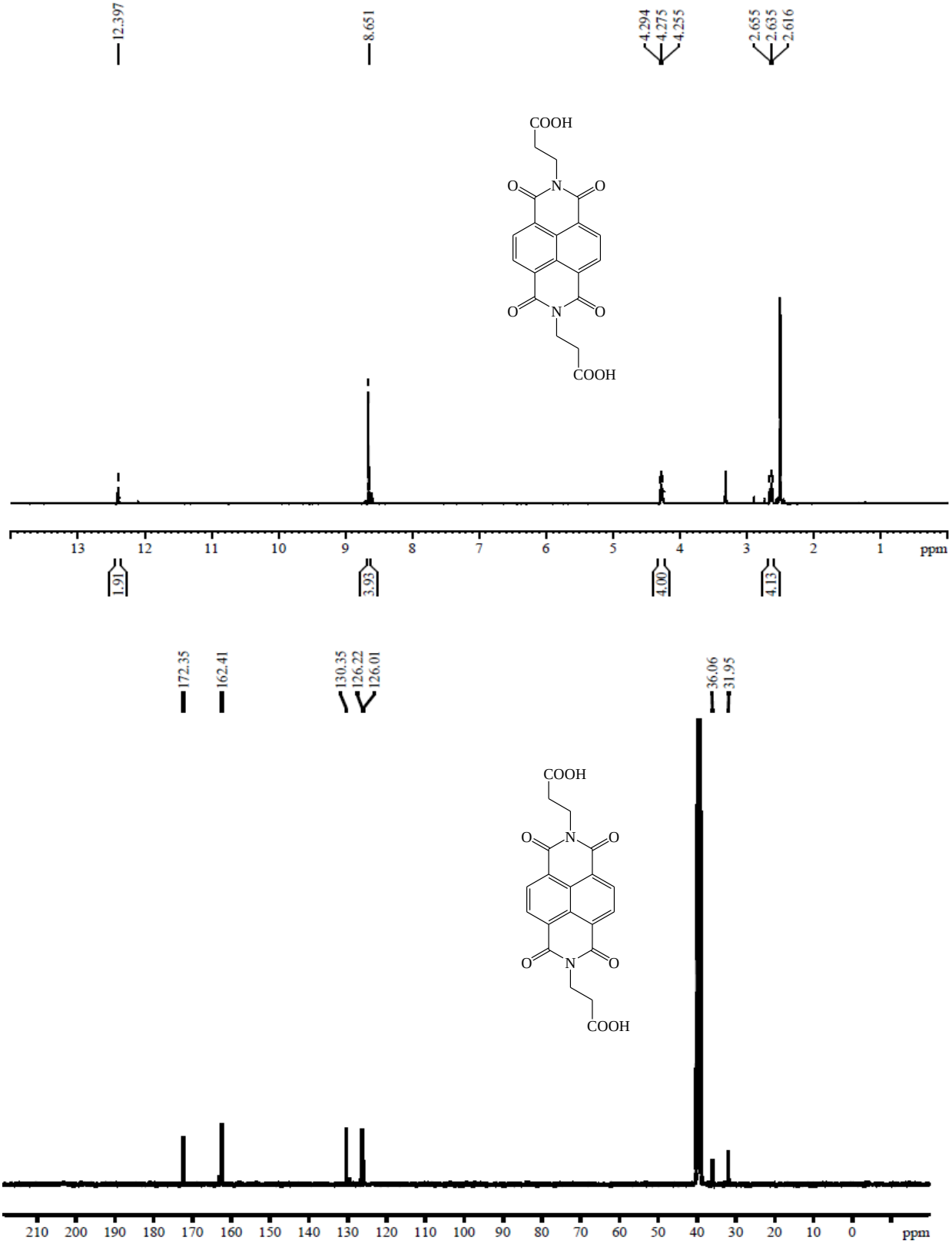
Ligand 7 In a single-necked round bottom (RB) flask, compound **3** (4.38 g, 10 mmol) was dissolved in 20 mL dry DMF at ambient temperature. The coupling reagents EDC.HCl (5.75 g, 30 mmol), HOBt (4.05 g, 30 mmol) were added and stirred for 5 min for dissolution. 2-(Aminomethyl) benzimidazole dihydrochloride (4.84 g, 22 mmol) and DIPEA (6.84 mL, 40 mmol) were added and stirred at room temperature for 12 h. The mixture was cooled to room temperature and added to water. The precipitated compound was filtered and washed with water and acetone thoroughly to remove DMF, and dried under vacuum to obtain **7** as dark brown color solid (5.29 g, 76 %). ^{1}H NMR (400 MHz, [D6] DMSO): δ_H ppm 12.09 (broad s, 2H, N-H), 8.64 (s, 4H, Ar-H), 8.46-8.44 (t, J = 5.6 Hz, 2H, N-H), 7.45 (broad s, 4H, Ar-H), 7.13-7.08 (m, 4H, Ar-H), 4.39-4.38 (d, J = 5.6 Hz, 4H, $2CH_2$), 4.14-4.10 (t, J = 7.2 Hz, 4H,$2CH_2$), 2.34-2.30 (t, J = 7.2 Hz, 4H, $2CH_2$), 2.01-1.92 (m, 4H, $2CH_2$); ^{13}C NMR (100 MHz, [D6] DMSO): δ_C ppm 171.9, 162.7, 152.1, 130.3, 126.3, 36.9, 32.8.

3.7. Appendix

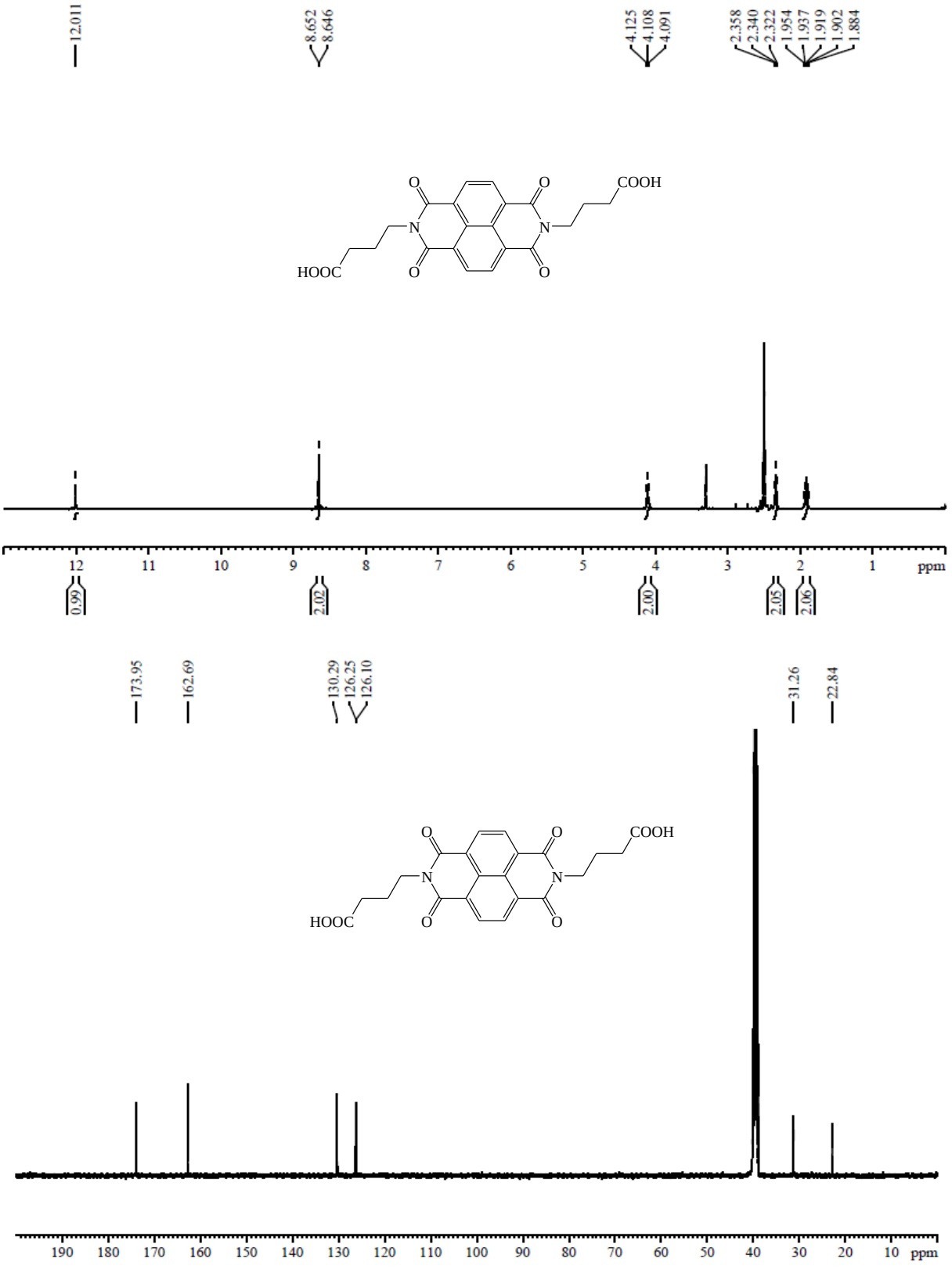
^{1}H and ^{13}C NMR spectrum of compound **1**



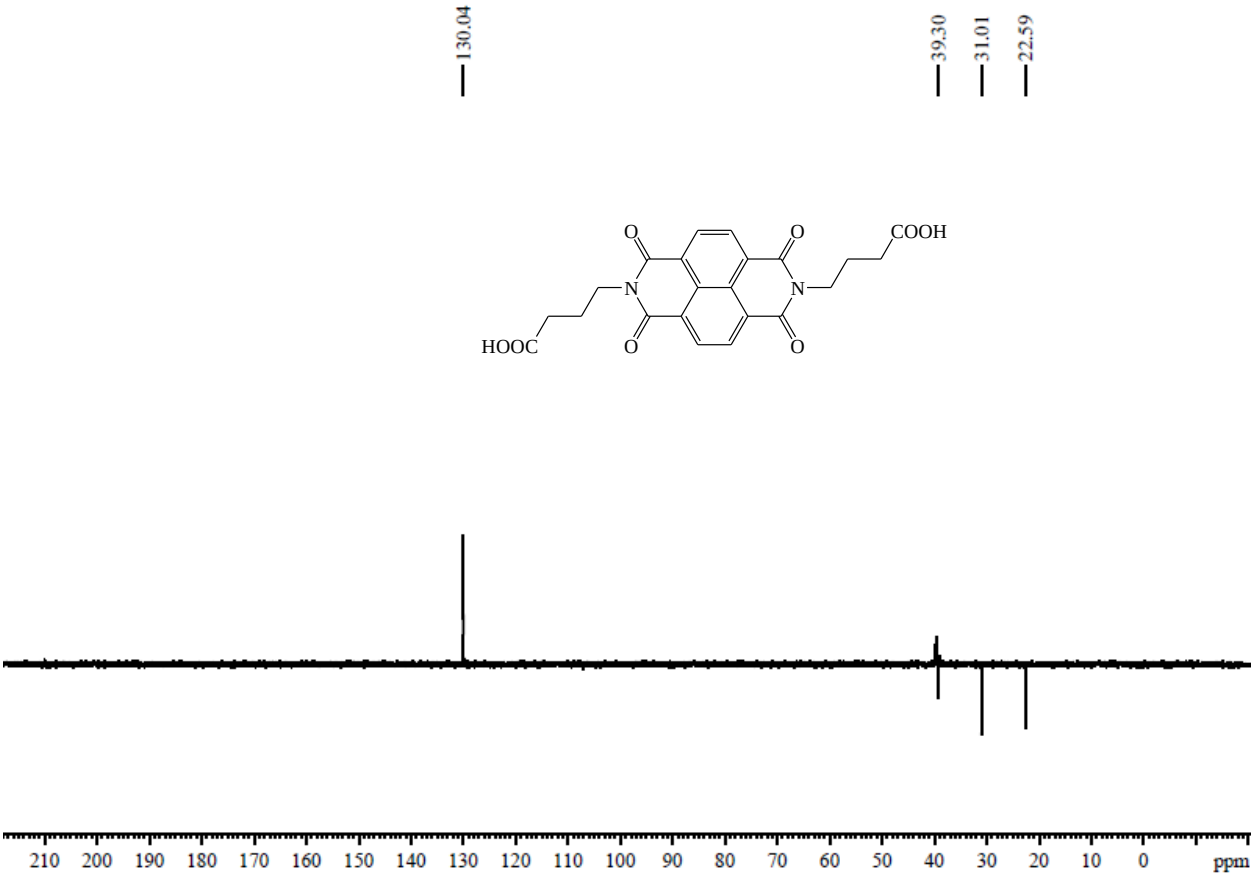
^{1}H and ^{13}C NMR spectrum of compound **2**



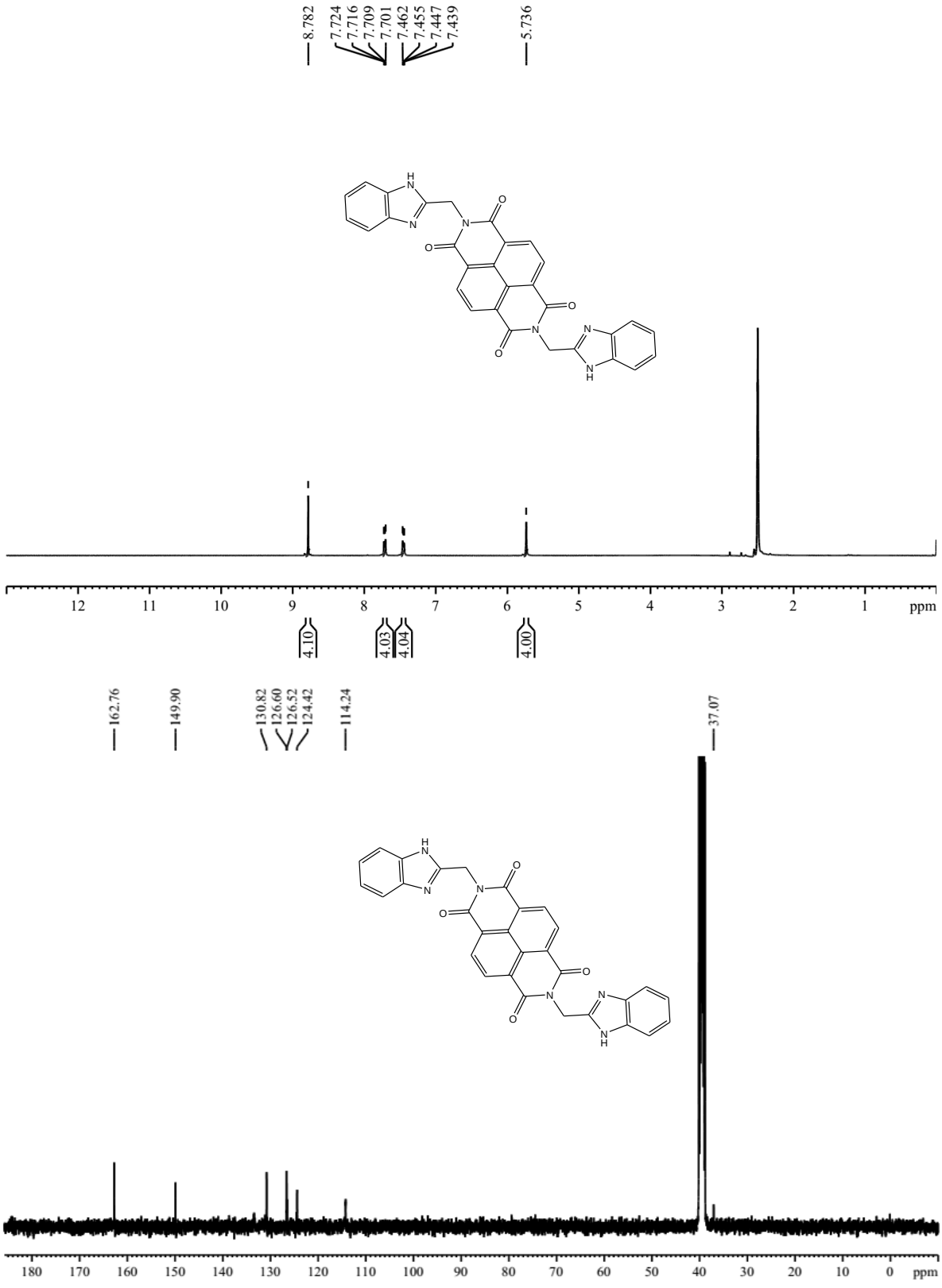
^{1}H and ^{13}C NMR spectrum of compound **3**



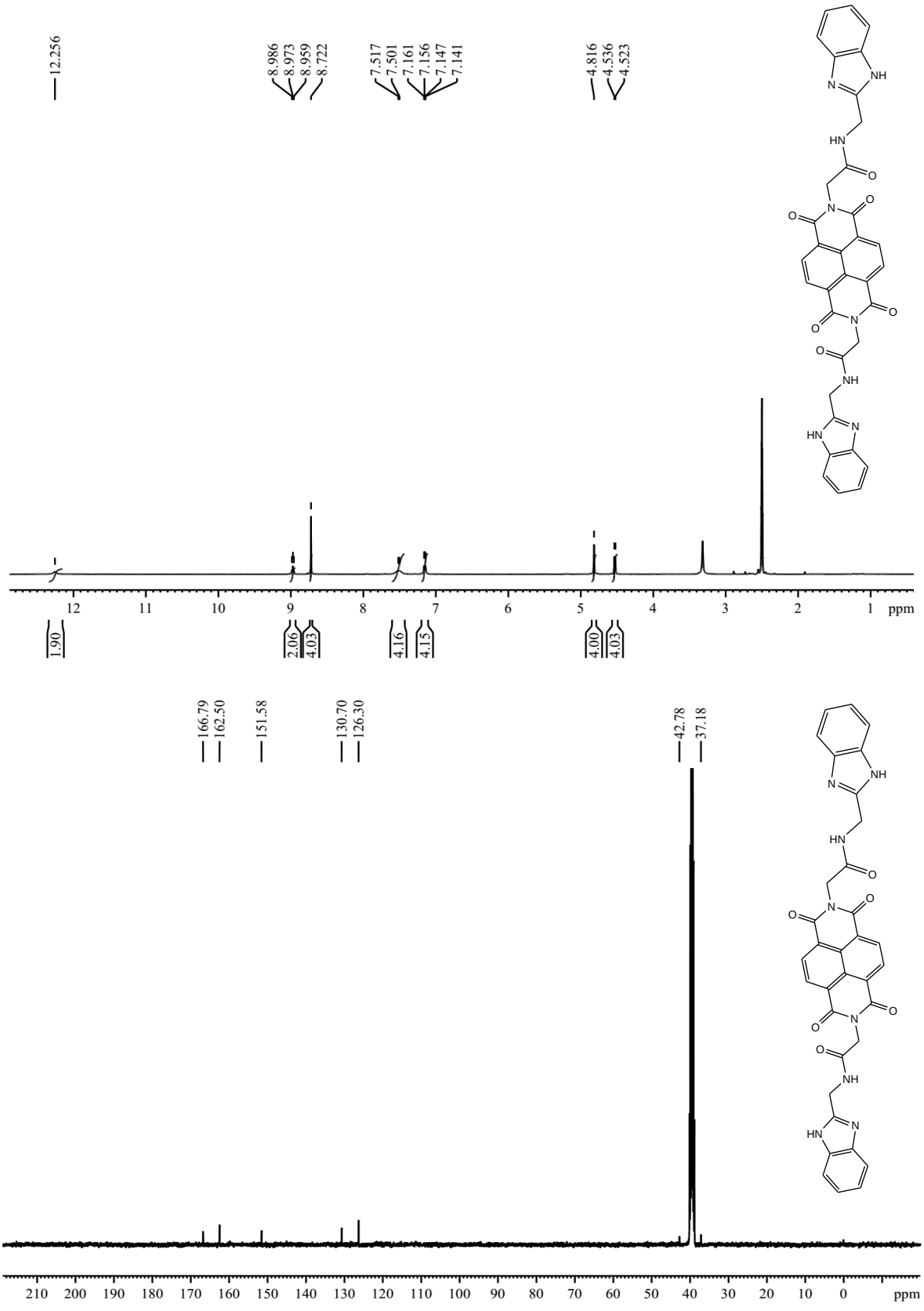
^{13}C DEPT spectrum of compound **3**



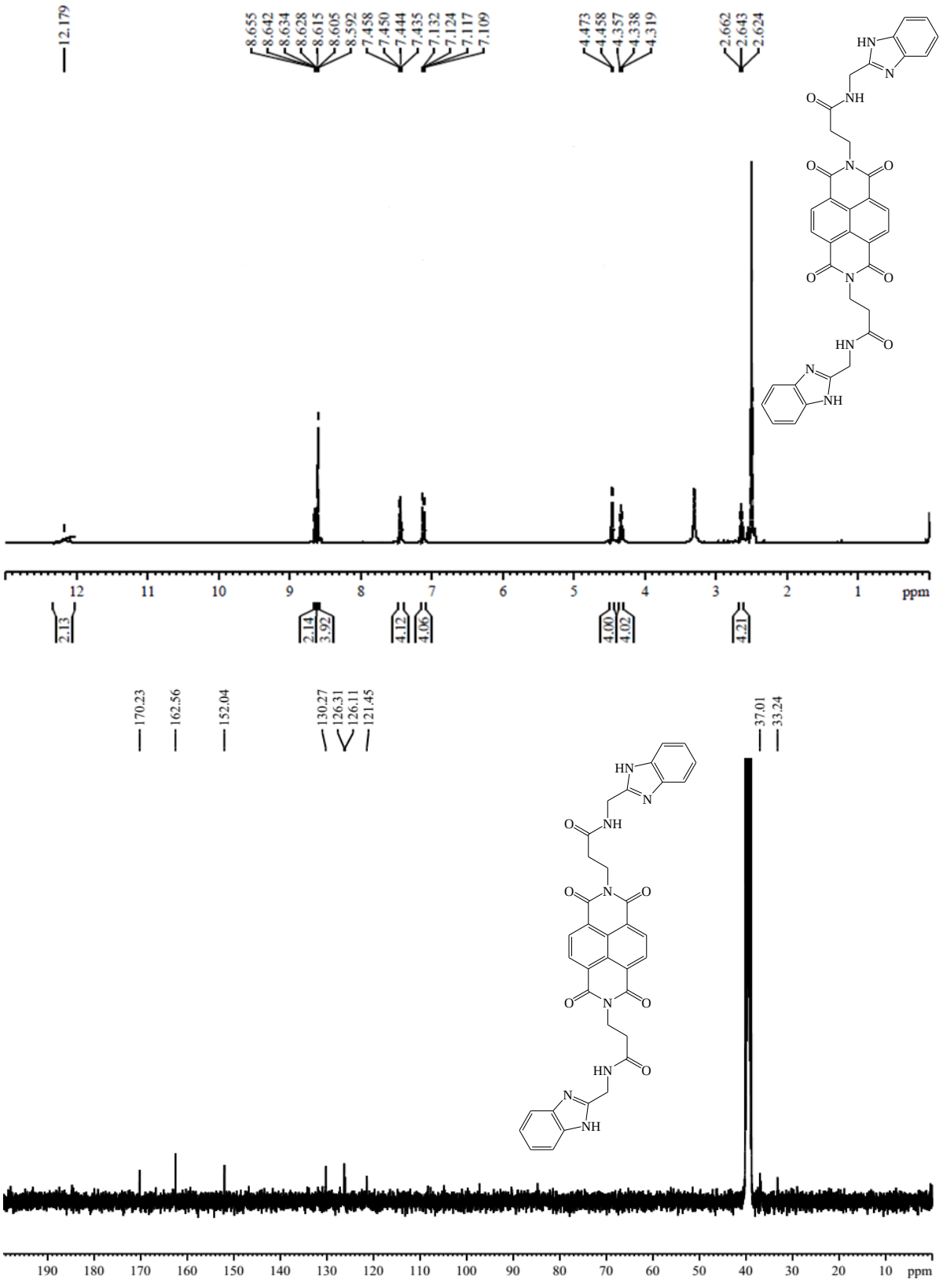
^{1}H and ^{13}C NMR spectrum of ligand **4**



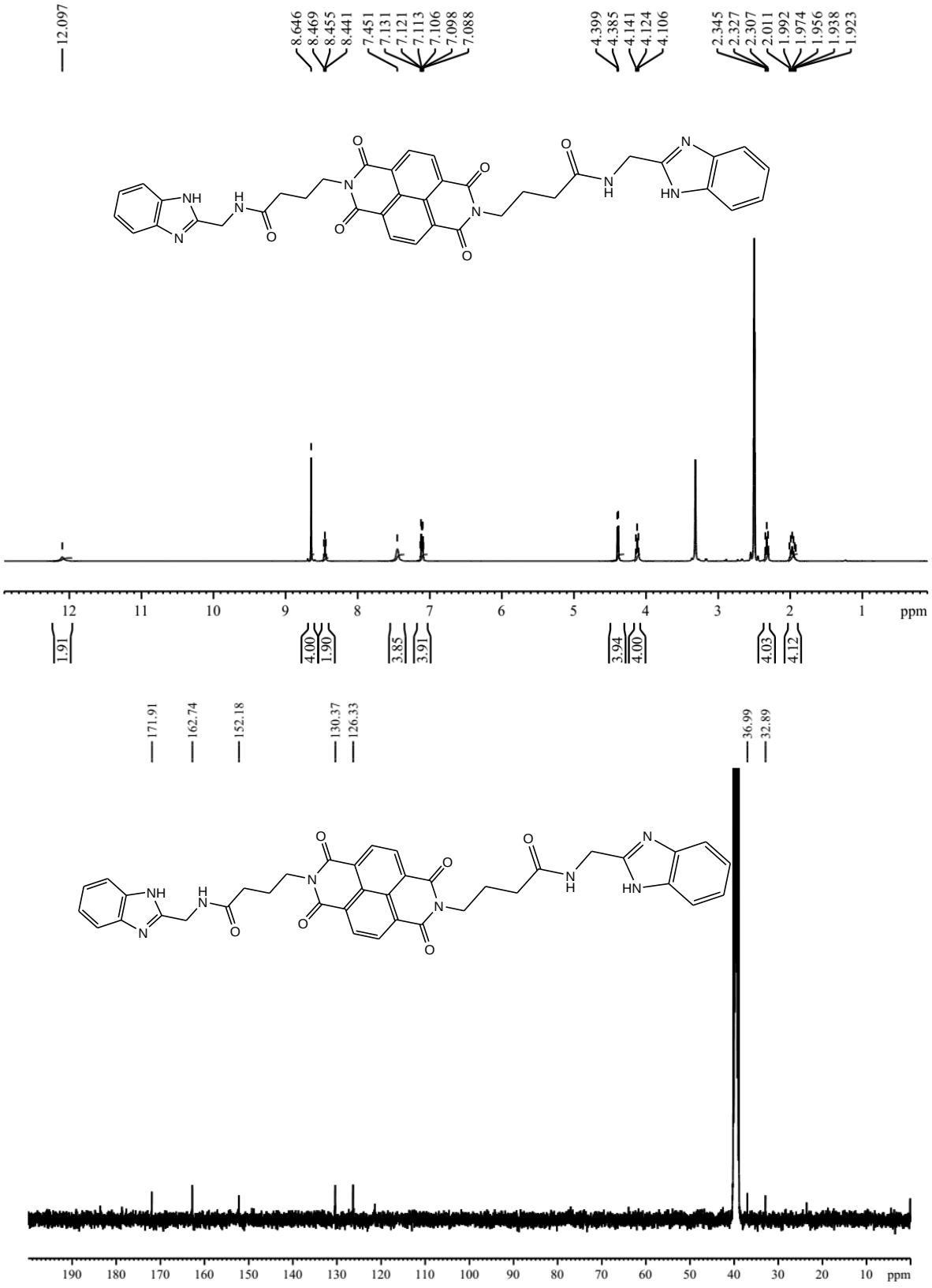
^{1}H and ^{13}C NMR spectrum of ligand **5**



^{1}H and ^{13}C NMR spectrum of ligand **6**



^{1}H and ^{13}C NMR spectrum of ligand **7**



3.8. References

1. Li, L.; Cao, W.; Fan, Z. C.; Chen, T., Ruthenium complexes containing 2,6-bis(benzimidazolyl)pyridine derivatives induce cancer cell apoptosis by triggering DNA damage-mediated p53 phosphorylation. *Dalton Trans*. **2012**, *41*, 12766-12772.
2. Chaires, J. B., Drug–DNA interactions. *Curr. Opin. Struct. Biol*. **1998**, *8*, 314-320.
3. Hurley, L. H.; Chaires, J. B., Advances in DNA-sequence specific agents. **1996**, Vol. *2*, JAI Press, Greenwich, CT.
4. Dervan, P. B., Design of sequence-specific DNA-binding molecules. *Science* **1986**, *232*, 464-471.
5. Nielsen, P. E., Design of sequence-specific DNA-binding ligands. *Chem. Eur. J*. **1997**, *3*, 505-508.
6. White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B., Recognition of the four Watson-Crick base pairs in the DNA minor groove by synthetic ligands. *Nature* **1998**, *391*, 468-471.
7. Trauger, J. W.; Baird, E. E.; Dervan, P. B., Recognition of 16 base pairs in the minor groove of DNA by a pyrrole-imidazole polyamide dimer. *J. Am. Chem. Soc.* **1998**, *120*, 3534-3535.
8. Dickinson, L. A.; Gulizia, R. J.; Trauger, J. W.; Baird, E. E.; Mosier, D. E.; Gottesfeld, J. M.; Dervan, P. B., Inhibition of RNA polymerase II transcription in human cells by synthetic DNA-binding ligands. *Proc. Natl Acad. Sci. USA* **1998**, *95*, 12890-12895.
9. Gowers, D. M.; Fox, K. R., Towards mixed sequence recognition by triple helix formation. *Nucleic Acids Res*. **1999**, *27*, 1569-1577.
10. Egholm, M., Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Nielsen, P. E., PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen bonding rules. *Nature* **1995**, *365*, 566-568.
11. Desjarlais, J. R.; Berg, J. M., Toward rules relating zinc-finger protein sequences and DNA binding site preferences. *Proc. Natl Acad. Sci. USA* **1992,** *89*, 7345-7349.
12. Bailly, C.; Chaires, J. B., Sequence-specific DNA minor groove binders. Design and synthesis of netropsin and distamycin analogues. *Bioconj. Chem*. **1998**, *9*, 513-538.

13. Leng, F., Priebe, W. Chaires, J. B., Ultratight DNA binding of a new bis-intercalating anthracycline antibiotic. *Biochemistry* **1998**, *37*, 1743-1753.
14. Stevenson, K. A.; Yen, S.; Yang, N.; Boykin, D. W.; Wilson, W. D., A substituent constant analysis of the interaction of substituted naphthalene monoimides with DNA. *J. Med. Chem.* **1984**, *27*, 1677-1682.
15. Braña, M. F.; Castellano, J. M.; Morán, M.; Pérez de Vega, M. J.; Romerdahl, C. R.; Qian, X. D.; Bousquet, P.; Emling, F.; Schlick, E.; Keilhauer, G., Bis-naphthalimides: a new class of antitumor agents. *Anti-Cancer Drug Des.* **1993**, *8*, 257-268.
16. Tanious, F. A.; Yen, S.; Wilson, W. D., Kinetic and equilibrium analysis of a threading intercalation mode: DNA sequence and ion effects. *Biochemistry* **1991**, *30*, 1813-1819.
17. Lamberson, C. Topologically constrained DNA intercalators: synthesis and DNA binding studies. **1991**, PhD thesis. pp 23-26. University of Illinois, USA.
18. Bailly, C.; Braña, M.; Waring, M. J., Sequence-selective intercalation of antitumour bis-naphthalimides into DNA. Evidence for an approach via the major groove. *Eur. J. Biochem.* **1996**, *240*, 195-208.
19. Liu, Z.; Hecker, K. H.; Rill, R. L., Selective DNA binding of (N-alkylamine)-substituted naphthalene imides and diimides to G + C rich DNA. *J. Biomol. Struct. Dyn.* **1996**, *14*, 331-339.
20. Smith, A. R.; Iverson, B., Threading polyintercalators with extremely slow dissociation rates and extended DNA binding sites. *J. Am. Chem. Soc*. **2013**, *135*, 12783−12789.

List of publications

1. Dibromohydantoin: a convenient brominating reagent for 1,4,5,8-naphthalenetetracarboxylic dianhydride.
   M. Sasikumar, **Y. V. Suseela**, and T. Govindaraju, *Asian J. Org. Chem.* **2013**, *2*, 779-785. (Cover page)
2. An effective and regioselective bromination of 1,4,5,8-naphthalenetetracarboxylic dianhydride using tribromoisocyanuric acid.
   Y. V. Suseela, M. Sasikumar and T. Govindaraju, *Tetrahedron. Lett.* **2013**, *54*, 6314-6318.