SYNTHESIS OF BIPYRIDINE-DERIVED LIGANDS FOR DNA BINDING AND SHAPE SWITCHING

by

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Abstract

The objective of this project is synthesizing bipyridine-derived ligands in order to study DNA conformational bending.

The synthesis of bipyridine derivatives has been investigated. 6,6'-Dibromo-2,2'-bipyridine and small scale of 6,6'-diformyl-2,2'-bipyridine have been successfully synthesized in the laboratory. The synthesis of large amount of a direct precursor to 6,6'-diformyl-2,2'-bipyridine in an multiple step way has been achieved.

The synthesis of mono functionalized pyrene derivatives and of 1,6-dissymmetrically functionalized pyrene derivatives has been heavily studied. Successfully methods have been reported in this thesis.

The complete assembly of bipyridine and pyrene units into the final ligands and their model has also been studied. Palladium borylation and Suzuki-Miyaura cross-coupling have been used to successfully connect the bipyridine with pyrene units.

In addition to Suzuki-Miyaura methodology, the direct coupling of N,N'-dioxide-2,2'-bipyridine with aromatic bromides under palladium catalysis has been investigated. This method could be an alternative way to access to mono-substituted 6-bipyridines, symmetrically or even asymmetrically 6,6'-disubstituted-2,2'-bipyridine derivatives.

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List of Symbols and Abbreviations

Ar	Aromatic
Bp(y)	2,2'-bipyridine
br	Broad
°C	degree Celsius
d	Doublet
dd	doublet of doublet
DMF	dimethyl formamide
DMSO	dimethylsulfoxide
EA	elemental analysis
EI	electron impact
ESI	electronspray ionization
hr	hour(s)
HRMS	high resolution mass spectra
IR	infrared spectroscopy
J	vicinal coupling constant
m	Multiplet
mg	Milligram

MHz	Megahertz
min	minute(s)
mL	milliliter(s)
mmol	millimole(s)
mol	Mole
MS	mass spectroscopy
NMR	nuclear magnetic resonance
ppm	part(s) per million
Py	Pyrene
rt	room temperature
s	Singlet
t	Triplet
UV	Ultraviolet
δ	chemical shift
λ	Wavelength

Chapter 1

Introduction

1.1 Importance of conformation in nature

1.1.1 General comments on the conformation of natural biomacromolecules

The cellular functions of biological macromolecules mainly depend on their primary structures, secondary or more advanced conformations. Thus studying their conformations leads to further understanding of their biological functions. DNA, one of the most important biological macromolecules, carries the genetic information that defines what we look like and how our body functions. The genetic information is expressed by both the primary structure of DNA (the sequence of bases) and by the different conformations that DNA adopts. The latter can be A, B, Z duplexes (Fig.1.1[22]), triplexes, quadruplexes, four-way junctions and many other forms. Those various conformations and their changes are known to regulate critical cellular events such as transcription, replication, and DNA packaging.[6] Thus studying the formation and changes of those conformations may provide advanced understanding in fundamental study and clinical diseases such as cancer.

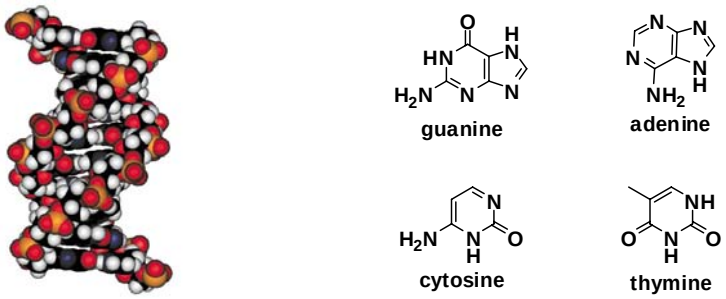


Figure 0.1 DNA duplex[22] and natural DNA bases

1.1.2 A focus on guanine quadruplex conformations and their biological relevance

Chemically, one DNA duplex consists of two anti-parallel strands made of nucleotide polymers brought together by hydrogen bonds between base pairs. However, all self-assembled DNA conformations are not necessarily in the duplex form. For instance, certain nucleic acid sequences rich in guanine are able to form four-stranded structures so called G-quadruplexes (Fig.1.2).[1] Four guanines self-assemble into a planar unit through hydrogen bonding, forming guanine quartets. Those are stabilized by the presence of cations (e.g. potassium ions) at the center of the hydrogen-bonded macrocycle.[27] Two quartets are believed to then stack to form an octamer.[27]Several octamers can then stack again into larger stacked architectures called guanine quadruplexes.[27] Recent interest has shown that the telomere sequences (found at the end of the chromosomes) are G-rich sequences that are able to form that G-quadruplex. Those are capped by proteins in vivo to protect the end of the chromosome.[1] Basic structures of G-quadruplexes are shown in Fig.1.2.

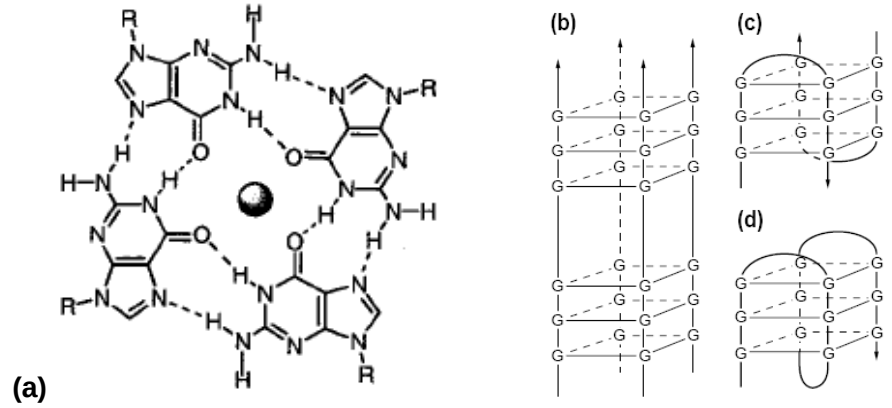


Figure 0.2 a) Four guanine forming a planar unit,[27] (b) A tetramolecular G-quadruplex can be formed from four single strands,[1] (c) and (d) Intermolecular G-quadruplexes.[1]

G quadruplexes seem to be biologically relevant and participate in nucleic acids processing, either in the core of the chromosome (e.g. regulation of gene transcription) or at its end (telomerase elongation, as detailed below).[1] Telomeres are the special regions at the end of the chromosomes composed of a double stranded tandem repeating sequence and a single strand guanine rich "tail"; the latter has been shown to form G quadruplexes when isolated from the appended duplex.[1] Telomeres are a unique part of the chromosome. In normal cells, cell division naturally induces the shortening of this region. As a result of the progressive shortening of the chromosome, and to prevent the loss of genetic information, normal cells have learned to stop dividing after a certain number of division cycles (of the order of 20 cell divisions). This is referred to as "cellular senescence" and "cellular clock" and is very important in the process of cell ageing. In cancer cells, however, where infinite division and growth are taking place, one main process is able to add up the "bit that is missing". In other words, the chromosome does naturally shorten upon cellular division, but the cell has the ability to elongate the single strand back to the length it had before division. As a result, nothing stops the cancer cells to divide again and again, and cancer cells are insensitive to ageing.[33] Because one particular enzyme (telomerase) is capable of elongating telomeres in more than 85% of all cancers, inhibiting this process has been a huge focus of research in order to selectively sensitize most cancers to ageing, and slow down or stop their progression. It turns out that the enzyme in question is only able to target free single strand DNA, but not guanine quadruplexes. So forcing a free single strand to change its conformation into a guanine quadruplex prevents the enzyme from elongating the telomere.[34] Thus G-quadruplexes have been targeted to develop anti-cancer drugs such as telomerase inhibitors.[5] Due to the special flat structure of G-quadruplexes, researchers have successfully adopted a structure-based approach to design small molecules that can interact and

stabilize the G4 structure, including, among others, 2,6-diamidoanthraquinones, cationic porphyrins and perylenes (Fig.1.3).[1]

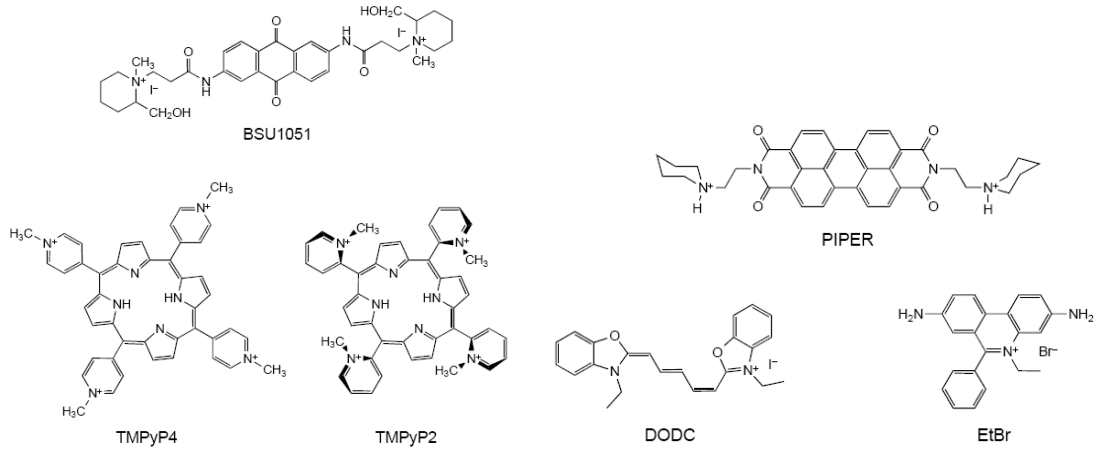


Figure 0.3 G-quadruplex-interactive compounds[1]

Significant efforts in industry have lead to the generation of very efficient telomerase inhibitors which, surely, will bring a new tool in the fight against cancer.[35]

The example of switching DNA telomere conformation from a single strand to a guanine quadruplex exemplifies that targeting DNA conformations may have significant benefits in clinical applications. We will see below that targeting the more abundant duplex of DNA also finds exciting applications.

1.1.3 Targeting DNA duplexes through minor groove binding

People have always been interested in using artificial agents to target DNA conformations to further control DNA events in both normal and diseased cells. One example is targeting DNA duplex via artificial DNA minor groove binders. Generally, B form DNA duplex has two grooves, one being the major groove which is 22Å wide and the minor groove which is 12Å wide.[21] The narrowness of the minor groove leads to the major groove being more accessible to

proteins such as transcription factors while small molecules usually access the minor groove.[20] PIP (Pyrrole-imidazole polyamide) is a family of small molecules that can bind to the minor groove of DNA duplex with high affinity and high sequence specificity, and inhibit gene expression.[2] Initially, Dervan et al. investigated natural polyamides such as ducarmycin A and distamycin A.[3] These were the source of inspiration for artificial polyamides, where pyrrole groups and imidazole groups are linked by amide bonds. Different heterocyclic groups such as N-methylpyrrole(Py), N-methylimidazole(Im) and Hydroxypyrrole(Hp) can be used, which provide high sequence specificity (Fig.1.4).[4] Indeed, Py, Im, and Hp are designed to specifically recognize particular base-pairs based on their own hydrogen bonding distribution (Fig.1.4 & 1.5).[22] Thus PIP type compounds can be designed and synthesized to bind specific DNA sequences and thus control specific gene events.[22] Also, PIPs are usually resistant to nucleases without additional delivery system and make it a good candidate as drugs or tool for biological medicine.[4]

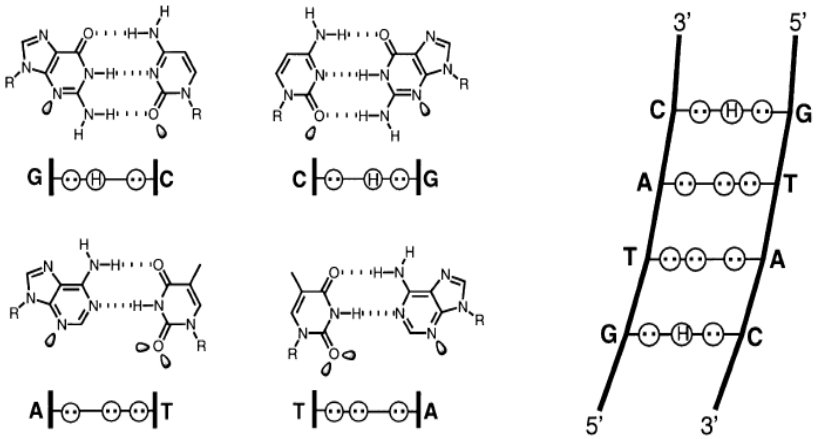


Figure 0.4 Minor groove hydrogen bonding pattern of base pairs (Circles with dots represent lone pairs of N(3) of purines and O(2) of pyrimidines, and circles containing an H represent the 2-amino group of guanine).[22]

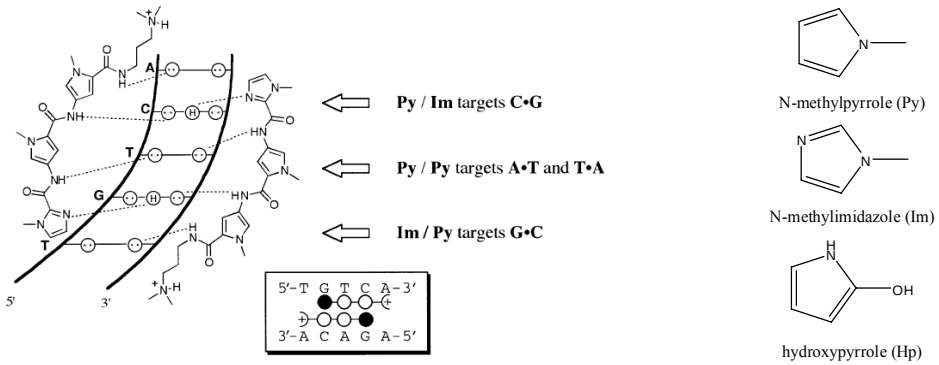


Figure 0.5 (Left) Model for antiparallel dimer PIP (ImPyPy) binding 5'-TGTCA-3' (Black circle represent Im and hollow circle represent Py)[22]; (Right) Core of Py, Im and Hp

One example is PIP-A and B designed by Hayashi *et al.* who showed that they are able to inhibit the gene encoding of Human aurora kinase-A (AURKA) and –B (AURKB) on chromosomes.[2] AURKA and AURKB are two types of human Aurora/|p|1p family protein kinases, which play very important roles in cell division and DNA replication.[2] The mRNA expressions of AURKA and AURKA are cell-cycle dependent.[2] The expression level is low during the G1/S period, is activated during the G2/M period and freezes again after cell division.[2] Studies show that their over-expression leads to chromosome irregularity and results in malignant cell conversion and high occurrence of cancer.[2] Also, over-expressions of AURKA and AURKB are found in tumor cells.[2] Therefore, inhibiting or regulating the expression of AURKA and AURKA becomes a target to develop anti-cancer drugs. The two synthetic PIPs can specially recognize and bind the known gene sequences encoding AURKA and AURKB (Fig.1.6).[2] The observation shows that the two PIPs can efficiently inhibit both mRNA expression and protein level of AURKA and AURKB and may even specifically target malignant cell line to induce cell death.[2]

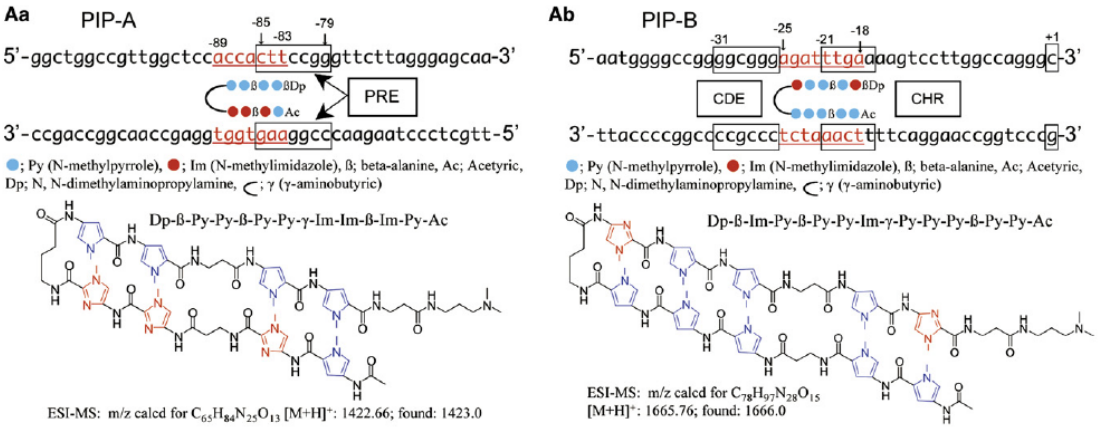


Figure 0.6 Chemical structure of PIP-A and PIP- B and binding model with chromosome[2]

1.1.4 A focus on DNA duplex bent conformations and their biological relevance

Recently, one class of DNA conformations, DNA bending, has become a topic of interest. DNA bending is a crucial cellar phenomenon to many critical life processes such as DNA packaging, transcriptional regulation[6] and DNA damage recognition and repair.[7]

DNA bending has been observed in DNA damage. In human cells, DNA damage can be caused by normal cell activities and many other environmental factors.[8] If not corrected, DNA damage can lead to mutagenesis and increase the risk of tumour formation. There are two types of DNA damage: endogenous damage induced by normal metabolism (e.g. oxidation leading to reactive oxygen species, oxidation of bases, alkylation of bases, hydrolysis of bases, adduct formation, and mismatch bases), and exogenous damage caused by external factors (e.g. radiations, chemicals…).

One example of exogeneous damage caused by radiation is the formation of thymine dimers. In thymine dimers, two adjacent thymines along one strand of a DNA duplex become linked by covalent bond via a 2+2 photo-cycloaddition into a cyclobutane-type pyrimidine photodimer (CPD).[8] The formation is induced both in frozen solution and in living cells by ~260 nm

ultraviolet light.[23] It is found that, in live cells, this process is partially reversible and dimers can be repaired by light.[23] The equilibrium between thymine and its dimer is related to the wavelength of ultraviolet light.[23] As a result, monomer is preferred at 240nm UV, while dimer is promoted at 280nm UV (Fig.1.7).[23] Solid state (crystal) and (NMR) solution structures have been reported for both (i) thymine dimer containing duplexes[24] and (ii) thymine dimer containing duplexes bound to natural proteins whose role is to recognize such damage.[23] In the former case (free of protein), the bending of DNA duplex induced by the dimer is found to be 27 º- 30 º.[24] Similarly, the thymine dimer containing duplex is recognized in a bent conformation by enzymes such as T4 endonuclease V, which has a sharp kink of 60 º.[8]

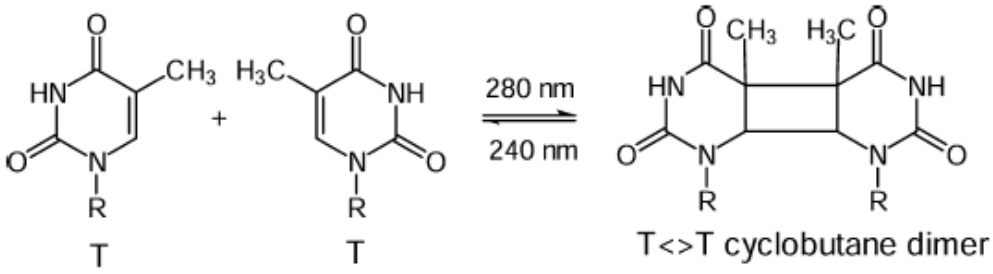


Figure 0.7 Equilibrium of thymine and its dimer[23]

In the reported protein-DNA complex crystal structures, the bases complementary to thymines involved in thymine dimers (e.g. adenine) may be flipped out of the duplex (Fig.1.8, 1.9, 1.10).[8] DNA bending caused by thymine dimer formation can assist enzymes in recognizing and repairing the damage.[8]

Figure 0.8 Bent DNA duplex induced by thymine dimer (red) compared to standard, straight, DNA duplex (green)[24]

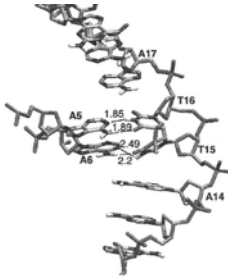


Figure 0.9 View of the single strand containing the thymine dimer (from the crystal structure of the duplex)[24]

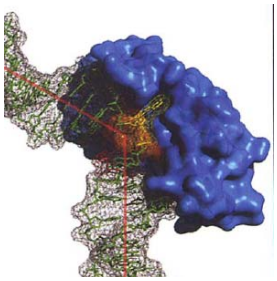


Figure 0.10 Structure of T4 endonuclease V enzyme bound to the DNA duplex containing a thymine dimer[24]

DNA damage can be recognized and repaired through different enzymes and strategies.[9] Base excision repair (BER) is a mechanism that repairs single damaged bases by removing the damaged nucleotides using a DNA glycosylase, followed by resynthesizing the appropriate missing nucleotide, and by sealing the nick with other enzymes.[9] Uracil DNA glycosylase (UDG) is one type of DNA glycosylase that can especially recognize and remove uracil in DNA duplex, through the activation of hydrolytic cleavage of the N-glycosidic bond of Uracil-containing nucleotide in DNA duplexes.[10] Recent studies have shown that the recognition process probably involves DNA bending.[10] The hypothesized model is that active UDG "intercalates" into the DNA duplex and forces DNA to bend (Fig.1.11 [25]).[10] One possibility is that uracil cannot form stable base pairs comparing to other DNA bases and is easier to flip out and to recognize by UDG.[10] Another possibility is that UDG will flip out and check every base sequentially until it finds the specific substrate-uracil fitting enzyme pocket.[9] Although the recognition process is still mysterious, it is believed that DNA bending is an essential conformation in UDG DNA damage recognition.

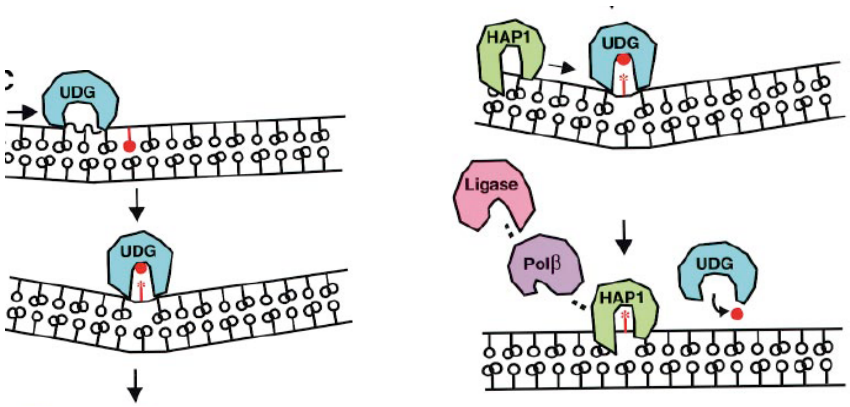


Figure 0.11 UDG recognizes and removes Uracil in double stranded DNA (HAP1, ligase and polβ are enzyme which resynthesize the appropriate nucleoside and seal the missing base)[25]

Additionally, DNA bending is thought to be key to the tremendous activity of certain drugs used in malignant tumour therapy. A successful example is cis-platin (cis diamminedichloro-platinum, left in Fig.1.12) which has turned testicular cancer into a curable disease.[12] Cisplatin's activity is believed to stem, at least in part, from DNA bending and its subsequent recognition by cell proteins (Fig.1.12).[12] Initially, chloride ligands of Cisplatin are slowly displaced by H_2O and thus the aquated platinum complex carries a positive charge.[12] This allows the complex to diffuse to the nucleus, approach negatively charged DNA, and coordinate to two adjacent guanines, forming the 1,2 intrastrand Cisplatin-DNA adduct as the most abundant product. NMR and X-ray studies show that the adducted DNA is bent 40 º towards the major groove at the guanine-cisplatin site. The bent adduct is known to be recognized by proteins (e.g. High Mobility Proteins), a process which is believed to trigger cell death.[12]

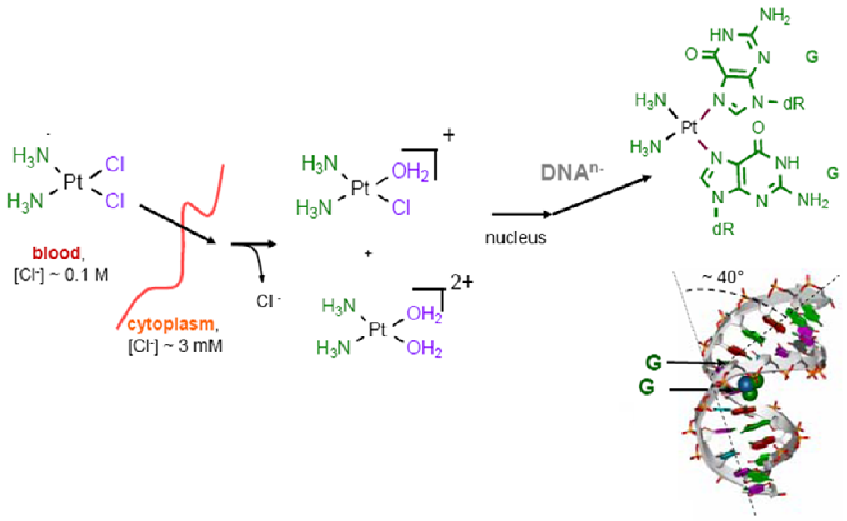


Figure 0.12 Cellular pharmacology of cisplatin.[26]

1.2 Inducing DNA bending using artificial systems

Due to the importance of bent DNA duplex conformations to many critical cellular processes, artificial systems aiming to induce DNA bending have been designed by different approaches. Those systems can aid further understanding DNA bending itself, as well as its relationship with DNA packaging, transcriptional regulation and DNA damage repair.

1.2.1 DNA coordination induced bending: Cisplatin

The earliest example of artificial bending was published by Lippard et al in 1988.[13] As previously mentioned, Cisplatin is able to induce DNA bending at the center of the two coordinated adjacent guanines. Short oligonucleotides (22 bp pair, Fig.1.13) may be treated with Cisplatin to form a bent adduct, and be submitted to ligases.[13] Ligases will join several oligonucleotides together into oligomers.[13] Due to the bent angle, the oligomers should end up cyclizing, and form a major large macrocycle, whose size should reflect the bent angle (e.g. sharp bending would lead to smaller ligated oligomers).[13] The resulting linear and circular oligomers are

separated by electrophoresis methods and the circular oligomer with the biggest contribution (C5 in this case) is further analyzed (Fig.1.13).[13] The study shows that the angle of DNA bending induced by Cisplatin is about 40°.[13]

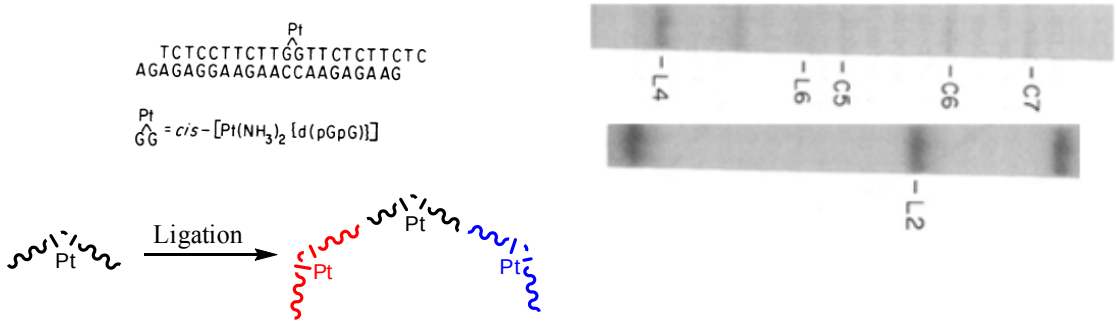
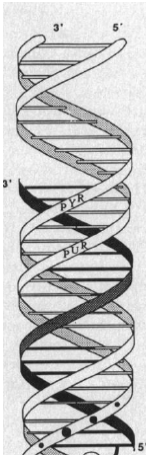


Figure 0.13 A Platinated 22bp DNA sequence,[13] cyclization of platinated sequence **B** Electrophoresis gel - C5, 6,7 represent circular products;L2,L4,L6 indicate linear products[13]

1.2.2 Inducing Bending at DNA groove: oligonucleotide triplexes and peptide nucleic acids invasion

Further than DNA bending through coordination of platinum to a DNA duplex, one may use the formation of triplex DNA to induce DNA bending. In this context, an additional short nucleic sequence is targeted at a DNA duplex, in order to form a final triplex architecture.[28] Such additional short nucleic sequence which promotes triplex formation is called a "Triplex Forming Oligonucleotide" (TFO) (Fig.1.14).[28] Similarly as with minor groove binders (see above), it is possible to design a TFO to target a specific short duplex section. Taking two TFOs which are specifically aiming for two noncontiguous duplex domains respectively, and linking them with a spacer, it is possible to perturb the duplex form. Indeed, choosing a spacer that is

shorter than the distance between the target domains will, upon triplex formation, force those two sections to come closer than in relaxed duplex, and lead to overall bending.[14,15,16]



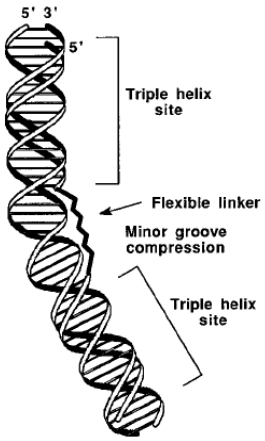


Figure 0.14A Simple triplex formation[28] **B** TFOs inducing DNA bending[15]

A TFO usually has 15 bases that can span a single turn of DNA helix.[14] The linker connecting the two TFOs are made from either propylene glycol phosphodiester subunits or triethylene glycol phosphodiester subunits.[14] The length of the linker is designed to be shorter than the distance between two binding sites to force DNA bending and can be adjusted to control the magnitude of the deformation (Fig.1.14 B).[14,15] The analysis and quantification of the induced DNA bending are performed via circular permutation and phase-sensitive electrophoresis, as explained below.[15] Three well known factors that affect linear DNA electrophoresis mobility are molecular weight, total carried charge and the mean square of the end-to-end distance.[15] Circular permutation method detects DNA electrophoresis mobility based on the distance from the end to the bending site.[15] Thus, the slowest mobility will occur when bending happens at the center of the DNA fragment (Fig.1.15).[15] Phase-sensitive electrophoresis is a more sensitive method used to detect DNA bending since it only detects the end to end distance of DNA duplex; thus it more accurately reveals the bending magnitude (Fig.1.16).[15] Those methods prove that the TFO systems induce DNA bending and the energy cost was calculated.[15]

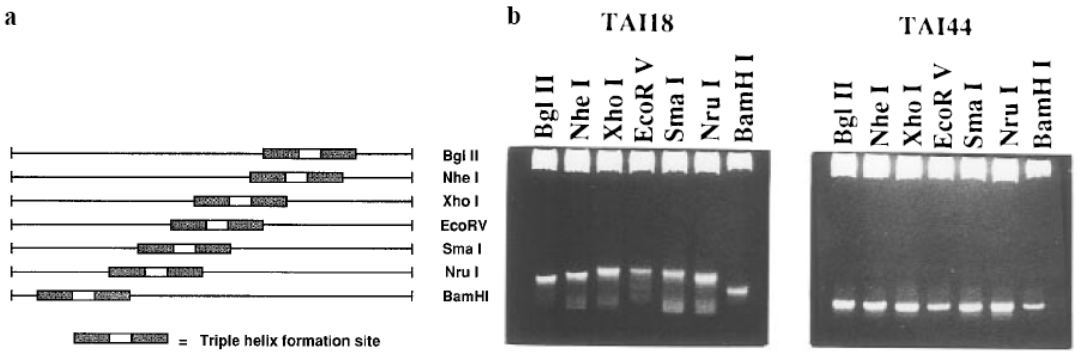


Figure 0.15A DNA fragment for circular analysis with TFO binding site at different location (each black box represents a TFO binding site while white hollow box indicates region between two sites).[15]

B Circular analysis: TAI18 and TAI44 represent two different TFO; TAI18 is designed to have a shorter linker than TAI44; For TA118 complexed DNA, EcoRV with the TFO binding sites in the middle has the slowest mobility, and BamH I with the binding sites closest to one end has the fastest mobility. For TAI44 with longer linker, there is not much difference between each complexed fragment.[15]

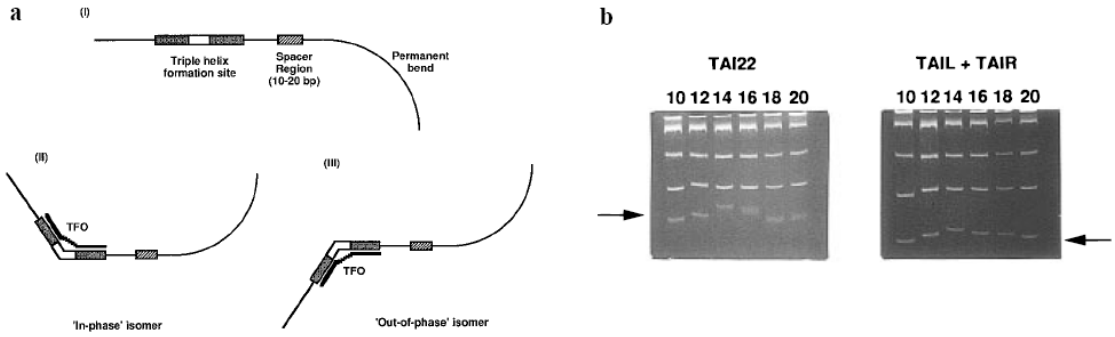


Figure 0.16 A DNA fragment for phase-sensitive analysis: the spacer shown can be varied from 10-20 bp and is used to reflect bending magnitude, the permanent bending results from an AT tract. Two extreme cases are shown as "in phase" and "out phase" isomer, two of which define the range of end to end distance.[15]

B Phase-sensitive analysis: for complexing with TA122 having a shorter linker, the different mobility is dependent on different spacer lengths (black arrow); For complexing with

two separated TFO- TAIL and TAIR without a linker, subtle mobility difference is shown. Those data can be further processed and calculated to determine the accurate bending angle.[15]

Peptide nucleic acids (PNA) appear as another class of oligonucleotide-based artificial system used for DNA bending. PNA is well known as a synthesized artificial polymer which mimics DNA or RNA.[17] Instead of having sugar backbone as in DNA or RNA, PNA uses repeating N-(2-aminoethyl)-glycine units to attach bases (Fig.1.17A).[17,18] Pseudocomplementary (pc) PNAs is a PNA duplex with two single strand of PNA designed to be complementary to each other.[18] pcPNAs can form stable pcPNA-ds (double strands) DNA complex and thus be investigated to induce DNA bending.[18] pcPNA carries natural the bases guanines(G) and cytosines(C), but uses artificial 2,6-diaminopurines (D) and 2-thiouracils(sU) instead of adenines and thymines.[18] As shown in Fig. 1.17B, the steric hindrance between 2,6-diaminopurines (D) and 2-thiouracils (sU) prevents their combination from forming stable double stranded pcPNA duplexes.[18] However, there is no hindrance between D (sU) with A and T and each single strand of pcPNA can form stable pcPNA-DNA complexes.[18] Thus, a short sequence of DNA duplex can be specifically targeted and DNA duplex invasion can be performed upon adding a pair of pcPNA duplex (Fig.1.17 A).[18] Upon invasion, DNA bending was found when two noncontiguous pcPNA binding sites are invaded with two pairs of sequence-specific pcPNAs duplex and the distance between two binding sites is a half-integer number of helical turn.[18] The magnitude of bending was also studied via circular permutation and phase-sensitive analysis as well as EM (electron microscope).[18] The proposed theory of pcPNA-induced bending is that two bulky pcPNA-DNA complexes will create relatively strained and tight distortion on the DNA duplex.[18] The extensive distortion causes DNA duplex bending in order to adapt to the new twisted conformation.[18]

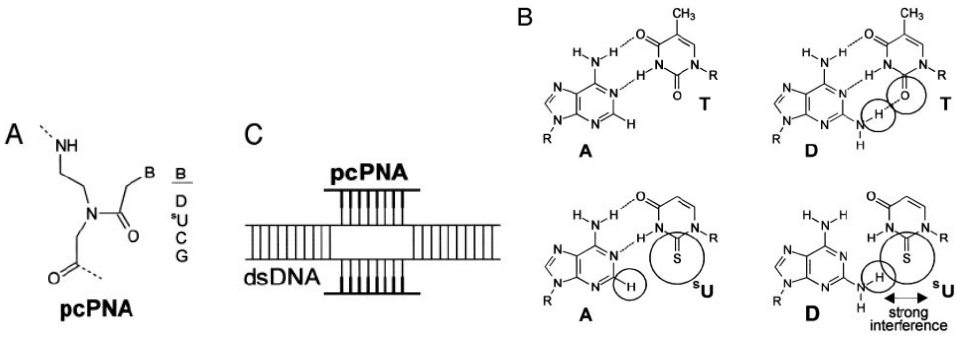


Figure 0.17 A Double duplex invasion[18] **B** pcPNA-DNA base pairs[18]

1.2.3 Bending by modifying charge distributions

Rather than short artificial nucleic acids, excess positive charge carriers have also been used to induce DNA bending. One contribution to the rigidity of DNA duplex is the repulsion between the negatively charged phosphates along the backbone.[19] Conceptually, by adding or reducing anion density on one side of DNA duplex, the asymmetric charge distribution should generate DNA bending.[19] Artificial systems based on this principle include DNA binding peptides and supramolecular cylinders, as exemplified below.

The carboxy-terminal basic leucine zipper (bZIP) domain composing of 60 amino acids from transcription factor Gcn4p of *Saccharomyces cereVisiae* has been well studied due to its simple secondary structure.[19] In 2007, Maher et al. reported using a homodimeric yeast bZIP domain as a positive charge carrier to approach a "U-shape" DNA duplex surface and induce DNA bending.[19] In-phase DNA bending has been observed in both opposite directions depending on anionic variants(EEE) or cationic variants (KKK) of the bZIP domain (Fig.1.18).[19] The results were confirmed by means of electrophoresis methods and fluorescence resonance energy transfer (FRET) - a powerful spectroscopic method to measure end to end distance of macromolecules.[19]

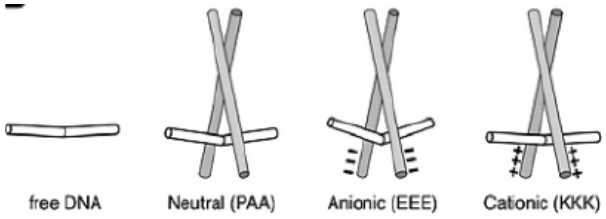


Figure 0.18 DNA binding protein inducing DNA bending through increasing or decreasing interphosphate repulsions[19]

Similarly, in 2001 Rodger et al. reported successfully employing positively charged metallo supramolecular cylinders to induce DNA bending.[20] Initially, the focus was to study cylinder binding to the DNA major groove; moreover, results showed that DNA bending is achieved through effective binding.[20] The designed supramolecular cylinder, shown in Fig.1.19 A, has two Fe^{2+} centers, and, thus, carries four positive charge.[20] Firstly, circular dichroism (CD) and linear dichroism (LD) studies proved that such cylinder does bind to DNA and also suggests the possibility of DNA bending at low cylinder loading.[20] Atomic force microscope (AFM) and NMR experiments further confirmed such DNA conformational changes (Fig.1.19B).[20]

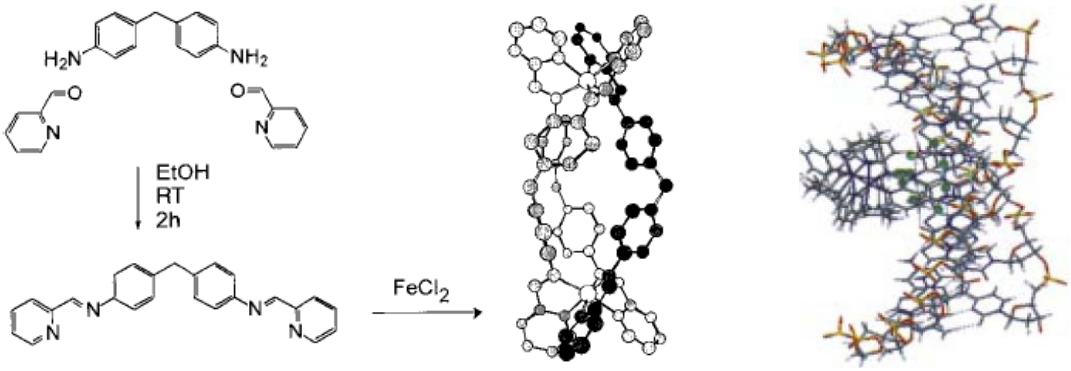


Figure 0.19 A Supramolecular cylinder [20] **B** DNA bending view based on NMR[20]

1.3 Design of bipyridine-derived ligands for DNA binding and shape switching

1.3.1 Design of artificial agents for reversible DNA bending

As described above, several methods have recently lead to "static" DNA bending, in the sense that there is little to no control on the bending event, except for the addition of the DNA bender itself. A further step closer to natural protein would consist of a system capable of reversibly transforming a "straight" duplex into a bent duplex, where the bending event would be controlled by an external trigger. Herein, we describe the design of two artificial agents that may be able to reversibly bend DNA. The artificial DNA benders are composed of (i) a switching spacer and (ii) two DNA anchors. The switching unit can be acted upon by a trigger. Conceptually, light, ions, small molecules can all be used as triggers, but we will focus on metal ions coordinating with two nitrogen binding sites on the switching unit. In the relaxed state (without trigger), only one anchor should be able to interact with DNA (Fig.1.10 left). Upon addition of the trigger, the switching unit should change from a trans conformation (relaxed state) to a cis conformation (tight state), allowing the two anchoring units to insert into DNA, put "pressure" on the DNA and force DNA to bend (Fig.1.20). By adding or removing triggers, the artificial DNA agents can hopefully achieve switchable DNA conformational changes.

These studies will hopefully provide useful information on the physical properties of DNA and on the mechanisms of DNA duplex bending. They may find applications as (i) ion-triggered DNA compaction agents for gene delivery, (ii) tools for molecular biology in order to study the dynamics of proteins targeting DNA bents and (iii) tools to study DNA damage recognition.

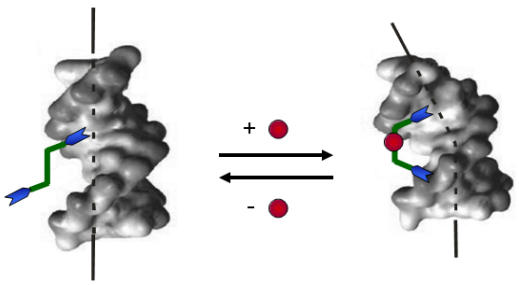
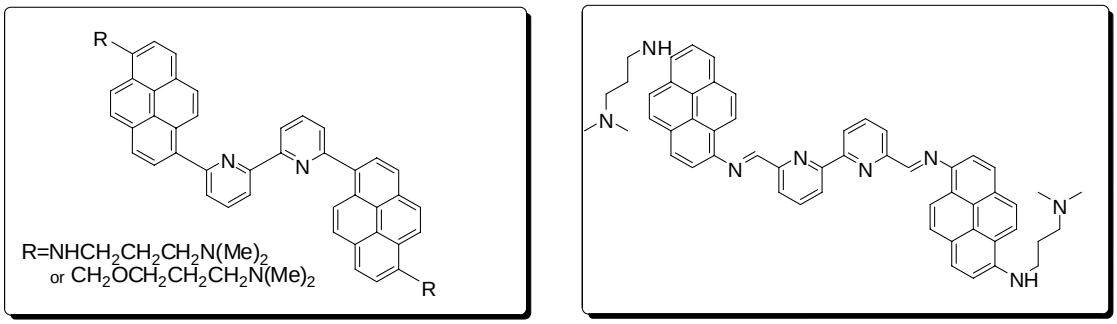


Figure 0.20 Cartoon representation of the concept behind reversible DNA bending

In the structures of the two artificial DNA bending agents shown in Fig.1.21, the bipyridine core serves as the switching spacer while pyrene units act as the DNA anchors.



Target A Target B

Figure 0.21 Chemical structures of the two artificial DNA bending agents considered herein

The rational design reasons are as following:

i) Bipyridine units can provide nitrogen atoms as coordination sites that can achieve shape switching by the addition or removal of metal cation triggers. Once the ligand coordinates with the metal cation, the additional positive charge will enhance the affinity of the agent towards DNA, which, as mentioned before, bears phosphate negative charges.

There are two coordination binding sites (N) in target A. Thus only a metal that adopts tetrahedron or planar geometry is considered for complex A. Due to the crowded coordination sites of target A, only metals with tetrahedron geometry are reasonable candidates; these include Cu+ and Ag+.

Target B has four binding sites and can coordinate with metal adopting square planar and/or octahedral geometries; these include Zn^{2+}, Pb^{2+}, Cd^{2+} and Ru^{2+} (although Ru would not be lead to reversibility, being a rather inert metal).[30,31,32]

ii) The C-C covalent bond connecting bipyridine and pyrene in target A and the C=N imine bond in target B are aiming to provide different coordination numbers (two in target A and 4 in target B) to adapt to various metal ions. They are also anticipated to have a different binding

strengths to metal ions, since more chelation sites in target B are likely to lead to larger binding constants to their corresponding metals compared to target A.

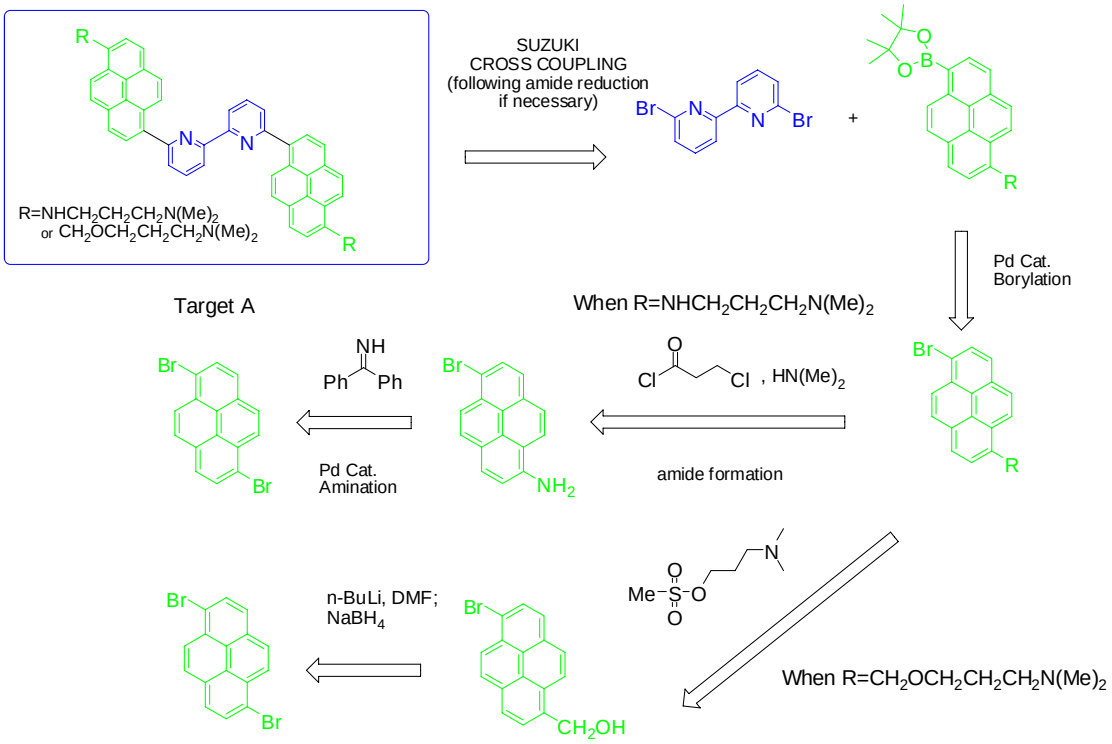
Since target A has shorter (or crowded) spacer compared to target B, it is hypothesized that target A may achieve sharper bending of the DNA duplex than target B.

iii). The amine/ammonium substituents on each pyrene unit aim at increasing solubility of the target molecules in aqueous solution. In addition, the two terminal nitrogen atoms should be protonated at pH 6-7 and will bear positive charges. The positive charge is proposed to also complement the negative charged DNA phosphate backbone and thus enhance DNA binding.

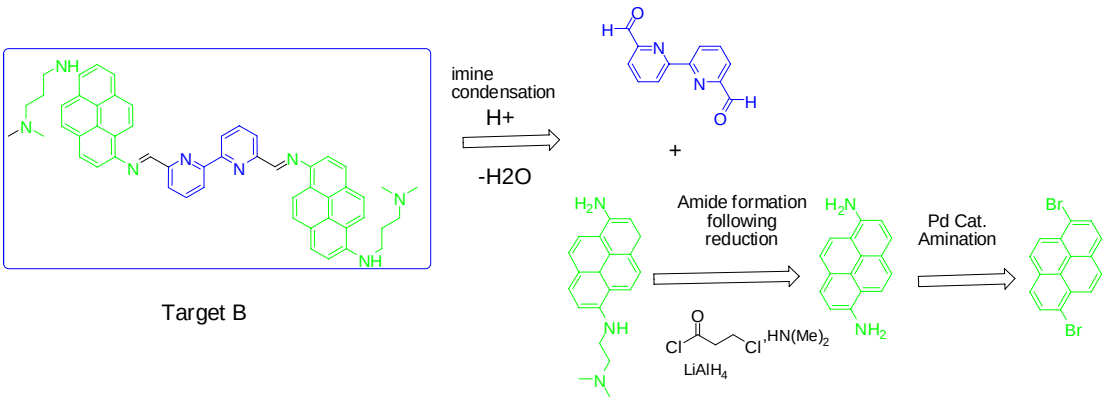
iv). The flat structure of pyrene is proposed to intercalate into the gap between the base pairs of DNA duplex sequences.[29] The intercalation is stabilized by π–π stacking between pyrene and base-pairs. Furthermore, substitutions on 1,6 positions of pyrene are proposed to mimic the twist angle of DNA duplexes.

1.3.2 Retrosynthesis routes to targets A and B

Herein, we reported the retrosynthesis routes to targets A and B respectively. Schemes 1 and 2 show how the bipyridine unit (blue) and pyrene unit (green) should be synthesized respectively. Upon Suzuki coupling or imine condensation, the complete assembly will give targets A and B. The synthesis pathways also indicate the sequence of the following parts of thesis (see below)



Scheme 0.1 Retrosynthesis route to target A



Scheme 0.2 Retrosynthesis route to target B

1.3.3 Plan of Thesis

Chapter 2: Synthesis of bipyridine unit

2.1 Short review of synthesis bipyridine derivatives

2.2 Synthesis of 2,6-dibromobipyridine

2.3 Synthesis of 2,6-diformylbipyridine

2.4 Investigation of new methodology of synthesis bipyridine derivative

Chapter 3: Pyrene functionalization

3.1 Short review of pyrene functionalization

3.2 Basic functional group on pyrene

3.3 Further functioning side chain on pyrene

Chapter 4: Fully assembly the bipyridine and pyrene unit

4.1 Model of target A

4.2 Target A assembly

4.3 Target A' assembly

Chapter 5 Experimental Section

Chapter 6 Conclusion

Appendix

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Chapter 2

Literature Review
Synthesis of bipyridine units

2.1 Short review on synthetic methods towards bipyridine motifs

2.1.1 Importance of 2,2' bipyridine derivatives in Chemistry

Bipyridine is a class of molecules in which two pyridine rings are coupled together. This class of molecules contains six isomers, among which 2,2'-bipyridine is the most prominent (Fig 2.1).[2] 2,2'-Bipyridine is well known as a bidentate chelating ligand and has been widely used as building blocks in supramolecular chemistry, macromolecular chemistry as well as nanoscience.[2] The 2,2'-bipyridine core has also been found in natural product such as caerulomycins and collismycins (Fig 2.2).[2,3]

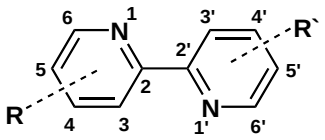


Figure 0.1 Structure of 2,2'-bipyridine

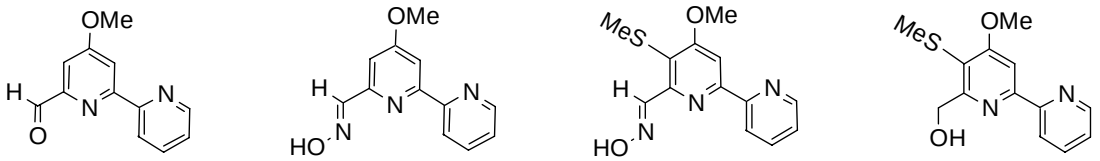


Figure 0.2 Examples of natural bipyridine motifs[2]

The very first synthesis of 2,2' bipyridine dates back more than 110 years ago, when the copper salt of the picolinic acid was used by Fritz Blau.[4] Since then, chemists have been interested in obtaining a variety of functionalized 2,2'-bipyridine derivatives, including

monosubstituted, symmetrically and dissymmetrically disubstituted derivatives.[2] Although the synthesis of 2,2'-bipyridine systems is one of a most challenging synthetic fields, certain reliable methods have been established to obtain the bipyridine scaffold, such as ring assembly, metal catalysis coupling and less traditional coupling.[2]

2.1.2 Synthesis of 2,2'-bipyridine derivatives via the ring assembly method

Krohnkes ring assembly can give simple monofunctionlized 2,2'-bipyridines from a pyridinium salt (a) with the treatment of an unsaturated ketone.[5] The pyridinium salt (a) is synthesized by reacting a bromomethyl ketone with pyridine. Then (a) is treated with an unsaturated ketone through a Michael addition and forms a 1,5-diketone intermediate (b). (b) is able to perform ring closure into the 2,2'-bipyridine product in the presence of ammonium acetate (Fig.2.3).[2] Based on this method, 5-methyl-2,2'-bipyridine was prepared in moderate yield.[6] In this case, the pyridinium salt (a) is generated by treating 2-acetylpyridine with iodine and pyridine. The salt then reacts with methacrolein to afford 5-methyl-2,2'-bipyridine in 72% yield (Fig.2.4).[2,6]

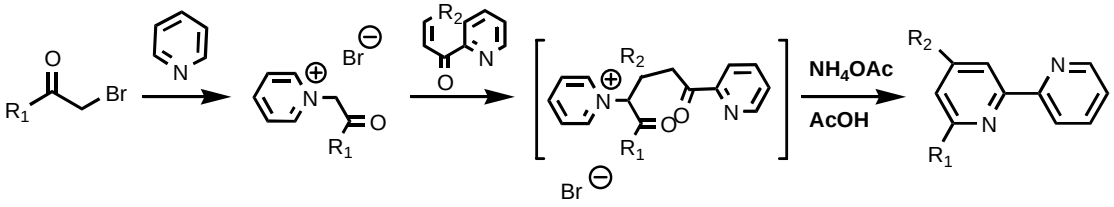


Figure 0.3 Krohnkes ring assembly[2]

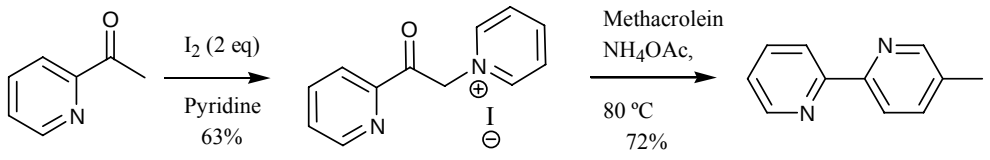


Figure 0.4 Preparation of 5-methyl-2,2'-bipyridine[2]

2.1.3 Synthesis of 2, 2'-bipyridine derivatives via « non-traditional » coupling

Using organophosphorus reagents, 6,6'-disubstituted-2,2'-bipyridine moieties can be obtained by coupling two 2-halopyridine reagents.[7] 2-Bromo- or 2-chloro-pyridine is treated with lithium phosphorus reagent followed by oxidation by hydrogen peroxide. The intermediate P-oxide affords the desired extrusion product in the presence of sodium ethoxide or sodium hydride in toluene at 100 ºC (Fig.2.5).[2]

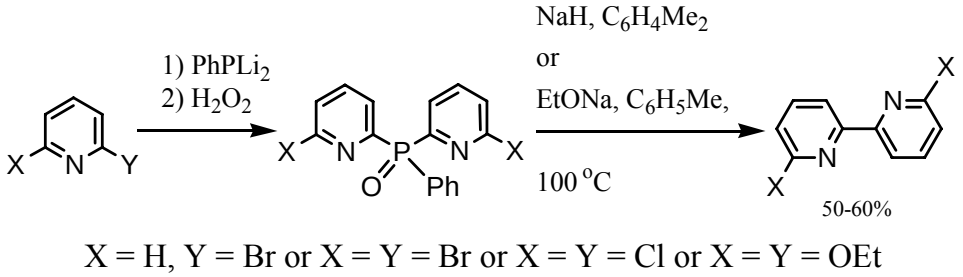


Figure 0.5 Extrusion of organophosphorus intermediate to afford 2,2'-bipyridine derivatives[2]

Alternatively, 6,(6')-substituted-2,2'-bipyridine can be obtained by ligand coupling of organosulfur compounds.[8] 2-(Alkylsulfinyl)pyridines treated with certain 2-pyridyl-magnesium or lithium organometallic species can give the desired coupling product (Fig 2.6).[8]

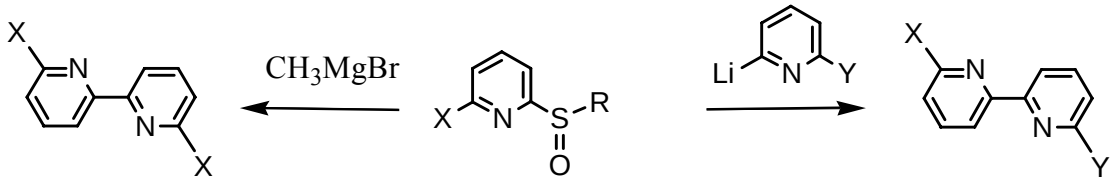
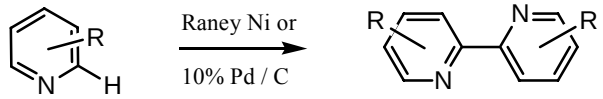


Figure 0.6 Ligand coupling of organosulfur to afford 2,2'-bipyridine derivatives[2]

2.1.4 Synthesis of 2,2' bipyridine derivatives via metal catalysis coupling

In the early work, difunctionalized 2,2'-bipyridine moieties could be synthesized through direct coupling of simple pyridines in the presence of Raney Ni or Pd / C as catalysts (Fig 2.7).[9] However, the yield of coupling from non-halogenated pyridines is generally low.[2]

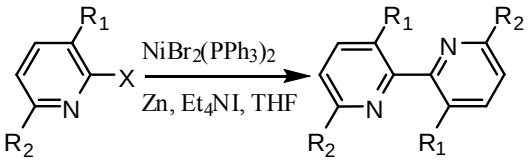


0.5-11%

R= H, CH_3, CH_2CH_3, C_6H_5, $COCH_3$, CN, CO_2Et

Figure 0.7 Raney Ni or Pd / C catalyzed[2] synthesis of 2,2'-bipyridine

Coupling of 2-halopyridine with Nickel catalyst and excess zinc as well as tetraethyl-



-ammonium iodide can afford symmetrically disubstituted 2,2'-bipyridine molecules in better yields (Fig 2.8).[10]

Figure 0.8 Ni catalyzed[2] synthesis of 2,2'-bipyridine

Recently, Negishi and Stille type couplings have emerged as efficient approaches to yield dissymmetrically substituted or monosubstituted 2,2'-bipyridine molecules.[2] Negishi type coupling employing palladium catalysis and organozinc reagents can provide monosubstituted 4,5,6-methyl 2,2'-bipyridine in good yields (Fig 2.9).[11] Stille type reactions couple 2-organotin pyridine with 2-halopyridine in the presence of palladium, and afford dissymmetrically substituted or monosubstituted 2,2'-bipyridines in relatively high yield (Fig 2.10).[12]

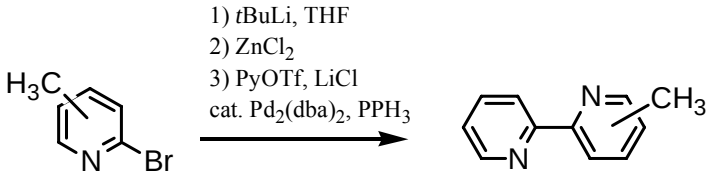


Figure 0.9 Negishi type coupling[2] to synthesize 2,2'-bipyridines

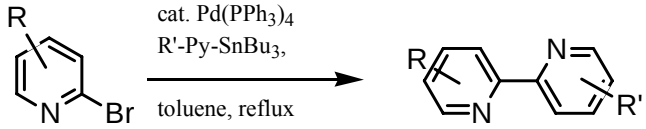


Figure 0.10 Stille type coupling to synthesize 2,2'-bipyridines

2.2 Synthesis of the 2,2'-bipyridine units as performed in this work

According to the retrosynthetic routes shown in the previous chapter (Schemes 1.1 and 1.2), 6,6'-dibromo-2,2'-bipyridine and 6,6'-diformyl-2,2'-bipyridine are required in order to synthesize the final artificial DNA bending agents.

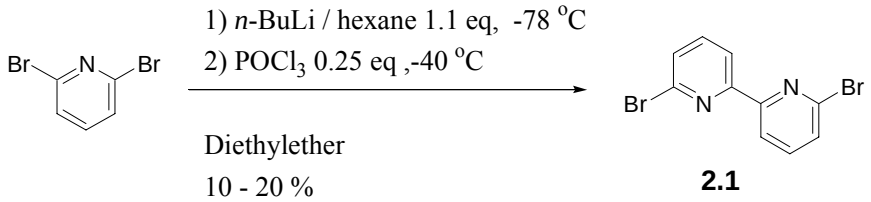
2.2.1 Synthesis of 6,6'-dibromo-2,2'-bipyridine (2.1)

6,6'-Dibromo-2,2'-bipyridine can be made via oxidative coupling of 2,6-dibromopyridine as reported in the literature.[13,14,15,16] Initially, the reaction was run in dry THF as solvent. *n*-BuLi (1.1 equiv.) was added slowly to 2,6-dibromopyridine (1.0 equiv.) in dry THF at -78 ºC and stirred for 2 h. Then the reaction was slowly warmed up to -40 ºC and 0.25 equivalent of phosphorus chloride oxide was added at -40 ºC; the mixture was stirred for another 2 h. Then the reaction was brought up to room temperature and quenched with water. The reaction was quenched by water at room temperature. In this case, 6,6'-dibromo-2,2'-bipyridine was not found as part of the products; the same outcome was obtained with one more attempt. Then, the reaction was performed in dry diethylether (the same solvent in the literature) instead of dry THF, while

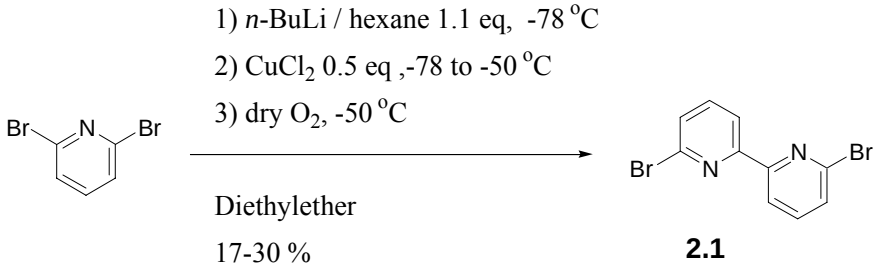
keeping other conditions the same (Scheme 2.1 Method A). In this case, 6,6'-dibromo-2,2'-bipyridine was successfully obtained upon careful purification of the crude by flash chromatography on silica. ^{1}H NMR confirmed that the product is the same one as in the literature. However, the yield of the reaction (10-20 %) is much lower compared to reported yields in the literature (40-70 %).[13,14] The suspected reason for low yields is the fact that proton is difficult to remove from phosphorus chloride oxide, and will consume the lithium reagent.

In other literature protocols,[15,16] 6,6'-dibromo-2,2'-bipyridine was also synthesized using Copper(II) chloride / O_2 (Scheme 2.2 Method B). Similarly, 1.0 equivalent of starting material in dry diethylether was treated with 1.2 equivalent of *n*-BuLi / hexane at -78 ºC and stirred for 2 h. At the same temperature, 0.5 equivalent of copper (II) chloride was added to the reaction mixture. The mixture was slowly warmed up to -50 ºC and dry oxygen was bubbled for 1 h at this temperature. During the bubbling, a greenish precipitate was observed, as reported in the literature. Then the reaction was warmed up to room temperature and quenched with an aqueous HCl solution. Since copper may bind to the formed bipyridine, we washed the filtered green precipitate with EDTA (NaOH solution) in order to free all possible bipyridine products. The final product was obtained as a white solid (as described in the literature) and its identity confirmed by ^{1}H NMR. This method gave a better yield of 30 % and can be compared to the reported yields (40%-50 %).[15,16]

Both methods require very strict conditions, which include very dry solvent, accurate concentration of *n*-BuLi reagent, and other dry reagents as well.



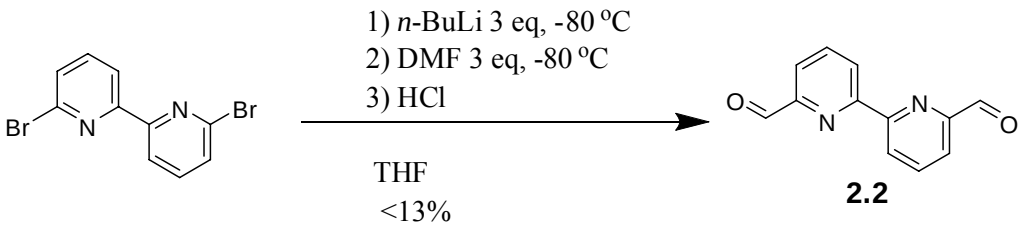
Scheme 0.1 Method A Synthesis of 6,6'-dibromo-2,2'-bipyridine (**2.1**) involving $POCl_3$



Scheme 0.2 Method B**.** Synthesis of 6,6'-dibromo-2,2'-bipyridine (**2.1**) involving $CuCl_2$ / O_2

2.2.2 Synthesis of 6,6'-diformyl-2,2'-bipyridine (2.2)

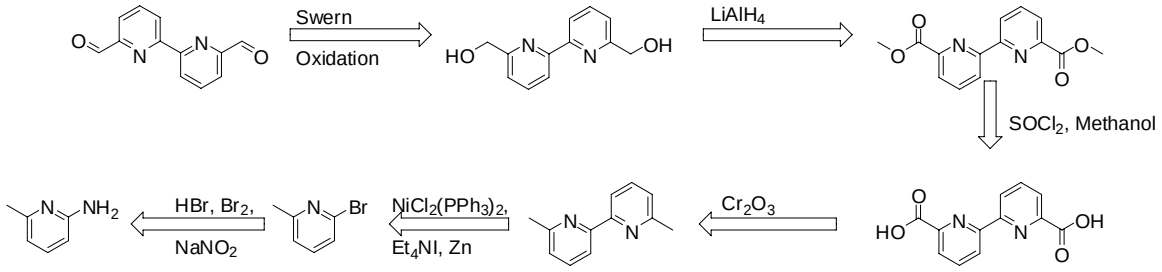
The synthesis of 6,6'-diformyl-2, 2'-bipyridine using *n*-BuLi / DMF is reported in the literature (Scheme 2.3).[17]



Scheme 0.3 Synthesis of compound **2.2** by using 6,6'-dibromo-2,2'-bipyridine

The same method has been attempted once in the laboratory on a scale of 200 mg of 2,6-dibromobipyridine. The desired pure product was obtained. However, due to the very poor yield (<13 %) compared to the reported yield of 70 % and difficulty of purification by chromatography (bipyridine systems tend to streak on silica columns) and by recrystallization,[17] a multiple-step

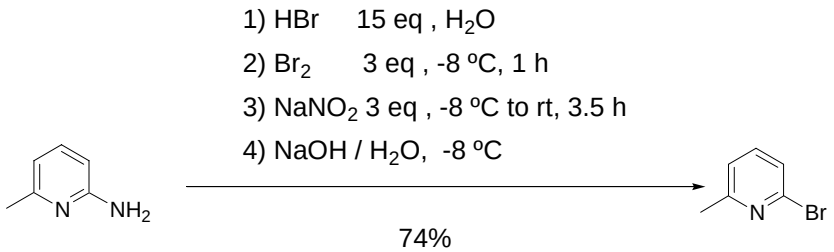
method (Scheme 2.4) was considered to be more reasonable and economical to obtain significant amounts of 6,6'-diformyl-2,2'-bipyridine. The retrosynthetic route is shown below. This work (from 2-aminopicoline to the bipyridine diol) was performed by Shawna Barker, a Chemistry 4th year student under my supervision in 2008-2009.



Scheme 0.4 Retrosynthetic route of 6,6'-diformyl-2,2'-bipyridine

Synthesis of 2-bromopicoline (2.3)

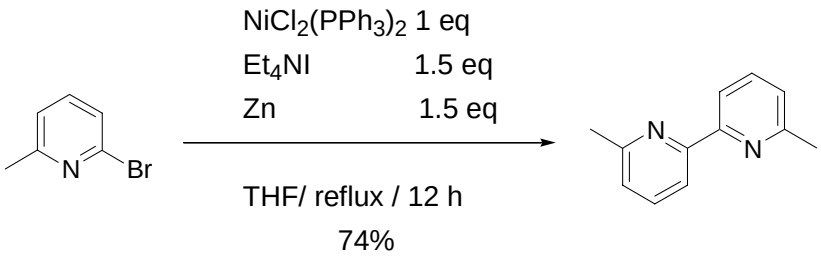
As shown above, inexpensive 2-aminopicoline was chosen as the starting point to synthesize 2-bromopicoline (Scheme 2.5). Following literature,[18] 10 g of 2-aminopicoline in aqueous hydrobromic acid underwent bromination by slow addition of bromine at -8 °C. After workup, the ^{1}H NMR analysis of the crude shows the desired product as well as a minor byproduct. An overall yield of 74 % of 2-bromopicoline was obtained upon purification by distillation.



Scheme 0.5 Synthesis of 2-bromopicoline

Synthesis of 6,6'-dimethyl-2,2'-bipyridine

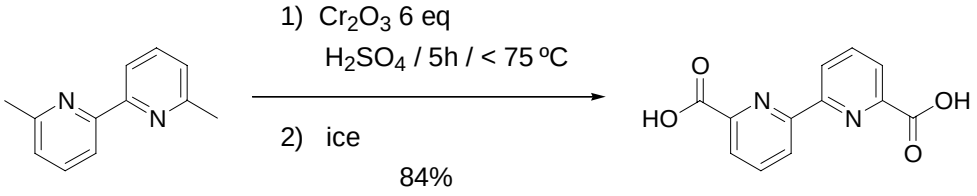
Formation of 6,6'-dimethyl-2, 2'-bipyridine can be done by homocoupling of 2-bromopicoline using a nickel catalyst (which was synthesized first from triphenylphosphine and nickel chloride in glacial acetic acid). The coupling itself followed a modified literature procedure (Scheme 2.6).[19] To a mixture of 1.0 equivalent of catalyst, 1.5 equivalents of tetraethylammonium iodide, and 1.5 equivalents of zinc dust in THF, was added a solution of 2-bromopicoline in THF. The reaction was refluxed for twelve hours. During the workup, the pH was adjusted to keep the desired bipyridine deprotonated. ^{1}H NMR shows the desired product. The crude was further purified by recrystallization in hexane and it gave a total yield 74 % of 6,6'-dimethyl-2,2'-bipyridine.



Scheme 0.6 Synthesis of 6,6'-dimethyl-2,2'-bipyridine

Synthesis of 6,6'-dicarboxy-2, 2'-bipyridine

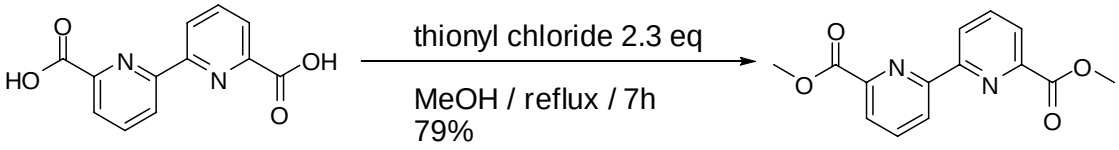
Transformation of the methyl groups into carboxylic acids was done through a modification of the procedure developed by Funeriu et al (Scheme 2.7).[20] To a solution of 6,6'-dimethyl-2,2'-bipyridine in neat sulfuric acid was slowly added chromium oxide as a powder. The temperature was monitored and kept under 75 ºC and the viscous reaction mixture was tentatively stirred overnight. The next day, the green mixture was poured onto crushed ice and kept in the fridge. The precipitate was filtered and washed with water, to give 78 % to 84 % yield of the desired product as confirmed by ^{1}H NMR analysis.



Scheme 0.7 Synthesis of 6,6'-dicarboxy-2,2'-bipyridine.

Synthesis of 6,6'-dicarboxy-2,2'-bipyridine dimethyl ester

The method to form 6,6'-dicarboxy-2,2'-bipyridine dimethyl ester was modified from a literature procedure (Scheme 2.8).[21] The previously synthesized dicarboxyl product was added to a solution of 2.3 equivalents of thionyl chloride in anhydrous methanol and the reaction was refluxed for seven hours. The precipitated formed at the end of the reaction was filtered (hydrochloride salt of the desired product), suspended in dichloromethane and basified to pH = 7 to deprotonate the bipyridine. Upon extraction with organic solvents, the desired product was obtained in a yield of 79-88 %. ^{1}H NMR analysis confirmed the positive result.



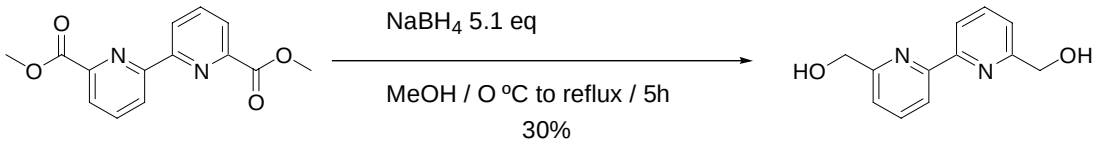
Scheme 0.8 Synthesis of 6,6'-dicarboxy-2,2'-bipyridine dimethyl ester

Synthesis of 6,6'-dihydroxymethyl-2,2'-bipyridine

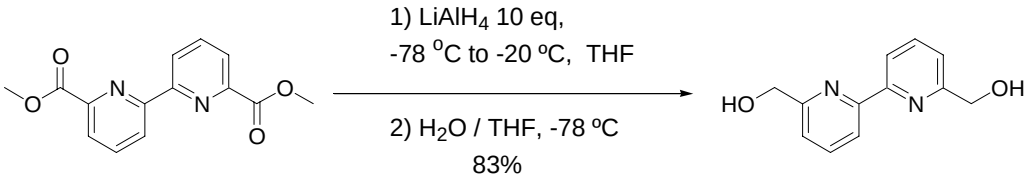
Although it is theoretically possible to directly transform a diester to a diol,[22] the presence of two ester functions combined with the possibility of over-reduction of each desired aldehyde to the alcohol prompted us to decompose this transformation in two steps: the two ester groups were firstly fully reduced to alcohols, then the two alcohols may be selectively oxidized to aldehydes (e.g. Swern oxidation[23]). The reduction was initially performed by modifying a protocol reported

in the literature involving a single pyridine (Scheme 2.9).[21] A mixture of diester and sodium borohydride (5.1 equivalents) was stirred in methanol at 0 ºC and refluxed for five hours. The reaction was then basified and refluxed for another two hours. During the workup, extraction with dichloromethane produced bubbles and a precipitate. The organic extractions gave only a yield of 30 % of the diol product as confirmed by ^{1}H NMR. The precipitate was filtered and ^{1}H NMR suggested some sort of boron-bipyridine polymer.

Secondly, reduction using lithium aluminum hydride was performed by modifying a literature method (Scheme 2.10).[24] A mixture of diester in dry THF was cooled to -78 ºC and 10 equivalents of lithium aluminum hydride in THF were added. The reaction was slowly warmed to -20 ºC to give a dark red solution. A methanol/water solution was carefully added at 0 ºC to quench the reaction. The crude was slowly warmed up to room temperature and left overnight. Upon extraction by dichloromethane, the desired 6,6'-dihydroxymethyl-2,2'-bipyridine was obtained (87 %) as confirmed by ^{1}H NMR.



Scheme 0.9 Reduction to 6,6'-dihydroxymethyl-2,2'-bipyridine via $NaBH_4$



Scheme 0.10 Reduction 6,6'-dihydroxymethyl-2,2'-bipyridine via $LiAlH_4$

The last step, namely the synthesis of 6,6'-diformyl-2,2'-bipyridine from the corresponding diol via Swern oxidation, is to be conducted soon.

2.3 Investigation of alternative methods to synthesize bipyridine derivatives via 2,2'-bipyridyl-N,N'-dioxide

2.3.1 Introduction

As indicated above, 6,6'-disubstituted-2,2'-bipyridine derivatives are usually very challenging to synthesize, especially when mono-substituted and dissymmetrically substituted moieties are targeted. The most popular functionalizing methods currently employ coupling procedures such as Suzuki type and Stille type cross-coupling reactions.[2] The former involves the challenging and costly preparation of poorly stable 2-pyridyl boronic acid derivatives, and the latter requires the use of organotin derivatives, which are easier to access but also toxic and difficult to remove entirely.

With our interest in developing 6,6'-aromatic disubstituted-2,2'-bipyridine derivatives, we wished to investigate an alternate method to introduce aromatic substituents onto a bipyridine system. Fagnou et al recently reported the regioselective direct arylation of pyridine N-oxides at the 2 position by employing palladium catalysis (Fig.2.11).[26]

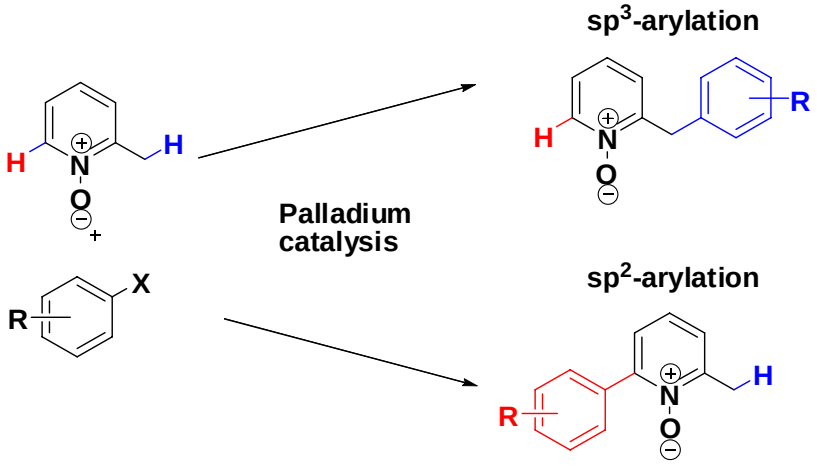


Figure 0.11 K. Fagnou's direct arylation of pyridine N-oxides[26]

Herein, we report the first investigation of unsubstituted 2,2'-bipyridine-N,N'-dioxide, as a coupling partner, with aromatic halides using palladium catalysis, in order to introduce substituents in the 6 (and 6') position(s) of the bipyridine core (Fig.2.12).Mild reduction of the N-oxide functions would then release the 6,6'-disubstituted-2,2'-bipyridine derivative. This method could be an alternate to boron or tin based cross-coupling reactions, allowing access to 6-mono-substituted, symmetrically or even dissymmetrically 6,6'-aromatic disubstituted 2,2'-bipyridine derivatives.

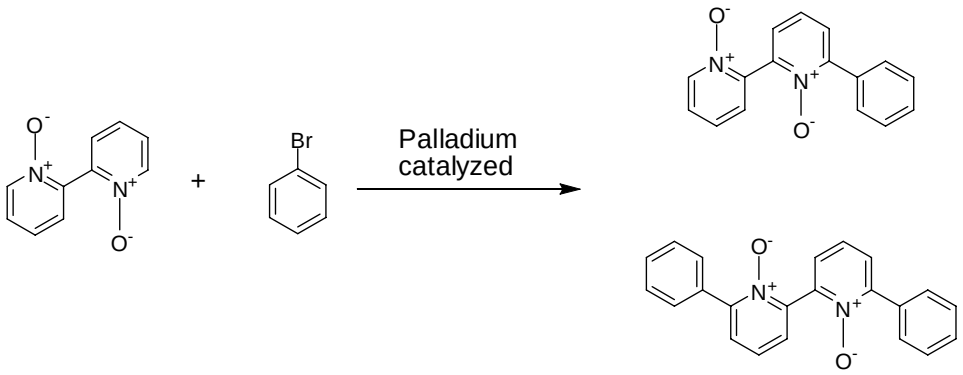
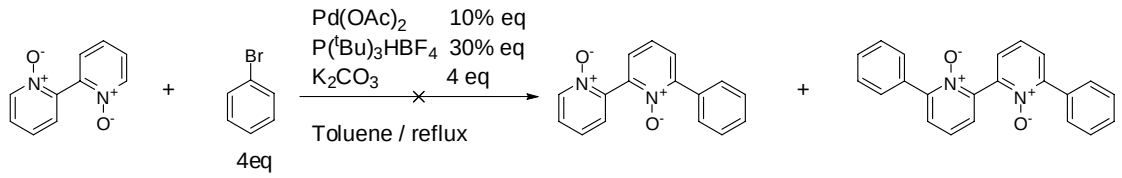


Figure 0.12 Proposed direct arylation of 2,2'-bipyridine-N,N'-dioxide

2.3.2 Synthesis of 6-phenyl-2,2'-bipyridine-N, N'-dioxide (2.3) and 6,6'-diphenyl-2,2'-bipyridine-N,N'-dioxide (2.4)

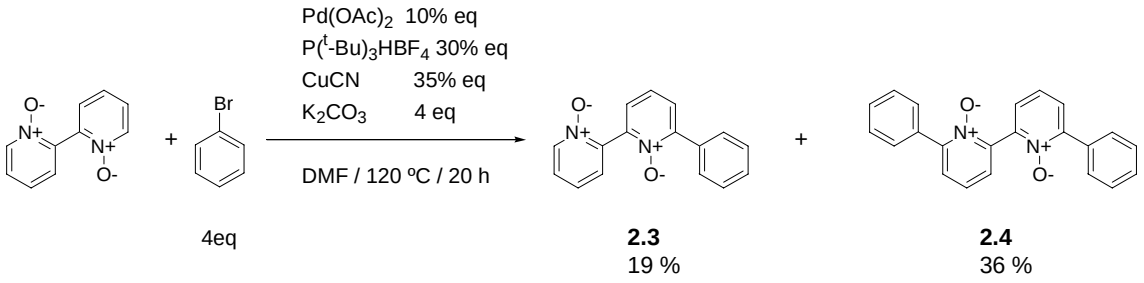
The same catalyst and solvent system as for the arylation of pyridine N-oxide ($Pd(OAc)_2$, tri-*tert*-butyl-phosphonium tetrafluoroborate($P(^t\text{-}Bu)_3HBF_4$), and toluene) were chosen as a starting point,[25,26] and bromobenzene was used in excess (four equivalents) to induce the formation of the bis coupled product (Scheme 2.11). The mixture of all the reagents was refluxed for 20 h in toluene under inert conditions. However, no coupling product was observed at this point. This condition system was then modified by increasing the amount of catalyst and ligand, extending reaction time and adding copper (I) cyanide (reported by Fagnou to increase the yields

for the simpler pyridine N-oxide),[26] but all cases failed to give either mono- or bis-coupling products.



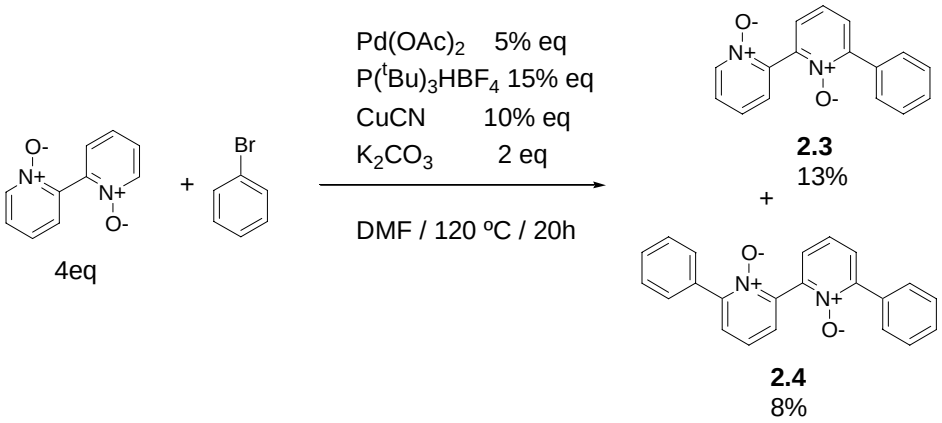
Scheme 0.11 Failed 2,2' bipyridine bis N-oxide coupling in toluene

Next, the coupling reaction was carried out in DMF, since (i) the reagents seemed more soluble in this solvent, and (ii) its boiling temperature is higher, allowing higher thermal activation of the reaction. The formation of both mono and bis coupling products were then successfully observed; the products were purified by column chromatography on silica gel, and isolated in 21 % and 9 % yields respectively. From this point, the conditions were optimized and copper(I) cyanide was found to increase the yield of bis coupling products to 36 % (Scheme 2.12). However, it seems as though the reaction got "saturated" at one point, since neither more catalyst, nor more starting material nor longer reaction time would force the reaction to completion.



Scheme 0.12 Optimized 2,2' bipyridine bis N-oxide coupling reaction at this stage

The reaction was also carried out in excess of 2,2'-bipyridine-N,N'-dioxide (four equivalents) in order to favour the synthesis of the mono coupling product (Scheme 2.13). The yield was lower than when an excess of bromobenzene was used, proving that the bis N-oxide is not a very active coupling partner.



Scheme 0.13 Coupling reaction in excess of bis N-oxide

2.4 Overall conclusion

In conclusion, we successfully synthesized 6,6'-dibromobipyridine in the laboratory. In addition, we investigated for the first time using 2,2'-bipyridine-N,N'-dioxide to obtain 6,(6') mono and bis substituted bipyridyl bis N-oxide scaffolds.º

In the future, the synthesis of 6,6'-diformyl-2, 2'-bipyridine from 6,6'-dihydroxymethyl-2,2'-bipyridine via Swern oxidation needs to be conducted. The conditions of palladium catalyzed bis N-oxide coupling need to be further optimized. Interesting work has already been reported on pyridine N-oxide showing that adding pivalic acid increases the yield and that aryl triflates are more active than aryl bromides.[27] Thus testing pivalic acid and aryl triflates is planned to further optimize the coupling reaction.

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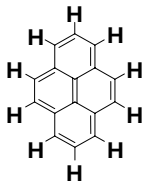
Chapter 3

Pyrene functionalization

3.1 Short review on pyrene functionalization



Pyrene is a poly aromatic hydrocarbon (PAH) compound. Recently, chemists have become more interested in pyrene chemistry because of the unique properties of the pyrene structure. So far, pyrene has been widely applied in biochemistry and cancer studies,[2] in supramolecular chemistry,[3, 4] and as a fluoroionophore.[5]

However, pyrene's reactivity is one of the most challenging due to the large amounts of possibility of isomers, steric hindrance of crowded protons, poor solubility, and high stability (low reactivity). In 1937, Vollmann *et al* published a review of various electrophilic aromatic substitutions and other basic functionlizations on pyrene.[1] So far, most pyrene chemistry has been focused on monosubstituted pyrene[3] or symmetrically disubstituted pyrene.[4] In 2000, Basu reported the successful synthesis of 1-amino 8-nitropyrene and 1-amino 6-nitropyrene.[6] The purification was obtained by HPLC[6] and indicated the difficulty to isolate pyrene isomers.

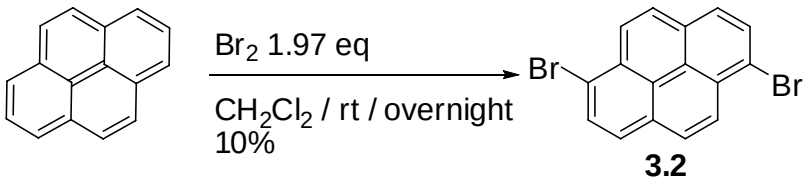
In this chapter, we reported the study of synthesis of dissymmetrically bisfunctionalized pyrene units

3.2 Synthesis of pyrene unit with diamino side chain

As shown in Scheme 1.1, 1,6-bisfunctional pyrene units are required to synthesize the final DNA bending agents.

3.2.1 Synthesis of 1,6-dibromopyrene (3.2)

1-Bromopyrene was found to be an unsuitable starting material due to the multiple isomers produced upon electrophilic aromatic substitution and difficulty of separation.[1] Thus known 1,6-dibromopyrene was chosen as a starting point. The bromo substituents should then be easily transformed into other desired functional groups without introducing isomers. 1,6-Dibromopyrene was synthesized from a protocol created by Grimshaw et al (Scheme 3.1).[7] The reaction was simply carried out in a suspension of pyrene in dichloromethane. Upon addition of a bromine/dichloromethane solution, bromination products formed and precipitated. The products were then filtered and ^{1}H NMR confirmed that the mixture was composed of two isomers: 1,6-dibromopyrene and 1,8-dibromopyrene. Upon fractional recrystallizations (6 to 15 times) in toluene, pure 1,6-dibromopyrene was then obtained. The yield was dependent on the scale of the reaction: Using 10g of pyrene, the yield of 1,6-dibromopyrene was 9.6% while bromination of 1g of pyrene only yielded 6% desired product.



Scheme 3. 1 Synthesis of 1,6-dibromo pyrene (**3.2**)

3.2.2 Retrosynthesis of diamino side chain for target A and B

Originally, the diamine substituent [$NHCH_2CH_2CH_2N(CH_3)_2$] was chosen as a water solubilizing side chain for the DNA bending agents (see also Chapter 1.3). Thus pyrene units A and B were considered towards targets A and B respectively (Fig. 3.1 & 3.2).

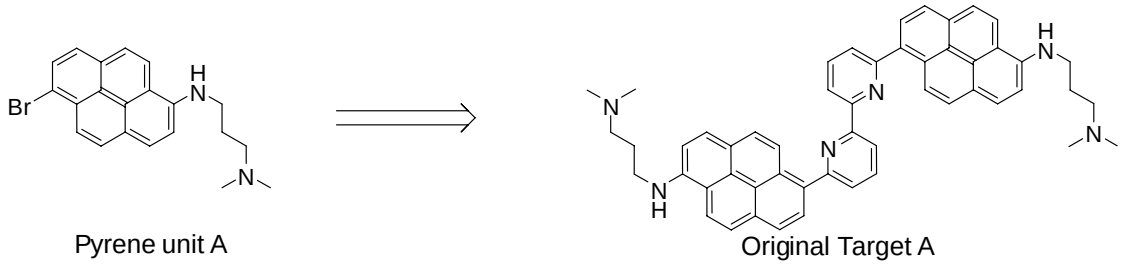


Figure 3.1 Structure of the original pyrene unit A

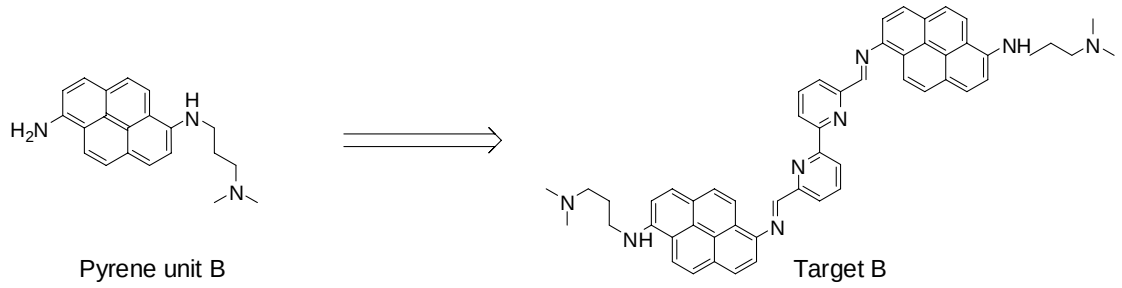


Figure 3.2 Structure of the pyrene unit B

At that stage, it was envisaged that the bromide group in pyrene unit A would be further converted into an amino group to give pyrene unit B (Fig.3.3).Thus the synthesis of pyrene unit A was heavily focused on and studied at the beginning of this work.

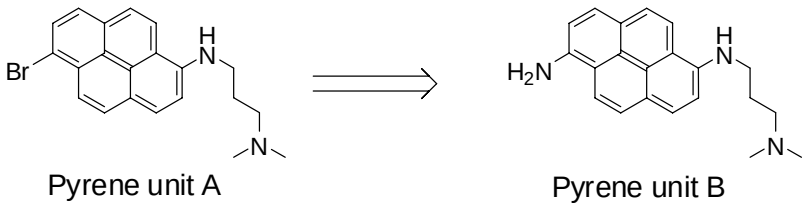
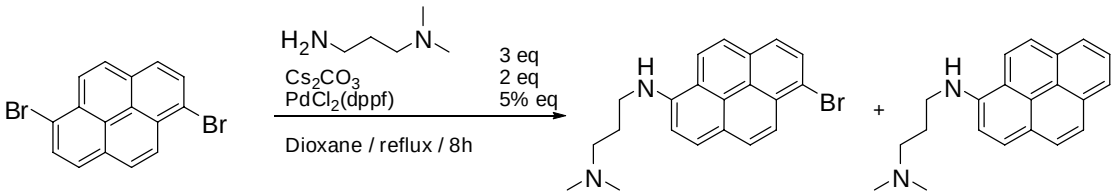


Figure 3.3 Proposed synthesis sequence of pyrene units A and B

3.2.3 Direct amination of 1, 6-dibromopyrene via a primary aliphatic amine

Direct amination of an aromatic halide with primary amines has become a topic of interest.[8] Thus the modified coupling conditions created by Yang and Buchwald[8] were used on 1, 6-dibromopyrene towards pyrene unit A. However, both desired coupling product and

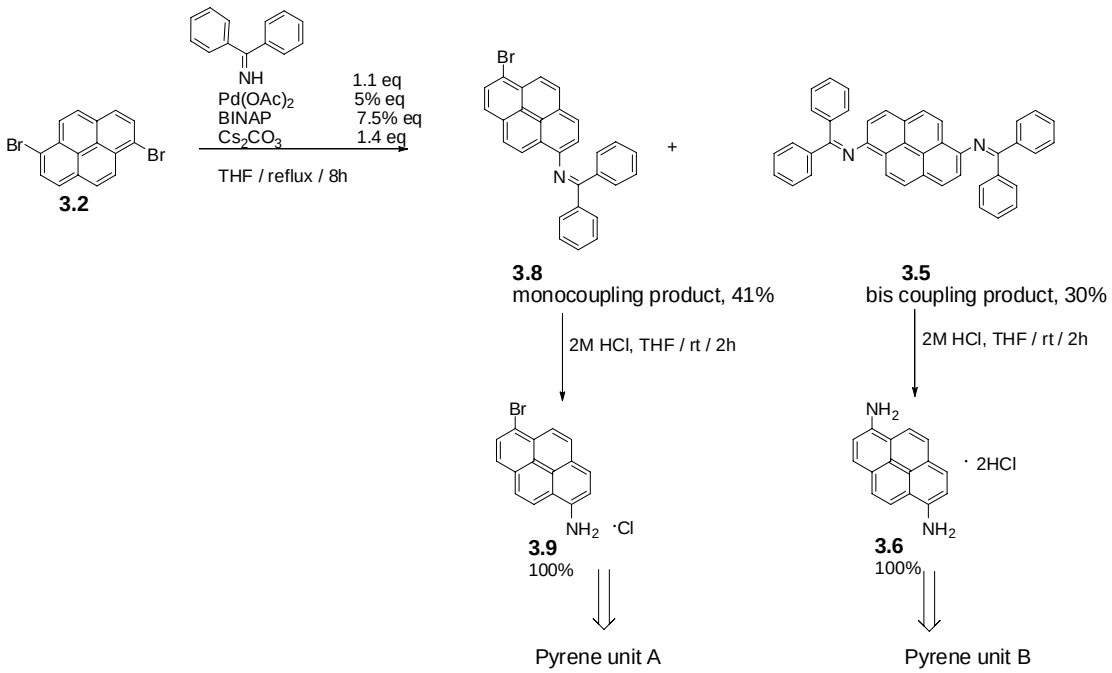
debromination byproduct are obtained as confirmed by mass spectrometry (Scheme 3.2). Due to their high polarity, separation of the two products though flash chromatography on silica gel failed. The reasons behind such a difficult reactivity are probably related to the fact that the primary amine coupling partner is not reactive enough and also may bind with the palladium catalyst, which slows down the reaction, requiring long reaction time, leading to significant debromination competition.



Scheme 3. 2 Amination of pyrene by coupling with a primary amine

3.2.4 Synthesis of 6-bromopyren-1-amine (3.8) and pyrene-1,6-diamine (3.5)

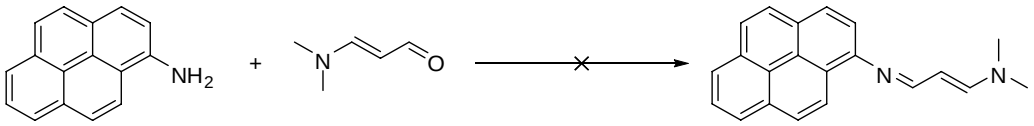
Thus an alternative two step method was employed to transform the bromo function into an amino group. Firstly, the coupling of 1,6-dibromopyrene with benzophenone imine was carried out based on the slightly modified procedure published by Beinhoff and Schluter *et al.*[9] Both the mono-coupling product (6-bromo-N-(diphenylmethylene)pyren-1-amine) and the bis-coupling product (N_1,N_6-bis (diphenylmethylene)pyrene-1,6-diamine) were produced and easily purified by flash chromatography on silica gel. Following hydrolysis, 6-bromopyren-1-amine and pyrene-1, 6-diamine were obtained as air stable hydrochloride salts. 6-Bromopyren-1-amine was considered for further transformation into pyrene unit A, while pyrene-1,6-diamine would be directly used to synthesize pyrene unit B. This divergent approach allows to save some pyrene unit A (Scheme 3.3, see also Fig.3.2).



Scheme 3. 3 Synthesis of 6-bromopyren-1-amine (**3.8**) and pyrene-1,6-diamine (**3.5**)

3.2.5 Reductive amination

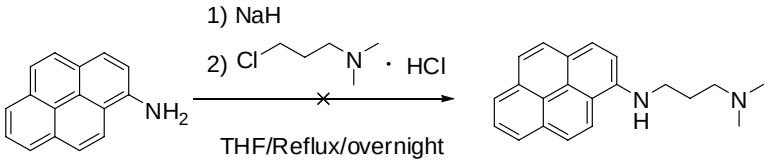
Reductive amination was considered in the first place to finish the rest of the side chain. 3-(Dimethylamino) acrylaldehyde was chosen as the aldehyde condensing partner while commercially available 1-aminopyrene was used as a test amine. 3-(Dimethylamino)-acrylaldehyde was synthesized following literature published by Menicagli *et al.*[10] The following imine condensation was operated following modified protocol reported by Jutz *et al*[11] in three different solvents: dichloromethane, ethanol and acetic acid (Scheme 3.4). However, the condensed imine could not be identified in any of those cases. The reasons are considered to be inert reactivity of amine group of 1-aminopyrene and stability of highly conjugated 3-(dimethylamino) acrylaldehyde.



Scheme 3. 4 Failed imine condensation

3.2.6 Alkylation of 1-amino pyrene

Literature shows the successful use of alkyl halides to alkylate amine group on benzene rings;[12] thus this method was attempted on 1-aminopyrene. 1-Aminopyrene was added to a suspension of two equivalents of sodium hydride in THF, followed by the addition of one equivalent of commercially available 3-chloro-N, N-dimethylpropan-1-amine hydrochloride salt (Scheme 3.5).[12] The mixture was refluxed overnight. The desired alkylation product failed to be identified through TLC and ^{1}H NMR. Alternative conditions using sodium hydroxide as a base and toluene/water as solvent were then attempted but failed. The reasons are considered that free 3-chloro-N, N-dimethylpropan-1-amine is able to process intermolecular condensation and the amine group of pyrene is a poor nucleophile, so condensation may happen before any alkylation takes place.



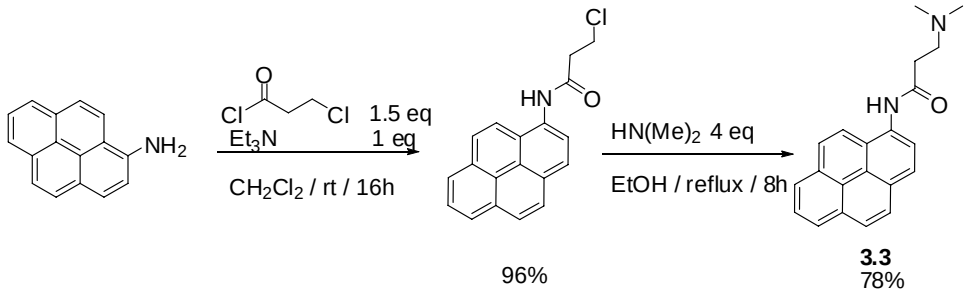
Scheme 3. 5 Failed alkylation of 1-aminopyrene

3.2.7 Amidation followed by reduction

Model test on 1-aminopyrene

An alternative three-step method was considered to synthesize the side chain. Similarly, 1-aminopyrene was used to test to the reactivity. In a solution of 1-aminopyrene and one

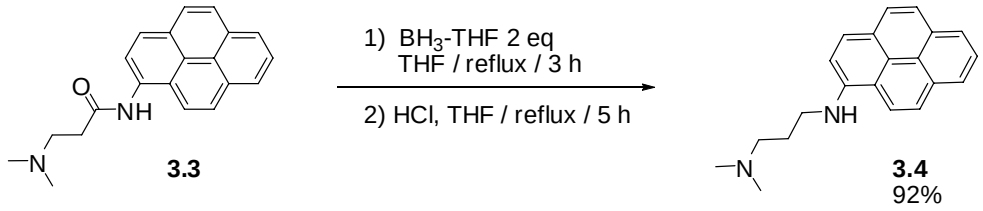
equivalent of triethylamine (Et_3N) in dichloromethane were added 1.5 equivalents of 3-chloropropanoyl chloride and it gave the amidation product -3-chloro-N-(pyren-1-yl)propanamide.[5] The chloride of 3-chloro-N-(pyren-1-yl)propanamide was then transformed into the desired dimethylamine group by refluxing with dimethylamine in ethanol[13, 14] and it gave 3-(dimethylamino)-N-(pyren-1-yl)propanamide with 78 % yield (Scheme 3.6).



Scheme 3.6 Synthesis of 3-(dimethylamino)-N-(pyren-1-yl)propanamide (**3.3**)

Borane tetrahydrofuran complex (BH_3-THF) was then chosen to reduce the amide bond. The reduction followed the procedure created by Park *et al*.[15] The solution of one equivalent of 3-(dimethylamino)-N-(pyren-1-yl) propanamide and two equivalents of 1.0 M Borane tetrahydrofuran complex solution was refluxed for 3 h and TLC shows the disappearance of the starting amide. Three spots were identified on TLC and were purified by flash chromagraphy on silica gel. ^{1}H NMR shows that all three spots have similar peaks pattern, the same proton integration of the desired aminopyrene and the third (the lowest on TLC) spot has the desired peak patterns. Due to the low polarity of first two spots compared to the anticipated high polarity of the desired diamine, those two spots were considered to be amine-boron complexes (the reduced amine has a nitrogen chelating site). Thus the two spots were taken up in THF again and refluxed with 2 M HCl solution. This process was monitored by TLC and after five hours it showed the disappearance of the original spots and appearance of a new spot which has the same

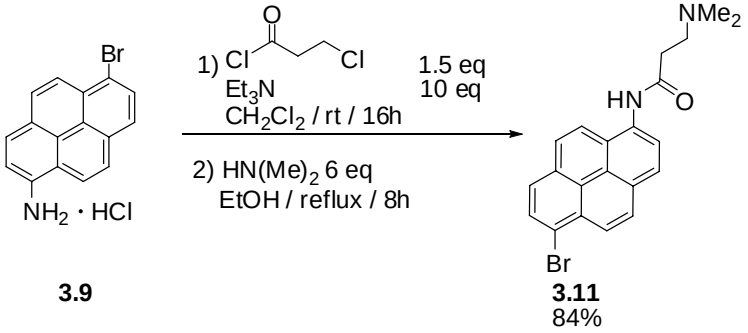
Rf as the third spot mentioned above. ^{1}H-NMR proved that the new spot was, indeed, the desired diamine as well (Scheme 3.7). The total yield of reduction was 92 %. However, it indicated that the diamine side chain can form a strong bond with complexed boron that requires long boiling time to break down. It was also observed that the reduced product was not very stable in air (the colour turned from fluorescent yellow to brown) and had to be kept in the freezer. The test method was judged reasonable enough to be applied on 6-bromopyren-1-amine and pyrene-1,6-diamine.



Scheme 3. 7 Reduction of amide in BH_3-THF

Synthesis of N-(6-bromopyren-1-yl)-3-(dimethylamino) propanamide (3.11)

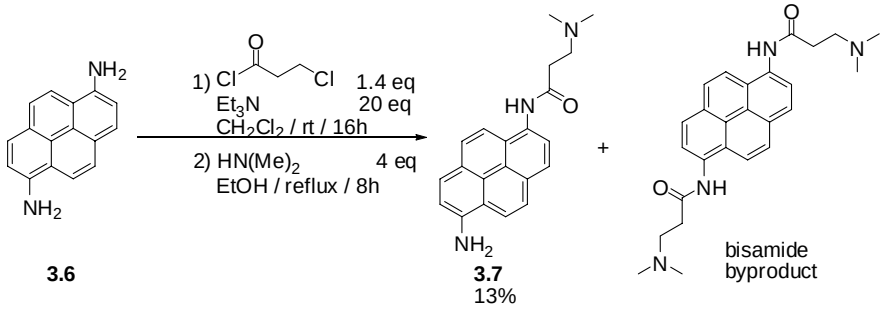
N-(6-Bromopyren-1-yl)-3-(dimethylamino) propanamide was synthesized from 6-bromopyren-1-amine hydrochloride salt. Similarly, 3-chloropropanoyl chloride was used to form the amide, followed by substitution of the chloride terminus using dimethylamine (Scheme 3.8).[5, 13, 14] The two steps gave a total yield of 82 %. Considering that the bromide function needed to be converted into a boron pinacol ester via palladium-catalyzed coupling, the reduction of the amide was not performed at this stage in order to limit the risk of having the free chelating diamine interfere with palladium catalysis.



Scheme 3. 8 Synthesis of N-(6-bromopyren-1-yl)-3-(dimethylamino) propanamide (**3.11**)

Synthesis of N-(6-aminopyren-1-yl)-3-(dimethylamino)propanamide (3.7)

Similarly, N-(6-aminopyren-1-yl)-3-(dimethylamino)propanamide was synthesized from 1,6-diamino pyrene hydrochloride salt using the method indicated above (Scheme 3.9).[5,13,14] The pyrene-1,6-diamine free base was found to be highly sensitive to oxygen and air because of the two electron donating amino groups on the pyrene core. Thus the reactions required deoxygenated conditions. The insoluble bisamide byproduct was filtered and the filtrate was quickly purified from flash chromatography on silica gel. The highly sensitive amide was kept under argon in the freezer. Due to the high sensitivity, the reduction step, which may require dry box conditions has not been performed.



Scheme 3.9 Synthesis of N-(6-aminopyren-1-yl)-3-(dimethylamino) propanamide (**3.7**)

3.3 Synthesis of pyrene unit with revised ether side chains

As shown in Chapter 3.2.7, the original diamine side chain can easily complex metal cations and disrupt metal coordination with the bipyridine unit. Also, the failure of reductive amination and alkylation of 1-aminopyrene indicate low pKa for the pyrene NH_2 group that may not be easily protonated in pH 7 DNA buffer. Ideally, the proposed DNA bending agents will be used in DNA buffer, with the side chain nitrogen fully protonated to allow optimal electrostatic interactions. Thus the diamine side chain was redesigned and one carbon inserted between the aromatic carbon and the first side chain nitrogen. Furthermore, it was considered that changing the "connecting" nitrogen for an oxygen may limit unwanted cation coordination to the side-chain (Fig.3.4). This leads to a new pyrene unit A' to be synthesized.

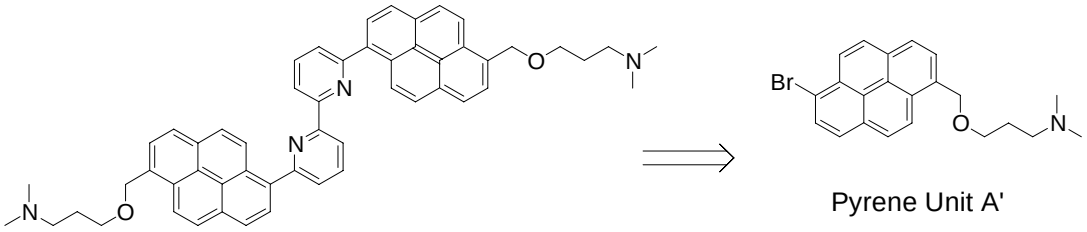


Figure 3.4 Structure of pyrene unit A' and target A' with revised side chain

3.3.1 Synthesis of revised pyrene unit A'

The proposed retrosynthesis route of pyrene unit A' is shown in Fig.3.5.

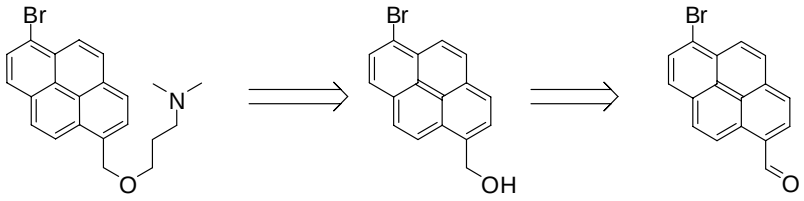
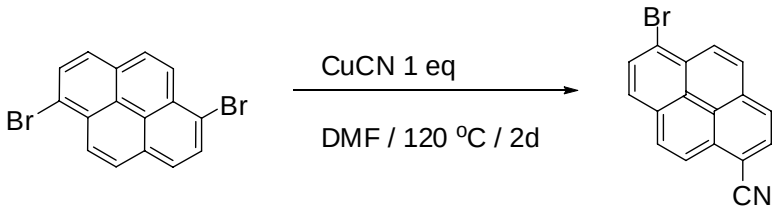


Figure 3.5 Proposed retrosynthesis route of pyrene unit A'

3.3.2 Monocyanation of 1,6-dibromopyrene

Monocyanation of 1,6-dibromo pyrene was considered in the first place. The nitrile group would then be reduced to an aldehyde group to give the desired 6-bromopyrene-1-carbaldehyde.

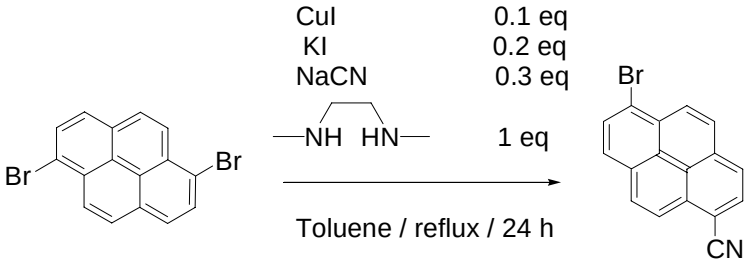
The first method to cyanate 1,6-dibromopyrene employed copper (I) cyanide. Due to the bisfunctional nature of 1,6-dibromopyrene, only one equivalent of copper (I) cyanide was used. A mixture of copper (I) cyanide and 1,6-dibromopyrene was simply heated in DMF at 120 °C for 2 days (Scheme 3.10).[16] The crude was purified through flash chromatography on silica gel and only gave desired the 6-bromopyrene-1-carbonitrile in a yield of 10 %. The low yield is considered to come from the low reactivity of both copper (I) cyanide and 1,6-dibromopyrene, and the fact that only one equivalent of copper (I) cyanide could be used (in order to limit the extent of bis-cyanation).



3.2 **3.12** 11%

Scheme 3. 10 Cyanation of 1,6-dibromo pyrene using CuCN

The cyanation method created by Buchwald was attempted to achieve better yield.[17] A mixture of 1,6-dibromopyrene, copper (I) iodide, potassium iodide, sodium cyanide and ligand *N, N'*-dimethylethylenediamine was refluxed in toluene for 24 h (Scheme 3.11). The crude was purified through flash chromatography on silica gel and it gave 18 % yield of 6-bromopyrene-1-carbonitrile.



3.2 **3.12** 18 %

Scheme 3. 11 Cyanation of 1,6-dibromopyrene using Buchwald conditions

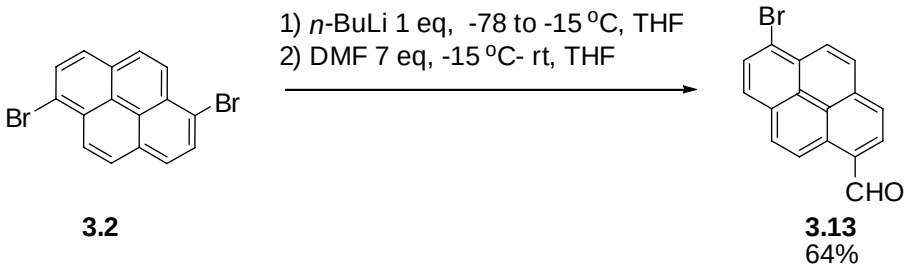
The reduction of 6-bromopyrene-1-carbonitrile by diisobutylaluminium hydride (DIBAL) was attempted once.[18] Due to the poor solubility of 6-bromopyrene-1-carbonitrile in both THF and dichloromethane and the crowded steric hindrance of pyrene protons, only a very small extent of reduction was observed by TLC and ^{1}H NMR of the crude.

Because of the poor solubility of 6-bromopyrene-1-carbonitrile, the low reactivity for both cyanation and reduction, and the toxicity of reagents and solvents, it is not practical to synthesize and purify large amounts of 6-bromopyrene-1-carbonitrile via chromatography for the following steps.

3.3.3 Synthesis of 6-bromopyrene-1-carbaldehyde (3.13)

A more straightforward way to convert one halide of 1,6-dibromopyrene into an aldehyde function was then applied to synthesize 6-bromopyrene-1-carbaldehyde via an organolithium nucleophile. One equivalent of *n*-butyllithium in hexane was added dropwise to a suspension of 1,6-dibromopyrene in THF at -78 ºC. Studies shows that the halogen-lithium exchange of 1(,6) position of pyrene requires higher temperatures.[3] Thus the reaction was warmed up to -15 ºC and stirred for 15 min and it gave a red solution. At the same temperature seven equivalents of DMF were added at once and the reaction was left to warm up to room temperature (Scheme 3.12). The

crude was recrystallized from chloroform / hexane. The filtrate from the recrystallization was then purified by flash chromatography on silica gel. It gave a total yield of 64 % of 6-bromopyrene-1-carbaldehyde. The reaction requires very strict conditions, including accurate concentration of *n*-butyllithium, very dry starting material, DMF, THF and glassware. Wet conditions will lead to large amount of the debromination byproduct (1-pyrene carbaldehyde) which was identified by GC/MS and will co-elute with the desired product on silica gel, leading to painful purification and poor yields.

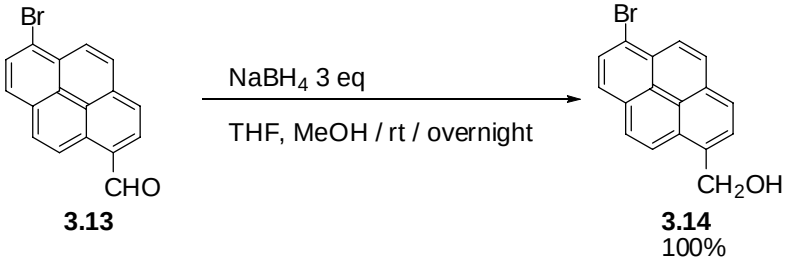


3.2 **3.13** 64%

Scheme 3. 12 Synthesis of 6-bromopyrene-1-carbaldehyde (**3.13**)

3.3.4 Synthesis of 6-bromopyrene 1-methanol (3.14)

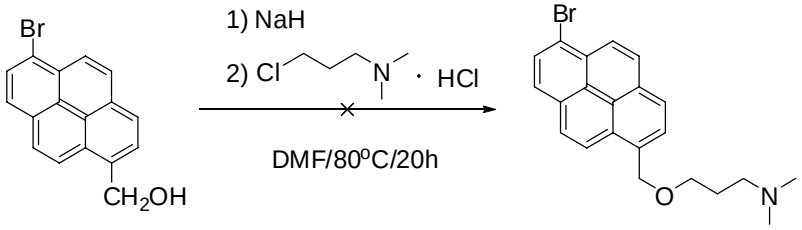
The reduction of 6-bromopyrene-1-carbaldehyde was followed the protocol published by Mckee.[19] To a solution of 6-bromopyrene-1-carbaldehyde in THF and methanol were added three equivalents of sodium borohydride and the reaction was left to stir overnight at room temperature. The crude was pure enough for next step and the conversation is 100 %. The choice of a mixture of solvent was made so that THF would solubilise 6-bromopyrene-1-carbaldehyde and methanol would assist the reduction reactivity.



Scheme 3.13 Synthesis of 6-bromopyrene-1-methanol

3.3.5 Synthesis of 3-((6-bromopyren-1-yl)methoxy)-N,N-dimethylpropan-1-amine (3.15)

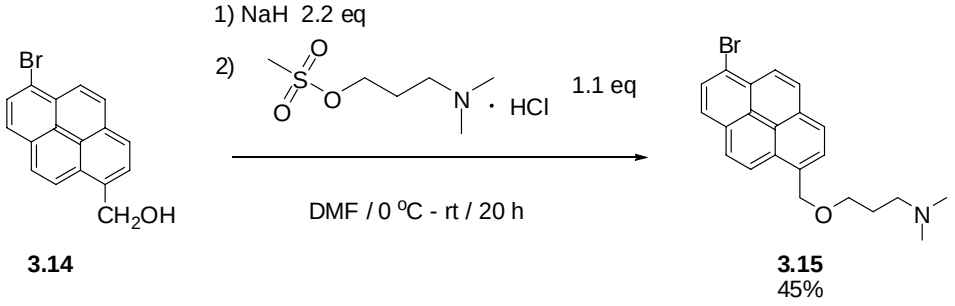
Initially, alkylation of 6-bromopyrene-1-methanol was attempted with 3-chloro-N,N-dimethylpropan-1-amine hydrochloride salt and sodium hydride (Scheme 3.14).[20] The reaction mixture was heated in DMF for 20 h. The crude showed multiple spots on TLC. Purification using the "protonation / deprotonation trick" failed because of the poor solubility of pyrene unit in aqueous solution. Purification through flash chromatography was attempted but the desired fraction failed to be identified. The reason is still considered to be self-condensation of free 3-chloro-N,N-dimethylpropan-1-amine, low reactivity of chloride, and steric hindrance from pyrene protons.



Scheme 3.14 Failed alkylation using 3-chloro-N,N-dimethylpropan-1-amine

A better leaving group - the Mesyl group- was then considered as a substitute for the chloride. Thus 3-(dimethylamino)propyl methanesulfonate hydrochloride salt was synthesized from reported literature and was freshly used.[4] To a mixture of 2.2 equivalents of sodium hydride

and 6-bromopyrene-1-methanol in dry DMF, was added 1.1 equivalents of 3-(dimethylamino)-propylmethanesulfonate hydrochloride salt portion-wise at 0 ºC.[4] Then the reaction was left over night at room temperature (Scheme 3.15). The crude was carefully purified through flash chromatography on silica gel and gave the desired ether in 45 % yield.



Scheme 3. 15 Synthesis of 3-((6-bromopyren-1-yl) methoxy)-N,N-dimethylpropan-1-amine (**3.15**)

3.4 Conclusion of Chapter 3

In this chapter, we report the study of synthesis the DNA anchor (pyrene units A and B) and the revised DNA anchor (pyrene unit A'). Both pre-pyrene unit A and B and revised pyrene unit A' were successfully obtained and are ready to complete the assembly of the bipyridine / pyrene conjugate.

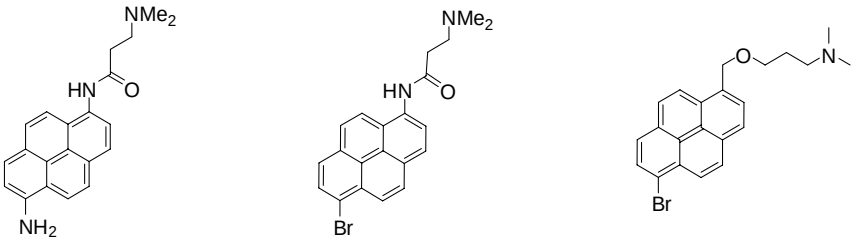


Figure 3.6 Structures of pre-pyrene unit B, pre-pyrene unit A, and revised pyrene unit A'

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Chapter 4

Fully assembling the bipyridine and pyrene units

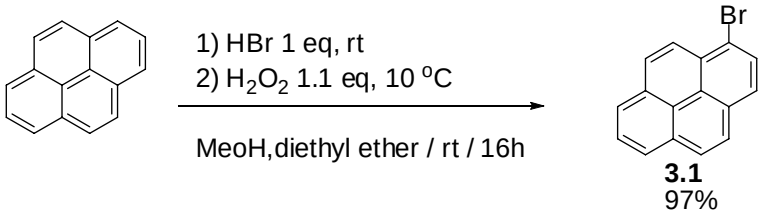
4.1 Study of model of target A

As shown in Scheme 1.1, the bromide function on bromo-substituted pyrene may be converted into a boronic ester(or acid), which is known to be an appropriate coupling partner for aryl bromides to form C-C bond, through what is referred to as a "Suzuki-Miyaura cross-coupling reaction". As a result, a pyrene-derived boronic ester (or acid) may cross-couple with 1,6-dibromobipyridine to complete the assembly of target A.

In order to test such coupling reaction, a model compound of target A (i.e. the same aromatic core as target A, but without the alkylammonnium side chain) was looked at first. This allowed the investigation of both the borylation and Suzuki cross coupling steps.

4.1.1 Synthesis of 1-bromopyrene (3.1)

1-Bromopyrene was synthesized in the laboratory following literature procedure.[1] As shown in Scheme 4.1, a suspension of pyrene in methanol and diethylether was treated with one equivalent of aqueous hydrobromic acid followed by 1.1 equivalent of aqueous hydrogen peroxide to yield the monobromination product. The crude was ready to use for the next step.

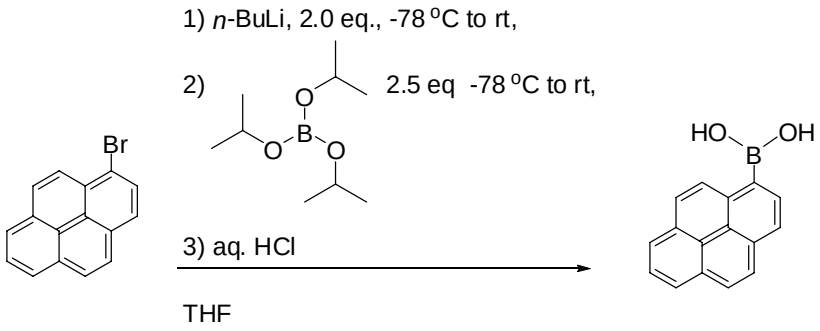


Scheme 4.1 Synthesis of 1-bromopyrene (**3.1**)

4.1.2 Borylation of 1-bromopyrene

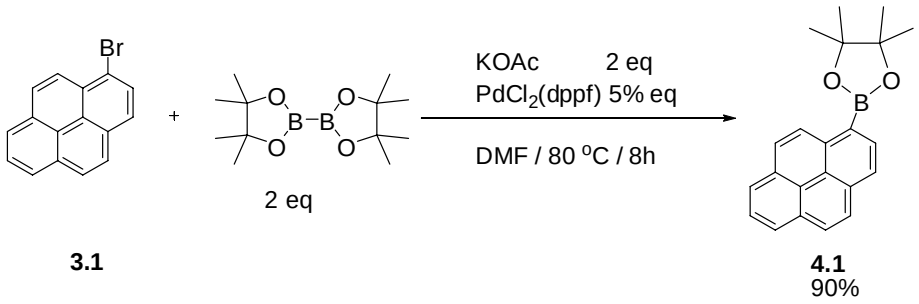
Both of common borylation methods, lithium halogen exchange and palladium catalyzed borylation, have been tested on 1-bromopyrene. However, only the palladium catalyzed borylation was considered for the actual substrate since it was aware that the amino group of pyrene unit side chain would interfere with lithiation.

Firstly, *n*-Butyllithium/triisopropyl borate was investigated for the synthesis of 1-pyreneboronic acid.[2] Two equivalents of *n*-butyllithium was added dropwise to a suspension of 1-bromopyrene in anhydrous THF at -78 ºC. The reaction was then warmed up to room temperature and stirred for another 2 h. Then the solution was cooled to -78 ºC and 2.5 equivalents of triisopropylborate were added (Scheme 4.2). The reaction was quenched by hydrochloride solution and worked up by dichloromethane. ^{1}H NMR of the crude showed the desired boronic acid peaks but purification was not successful. The reason is considered to be wet triisopropyl borate [B(O-iPr)$_3$] reagent. Since this method was not to be used on the actual substrate, we then focused on palladium catalyzed borylation.



Scheme 4.2 Failed borylation of 1-bromopyrene

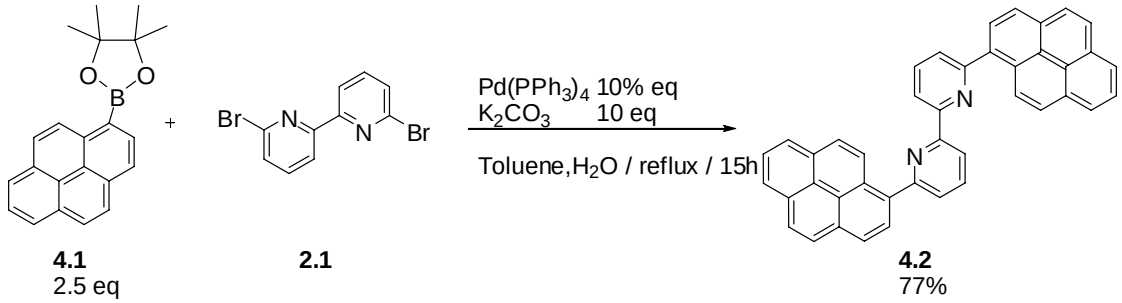
Successful borylation of 1-bromopyrene was performed following protocols created by Ishiyama and Miyaura et al (Scheme 4.3).[3] A mixture of 1-bromopyrene, two equivalents of bis(pinacolato)diboron (B_2Pin_2), three equivalents of potassium acetate, 5 % equiv. of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane was heated at 80 °C in DMF under argon for 8 h. The reacted mixture was washed with water and the aqueous layer was extracted with diethylether. The crude was purified via flash chromatography on silica gel and yielded the desired 1-pyrene boronic pinacol ester (1-BpinPyrene) in 72.5 %.



Scheme 4. 3 Palladium catalyzed borylation of 1-bromopyrene leading to 1-BpinPyrene

4.1.3 Synthesis of model of target A (4.2)

Suzuki type cross coupling reaction between 6,6'-dibromo-2,2'-bipyridine and 1-BpinPyrene was carried out following a modified procedure (Scheme 4.4).[4] A mixture of 2.5 equivalents of 1-pyrene boronic pinacol ester, one equivalent of 6,6'-dibromobipyridine, 10 equivalents of potassium carbonate, and 10 % equiv. of tetrakis(triphenylphosphine)palladium (0) was refluxed in a toluene / water mixture for 15 h under argon. The crude was taken up in dichloromethane and filtered. The precipitate was confirmed later as being the desired coupled product. The filtrate was purified through flash chromatography on silica gel and then recrystallized from chloroform. It gave a total yield of 51 % of the desired coupling product.



Scheme 4. 4 Synthesis of model of target A (**4.2**)

Thus, palladium borylation and Suzuki coupling are considered to be used to assemble the actual bipyridine and functionalized pyrene fragments into target A.

4.1.4 Switching Studies of the target A model

As mentioned in Chapter 1.3, metal ions such as Cu^{+} are considered as ionic switching triggers for target A. Thus the binding of Cu^{+} has been investigated through a preliminary study based on model A via ^{1}H NMR.

To a suspension of 2.0 mg (3.6×10^{-3} mmol) of ligand Model A in 300 μL degassed $CDCl_3$ and 300 μL degassed CD_3CN was added a solution of 0.5 equivalent of $Cu^{I}(CH_3CN)_4BF_4$ salt in 100 μL degassed CD_3CN and 100 μL degassed $CDCl_3$ (Scheme 4.5). ^{1}H NMR (400MHz) indicated that binding occurred, as the bipyridine conformation changed (the protons on the 3 and 3' positions moved from δ 8.64 ppm to δ 8.54 ppm, Fig.4.1 & 4.2). Coordination was also confirmed by a change in colour from pale yellow to light orange. A white solid precipitated after the colour changed. The binding ratio Cu^{+} / Ligand 1:1 of the final mixture was confirmed by ESI^{+}-MS. This 1:1 binding ratio, even at low copper loading, is reasonable: the two pyrene units which are directly linked to the bipyridine core are bulky substituents and would block the access of a second bipyridine ligand. Sauvage et al reported in 1984 the study of Cu^{+} coordination by

6,6'-bisphenyl-substituted bipyridines and phenanthrolines;[5] later on, Ward et al reported the crystal structures of such species.[6] Briefly, when the 6 and 6'substituents on bipyridine (or the corresponding phenanthroline) are phenyl groups, copper (I) does still bind (no steric hindrance for copper ions) and the 2:1 complex may still form. In the crystal structures,[6] the phenyl ring of one ligand covers one pyridine of the other ligand through interactions.[5] All phenyl substituents provide some steric bulk around the complex, resulting in efficient shielding of the copper ion, and favoring long lived charge transfer excited states.[5] Sauvage also reported that a 1:1 complex forms when the phenyl substituents on a phenanthroline ligand are "strapped" into a macrocycle, preventing further coordination of an additional phenanthroline.[6] Those 1:1 complexes may therefore form, although they are not as stable as the 2:1 complexes. In our particular case, pyrene substituents are much bulkier than phenyl rings, so it is not surprising not to detect any 2:1 complexes (absent by ESI-MS).

The observation that only the 1:1 complex is formed is promising for DNA studies, as the $Cu(bpy)_2$ complex would not allow pyrene intercalation anymore. In addition, the two free metal coordination sites (probably filled by CD_3CN in this experiment) may be useful for additional DNA base coordination to the copper center.

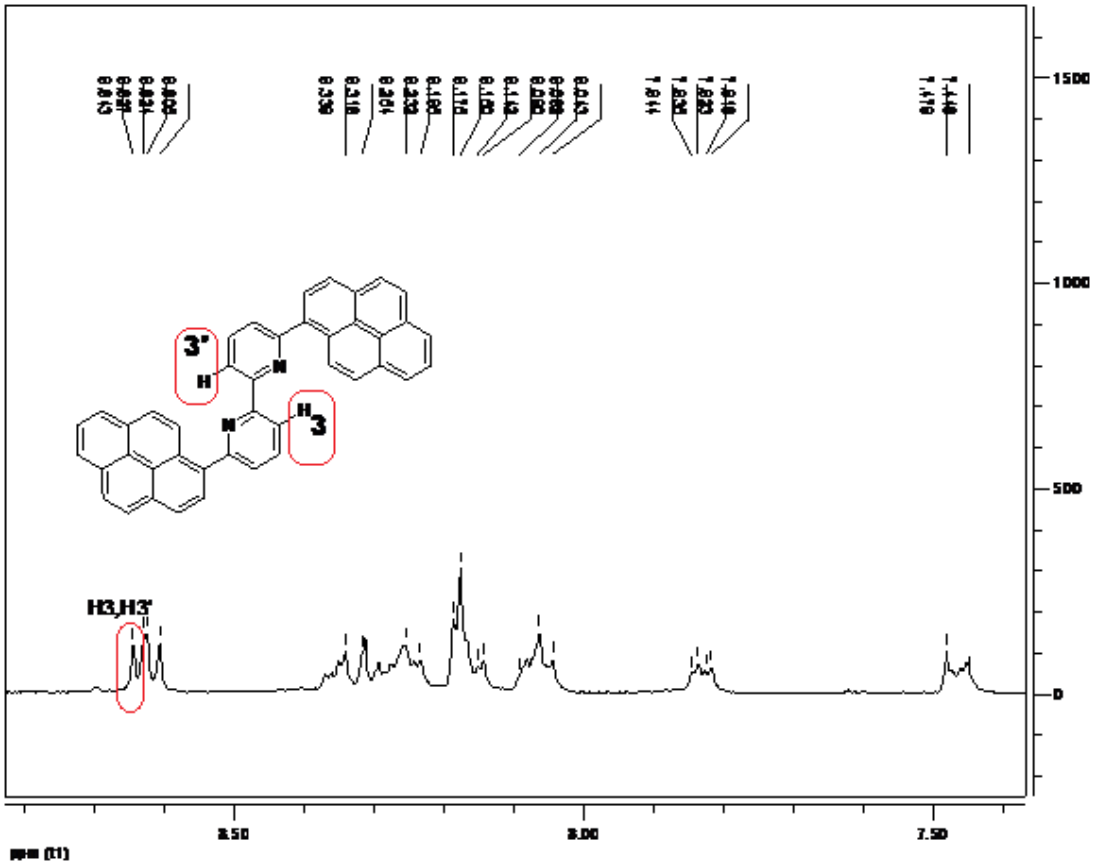


Figure 4.1 ^{1}H NMR of Model A before adding Cu^{+} in $CDCl_3$ and CD_3CN

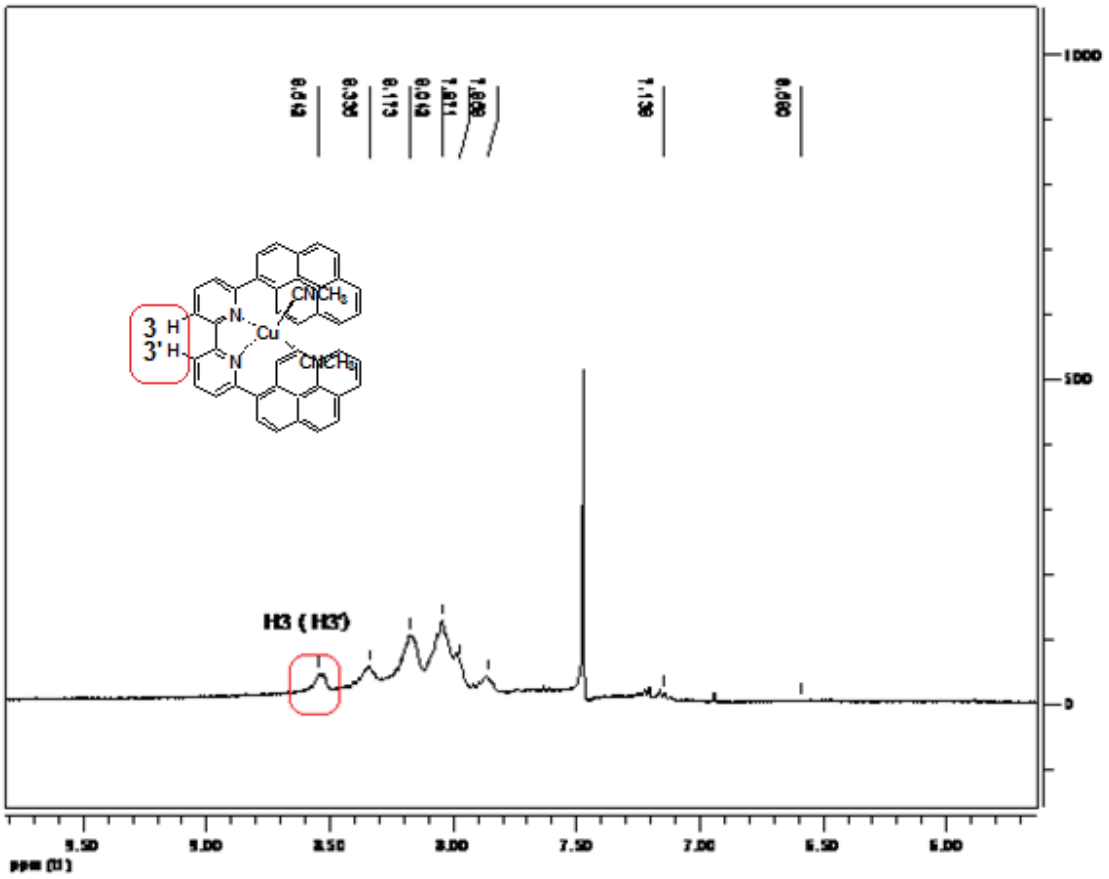
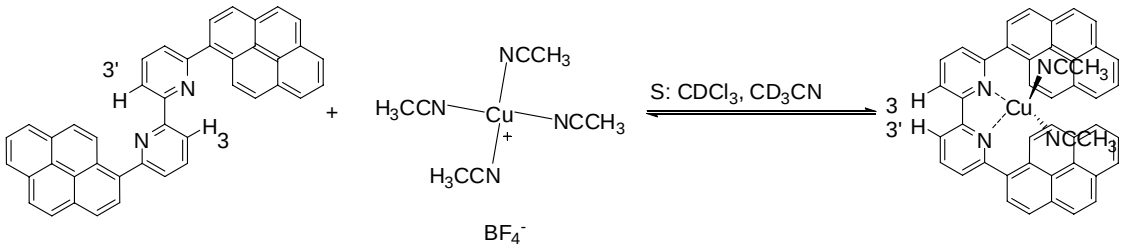
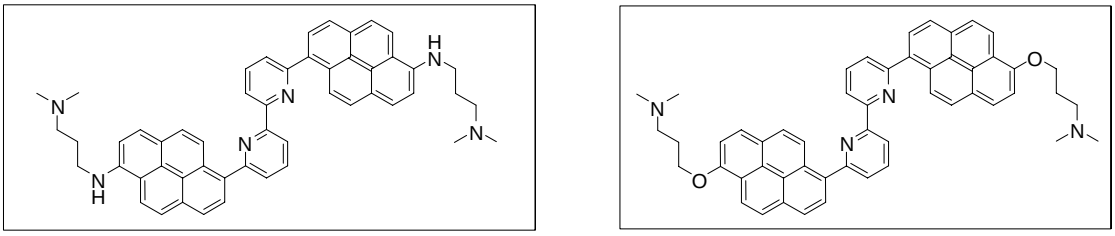


Figure 4.2 ^{1}H NMR of Model A binding with Cu^{+} in $CDCl_3$ and CD_3CN



Scheme 4.5 Cu^{+} coordinating with model A

4.2 Synthesis of original Target A



Target A with original diamino side chain

Target A with revised ether side chain

Figure 4.3 Chemical structures of target A and revised target A'

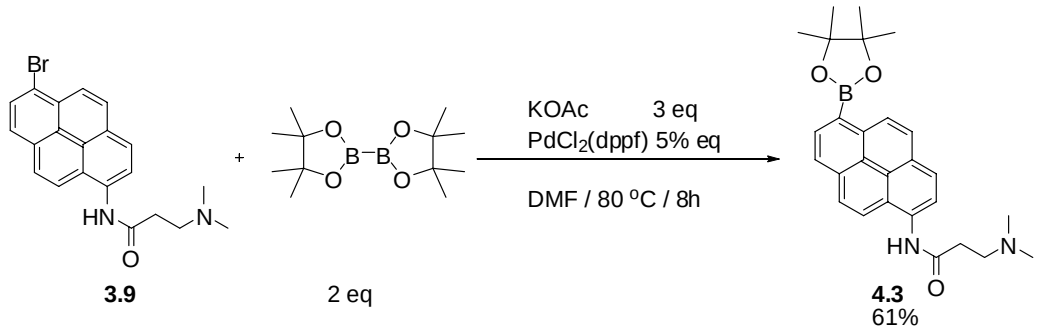
The chemical structures of the original target A with diamino side chain and revised target A with ether side chain are shown in Fig.4.3. At the beginning, the original target A was the focus of the synthesis.

As the retrosynthesis route to target A indicates (chapter 1.3), the bromide function of Pre-pyrene unit A (see also chapter 3) first needed to be transformed into a boronic pinacol ester, which was to cross-couple with 6,6'-dibromo-2,2'-bipyridine. Further amide reduction should give access to the original target A.

4.2.1 Borylation of pre pyrene unit A: N- (6-bromopyren-1-yl)-3 (dimethylamino)propanamide

Borylation of pre-pyrene unit A- N-(6-bromopyren-1-yl)-3 (dimethylamino)propanamide also employed the palladium catalyzed method developed for 1-bromopyrene (Scheme 4.6).[3] Similarly, a mixture of N-(6-bromopyren-1-yl)-3 (dimethylamino)propanamide, two equivalents of bis(pinacolato)diboron (B_2Pin_2), three equivalents of potassium acetate, 5 % equiv. of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane was heated at 80 °C in DMF under argon for 8 h. The reacted mixture was washed with water and the

aqueous layer was extracted with ethyl acetate. The crude was purified via flash chromatography on silica gel and yielded 61 % the desired product- 3-(dimethylamino)-N-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1 yl)propanamide.

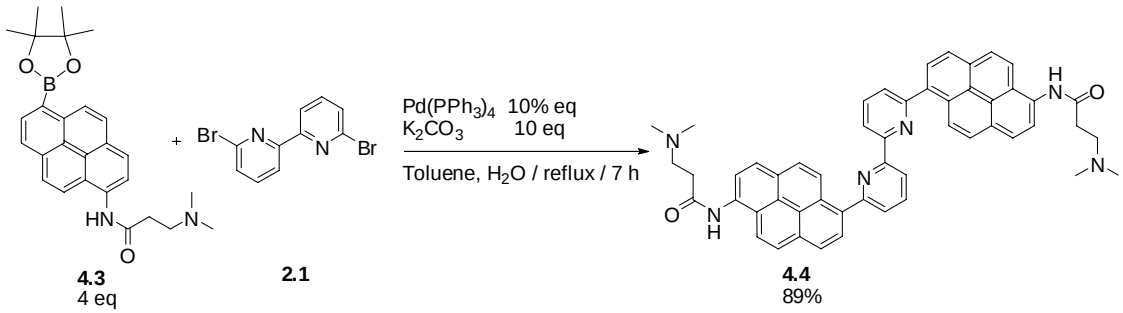


Scheme 4. 6 Synthesis of 3-(dimethylamino)-N-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)propanamide (**4.3**)

4.2.2 Synthesis of N,N'-(6,6'-(2,2'-bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide (4.4)

The above borylation product was then cross-coupled with 6,6'-dibromo-2,2'-bipyridine using typical Suzuki coupling conditions. The reaction was carried out in the similar procedure as the synthesis of model A (see above, Scheme 4.7).[4] A mixture of four equivalents of 3-(dimethylamino)-N-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)propanamide, 1 equivalent of 6,6'-dibromobipyridine, ten equivalents of potassium carbonate, and 10 % equivalents of tetrakis(triphenylphosphine)palladium(0) was refluxed in a toluene / water mixture under argon for 7 h. During the first hour, a large amount of a greenish solid was observed in the reflux condenser, and was unable to reflux back into the reaction flask. The reaction was left to reflux another 6 h. The resulted mixture was then taken up in dichloromethane and filtered. The precipitate was then confirmed to be desired product. The filtrate was washed with water and extracted with dichloromethane. Both precipitate and filtrate had to be purified to remove any

palladium and other unwanted matter. The purification wouldn't be carried out on silica gel because of the high polarity and many nitrogen basic sites that streak on silica. The purification was done via chromatography on aluminum oxide. It gave a final yield of 89 % of desired coupling product.

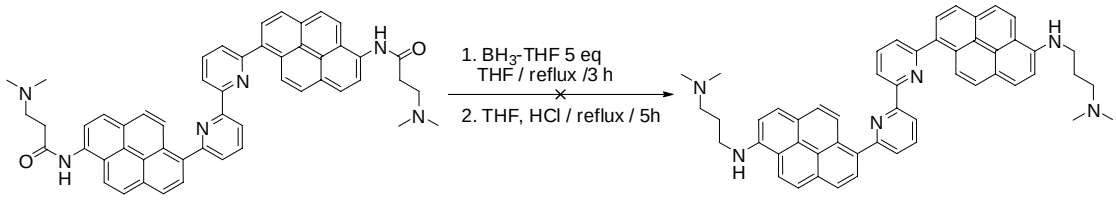


Scheme 4. 7 Synthesis of N,N'-(6,6'-(2,2'-bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide (**4.4**)

4.2.3 Reduction of N,N'-(6,6'-(2,2'-bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide

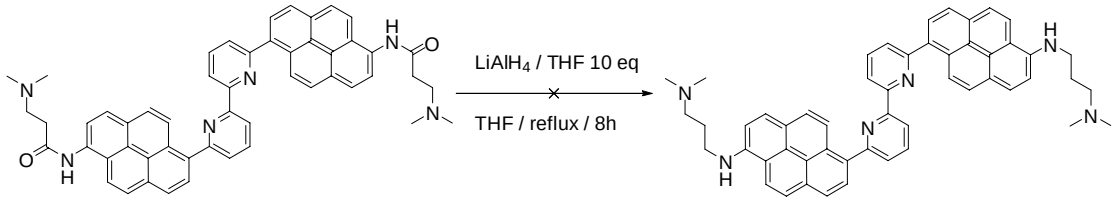
Initially, the borane tetrahydrofuran complex, tested on 1-aminopyrene (see Chapter3), was employed to reduce the previous coupling product. The solution of one equivalent of N,N'-(6,6'-(2,2'-bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide and 5 equivalents of borane-THF adduct was refluxed in THF for 3h under argon (Scheme 4.8).[4] The reaction was then quenched by methanol and worked up by dichloromethane. The crude ^{1}H NMR was messy and multiple spots were observed on TLC. It was then considered that the boron adduct may still be present. The crude was taken up THF and 2 M HCl, and refluxed for 5 h. The resulted crude still showed multiple spots on TLC. Flash chromatography on silica gel was

attempted to purify the crude but was not successful in isolating nor identifying the desired product.



Scheme 4.8 Attempted reduction using borane tetrahydrofuran complex

Next, lithium aluminum hydride was attempted to reduce the amide bond. A solution of amide product and ten equivalents of lithium aluminum hydride was refluxed in THF for 8 h under argon (Scheme 4.9).[7] ^{1}H NMR of the resulted crude did not clearly indicate the presence of the desired product. Flash chromatography on silica gel was still attempted to purify the crude, but failed. None of the fractions was identified as the desired product.



Scheme 4.9 Attempted reduction using lithium aluminum hydride

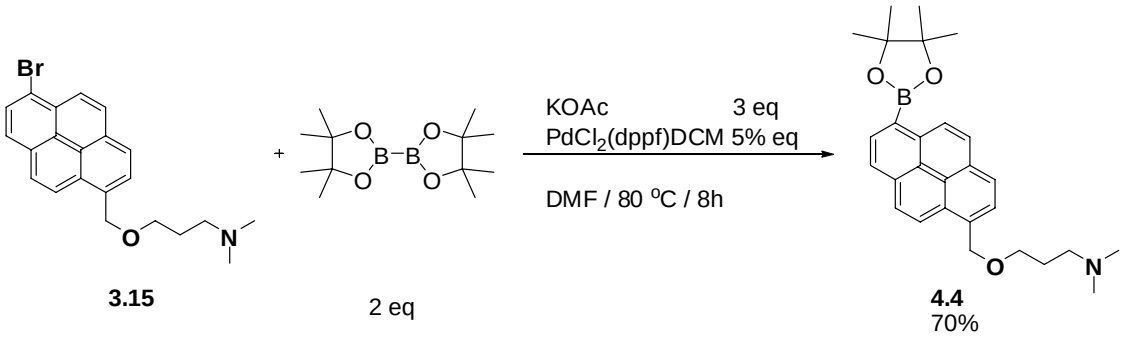
The reasons for the failed reduction of N,N'-(6,6'-(2,2'-bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide may be the possibility of reducing the bipyridine itself in the presence of above reducing reagents.[8] Also, two free diamino side chain and bipyridine may bind to the reducing reagent and/or by-products. It has also been observed that the crude was not very stable in air – the colour of the crudes from the above two trials would slowly turn from yellow to dark brown with time. This observation was not surprising since a similar phenomenon has been observed on N_1,N_1-dimethyl-N_3-(pyren-1-yl) propane-1,3-diamine (see

also section 3.2.7). Due to the above reasons, it was concluded that the originally d esigned target A may not be a proper candidate for DNA binding. Thus, the synthesis work was focused on a revised target A with ether side chains.

4.3 Synthesis towards revised target A

4.3.1 Borylation of pyrene unit A': 3-((6-bromopyren-1-yl)methoxy)-N, N-dimethylpropan-1-amine

This step followed the same palladium catalyzed borylation procedure as mentioned before (Scheme 4.10).[3] A mixture of 3-((6-bromopyren-1-yl)methoxy)-N,N-dimethylpropan-1-amine, two equivalents of bis(pinacolato)diboron (B_2Pin_2), three equivalents of potassium acetate, 5 % equivalents of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane was heated at 80 °C in DMF under argon for 8 h. The reacted mixture was washed with water and the aqueous layer was extracted with ethyl acetate. The resulted crude was purified through flash chromatography on silica gel. It gave 70 % of the desired product-N,N-dimethyl-3-((6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)methoxy)propan-1-amine.



Scheme 4. 10 Synthesis of N, N-dimethyl-3-((6-(4, 4, 5, 5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)methoxy)propan-1-amine (**4.4**)

4.3.2 Synthesis of revised target A

This step has not been performed yet.

4.4 Conclusion of chapter 4

In this chapter, we reported the successful borylation of pre-pyrene unit A and the following Suzuki cross coupling. The reduction of this coupling product was attempted using borane tetrahydrofuran complex or lithium aluminum hydride but failed. The reasons are considered to be undesired reduction of bipyridine as well as the multiple nitrogen binding sites. The crude was also observed to be unstable in air. Thus designed original target A is not proper to study DNA binding.

We also reported successful borylation of revised pyrene unit A'. In the future, the final cross-coupling will be performed.

Although the actual final target has not yet been completed, tests run on a model bipyridine-pyrene conjugate show promising results with regards to Cu^{+} binding. NMR and ESI-MS indicate the formation of the 1:1 complex of interest. Further investigation with the model system are considered, such as UV-vis and fluorescence titrations (both requiring much lower concentrations than NMR) on the Cu^{+} and Ag^{+} salts. Such spectroscopic studies should give some insight into the binding constants and stoechiometries. However, only the water-soluble actual system will give true indications on the behaviour of this system in solutions relevant to DNA binding.

Once large amount of 6,6'-diformyl-2, 2'-bipyridine are obtained (see Chapter 2), the assembly of target B and its model will be studied.

References

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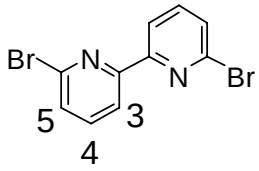
Chapter 5
Experimental Section

5.1 General methods

Commercially available compounds were purchased from Aldrich and Alfa Aesar and were used as received. Anhydrous diethylether, dichloromethane, THF, toluene were dried by passing through an activated alumina column according to the manufacturer's instructions (Innovative Technology). Anhydrous DMF was purchased from EMD (DriSolv) and was used as received. All reactions were performed under Argon (Ar) unless stated otherwise. Deuterated solvents (Cambridge Isotopes) were used as received.^{1}H NMR and ^{13}C NMR was performed using a 300 MHz and 400 MHz Bruker instruments. Peak listings for all NMR spectra are given in ppm and referenced against the solvent residual signal. Thin layer chromatography (TLC) analysis was performed on *Fluka silica gel* with a pore diameter of 60 Angstroms or activated basic Aluminium oxide with a pore diameter of 58 Angstroms. Column chromatography was performed with *Silia-P flash silica gel* with a particle size of 40-63 μm and a pore diameter of 60 Angstroms. Melting points were recorded on a MEL-TEMP (laboratory Device.USA) apparatus. Mass spectrometry was performed on a Waters/Micromass GC-TOF EI-MS or ESI –MS spectrometer. Elemental analyses were conducted at the Elemental Analysis service at the Université de Montréal.

5.2 Experimental details

6,6'-Dibromo-2, 2'-bipyridine (2.1)

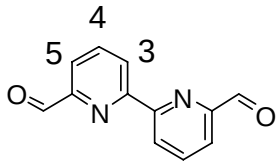


Method A: Method A is slightly modified from literature protocols.[1, 2] To a mixture of 4.0 g (0.017 mol) of commercially available 2,6-dibromopyridine in 80 mL dry diethylether at -78 ºC, were slowly added 6.80 mL (0.017 mol) of 2.5 M *n*-BuLi / hexane. The suspension was stirred at this temperature for 0.5 h.The mixture was stirred another 2 h at -40 ºC, giving a dark green suspension. 0.40 mL (4.25 mmol) $POCl_3$ in 14 mL dry diethylether was added slowly at -40 ºC. After stirring another 2 h at this temperature, the orange mixture was slowly brought to room temperature and quenched with 20 mL water. The aqueous layer was extracted by diethylether. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. The crude was purified by careful flash chromatography on silica gel with a 1:0.7 hexane / CH_2Cl_2 mixture as an eluent, yielding 540 mg (1.72 mmol, 20 %) of a white solid identified as 6,6'-dibromobipyridine.

Method B: Method B follows a procedure developed by Weber *et al*[3] and Parks, J. E. *et al*[4].To a mixture of 8.12 g (33.7 mmol) of 2,6-dibromopyridine in 80 mL dry diethylether at -78 ºC**,** 16.4 mL (41.1 mmol) of 2.5 M *n*-BuLi / hexane was added slowly and stirred at this temperature for 2 h. 2.27 g (16.9 mmol) dry $CuCl_2$ was added to the dark green suspension at the same temperature and gave a brown suspension. The reaction mixture was brought slowly to -50 ºC and stirred for another 0.5 h. Then dry oxygen was bubbled at -50 ºC for 1 h and precipitate formed in the green suspension. Then the reaction was brought slowly to room temperature and quenched with 40 mL of 6 M aq. HCl. The mixture was filtered and washed with 0.5 M aq. HCl. The green precipitate was washed with EDTA solution (pH = 9) and gave 1.60 g (5.10 mmol) of a white solid identified as 6,6'-dibromobipyridine in a yield of 30 %.

Rf = 0.39 (Hexane : CH_2Cl_2 = 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 8.38 (d, ^3J = 7.8 Hz, 2H, H_3), 7.67(t, ^3J = 7.8 Hz, 2 H, H_4), 7.51 (d, ^3J = 8.4 Hz, 2H, H_5).

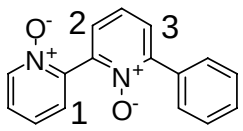
6,6'-Diformyl-2,2'-bipyridine (2.2)



This procedure follows the same protocol reported by Ilyashenko *et al*.[5] To a solution of 1.47 mL (2.10 mmol) 1.43 M of *n*-BuLi in 14 mL dry THF at -78 ºC, was added a hot solution of 200 mg (0.637 mmol) of 6,6'-dibromo-2,2'-bipyridine (10 mL dry THF) in one shot. The dark red solution was then stirred for extra 45 min at -78 ºC. Then 0.2 mL (2.5 mmol) of dry DMF was added. The reaction was warmed up to -30 °C and quenched by 4 mL of 4 M aq. HCl. After warming up to room temperature, the aqueous layer was separated from THF. It was then basified to pH = 14 by sat. KOH solution and was extracted with warm chloroform. The combined THF and chloroform organic layers were dry with Na_2SO_4, filtered and evaporated in *vacuo*. The crude was then washed by MeOH and recrystallized from toluene, giving 18.2 mg (0.085 mmol) of a white solid with a yield of 13 %. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): δ.10.19 (s, 2H, *CHO*), 8.83 (dd, ^3J = 6.9 Hz, ^4J = 2.1 Hz, 2H, H_5), 8.06 (m, 4H, H_3, H_4).

For the Shawna's synthesis (Chapter 2), please see her Honour's project report (2009).

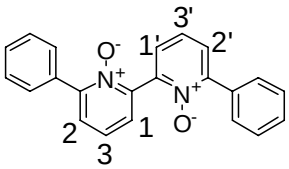
6-Phenyl-2,2'-bipyridine-N,N'-dioxide (2.3)



This procedure is modified from general palladium N-oxide coupling protocols reported by Fagnou *et al*.[6] 179.0 mg of potassium carbonate (1.30 mmol), 40.3 mg (0.143 mmol) of tri-tert-butylphosphine tetrafluoroborate ($P(^tBu)_3 – HBF_4$, 0.143 mmol), 10.3 mg of palladium acetate (0.048 mmol), 80.0 mg of copper (I) cyanide (0.124 mmol), and 66.7 mg of N,N-dioxide-2,2'-bipyridine (0.354 mmol) were weighed in air and placed in a round bottom flask with a magnetic stir bar. The flask was evacuated and backfilled with argon (x 3). Bromobenzene (0.187 mL, 1.77 mmol) and 2 mL DMF (~0.1M) were then

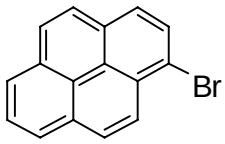
added via a syringe. The mixture was then heated to 120 ºC for 20 h.The reaction mixture was diluted with acetone and stirred overnight in order to precipitate unreacted N, N-dioxide-2, 2'-bipyridine. The crude was then filtered through celite, the filtrate evaporated under reduced pressure and purified by flash chromatography on silica gel using a 20:1 CH_2Cl_2 / MeOH mixture as an eluent. If necessary, the oily fractions are treated with Et_2O to yield 17.9 mg (0.068 mmol, 19 %) white-yellow solids. Rf= 0.18 (CH_2Cl_2 : MeOH = 20:1). ^{1}H NMR (300MHz, $CDCl_3$, 25 ºC): δ 7.32-7.65 (m, 12H, Ar-H), 7.85 (m, 2H, H_2, H_3), 8.34 (d, ^3J= 6.0 Hz, 1H, H_1). ESI^+-MS: calculated for $C_{16}H_{13}N_2O_2$ (MH^+) 265.10; found: 264.84.

6,6'-Diphenyl-2,2'-bipyridine N, N'-dioxide (2.4)



6, 6'-Diphenyl-2,2'-bipyridine N,N'-dioxide was synthesized by the same procedure as **2.3**[6] and purified by flash chromatography on silica gel using a 20:1 CH_2Cl_2 / MeOH mixture as an eluent. It yielded 44 mg (0.13 mmol, 36 %) of white-yellow oily solid **2.9**. Rf = 0.38 (CH_2Cl_2 : MeOH = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): δ 7.26-7.55 (m, 10H, Ar-H), 7.63 (d, ^3J = 8.7 Hz, 2H, H_3), 7.85 (d, ^3J = 5.0 Hz, 4H, H_1, H_2). ESI^+-MS: calculated for $C_{22}H_{17}N_2O_2$ (MH^+) 341.13; found: 340.86.

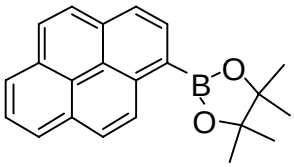
1-Bromopyrene (3.1)



3.1 was synthesized following slightly modified procedure from Vyas and Bedekar *et al*.[7] Under air condition, 0.28 mL (2.52 mmol) hydrobromic acid (HBr) aqueous solution was added to a pale yellow solution of 0.51 g (2.52 mmol) pyrene in 11 mL methanol and 11 mL diethylether. Hydrogen peroxide (0.27 mL, 2.60 mmol) was added at 10 ºC. After stirring at room temperature for 16 h, the reaction mixture was concentrated. The crude was taken up in ethyl acetate and washed with 15 mL water and 5 mL brine. The aqueous layer was extracted with ethyl acetate. The combined organic layers were

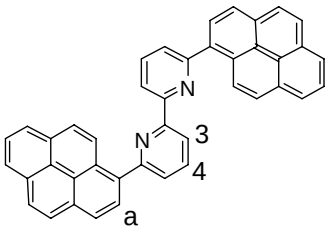
dried on sodium sulphate and concentrated under reduced pressure. It gave 0.617 g (2.19 mmol, 87 %) pale yellow solid which was used in the next step without further purification. Rf = 0.5 (Hexane). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): δ 8.44 (d, ^{3}J = 9.3 Hz, 1H), 8.17-8.26 (m, 4H), 7.99-8.12 (m, 4H).

4, 4, 5, 5-Tetramethyl-2-(pyren-1-yl)-1, 3, 2 dioxaborolane (4.1)



This method was modified from general coupling procedure created by Ishiyama and Miyaura *et al*.[8] Under the atmosphere of argon, 1-bromopyrene (564 mg, 2.00 mmol), bis(pinacolato)diboron (762 mg, 3.00 mmol), potassium acetate (589 mg, 6.00 mmol) and bis(diphenylphosphino)ferrocene]-dichloropalladium(II) complex with dichloromethane (73.2 mg, 0.10 mmol) were mixed in 13 mL dry degassed DMF. After stirring at 80 ºC for 8 h, the reaction was stopped (complete disappearance of starting material by TLC). The mixture was cooled to room temperature and quenched with 20 mL water. The aqueous layer was extracted by diethylether. The combination of organic layers was dried on sodium sulphate and concentrated under reduced pressure. Purification by careful flash column chromatography on silica gel using a 2:0.25 Hexane / EtOAc mixture as eluent gave 0.41 g (1.24 mmol, 62 %) light yellow solid of 4,4,5,5-tetramethyl-2-(pyren-1-yl)-1,3,2-dioxaborolane. Rf = 0.47 (Hexane: EtOAc = 2:0.25). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 9.16 (d, ^{3}J = 9 Hz, 1H), 8.63 (d, ^{3}J = 7.5 Hz, 1H), 8.01-8.27 (m, 7H).

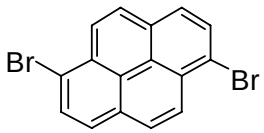
6,6'-Dipyrene-2,2'-bipyridine (4.2)



This procedure was modified from reported protocol by Beinhoff and Schluter *et al*.[9] A mixture of **4.1** (260 mg, 0.92 mmol), **2.1** (116 mg, 0.37 mmol), potassium carbonate (511 mg, 3.70 mmol), tetrakis(triphenylphosphine)palladium (0) (11.4 mg, 0.037 mmol) was refluxed under argon in 3.7 mL toluene and 3 mL H_2O for 15 h. The reaction mixture was

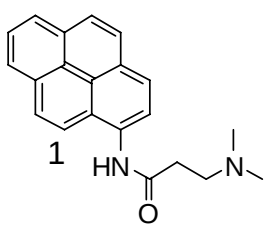
concentrated, taken up in CH_2Cl_2 and washed with water and brine. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. The crude was taken up in CH_2Cl_2 and filtered. The precipitate was later confirmed as being the product. The filtrate was purified from flash column chromatography on silica gel using a 1.5:1 Hexane / CH_2Cl_2 1.5:1 mixture as eluent and then recrystallized in chloroform, giving a total of 103 mg (0.19 mmol, 51 %) solid of compound **4.2**. ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ 8.72 (d, ^3J = 7.5 Hz, 2H, H_3), 8.64 (d, ^3J = 9.3 Hz, 2H, H_a), 8.38-7.99 (m, 18H, *Py-H, Bpy-H*), 7.80 (d, ^3J = 7.2 Hz, 2H, H_4); ^{13}C NMR is not possible due to poor solubility; ESI$^+$-MS: calculated for $C_{42}H_{25}N_2$ (MH^+) 557.20; Found: 557.17; EA: $C_{42}H_{24}N_2$. 1.06 $CHCl_3$, calculated: %C 75.65, %H 3.69, %N 4.10, found: %C 75.69, %H 3.40, %N 4.52; mp: 279.5-281.0 ºC (decomposed).

1,6-Dibromopyrene (3.2)



3.2 Dibromide was synthesized from protocol created by Grimshaw et al.[10] To a pale yellow solution of 10.0 g (49.4 mmol) pyrene in 250 mL CH_2Cl_2, was added dropwise 5 mL bromine / 250 mL CH_2Cl_2 under air condition in 5 h. The mixture was allowed to stir at room temperature for 16 h. The pale green suspension was then filtered. The precipitate was purified by fractional recrystallization in toluene and gave 1.70 g (4.74 mmol, 10 %) solid of 1,6-dibromopyrene. Rf = 0.63 (Hexane); ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 8.46 (d, ^3J = 9.3 Hz, 2H), 8.27 (d, ^3J = 8.1 Hz, 2H), 8.11 (d, J = 9.3 Hz, 2H), 8.05 (d, J = 8.1 Hz, 2H).

3-(Dimethylamino)-N-(pyren-1-yl)propanamide (3.3)

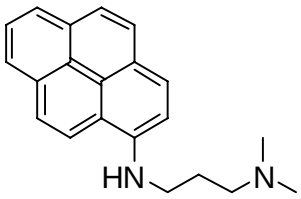


This step followed the reported procedure by Kim and Chang.[11] To a solution of 50.2 mg (0.23 mmol) of 1-aminopyrene in 3.20 mL anhydrous CH_2Cl_2, was added a mixture of 0.03 mL (0.23 mmol) Et_3N

with 1.40 mL CH_2Cl_2. 0.40 mL (0.35 mmol) of 3-chloropropionyl chloride was added to the reaction mixture and a white precipitate was formed. The reaction was left to stir overnight at room temperature. The mixture was then diluted with CH_2Cl_2 and washed with water. The aqueous layer was then extracted with CH_2Cl_2. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. The crude was recrystallized in methanol and the precipitate gave 69.6 mg (0.22 mmol) white solid in a yield of 96 %.

This step was modified from protocols created by Kostakis, Kletsas *et al* and Philips, Sullivan *et al*.[12, 13] A mixture of 56.2 mg (0.18 mmol) of 3-chloro-N-(pyren-1-yl) propanamide, 0.092 mL (0.73 mmol) of 40 % dimethylamine in water in 9 mL EtOH was refluxed for 9 h. Removing the volatiles in *vacuo* gave 67.0 mg (0.18 mmol) light yellow solid of compound **3.3**. The total yield of two steps is 78 %. The crude is ready to use for next step. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 11.75 (s, 1H, N*H*), 8.81 (d, ^3J = 8.4 Hz, 1H, *H*$_1$), 8.18-7.96 (m, 8H, *Py-H*), 2.86 (t, ^3J = 5.1 Hz, 2H, $COCH_2CH_2N(CH_3)_2$), 2.77 (t, ^3J = 6 Hz, 2H, $COCH_2CH_2N(CH_3)_2$), 2.57 (s, 6H, $COCH_2CH_2N(CH_3)_2$).

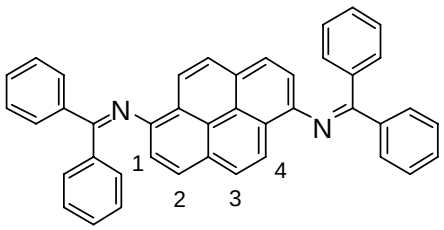
N_1,N_1-Dimethyl-N_3-(pyren-1-yl)propane-1,3-diamine (3.4)



This reaction followed procedure reported by Park and Roundhill *et al*.[14] To a solution of 76.0 mg (0.24 mmol) of **3.3** in 5 mL dry THF, was added a solution of 1 M borane tetrahydrofuran complex solution (0.48 mL, 0.48 mmol). The reaction was refluxed for 3 h (TLC showed disappearance of starting material). MeOH (5 mL) was added to the cool solution and the solvent was evaporated in *vacuo*. The crude was taken up in CH_2Cl_2 and washed with 1 M K_2CO_3 solution. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel with gradient elution CH_2Cl_2 to CH_2Cl_2 / MeOH 10:1 to CH_2Cl_2 / MeOH 5:1. It gave three fractions: F1 (Rf =

0.69 in CH_2Cl_2 / MeOH = 5:1), F_2, (Rf = 0.63 in CH_2Cl_2 / MeOH = 5:1), F_3 (Rf = 0.19 in CH_2Cl_2 / MeOH = 5:1). F_1 and F_2 were taken up in 5 mL of pre-degassed THF and 7 mL of pre-degassed 2 M aq. HCl solution and refluxed for 6 h. The cool mixture was washed by 10 % aq. NaOH solution and extracted with CH_2Cl_2. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. It gave 62.6 mg of fraction wash. Combining with F_3, the total yield is in 92 % with 67.9 mg (0.22 mmol) oily product of light-air sensitive yellow oil **3.4**. Rf = 0.19 (CH_2Cl_2 : MeOH = 5:1); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 8.04-7.85 (m, 7H, *Py-H*), 7.71 (d, ^3J = 9 Hz, 1H, *Py-H*), 7.27 (d, ^3J = 6.9Hz, 1H, *Py-H*), 3.54 (t, ^3J = 6 Hz, 2H, NH*C*$H_2$$CH_2CH_2N(CH_3)_2$), 2.18 (t, ^3J = 6 Hz, 2H, $NHCH_2CH_2$*C*$H_2$$N(CH_3)_2$), 2.39 (s, 6H, $NHCH_2CH_2CH_2N$*(C*H_3*)*$_2$), 2.01 (q, ^3J = 6 Hz, 2H, $NHCH_2$*C*$H_2$$CH_2N(CH_3)_2$); ESI$^+$-MS: calculated for $C_{21}H_{23}N_2$ (MH^+) 303.19; found: 302.875.

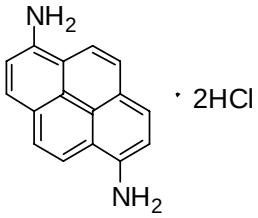
N_1,N_6-Bis (diphenylmethylene) pyrene-1,6-diamine (3.5)



This reaction was carried out by modifying reported procedure from Charaborti and Basu *et al.*[15] Under Argon atmosphere, 0.61 mL (3.66 mmol) of benzophenone imine was added to a mixture of 1.20 g (3.33 mmol) of **3.2**, 37.4 mg (0.17 mmol) of palladium acetate, 155.4 mg (0.25 mmol) of (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (BINAP), and 1.52 g (4.66 mmol) of cesium carbonate in 40 mL pre-dry degassed THF. The reaction was then refluxed under argon for 13 h. After cooling to room temperature, THF was removed under reduced pressure. The residue was taken up in 50 mL CH_2Cl_2 and unreacted 1,6-dibromopyrene was filtered off. The filtrate was washed with water and brine, and the aqueous layers were extracted with 5 × 20 mL CH_2Cl_2. The combined organic layers were dried on sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel eluted with gradient elution CH_2Cl_2

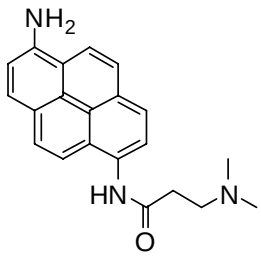
/ Hexane 1:1 to 3:1 and it gave 565 mg (1.01 mmol) orange solid of **3.5** in a yield of 30 %. Rf = 0.15 (CH_2Cl_2 : Hexane = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 8.14 (d, ^3J = 9.3 Hz, 2H, *H*$_1$), 7.95 (d, ^3J = 6.9 Hz, 4H, *H*$_4$, *Ph-H*), 7.86 (d, ^3J = 9.3 Hz, 2H, *H*$_3$), 7.79 (d, ^3J = 8.1 Hz, 2H, *H*$_2$), 7.57-7.56 (m, 6H, *Ph-H*), 7.15-7.12 (m, 10H, *Ph-H*), 7.03 (d, ^3J = 8.1 Hz, 2H, *Ph-H*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 168.83, 145.54, 139.57, 136.40, 130.84, 129.56, 129.08, 128.61, 128.27, 127.92, 126.73, 125.51, 124.43, 123.36, 121.60, 117.52; EI$^+$-MS: calculated for $C_{42}H_{28}N_2$: 560.23; found: 560.22 $[M]^+$, 483.19 $[M\text{-}C_6H_5]^+$; EA: $C_{42}H_{28}N_2$. 0.19 CH_2Cl_2, calculated: %C 87.87, %H 4.96, %N 4.86, found: %C 87.87, %H 4.95, %N 4.72; mp: 293.1-294.0 °C.

Hydrochloride salt of pyrene-1,6-diamine (3.6)



This hydrolysis procedure followed the method created by Purohit and Basu.[16] To a mixture of 714 mg (1.27 mmol) of **3.5** in 33 mL THF, 1.7 mL (5 % v/v) 2 M aq. HCl solution was added. The reaction was protected under argon and was stirred for 2 h at room temperature. The formed precipitate was filtered and washed by 3 × 5mL THF. It gave a 370 mg (1.23 mmol) pink solid of **3.6** in a yield of 97 %. The product was kept under argon and used without further purification.

N-(6-Aminopyren-1-yl)-3-(dimethylamino) propanamide (3.7)



This step followed the reported procedure by Kim and Chang.[11] To a mixture of 281 mg (0.92 mmol) of compound **3.6** in 25 mL pre-degassed dry dichloromethane under argon, 2.6 mL (18.4 mmol) of triethylamine (pre-stir with K_2CO_3) was added. Then a solution of 0.12 mL (1.29 mmol) of 3-chloropropionyl chloride in another 20 mL pre-degassed dry dichloromethane was

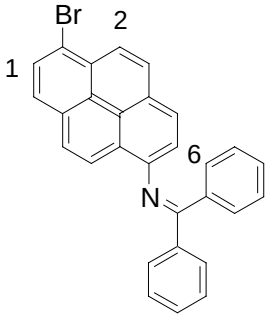
slowly added over 2 h. The reaction was left to stir overnight at room temperature. Then the solvent was removed under reduced pressure and the light air sensitive yellow crude was kept under argon without further purification.

This step was modified from protocols created by Kostakis, Kletsas *et al* and Philips, Sullivan *et al*.[12,13] To a solution of previous crude in 57.5 mL degassed ethanol, was added 0.47 mL (3.68 mmol) of 40 % dimethylamine in water. The reaction was refluxed under argon for 5 h and was cooled to room temperature. The formed precipitate was filtered which was later confirmed as the bis-amide byproduct. The filtrate was concentrated in *vacuo*. The crude was taken up in 30 mL CH_2Cl_2, washed with 1 M K_2CO_3 solution and extracted by 5 × 20 mL CH_2Cl_2. The combined organic layers were dried on sodium sulphate, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel eluted with gradient elution CH_2Cl_2 / MeOH 10:1 to 5:1 to CH_2Cl_2 / MeOH / NH_4OH = 30:8:1. It gave 40 mg (0.12 mmol) yellow solid of light-air sensitive compound **3.7**.The total yield of two steps is 13 %. Rf = 0.58 (CH_2Cl_2 : MeOH : NH_4OH = 8:4:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 11.64 (s, 1H, N*H*), 8.71(d, ^3J = 8.4 Hz, 1H, *Py-H*), 8.03 (d, ^3J = 8.4 Hz, 1H, *Py-H*), 7.95- 7.80 (m, 5H, *Py-H*), 7.36 (d, ^3J = 8.1 Hz, 1H, *Py-H*), 2.85 (t, ^3J = 5.1 Hz, 2H, CO$CH_2$$CH_2N(CH_3)_2$), 2.7 (t, ^3J = 6.3 Hz, 2H, COCH_2CH_2N$(CH_3)_2$), 2.54 (s, 6H, COCH_2CH_2N$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 171.044 (NH_2CO), 141.04, 131.24, 127.70, 126.10, 125.66, 123.96, 120.59, 118.86, 117.00, 116.45, 114.56, 114.11, 98.49, 97.10, 55.14 (CO$CH_2$$CH_2N(CH_3)_2$), 44.52 (CO$CH_2CH_2N(CH_3)_2$), 33.73 (CO$CH_2CH_2N(CH_3)_2$); ESI$^+$-MS: calculated for $C_{21}H_{22}N_3O$ (MH^+) 332.176; found: 331.830.

6-Bromo-N-(diphenylmethylene)pyren-1-amine (3.8)

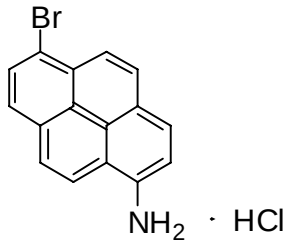
Compound **3.8** was synthesized in the same procedure as compound **3.5.**[15] It was purified by flash chromatography on silica gel eluted with gradient elution- a mixture of CH_2Cl_2 / Hexane 1:1 to

3:1 and gave 630 mg (1.37 mmol) orange solid of **3.8** in a yield of 41 %. Rf = 0.58 (CH_2Cl_2 : Hexane = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 8.30 (m, 2H, *H_1, H_2),* 8.19 (d, ^3J = 8.1 Hz,



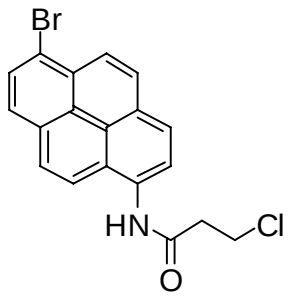
1H, *H_6*), 8.02-7.89 (m, 6H, *Py-H*), 7.57-7.48 (m, 3H, *Ph-H*), 7.16-7.10 (m, 6H, *Ph-H*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 169.30, 146.70, 139.25, 136.16, 131.01, 130.10, 129.61, 128.97, 128.74, 128.33, 127.91, 127.21, 126.38, 126.23, 125.59, 124.92, 124.56, 124.13, 123.67, 119.37, 118.14; EI$^+$-MS: calculated for $C_{29}H_{18}BrN$:461.061; found 461.056,459.06 $[M]^+$, 382.022, 384.02 $[M\text{-}C_6H_5]^+$; EA: $C_{29}H_{18}BrN$. 0.08 CH_2Cl_2, calculated: %C 74.79, %H 3.90, %N 3.00, found: %C 74.79, %H 3.57, %N 2.95; mp: 204.0-206.1 ºC.

6-Bromopyren-1-amine (3.9)



This hydrolysis procedure followed the method created by Purohit and Basu.[16] A solution of 2 M aqueous hydrochloric acid (1 mL, 5 % v/v) was added to a suspension of **3.8** (597 mg, 1.3 mmol) in 13 mL THF at room temperature. The reaction was then stirred for 2 h. The formed precipitate was filtered and it gave 395 mg (1.19 mmol) white-pink solid of **3.9** in a yield of 91 %. The precipitate was ready to use for next step and without further characterization.

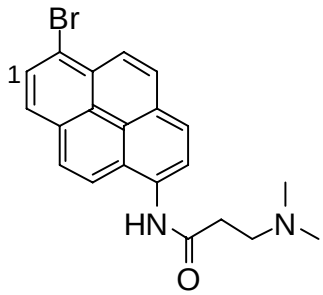
N-(6-Bromopyren-1-yl)-3-chloropropanamide (3.10)



This step followed the reported procedure by Kim and Chang.[11] To a solution of 395 mg (1.19 mmol) compound **3.9** in 20 mL pre-degassed anhydrous CH_2Cl_2, 1.65 mL (11.9 mmol) of Et_3N was added at room temperature. Then a solution of 0.17 mL (1.78 mmol) of 3-

chloropropionyl chloride in 3 mL of pre-degassed anhydrous CH_2Cl_2 was added to the reaction. The mixture was left to stir over night at room temperature. The formed yellow precipitate was filtered and gave 381 mg (0.98 mmol) white solid of **3.10** with a yield of 82 %. The precipitate was used directly without further purification. ^{1}H NMR (400 MHz; DMSO-d_6, 25 ºC): δ 10.63 (s, 1H, N*H*CO), 8.46-8.22 (m, 8H, *Py-H*), 6.82 (m, 1H, CO*CH*$_2$CH$_2$Cl), 6.41 (dd, ^3J = 12.9 Hz, ^4J = 1.2 Hz, 1H, CO*CH*$_2$CH$_2$Cl), 5.90 (d, ^3J = 7.5 Hz, 1H, COCH$_2$*CH*$_2$Cl), 3.07 (d, *J*= 4.8 Hz, 1H, COCH$_2$*CH*$_2$Cl).

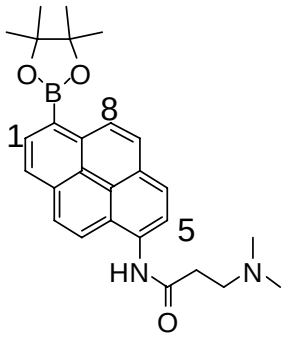
N-(6-Bromopyren-1-yl)-3-(dimethylamino)propanamide (3.11)



This step was modified from protocols created by Kostakis, Kletsas *et al* and Philips, Sullivan *et al*.[12,13] Dimethylamine 40 % in water (0.75 mL, 5.92 mmol) was added to a mixture of 381 mg (0.99 mmol) of **3.10** in 52 mL 95 % ethanol. The reaction was then refluxed overnight. After cooling to room temperature, ethanol was evaporated in *vacuo*. The residue was taken up in CH_2Cl_2, washed with brine and 10 % NaOH solution, and extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. It gave 390 mg (0.99 mmol) yellow solid of compound **3.11** in a yield of 100 %. Rf = 0.2 (CH_2Cl_2: MeOH = 10:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 11.89 (s, 1H, N*H*), 8.87 (d, ^3J = 8.4 Hz, 1H, *H*$_1$), 8.33-7.91 (m, 7H, *Py-H*), 2.85 (t, ^3J = 6.3 Hz, 2H, CO*CH*$_2$CH$_2$N(CH$_3$)$_2$), 2.75 (t, ^3J = 6.6 Hz, 2H, COCH$_2$*CH*$_2$N(CH$_3$)$_2$), 2.56 (s, 6H, COCH$_2$CH$_2$N*(CH*$_3$*)*$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 171.09 (NH*CO*), 133.04, 130.25, 130.17, 129.95, 129.02, 127.61, 127.03, 126.12, 126.09, 124.93, 124.56, 124.52, 121.48, 120.74, 120.40, 119.93, 55.25 (CO*CH*$_2$CH$_2$N(CH$_3$)$_2$), 44.68 (COCH$_2$*CH*$_2$N(CH$_3$)$_2$), 33.77 (COCH$_2$CH$_2$N*(CH*$_3$*)*$_2$); EI$^+$-MS: calculated for $C_{21}H_{19}BrN_2O$: 394.07; found: 396.80, 394.07 $[M]^+$,

351.01 [M-C_2H_6N]$^+$, 296.00 [M-C_5H_8NO]$^+$.EA: $C_{21}H_{19}N_2OBr$. 0.26 CH_2Cl_2, calculated: %C 61.14, %H 4.71, %N 6.71, found: %C 61.14, %H 4.37, %N 6.87; mp: 164.5-166.1 °C.

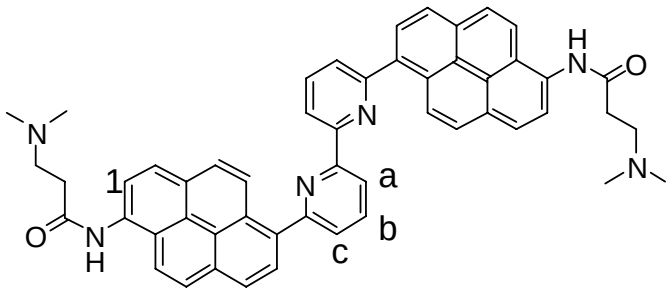
3-(Dimethylamino)-N-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1 yl)propanamide (4.3)



This method was modified from general coupling procedure created by Ishiyama and Miyaura *et al.*[8] 505 mg (1.28 mmol) of compound **3.9**, 650 mg (2.56 mmol) of bis(pinacolato)diboron, 377 mg (3.84 mmol) of potassium acetate, and 47 mg (0.064 mmol) of bis(diphenyl-phosphino)ferrocenedichloropalladium(II) complex with dichloromethane were weighed in air and placed in a round bottom flask with a magnetic stir bar. The flask was evacuated and backfilled with argon (x 3). DMF (6 mL, ~0.2 M) were then added via a syringe. The mixture was then heated to 80 ºC for 8 h. The residue was poured into 60 mL of EDTA / NaOH solution and extracted with ethyl acetate. The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel eluted with gradient elution- a mixture of EtOAc / MeOH 20:1 to 10:1 to 5:1 and it gave 348 mg (0.79 mmol) yellow solid of **4.3** in a yield of 61 %. Rf = 0.4 (EtOAc : MeOH = 5:1) ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 11.74 (s, 1H, N*H*), 8.99 (d, ^3J = 6.9 Hz, 1H, H_1), 8.78 (d, ^3J = 6.3 Hz, 1H, H_8), 8.52 (d, ^3J = 5.7 Hz, 1H, H_5), 8.21-8.05 (m, 5H, *Py-H*), 2.85 (t, ^3J = 3.9 Hz, 2H, CO$CH_2$$CH_2N(CH_3)_2$), 2.75 (t, ^3J = 4.2 Hz, 2H, CO$CH_2$$CH_2N(CH_3)_2$), 2.54 (s, 6H, CO$CH_2$$CH_2N(CH_3)_2$), 1.49 (s, 12H, B[OC$(CH_3)_2$]$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 171.01 (NH*CO*), 136.77, 133.96, 133.06, 132.30, 127.83, 127.64, 127.48, 126.73, 125.88, 125.07, 124.63, 123.62, 121.77, 121.29, 120.33, 112.97, 83.86, 60.36, 55.17, 49.81, 44.57, 33.66, 25.03, 14.17; EI$^+$-HRMS: calculated for $C_{27}H_{31}BN_2O_3$: 442.2433; found: 442.2430 [M]$^+$, 307.1795 [M- HN$(Me)_2$]$^+$, 342.1662 [M-

$HN(Me)_2$- $C_3H_2O]^+$, 242.0806 [M- $HN(Me)_2$- C_2H_3- $C_6H_{13}BO_2]^+$; 215.0688 [M- $HN(Me)_2$- C_2H_3- $C_6H_{13}BO_2$-CO]$^+$.

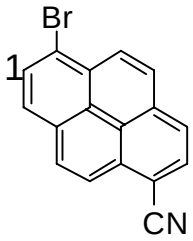
N,N'-(6,6'-(2,2'-Bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide) (4.4)



This procedure was modified from reported protocol by Beinhoff and Schluter *et al.*[9] 200 mg (0.45 mmol) of compound **4.3**, 35 mg (0.112 mmol) of **2.1**, 4.0 mg (0.01 mmol) of tetrakis(triphenylphosphine)palladium(0) and 155 mg (1.13 mmol) of potassium carbonate were weighed in air and placed in a round bottom flask with a magnetic stir bar and reflux condenser. The flask is evacuated and backfilled with argon (x 3). Pre-degassed toluene (2 mL) and pre-degassed H_2O (1 mL) were then added via a syringe. The mixture was then reflux for 7 h. Toluene was removed under reduced pressure and the residue was taken up in CH_2Cl_2, was washed with 1 M NaOH solution and brine, and was then filtered. The filtrate was extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtered and evaporated in *vacuo* and it gave F_1. The precipitate was careful washed by CH_2Cl_2 multiple times and the washed organic layers were concentrated in *vacuo* to give F_2. Both F_1 and F_2 were purified by chromatography on basic aluminum oxide using a 40:1 CH_2Cl_2 / MeOH mixture as eluent. It gave a final yield of 89 % of 80 mg (0.10 mmol) pale-white solid of compound **4.4**. Rf = 0.5 (CH_2Cl_2 : MeOH = 40:1 on Al_2O_3 TLC). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 11.95 (s, 2H, N*H*), 8.89 (d, ^3J = 6.3 Hz, 2H, *Ha*), 8.70 (d, ^3J = 5.7 Hz, 2H, *Hc*), 8.53 (d, ^3J = 9Hz, 2H, *H1*), 8.29-8.00 (m, 14H, *Py-H*), 7.79 (d, ^3J = 5.7 Hz, 2H, *Hb*), 2.89 (t, ^3J = 3.9 Hz, 4H, CO*CH*$_2$CH$_2$N(CH$_3$)$_2$), 2.78 (t, ^3J = 4.2 Hz, 4H, COCH$_2$*CH*$_2$N(CH$_3$)$_2$), 2.60 (s, 12H, COCH$_2$CH$_2$N*(CH$_3$)$_2$*); ^{13}C NMR not possible

due to very poor solubility; ESI^+-HRMS (20 V): calculated for $C_{52}H_{45}N_6O_2$ (MH^+) 785.3598; found: 785.3593.

6-Bromopyrene-1-carbonitrile (3.12)



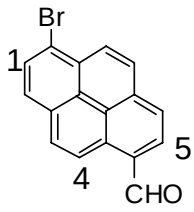
Method A: The method followed procedure developed by Goldsmith and Wasielewski.[17] A mixture of 509 mg (1.41 mmol) of **3.2**, 131 mg (1.04 mmol) of copper (I) cyanide in anhydrous DMF (12 mL) was heated at 120 °C for two days. The reacted mixture was poured into NH_4OH solution and stirred for 40 min. It was then filtered through celite and the filtrate was extracted with ethyl acetate. The combined filtrates were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified with flash chromatography on silica gel using a 1:1 Hexane / CH_2Cl_2 mixture as eluent and it gave 45 mg (0.15 mmol) white-yellow solid of **3.12** in a yield of 11 %.

Method B: This method followed procedure developed by Zanon and Buchwald.[18] 312 mg (0.87 mmol) of compound **3.2**, 165 mg (0.086 mmol) of copper (I) iodide 125 mg (0.17 mmol) of potassium iodide and 51 mg (1.04 mmol) of sodium cyanide were weighed in air and placed in a round bottom flask with a magnetic stir bar and reflux condenser. The flask is evacuated and backfilled with argon (x 3). *N, N'*-Dimethylethylenediamine (0.093 mL) and 3 mL of pre-degassed toluene were then added via a syringe. The reaction was then refluxed for 24 h. The reacted residue was washed with 5 mL of 30 % ammonium solution and extracted by CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified with flash chromatography on silica gel using a 1:1 hexane / CH_2Cl_2 mixture as eluent. It gave a 50 mg (0.16 mmol) of solid **3.12** in a yield of 18 %.

Rf = 0.2 (Hexane : CH_2Cl_2 = 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 8.60 (d, ^3J = 9.3 Hz, 1H, H_5), 8.48 (d, ^3J = 8.7 Hz, 1H, H_1), 8.36-8.14 (m, 6H, *Py-H*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C):

δ 134.06, 131.28, 130.38, 130.25, 130.00, 129.18, 127.55, 125.04, 124.37, 122.32, 112.85, 112.70; IR (V_{max}/cm^{-1}) :2926, 2364, 2220, 1586, 1075, 842, 712, 528; EI$^+$-MS: calculated for $C_{17}H_8BrN$: 304.98; found: 306.98, 304.98 $[M]^+$, 226.07 $[M\text{-}Br]^+$, 199.06 $[M\text{-}Br\text{-}CN]^+$.

6-Bromopyrene-1-carbaldehyde (3.13)

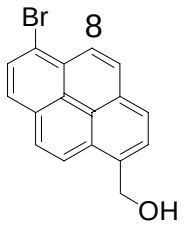


This step was referenced in the method reported by Suzuki and Ueno.[19] To a mixture of 500 mg (1.39 mmol) of **3.2** in dry THF (35 mL) at -78 ºC, was added dropwise 0.63 mL (1.39 mmol, 2.2 M) *n*-BuLi / hexane over a period of 5 min and stirred at this temperature for 10 min. The mixture was then warmed up to -15 ºC and stirred for another 15 min (red solution). DMF (0.7 mL, 9.04 mmol) was added at the same temperature. The reaction was warmed up to room temperature in a period of 20 min. The yellow solution was then quenched with 5 mL of 2 M aq. HCl solution and 5 mL ammonium chloride solution. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The crude was recrystallized from $CHCl_3$ / hexane, giving 214 mg of precipitate. The filtrate was purified by flash chromatography on silica gel eluted with a 2:1 Hexane / CH_2Cl_2 mixture. It gave an overall yield of 64 % (277 mg, 0.90 mmol) of a yellow puffy solid **3.13.** Rf = 0.5 (Hexane : CH_2Cl_2 = 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 10.79 (s, 1H, C*H*O), 9.45 (d, ^3J = 9 Hz, 1H, H_4), 8.60 (d, ^3J = 9 Hz, 1H, H_1), 8.49 (d, ^3J = 8.1 Hz, 1H, H_5), 8.33-8.12 (m, 5H, *Py-H*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 192.91 (*CHO*), 135.15, 131.95, 130.81, 129.17, 128.63, 127.81, 127.25, 125.23, 125.02, 123.27, 122.14; EI$^+$-HRMS: calculated for $C_{17}H_9BrO$: 307.9837; found: 309.9821, 307.9833 $[M]^+$, 280.9814, 278.9831 $[M\text{-}CO]^+$, 200.0660 $[M\text{-}Br\text{-}CO]^+$; mp: 118.1-120.0 °C.

(6-Bromopyren-1-yl)methanol (3.14)

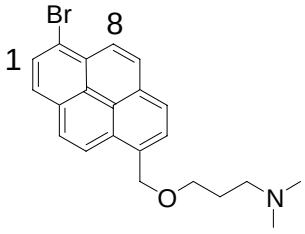
This step was slightly modified from reported protocol by Bair and Mckee.[20]

To a mixture of 434 mg (1.40 mmol) of **3.13** in 4 mL THF and 20 mL methanol, was added sodium borohydride (159 mg, 4.21 mmol) as a solid at room temperature. The reaction was then stirred for 24 h under argon atmosphere at room temperature. Then water was added at 0 ºC to quench the reaction. Organic solvents were evaporated in *vacuo.* The aqueous layer was acidified by 2 M aq. HCl to pH = 2 and extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. It gave 437 mg (1.40 mmol) pale-white solid of compound **3.14** in a 100 % yield.



^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 8.46-8.40 (m, 2H, *H_1, H_8*), 8.27-8.03 (m, 6H, *Py-H*), 5.43 (s, 2H, C*H_2*OH), 1.91 (brs, 1H, CH_2*OH*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 131.08, 130.24, 129.78, 128.99, 127.61, 126.61, 125.80, 125.32, 123.33, 63.84 (*C*H_2OH); EI^+-MS: calculated for $C_{17}H_{11}BrO$: 310.00; found: 312.01, 309.99 $[M]^+$, 202.08 $[M\text{-}CO\text{-}Br]^+$.

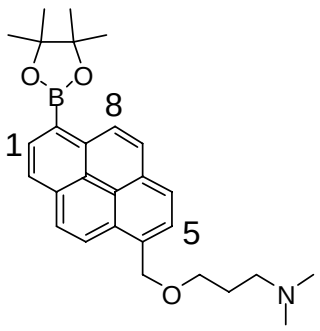
3-((6-Bromopyren-1-yl) methoxy)-N, N-dimethylpropan-1-amine (3.15)



Firstly, 3-(dimethylamino) propyl methanesulfonate hydrochloride salt was synthesized in the same procedure reported by Inouye and Nakazumi.[21] This step follows procedure published by Inouye and Nakazumi.[21] A solution of 437 mg (1.40 mmol) **3.14** in 4 mL anhydrous DMF was added to 122 mg (3.09 mmol, 60% in mineral oil) sodium hydride in 1.2 mL anhydrous DMF at 0 °C. The red solution was then stirred for 1 h at 0 °C. 3-(Dimethylamino)propyl methanesulfonate hydrochloride salt (311 mg, 1.54 mmol) was added to the reaction solution at 0 °C and was warmed up to room temperature overnight. The reaction was quenched by 80 mL water and adjusted to pH to 11 using aq. NaOH solution. The aqueous layer was then extracted with ethyl acetate. The combined organic layers

were dried with Na_2SO_4, filtered, and evaporated in *vacuo*. The crude was careful purified with flash chromatography on silica gel eluted with a mixture of CH_2Cl_2 / MeOH=50:1 to 40:1 to 20:1. It gave 250 mg (0.63 mmol) solid of **3.15** in a yield of 45 %. Rf = 0.23 (CH_2Cl_2 : MeOH = 5:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC): δ 8.43-8.34 (m, 2H, *H_1*, *H_8*), 8.24-8.00 (m, 6H, *Py-H*), 5.20 (s, 2H, Py-*CH_2*OH), 3.68 (t, ^3J = 6 Hz, 2H, OC*H_2*$CH_2CH_2N(CH_3)_2$), 2.51 (t, ^3J = 7.2 Hz, 2H, $OCH_2CH_2CH_2$*H_2*$N(CH_3)_2$), 2.31 (s, 6H, $OCH_2CH_2CH_2N(CH_3)_2$), 1.89 (q, ^3J = 6.9 Hz, 2H, OCH_2CH_2*H_2*$CH_2N(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 132.33, 131.03, 130.24, 130.13, 129.71, 129.34, 128.99, 127.50, 127.34, 125.90, 125.70, 125.07, 124.30, 123.73, 120.16, 68.54 (Py*CH_2*O), 56.56 (OC*H_2*$CH_2CH_2N(CH_3)_2$), 45.04 ($OCH_2CH_2CH_2$*H_2*$N(CH_3)_2$), 29.72 (OCH_2CH_2-$CH_2N(CH_3)_2$), 27.49 (OCH_2CH_2*H_2*$CH_2N(CH_3)_2$); EI$^+$-HRMS: calculated for $C_{22}H_{22}NOBr$: 395.0885; found: 397.0923, 395.0898 $[M]^+$, 295.0009, 293.0024 $[M\text{-}C_5H_{10}NO]^+$, 213.0764 $[M\text{-}C_5H_{10}NO\text{-}Br]^+$, 200.07 $[M\text{-}Br\text{-}C_6H_{14}NO]^+$; EA: $C_{22}H_{22}NOBr$. 1.05 CH_2Cl_2, calculated: %C 57.06, %H 5.21, %N 3.13, found: %C 57.06, %H 5.01, %N 2.89; mp: 59.0-60.0 ºC.

N,N-Dimethyl-3-((6-(4, 4, 5, 5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)methoxy)propan-1-amine (4.4)



4.4 was synthesized from general coupling procedure created by Ishiyama and Miyaura *et al*.[8] Compound **3.15** (39.4 mg, 0.10 mmol), bis(pinacolato)diboron (51 mg, 0.20 mmol), potassium acetate (29.4 mg, 0.30 mmol), and bis(diphenylphosphino)-ferrocene]dichloropalladium(II) complex with dichloromethane (0.4 mg, 5 μmol) were weighed in air and placed in a round bottom flask with a magnetic stir bar. The flask was evacuated and backfilled with argon (x 3). DMF (1 mL, ~0.1 M) were then added via a syringe. The mixture was then heated to 80 ºC for 8 h. The residue was poured into 60 mL of EDTA / NaOH solution and extracted with ethyl acetate. The

combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel eluted with a mixture of CH_2Cl_2 / MeOH 20:1 and it gave 30 mg (0.07 mmol) yellow solid of **4.4** in a yield of 70 %. Rf = 0.17 (CH_2Cl_2 : MeOH = 8:1); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 9.05 (d, ^3J = 9.3 Hz, 1H, H_1), 8.54 (d, ^3J = 7.5 Hz, 1H, H_8), 8.41 (d, ^3J = 9.3 Hz, 1H, H_5), 8.19-8.01 (m, 5H, *Py-H*), 5.22 (s, 2H, Py-CH_2OH), 3.68 (t, ^3J = 6.3 Hz, 2H, OC$H_2$$CH_2CH_2N(CH_3)_2$), 2.42 (t, ^3J = 7.2 Hz, 2H, OCH_2-$CH_2CH_2N(CH_3)_2$), 2.24 (s, 6H, $OCH_2CH_2CH_2N(CH_3)_2$), 1.86 (q,2H, $OCH_2CH_2CH_2N(CH_3)_2$), 1.52 (s, 12H, $B[OC(CH_3)_2]_2$);^{13}C NMR (100 MHz, $CDCl_3$, 25 ºC): δ 136.54, 133.84, 133.04, 131.65, 130.88, 129.33, 127.96, 127.79, 127.73, 126.85, 124.88, 124.53, 124.28, 83.91, 71.68, 68.53, 56.48, 45.04, 29.69, 27.66, 25.05; EI^+-HRMS: calculated for $C_{28}H_{34}BNO_3$: 443.2637; found: 443.2619 $[M]^+$, 342.1826 $[M- C_5H_{11}NO]^+$, 241.0920 $[M- C_6H_{14}NO- C_6H_{14}]^+$.

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Chapter 6
Conclusion

6.1 Summary and conclusion

The project started with synthesis of bipyridine unit. In Chapter 2, we reported, for the first time, the use of 2,2'-bipyridine-N,N'-dioxide as a reactant to access 6,(6') mono and bis substituted bipyridyl bis N-oxide scaffolds (Fig.6.1).

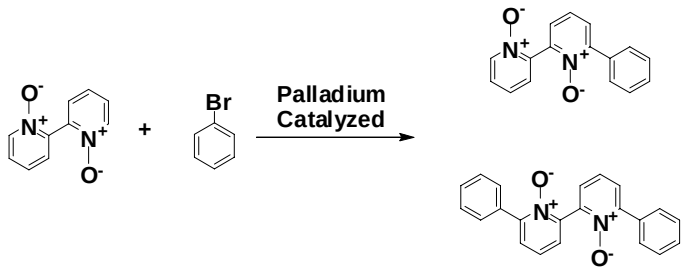


Figure 6.1 Direct arylation of 2,2'-bipyridine-N, N'-dioxide**.**

In Chapter 3, we reported the study of synthesis the DNA anchor (pyrene units A and B) and the revised DNA anchor (pyrene unit A'). Both pre-pyrene unit A and B and revised pyrene unit A' were successfully obtained and are ready to complete the assembly of the bipyridine / pyrene conjugate in a final step (Fig.6.2).

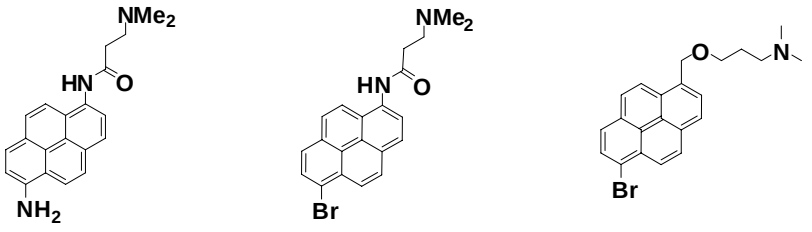


Figure 6.2 Structures of pre-pyrene unit B, pre-pyrene unit A, and revised pyrene unit A'

In Chapter 4, we reported the successful borylation of pre-pyrene unit A and the following Suzuki cross coupling (Fig. 6.3). The reduction of the coupling product was attempted using borate tetrahydrofuran or lithium aluminum hydride, but failed. The reasons are considered to be the undesired reduction of bipyridine as well as the multiple nitrogen binding sites. The crude was observed not to be very stable in air, thus the designed original target A was judged not to be appropriate for DNA binding. We also reported the successful borylation of revised pyrene unit A', which is now ready for a final coupling step.

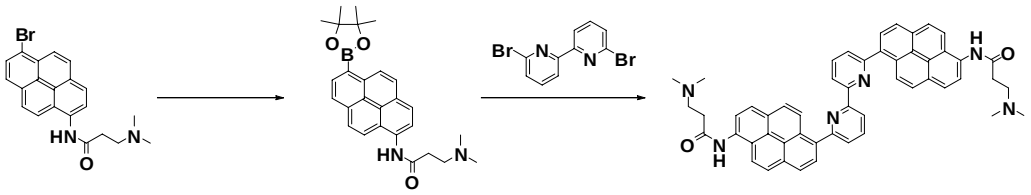


Figure 6.3 Assembly of pre-pyrene unit A with bipyrdine unit

In Chapter 4, we also reported the successful synthesis of a bipyridine-pyrene conjugate model for target A. Although the actual final target has not yet been completed, tests run on the model show promising results with regards to Cu^{+} binding (Fig.6.4). NMR and ESI-MS indicate the formation of the 1:1 complex of interest. Further investigations with the model system are considered, such as UV-vis and fluorescence titrations (both requiring much lower concentrations than NMR) on the Cu^{+} and Ag^{+} salts. Such spectroscopic studies should give some insight into the binding constants and stoechiometries. However, only the water-soluble actual system will give true indications on the behaviour of this system in solutions relevant to DNA binding.

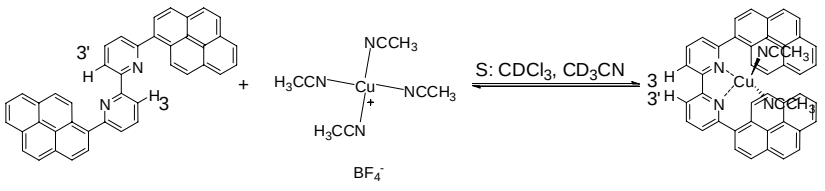


Figure 6.4 Cu^{+} coordinating with model A.

6.2 Future directions

For the synthesis part, the synthesis of 6,6'-diformyl-2,2'-bipyridine from 6,6'-dihydroxymethyl-2,2'-bipyridine via Swern oxidation needs to be conducted. Once large amount of 6,6'-diformyl-2,2'-bipyridine are obtained, the assembly of target B and its model will be studied.

The conditions of palladium catalyzed bis-N-oxide coupling need to be further optimized. Interesting work has already been reported on pyridine N-oxide showing that adding pivalic acid increases the yield and that aryl triflates are more active than aryl bromides. Thus testing pivalic acid and aryl triflates is planned to further optimize the coupling reaction.

The assembly of the revised pyrene unit A' with the bipyridine unit need to be completed.

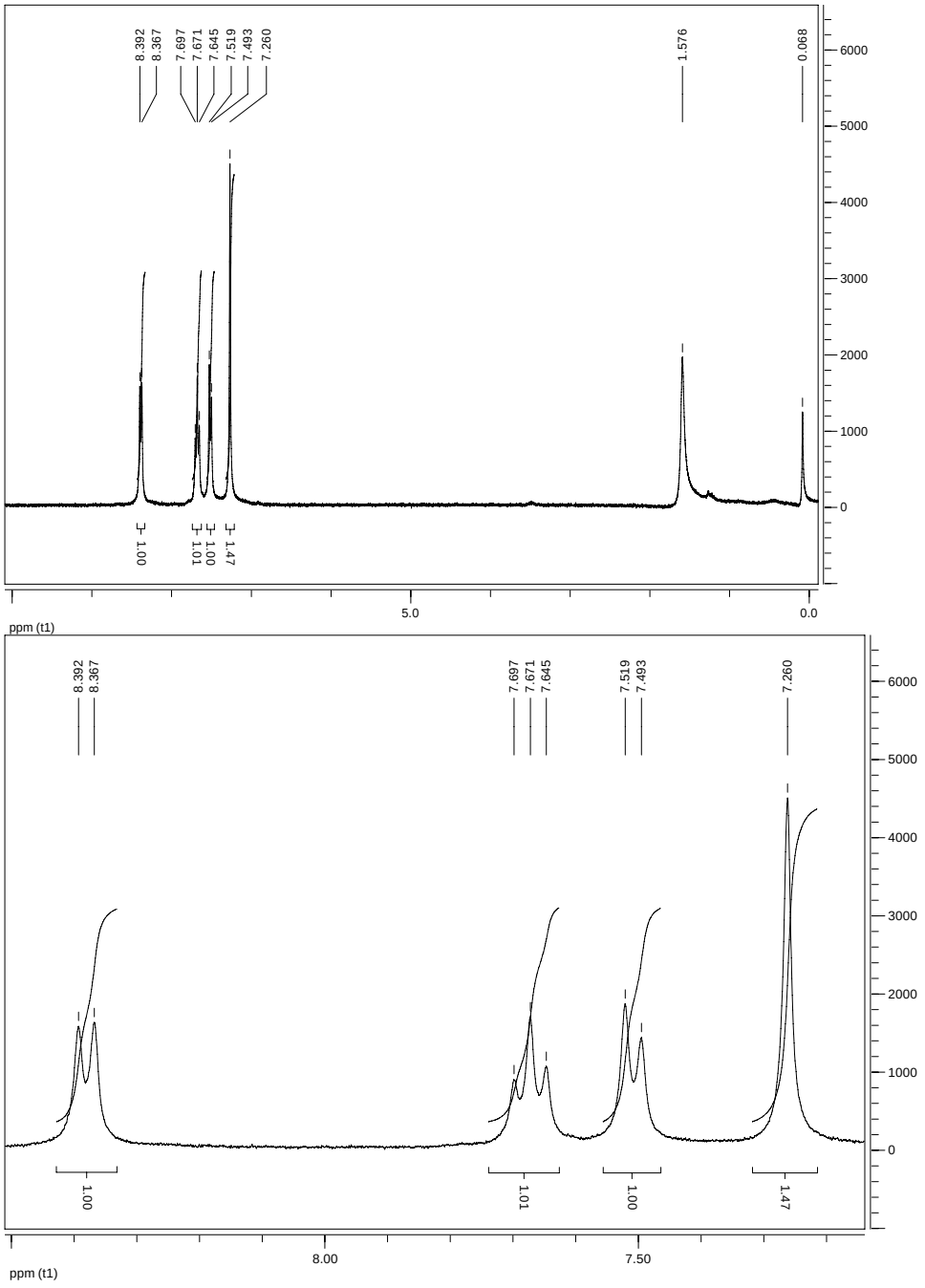
Once targeting ligands are obtained, further studies on ligand coordinating with metal cations and their complex impacting DNA binding and shape switching need to be conducted.

Appendix A

NMR and MS spectra

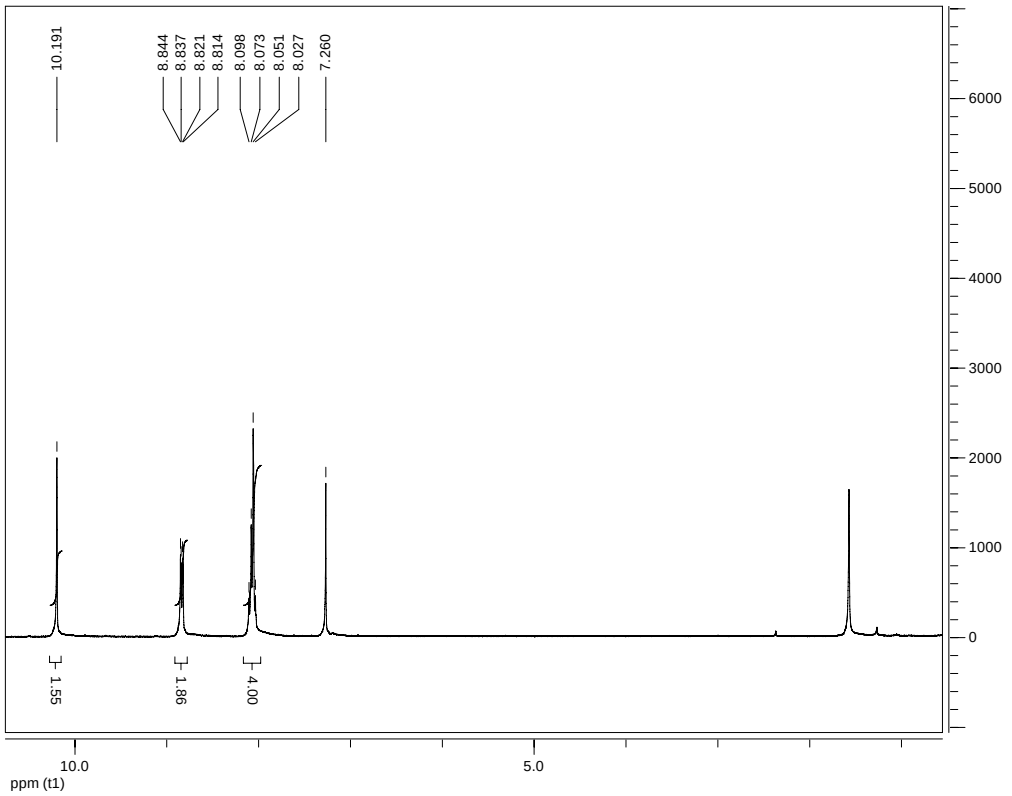
6,6′-Dibromo-2,2′-bipyridine (**2.1**)

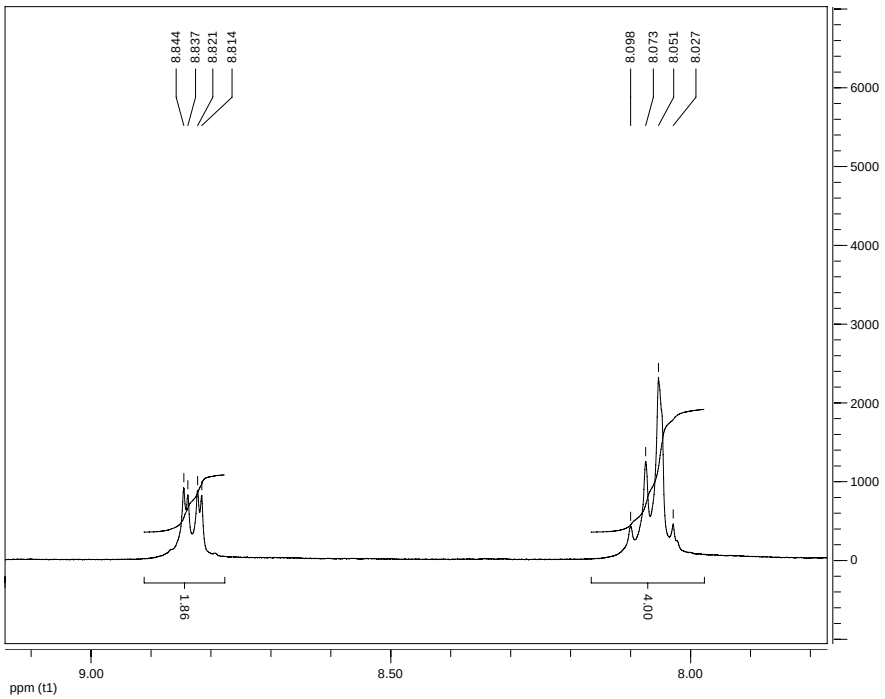
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)



6,6′-Diformyl-2,2′-bipyridine (**2.2**)

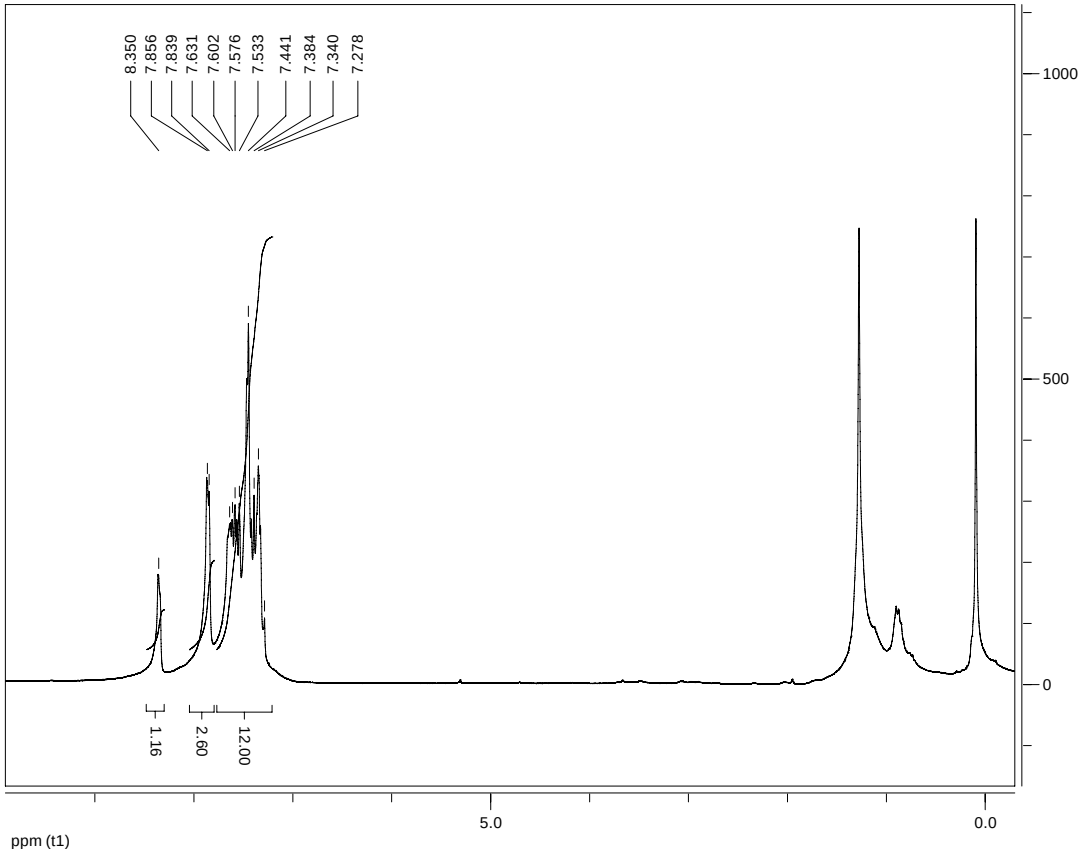
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)

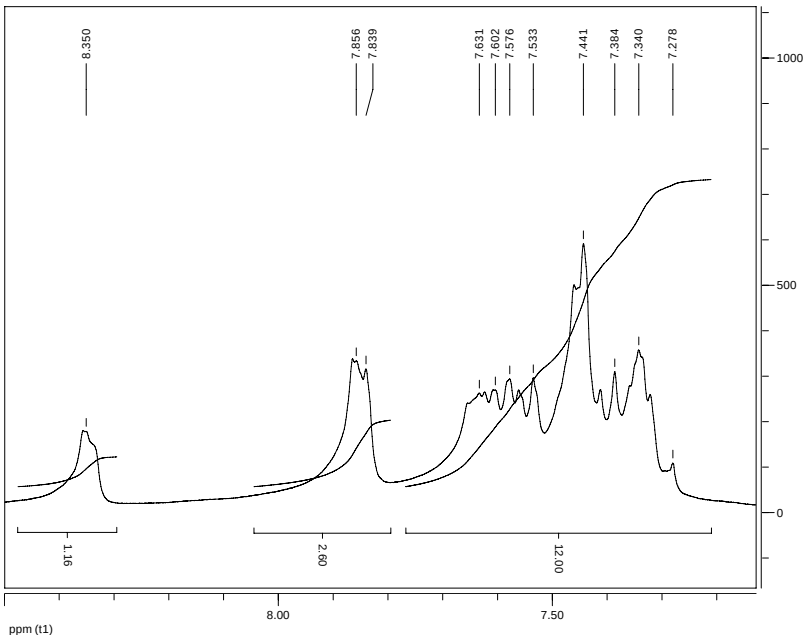




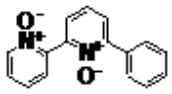
6-Phenyl-2,2'-bipyridine-N,N'-dioxide (**2.3**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)

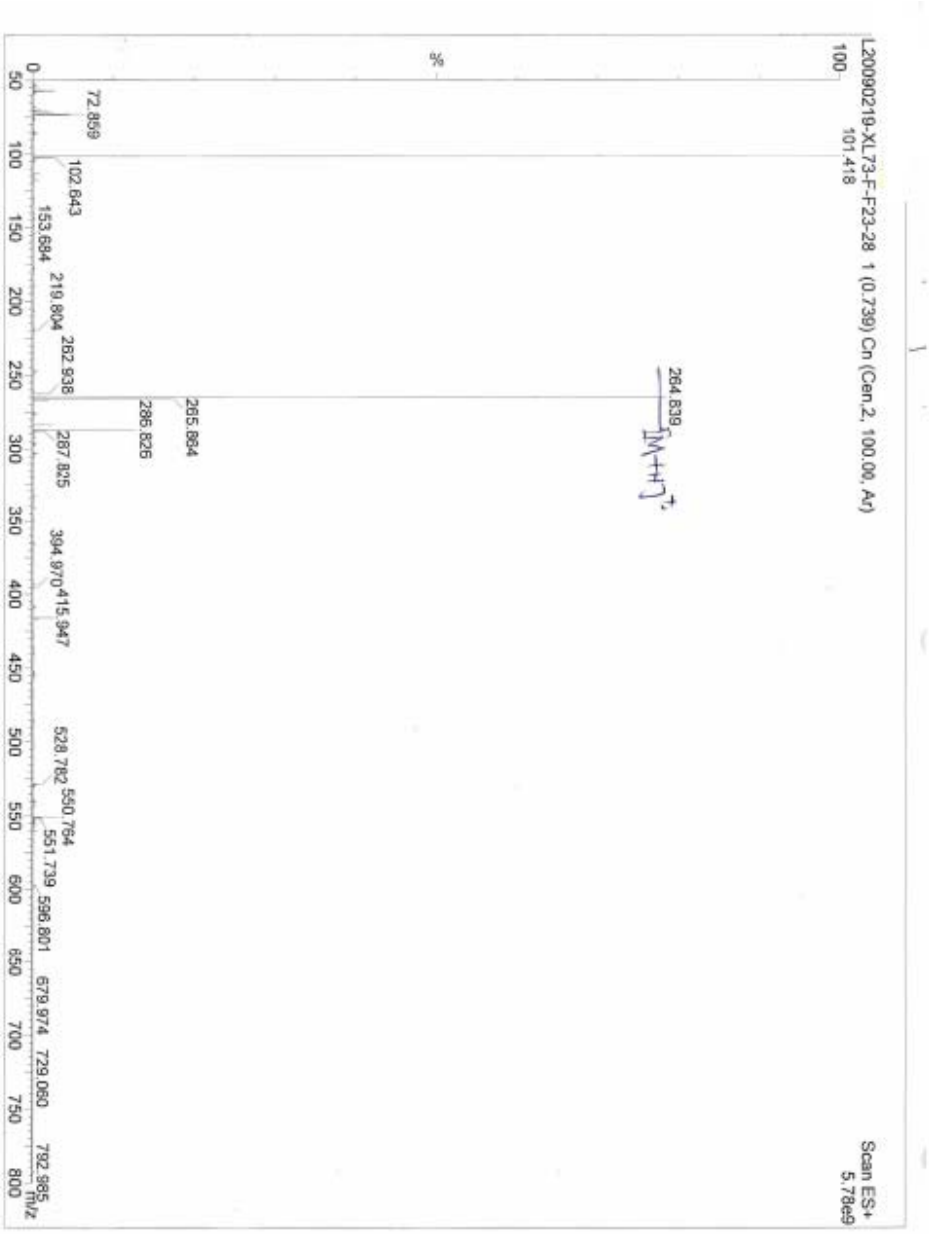




6-Phenyl-2,2'-bipyridine-N,N'-dioxide (**2.3**)

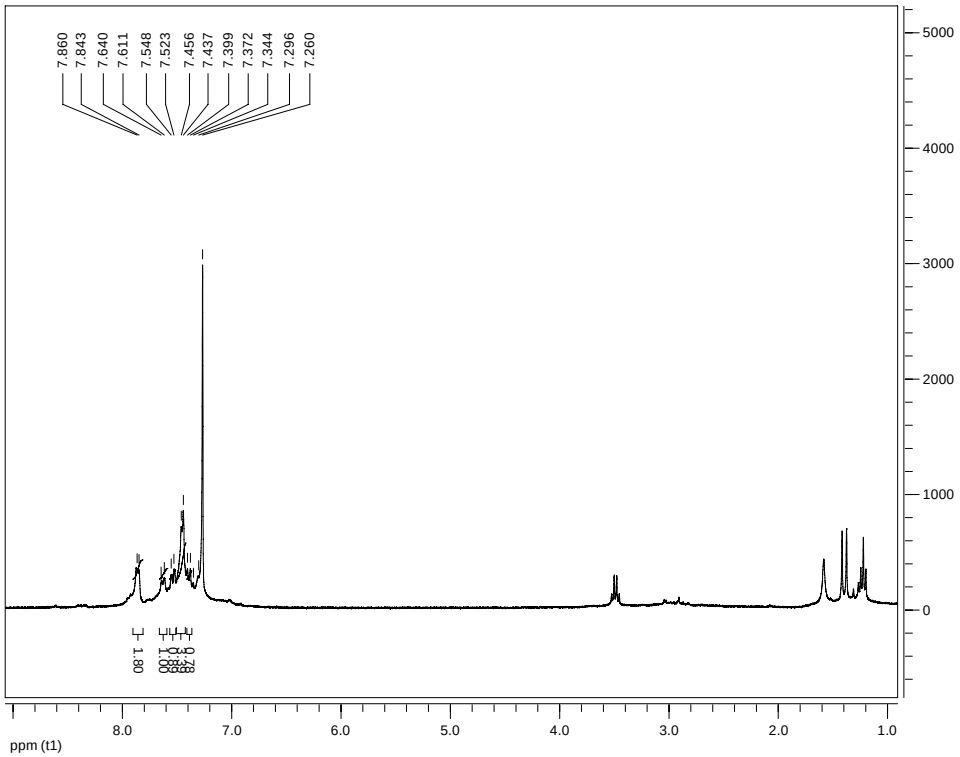


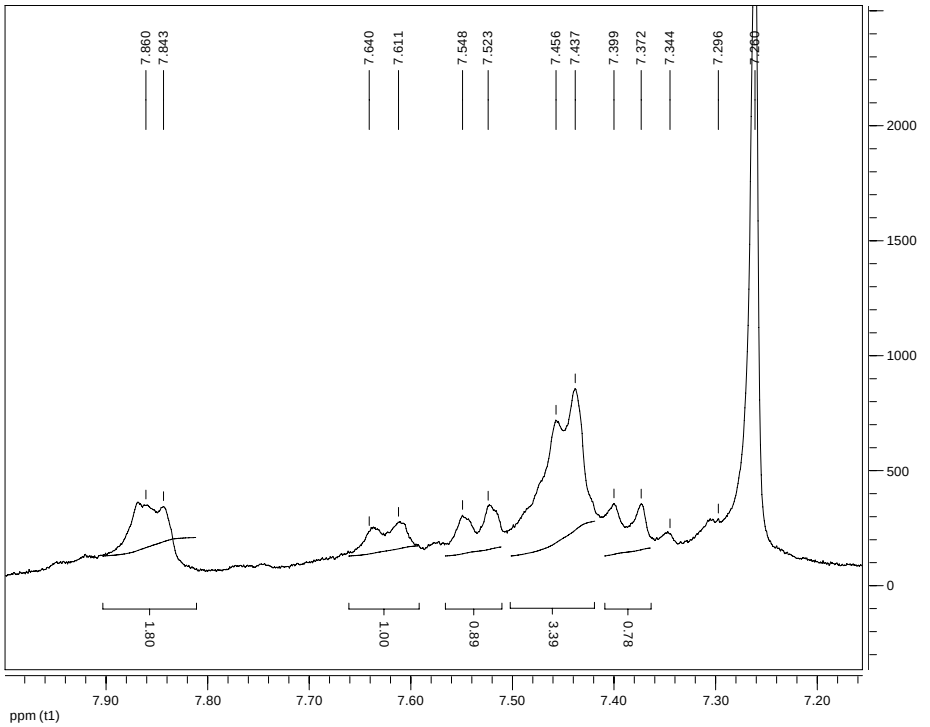
ESI^+-MS



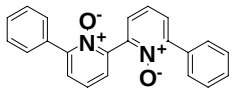
6,6'-Diphenyl-2,2'-bipyridine-N,N'-dioxide (**2.4**)

^{1}H NMR (300MHz, $CDCl_3$, 25 ^oC)

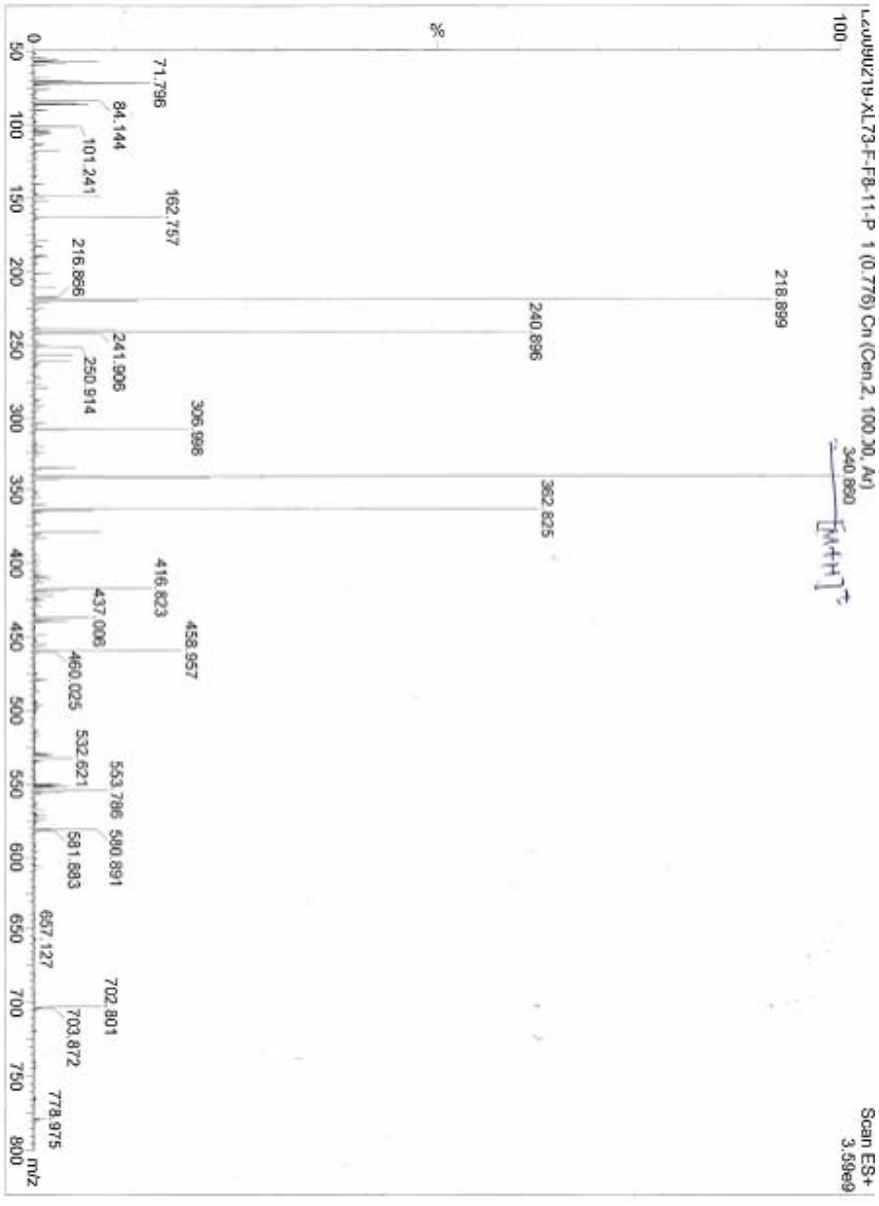




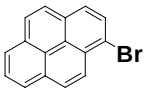
6,6'-Diphenyl-2,2'-bipyridine-N,N'-dioxide (**2.4**)



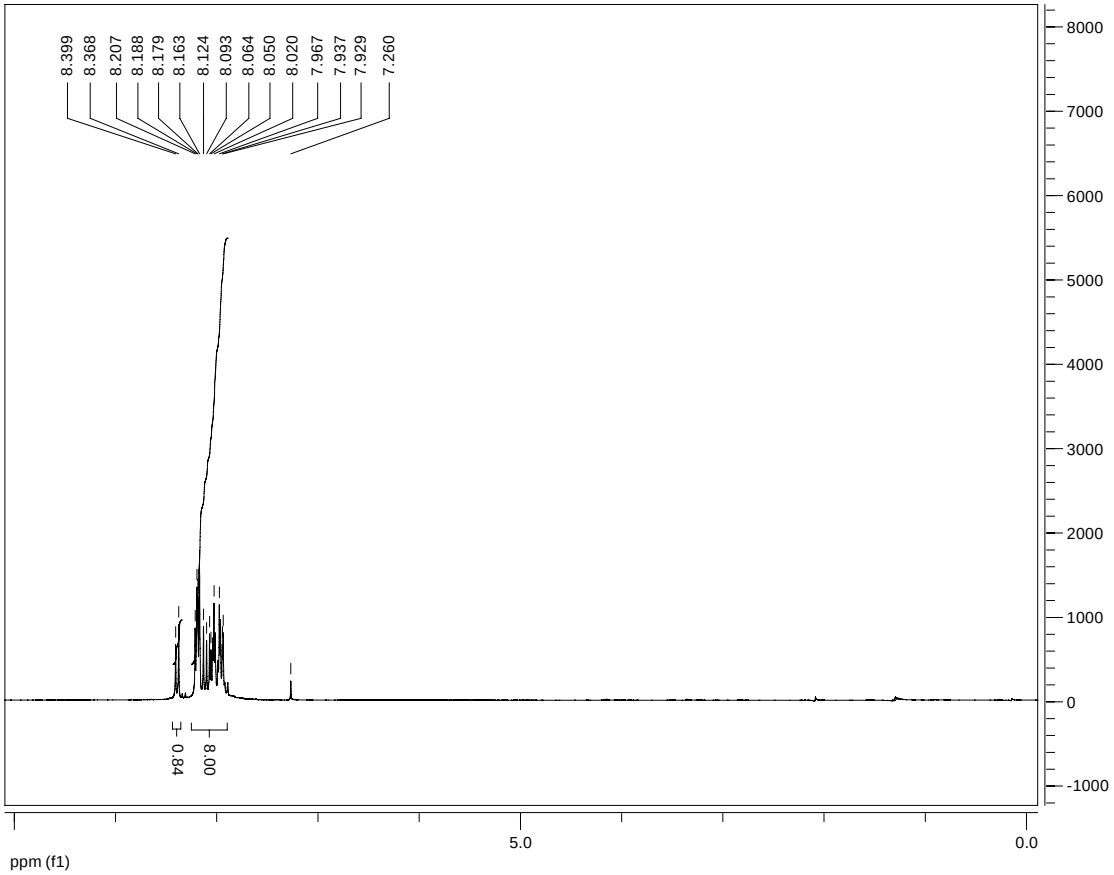
ESI^+-MS

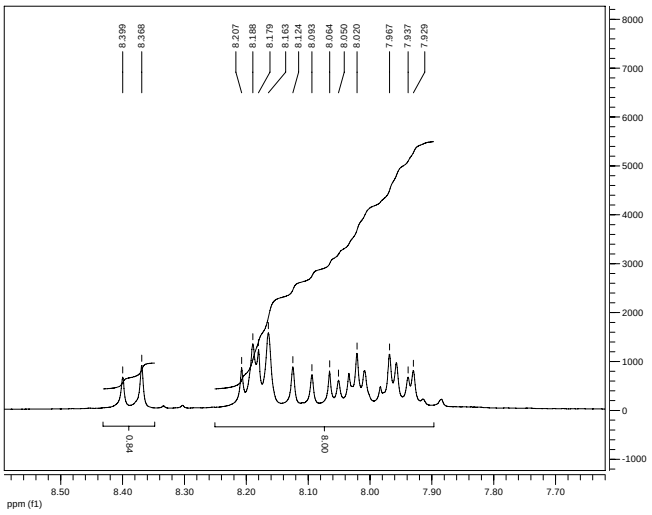


1-Bromopyrene (**3.1**)



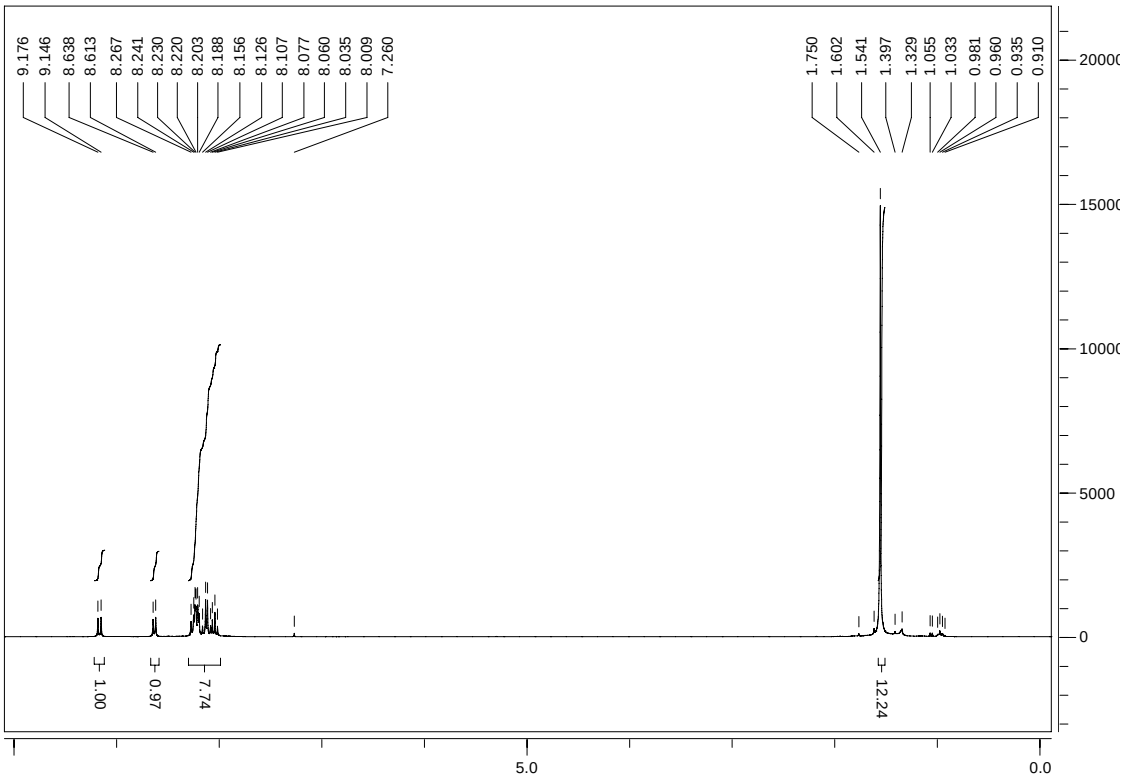
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C):

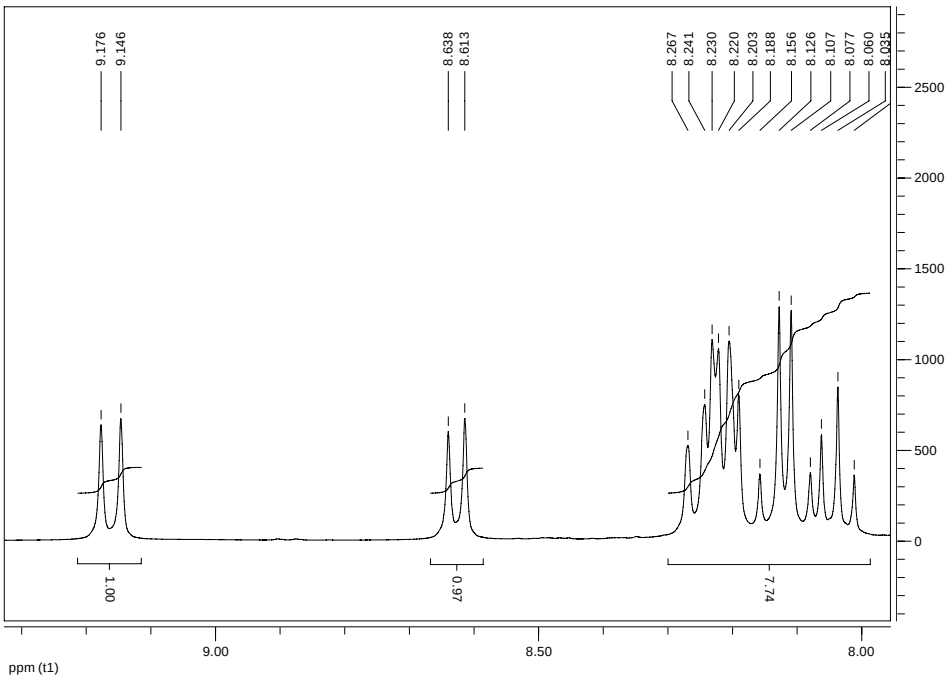




4,4,5,5-Tetramethyl-2-(pyren-1-yl)-1,3,2-dioxaborolane (**4.1**)

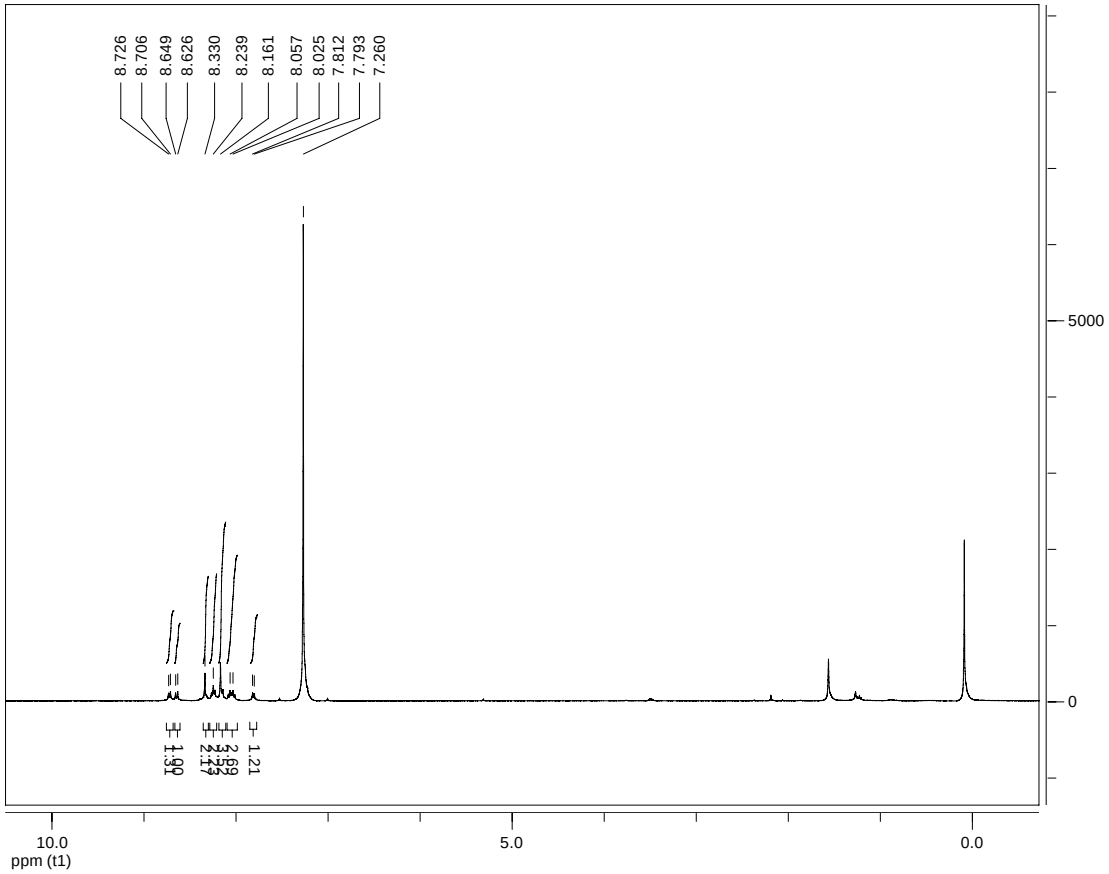
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

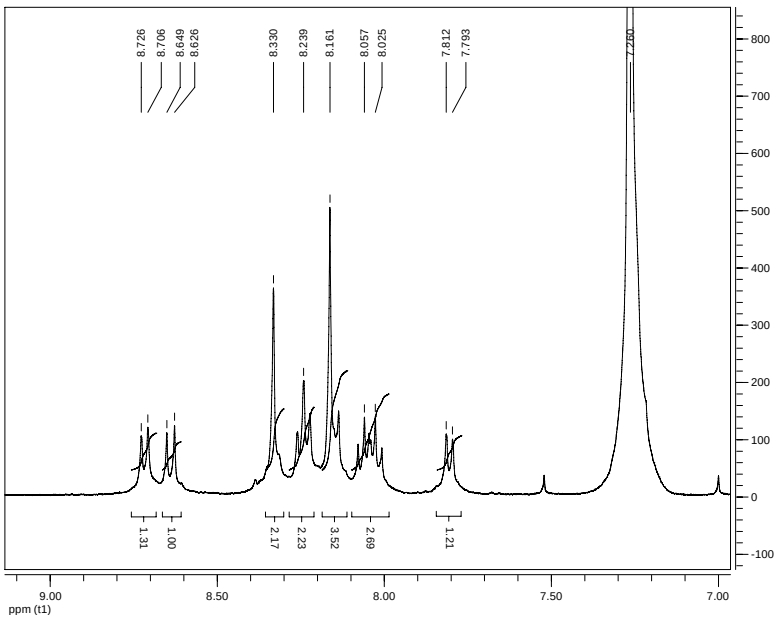




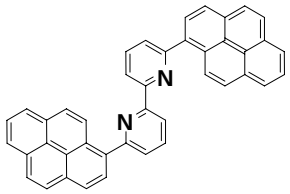
6,6′-Dipyrene-2,2′-bipyridine (**4.2**)

^{1}**H NMR** (400 MHz, $CDCl_3$, 25 ^{o}C)

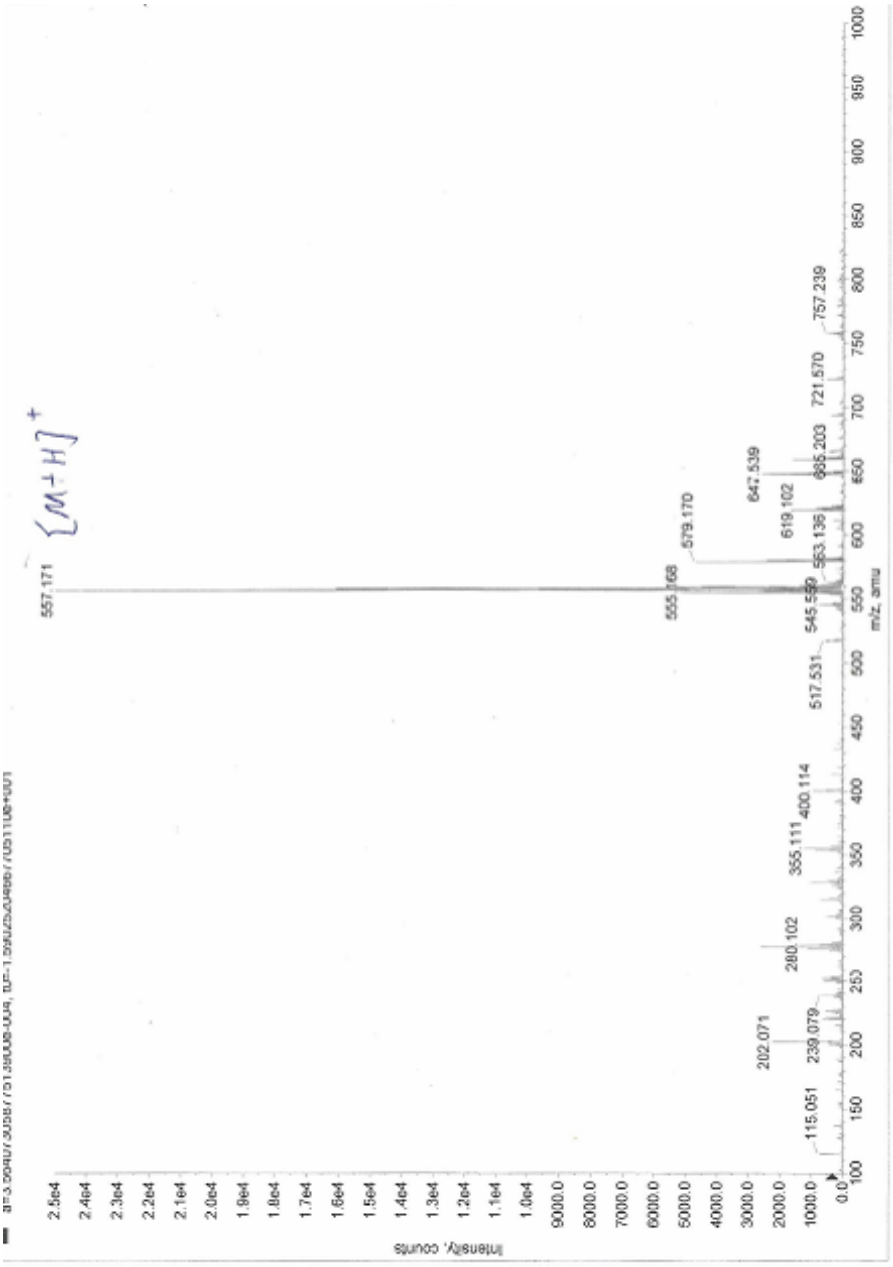




6,6′-Dipyrene-2,2′-bipyridine (**4.2**)

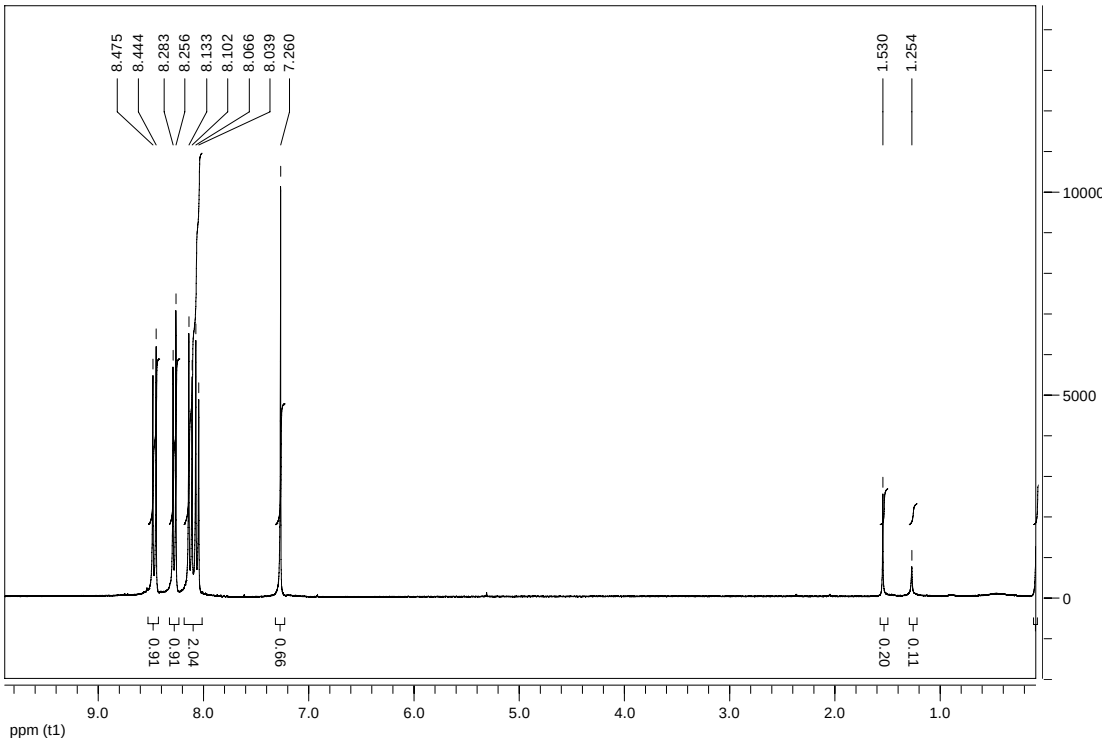


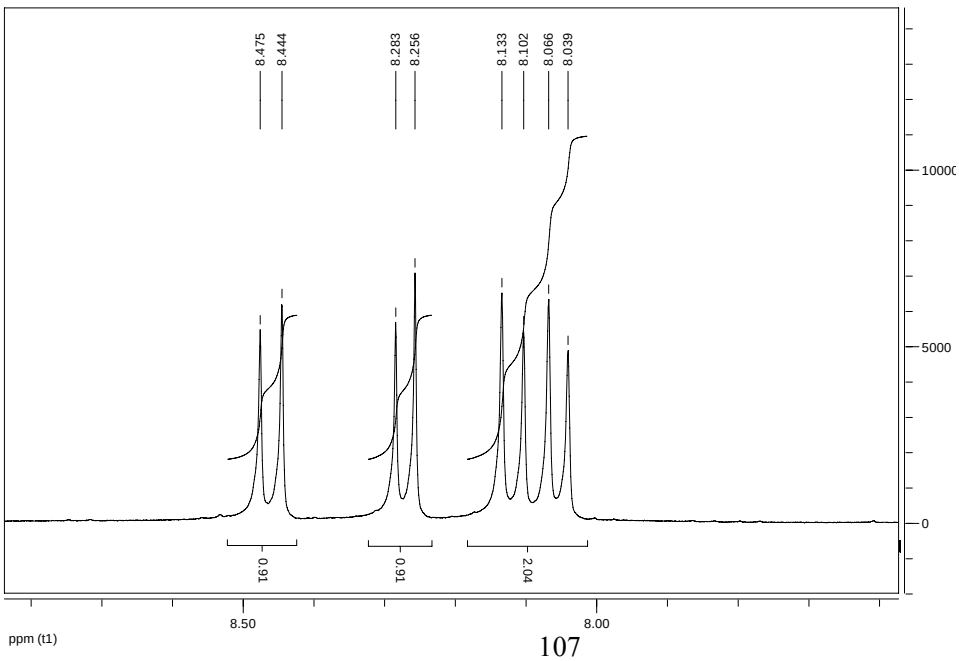
ESI^+-MS



1,6-Dibromopyrene (**3.2**)

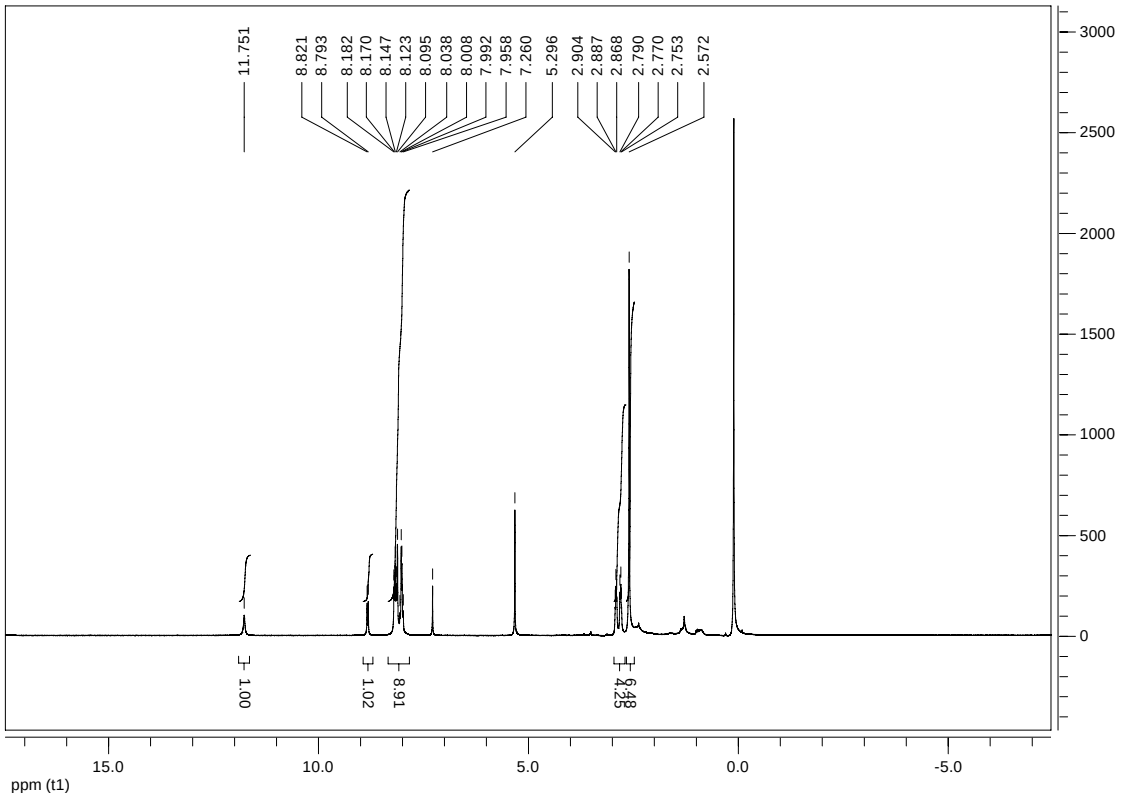
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)

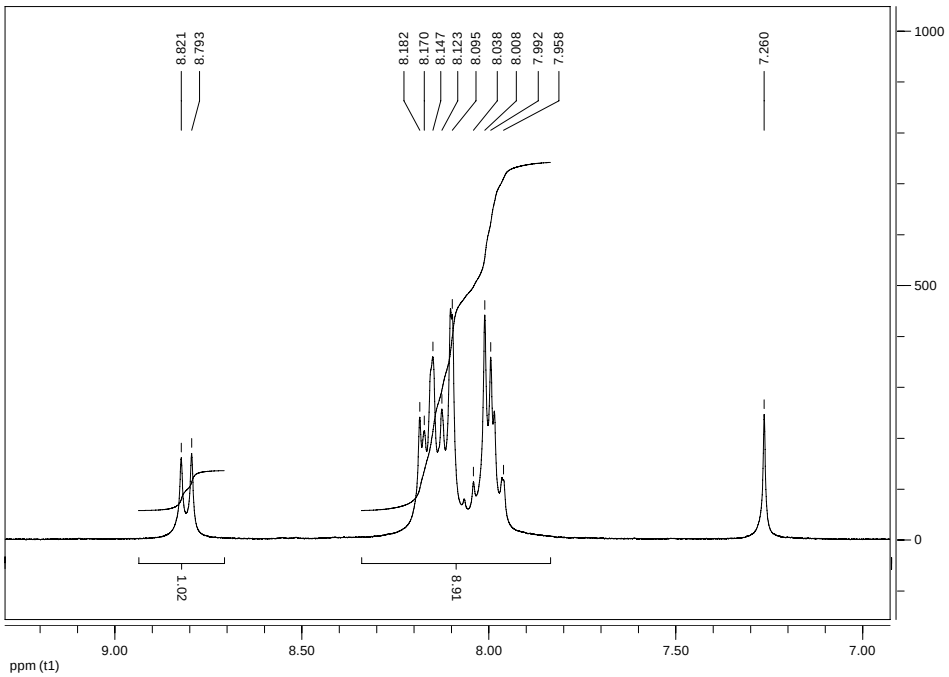




3-(Dimethylamino)-N-(pyren-1-yl)propanamide (**3.3**)

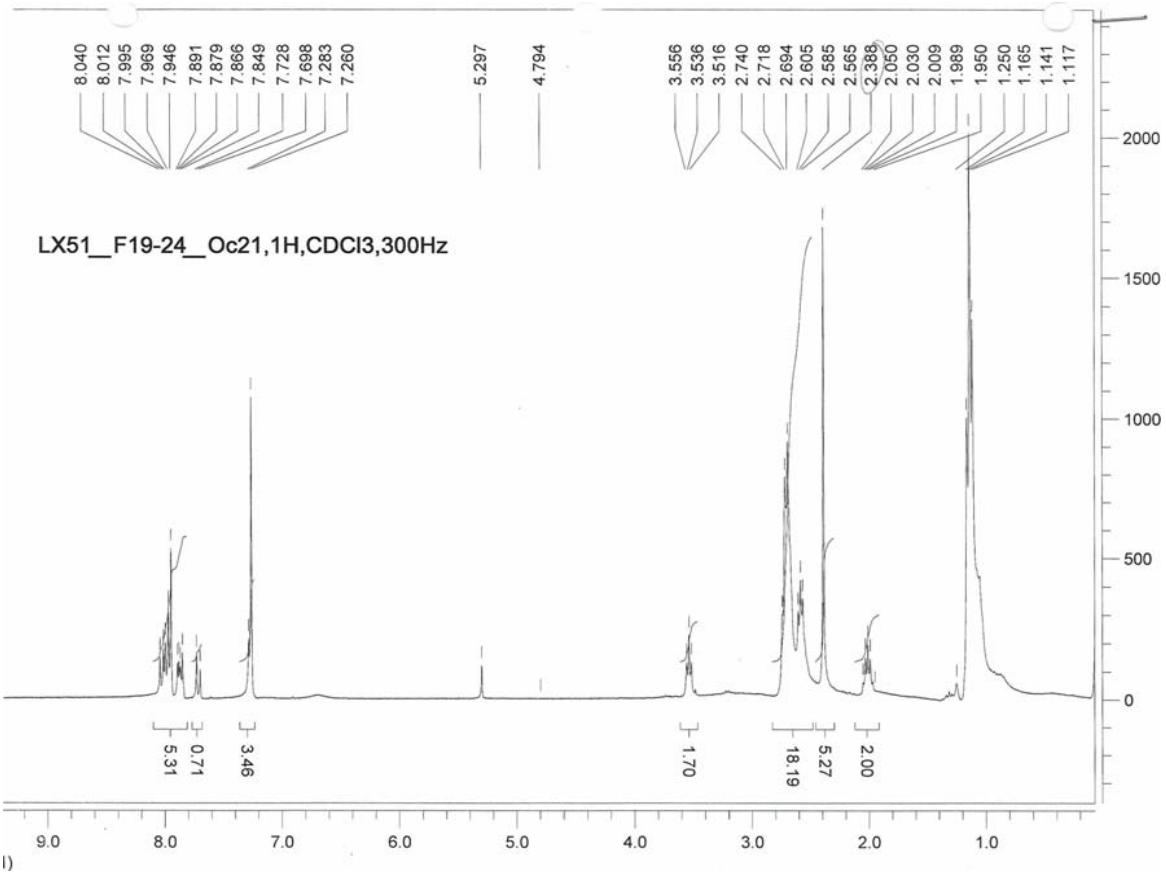
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)



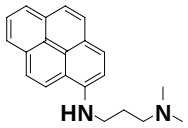


N_1,N_1-Dimethyl-N_3-(pyren-1-yl)propane-1,3-diamine (**3.4**)

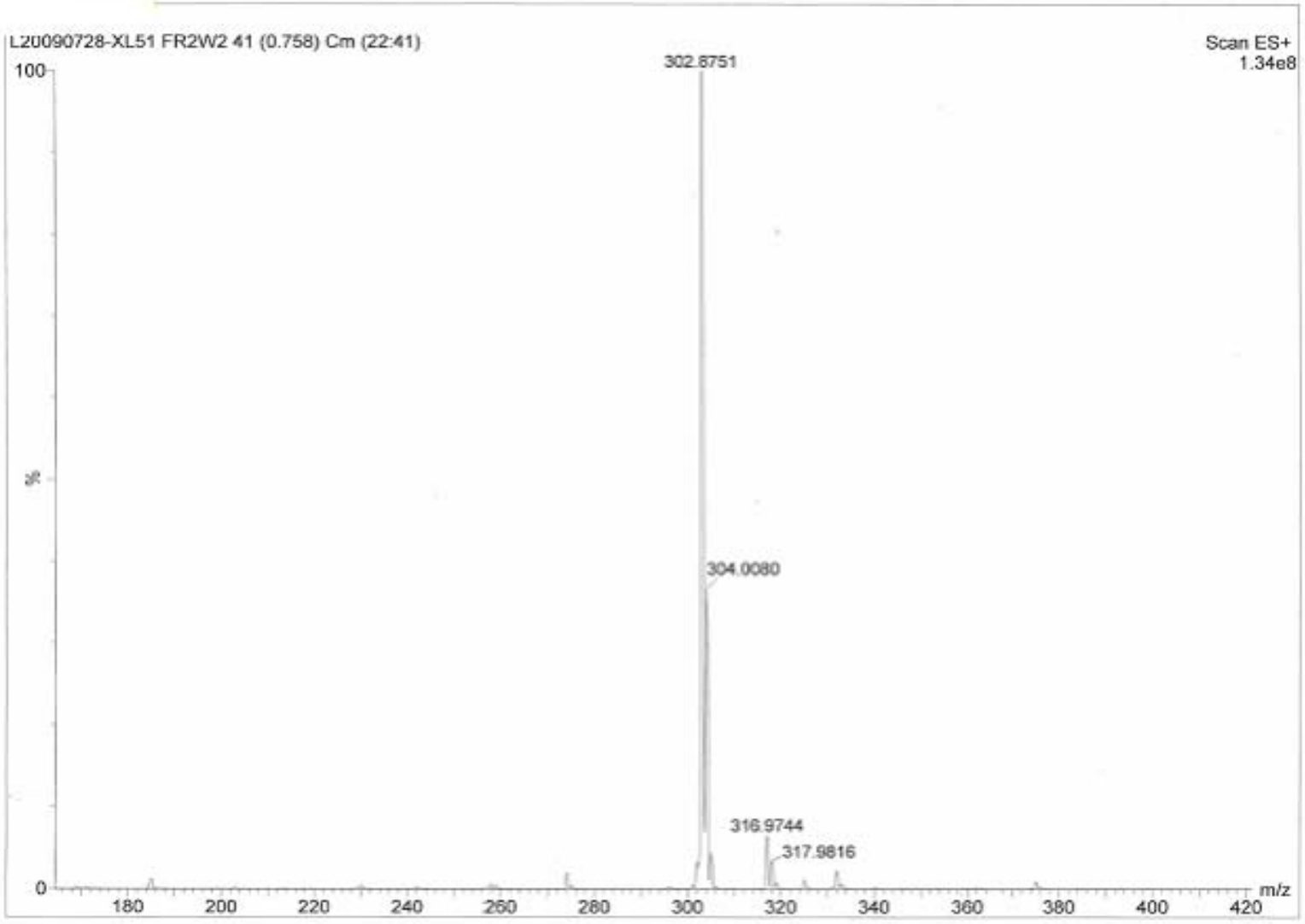
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)



N_1,N_1-Dimethyl-N_3-(pyren-1-yl)propane-1,3-diamine (**3.4**)



ESI^+-MS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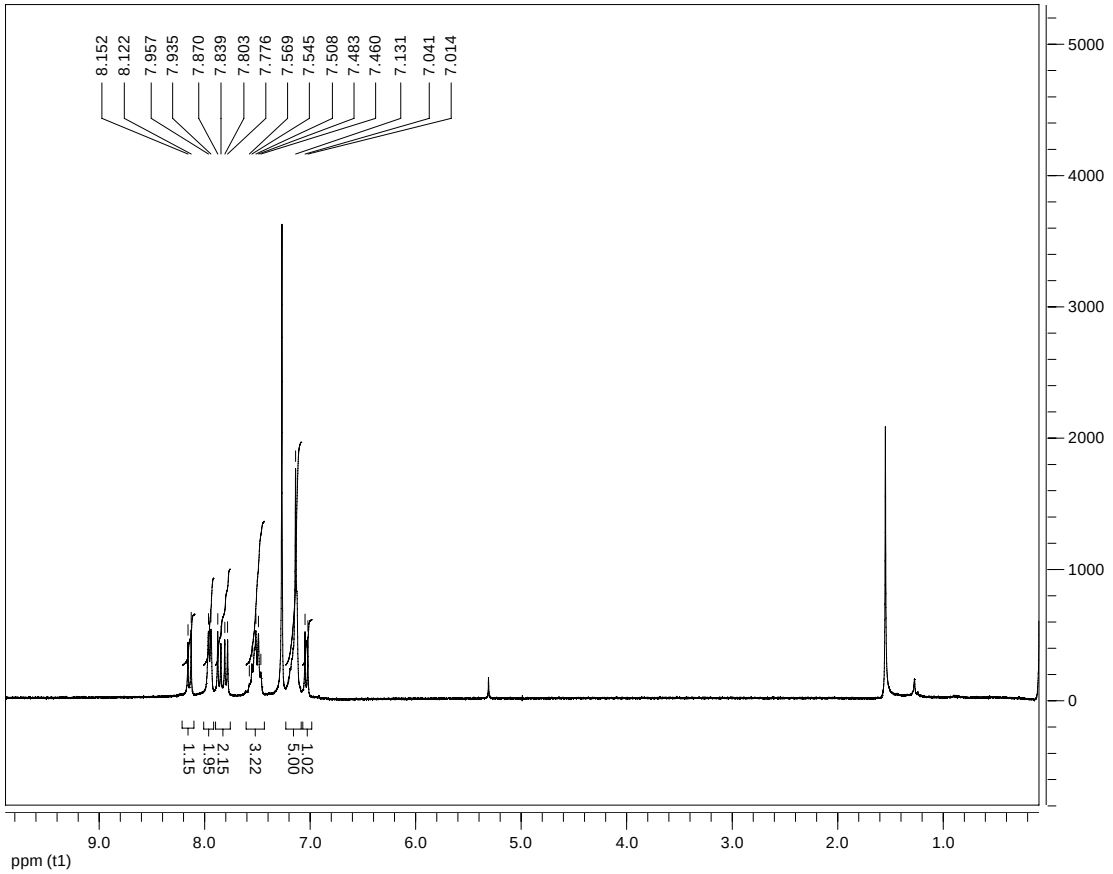
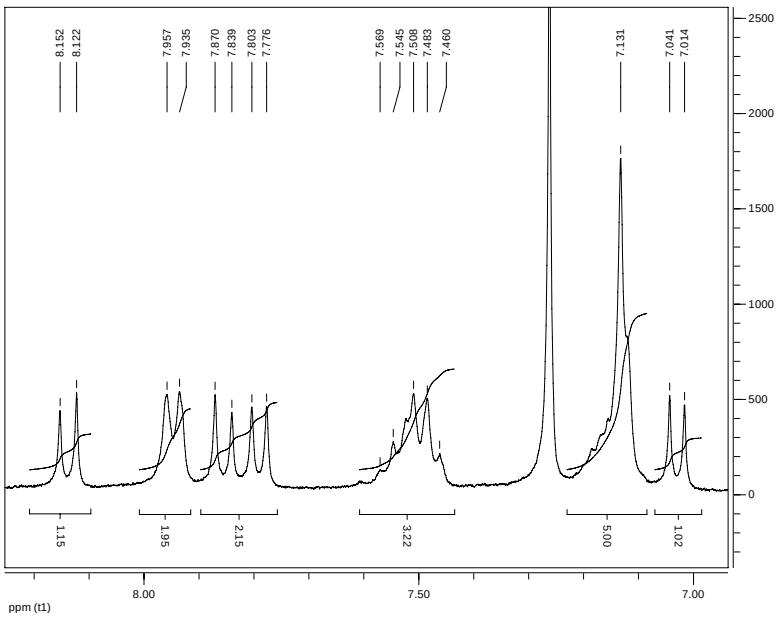
320
340
360
380
400
420
m/z

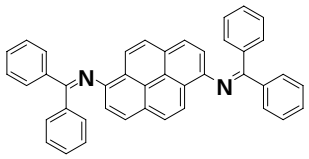
N_1,N_6-Bis(diphenylmethylene)pyrene-1,6-diamine (**3.5**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

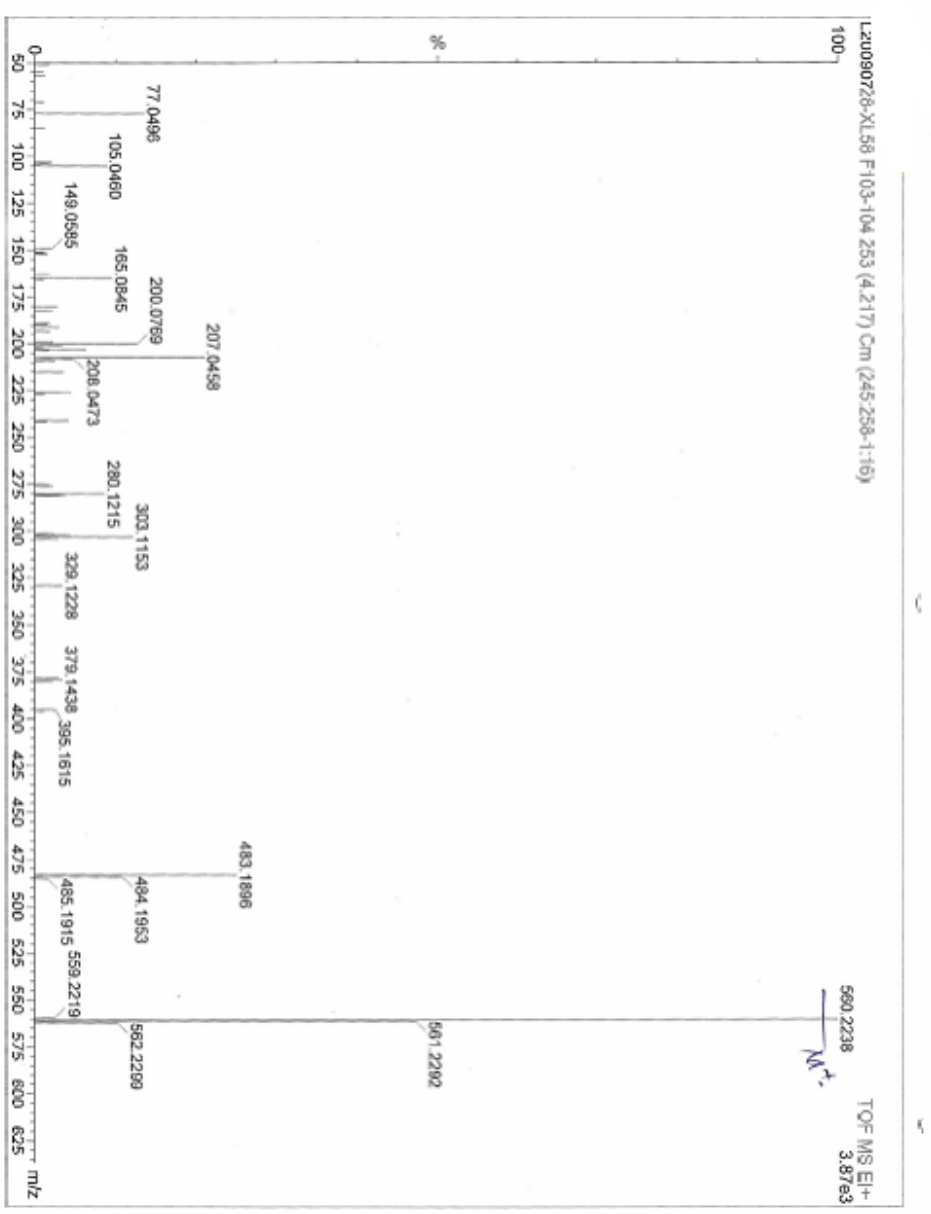




N_1, N_6-Bis(diphenylmethylene)pyrene-1,6-diamine (**3.5**)

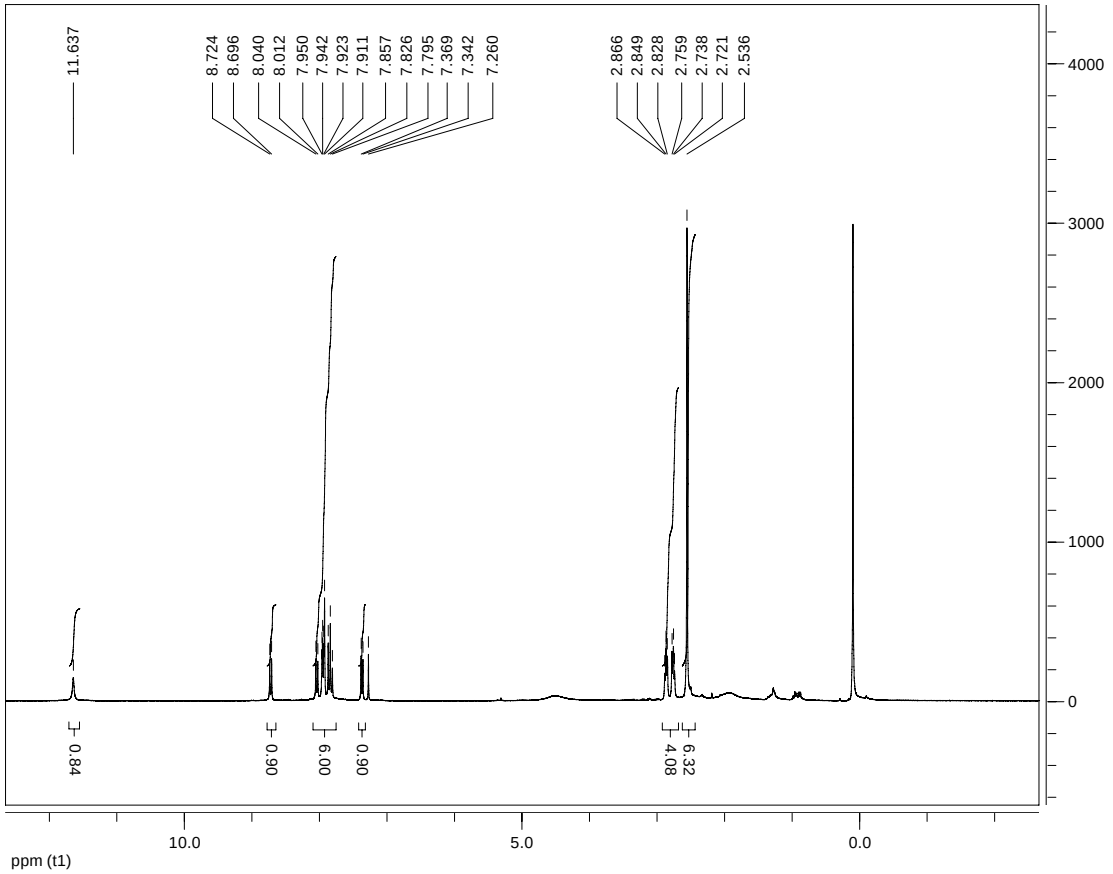


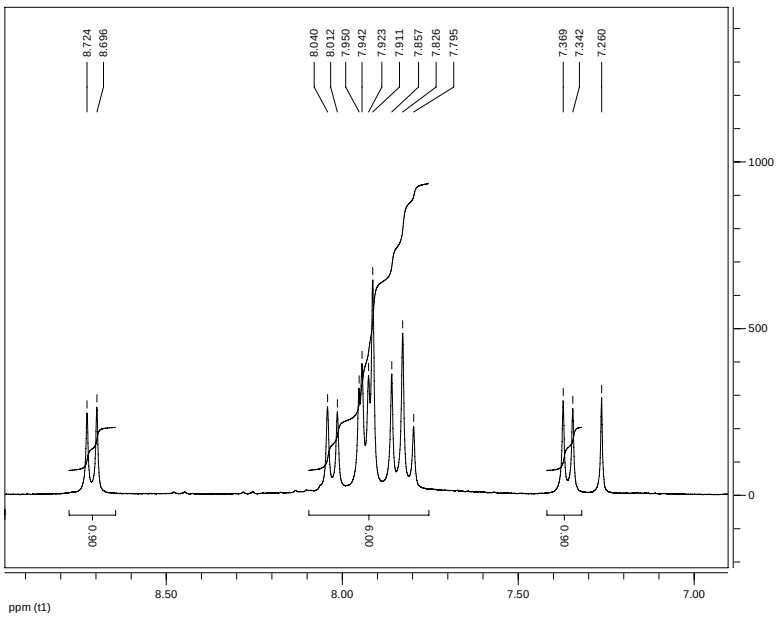
EI^+-MS



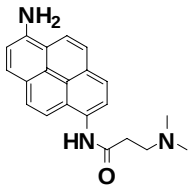
N-(6-Aminopyren-1-yl)-3-(dimethylamino)propanamide (**3.7**)

^{1}H NMR (300 MHz, $CDCl_3$, 25 ^oC)

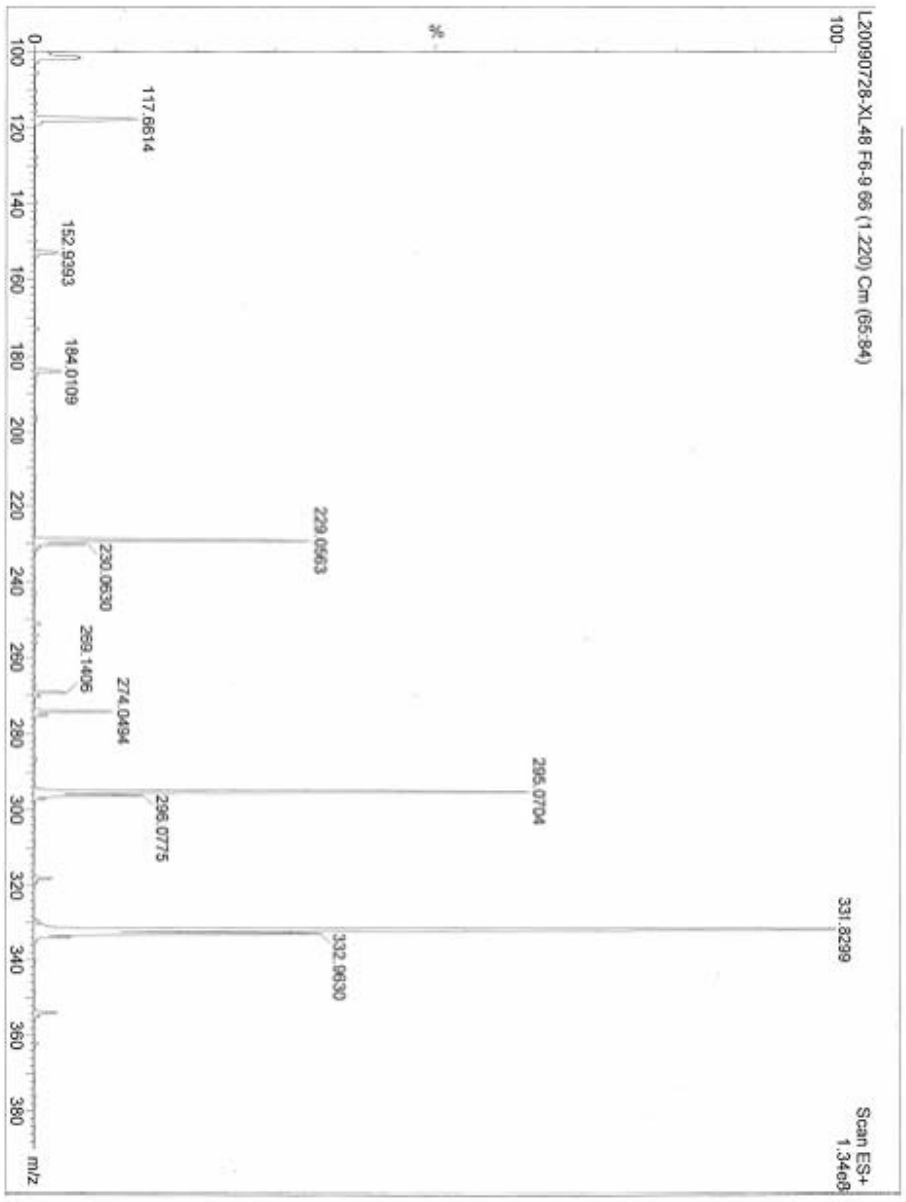




N-(6-Aminopyren-1-yl)-3-(dimethylamino)propanamide (**3.7**)

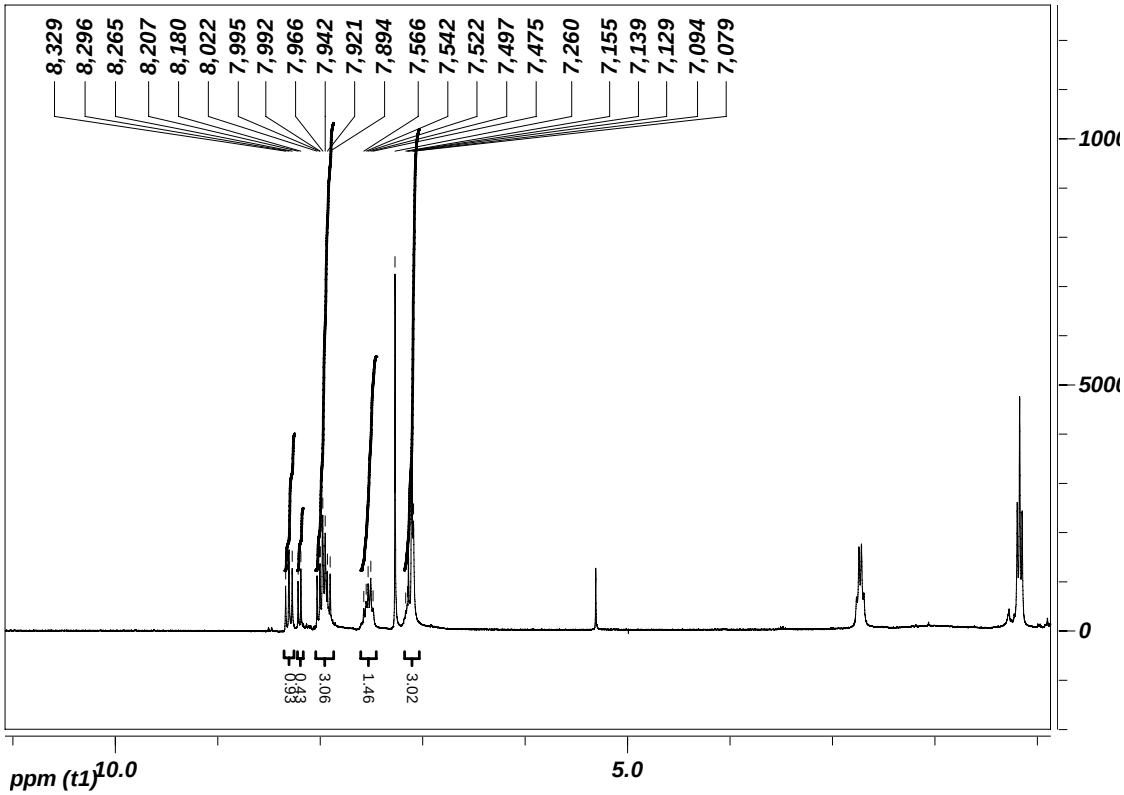


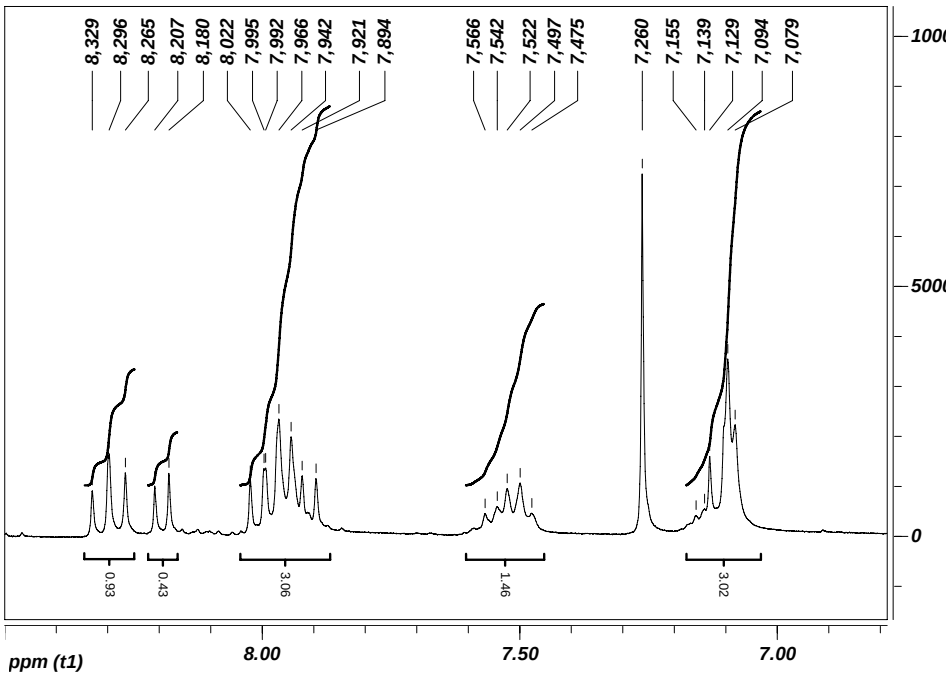
ESI^+-MS



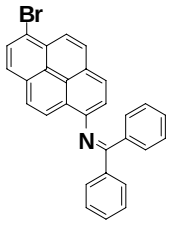
6-Bromo-N-(diphenylmethylene)pyren-1-amine (**3.8**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)

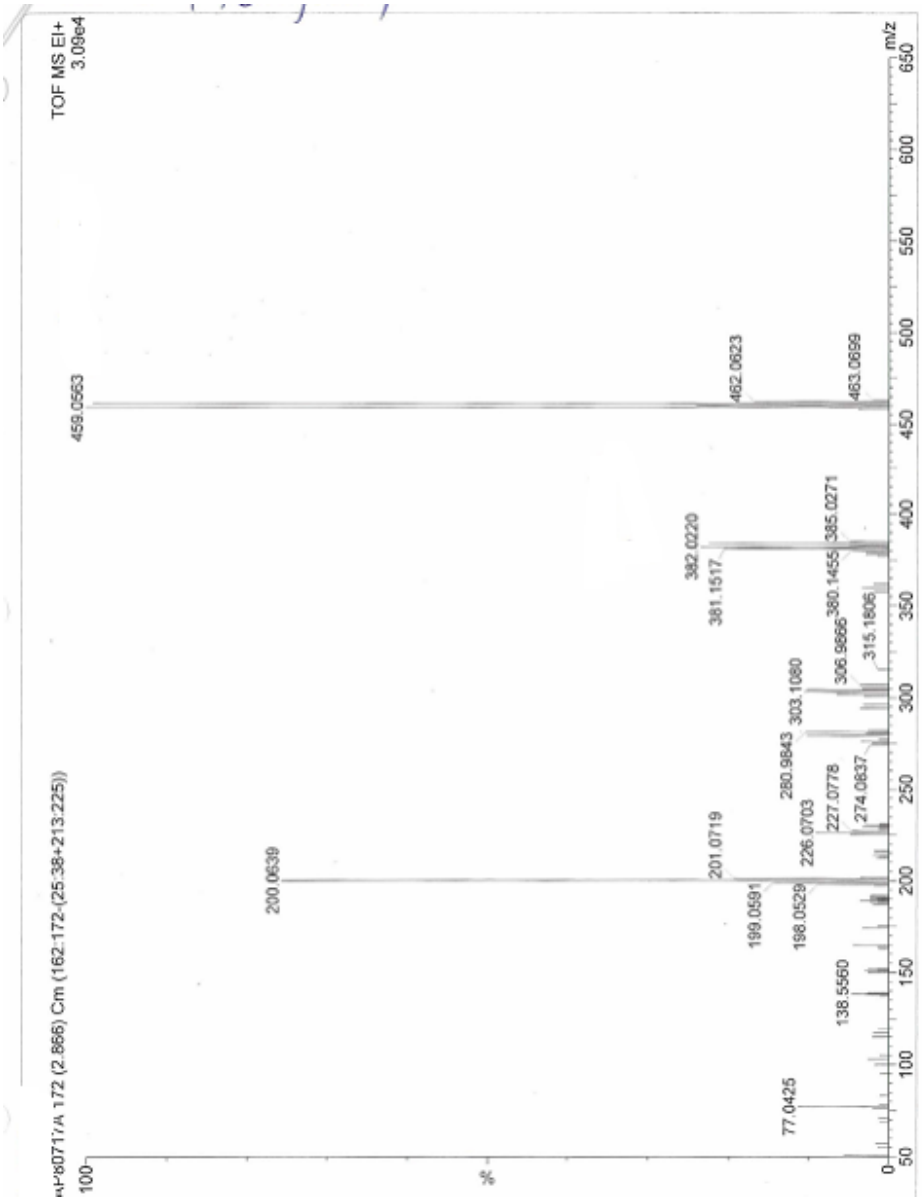


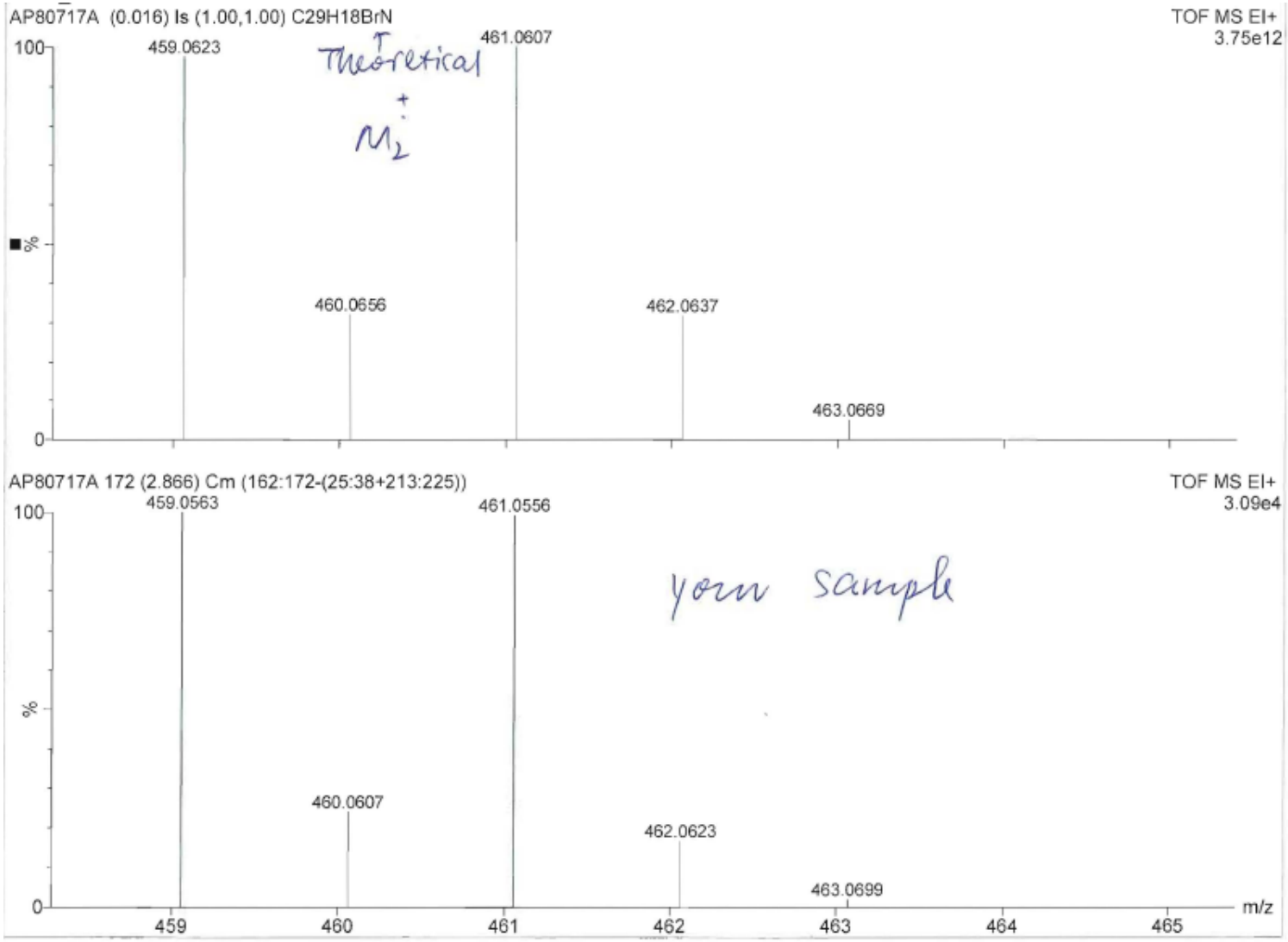


6-Bromo-N-(diphenylmethylene)pyren-1-amine (**3.8**)



EI^+-MS:

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%
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TOF MS EI+
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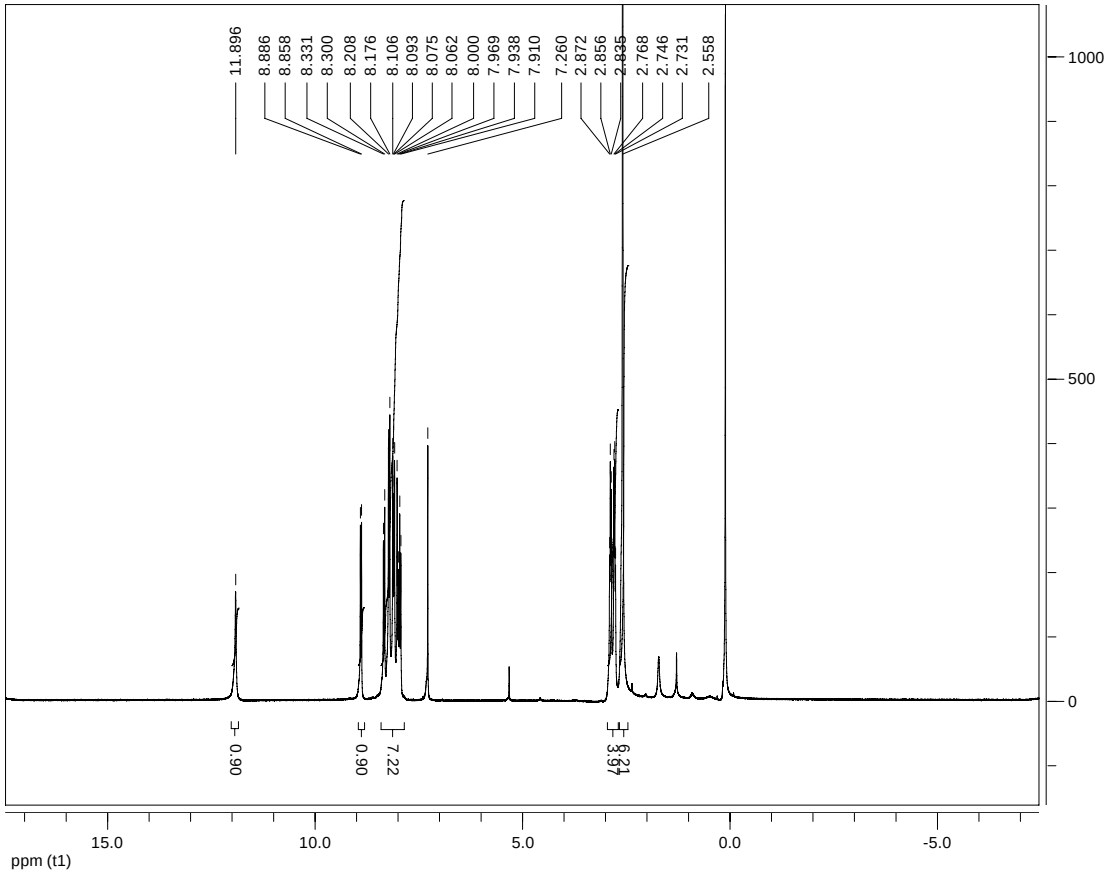
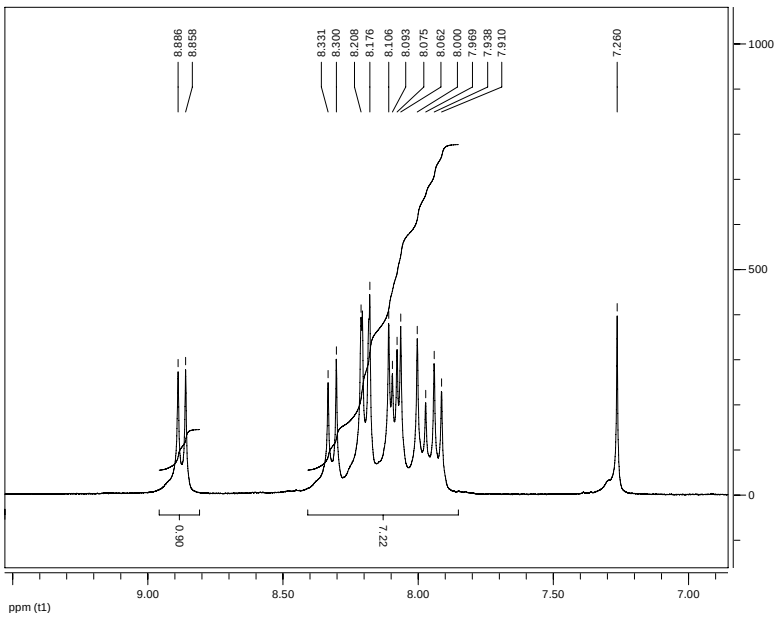
100
459.0563
461.0556
your sample
%
460.0607
462.0623
463.0699
0
m/z
459
460
461
462
463
464
465

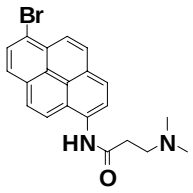
N-(6-Bromopyren-1-yl)-3-(dimethylamino)propanamide (**3.11**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

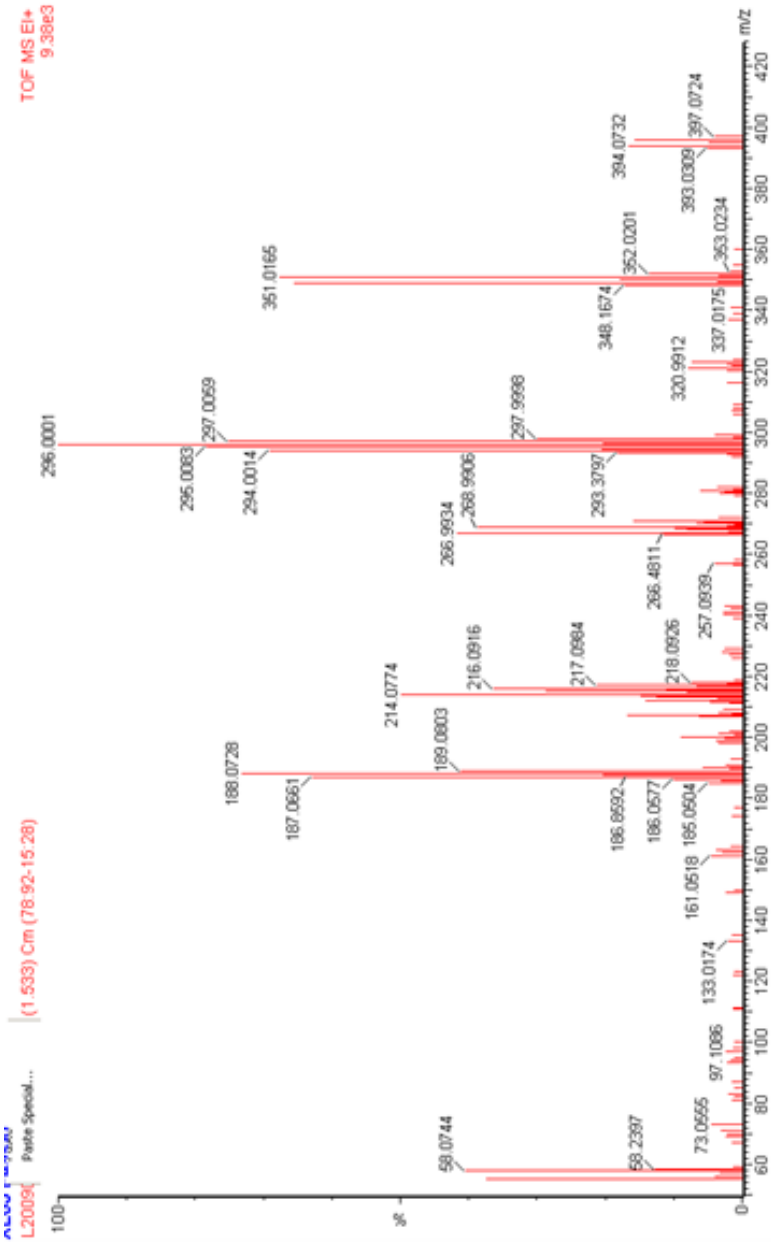




N-(6-Bromopyren-1-yl)-3-(dimethylamino)propanamide (**3.11**)

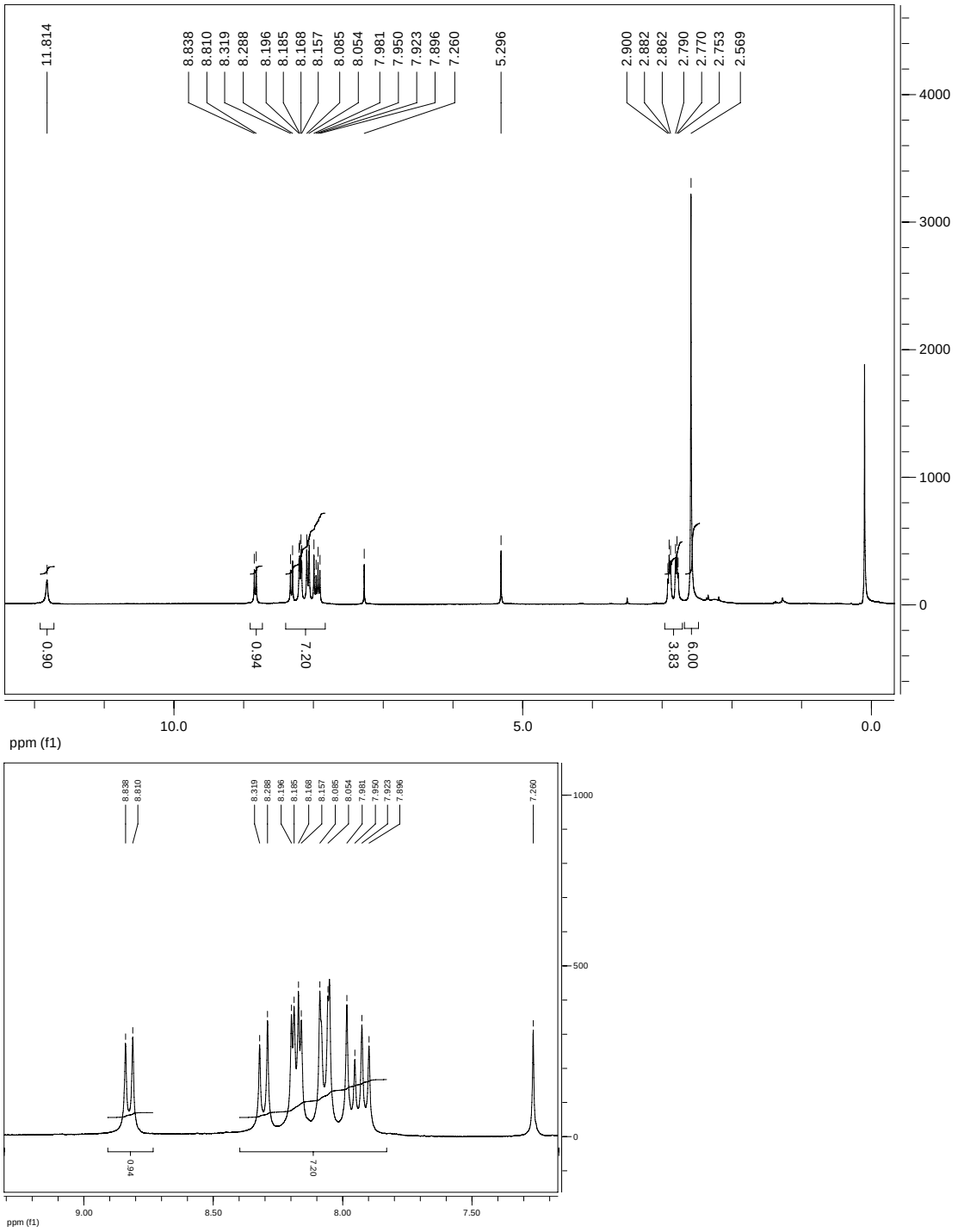


EI^+-MS



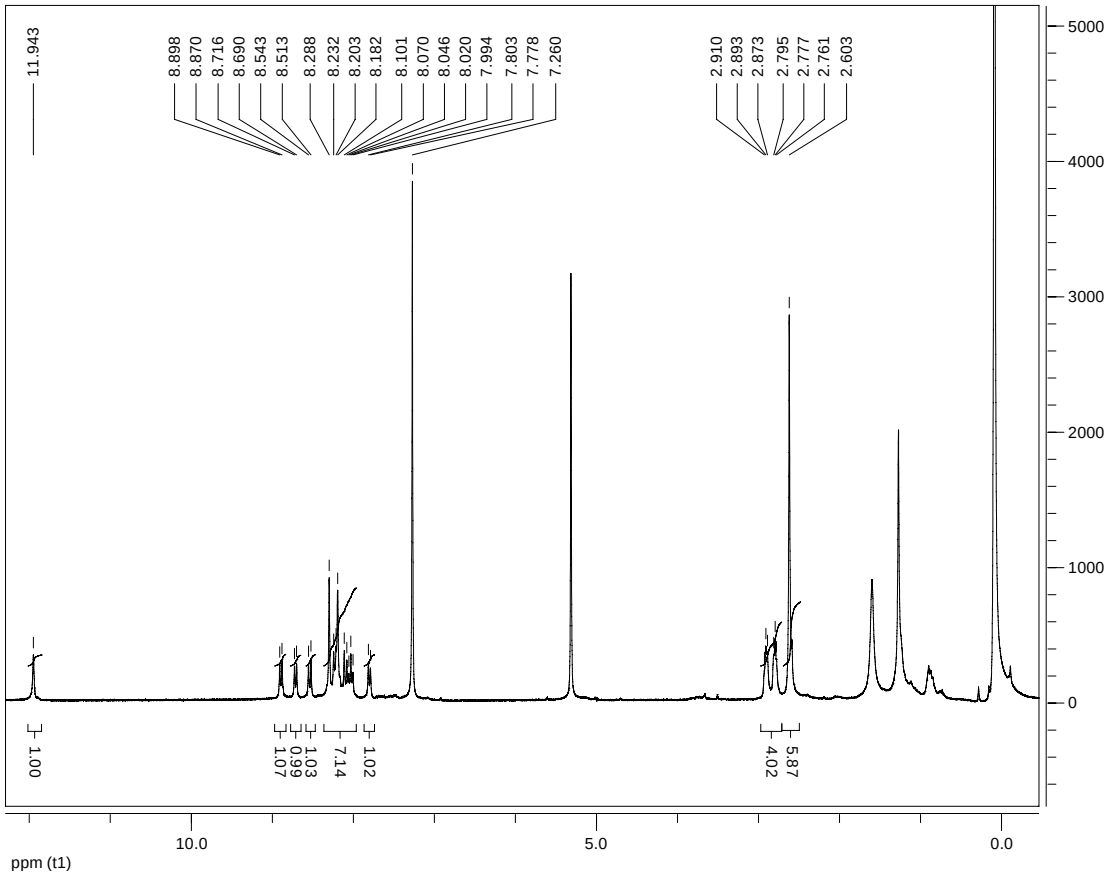
3-(Dimethylamino)-N-(6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)propanamide (**4.3**)

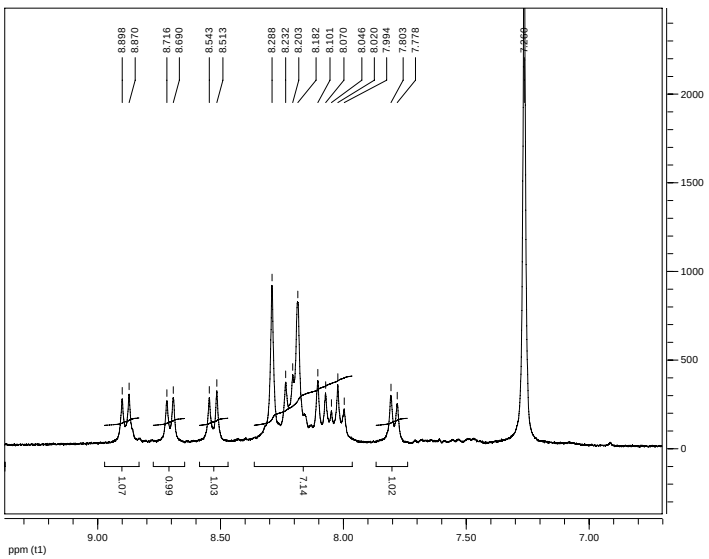
^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)



N,N'-(6,6'-(2,2'-Bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide)(**4.4**)

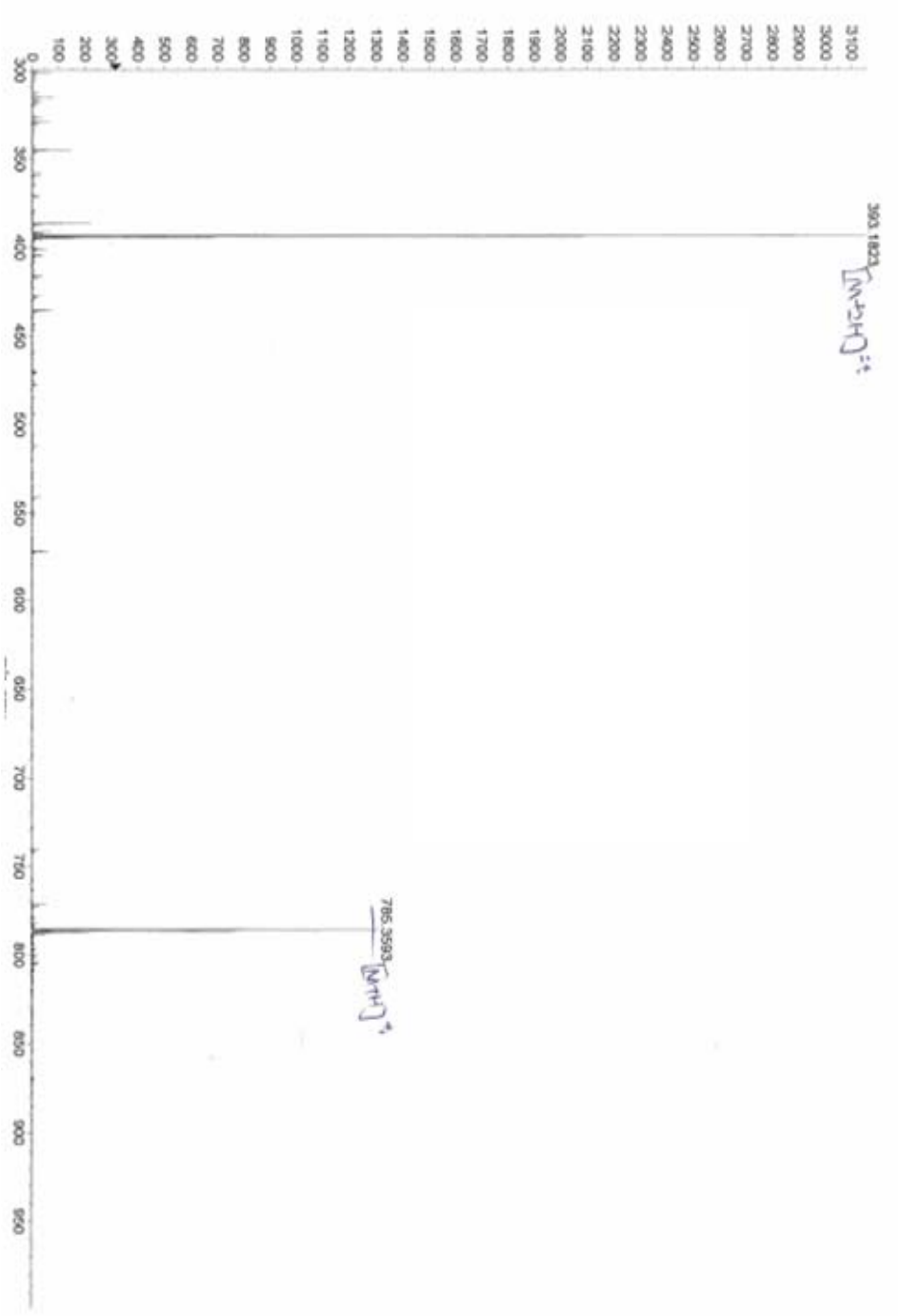
^{1}**H NMR** (300 MHz; $CDCl_3$, 25 ^{o}C)





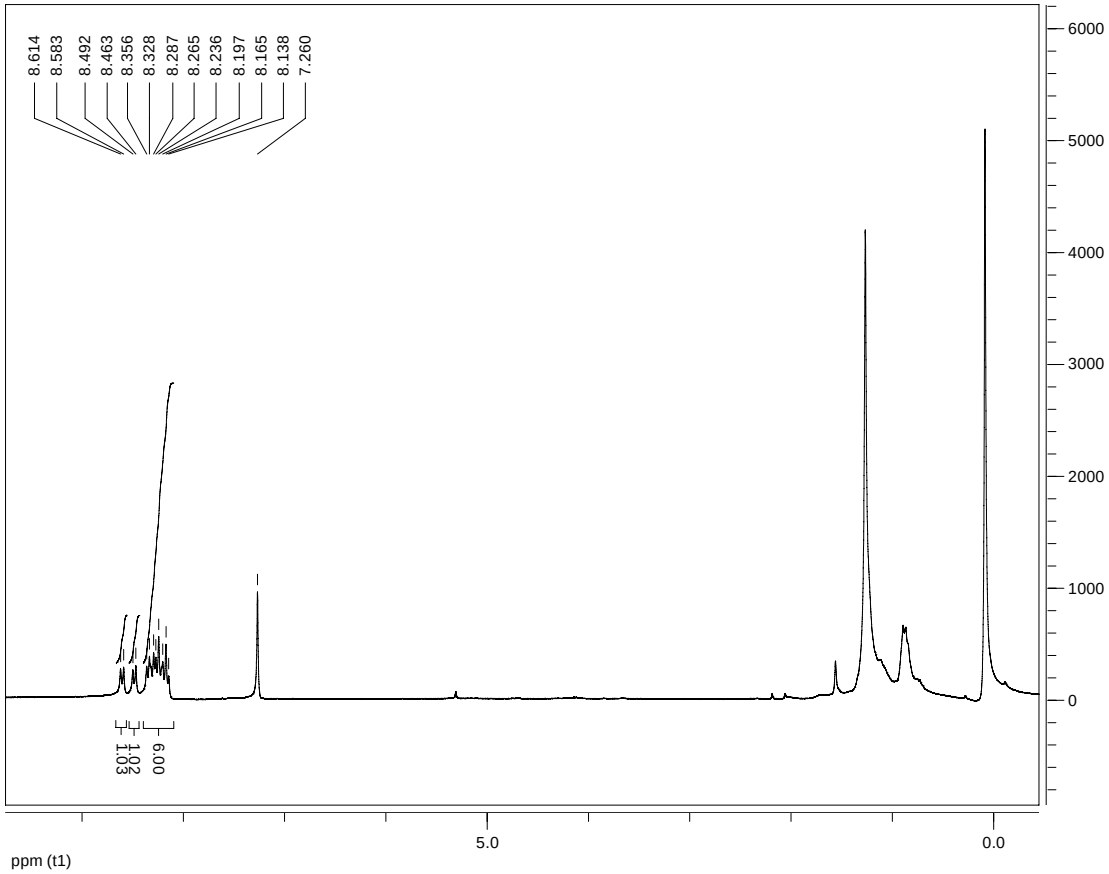
N,N'-(6,6'-(2,2'-Bipyridine-6,6'-diyl)bis(pyrene-6,1-diyl))bis(3-(dimethylamino)propanamide)(**4.4**)

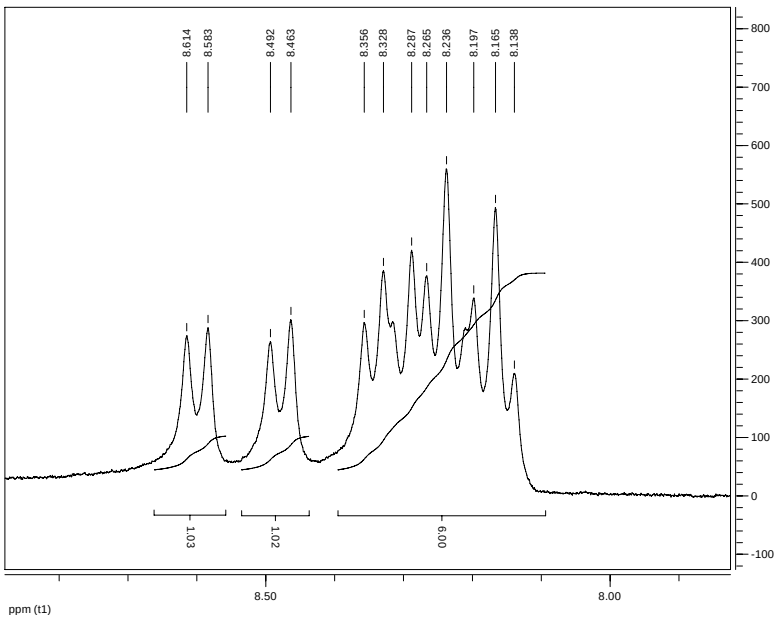
ESI^+-HRMS



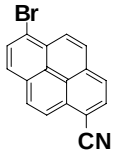
6-Bromopyrene-1-carbonitrile (**3.12**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

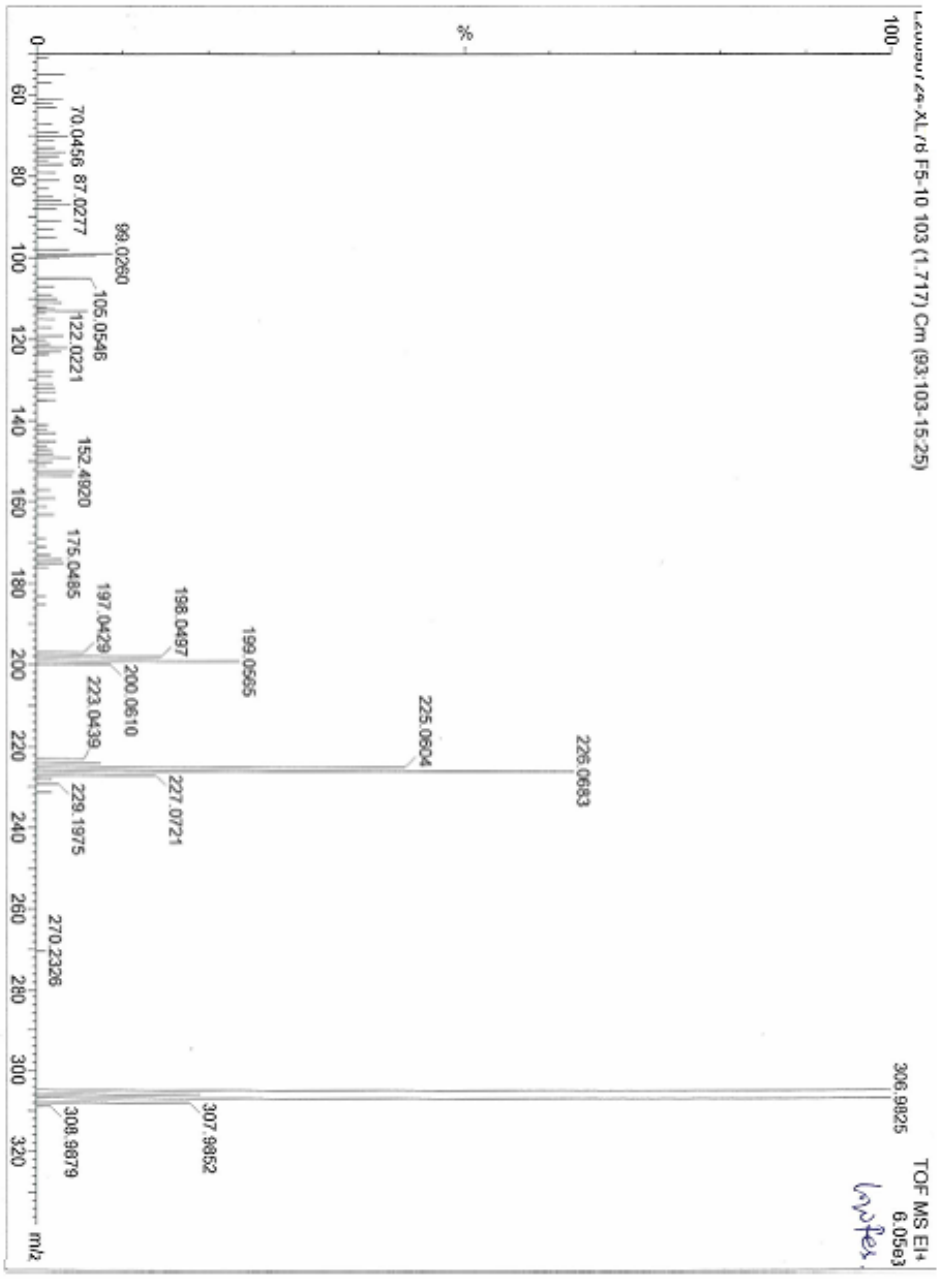


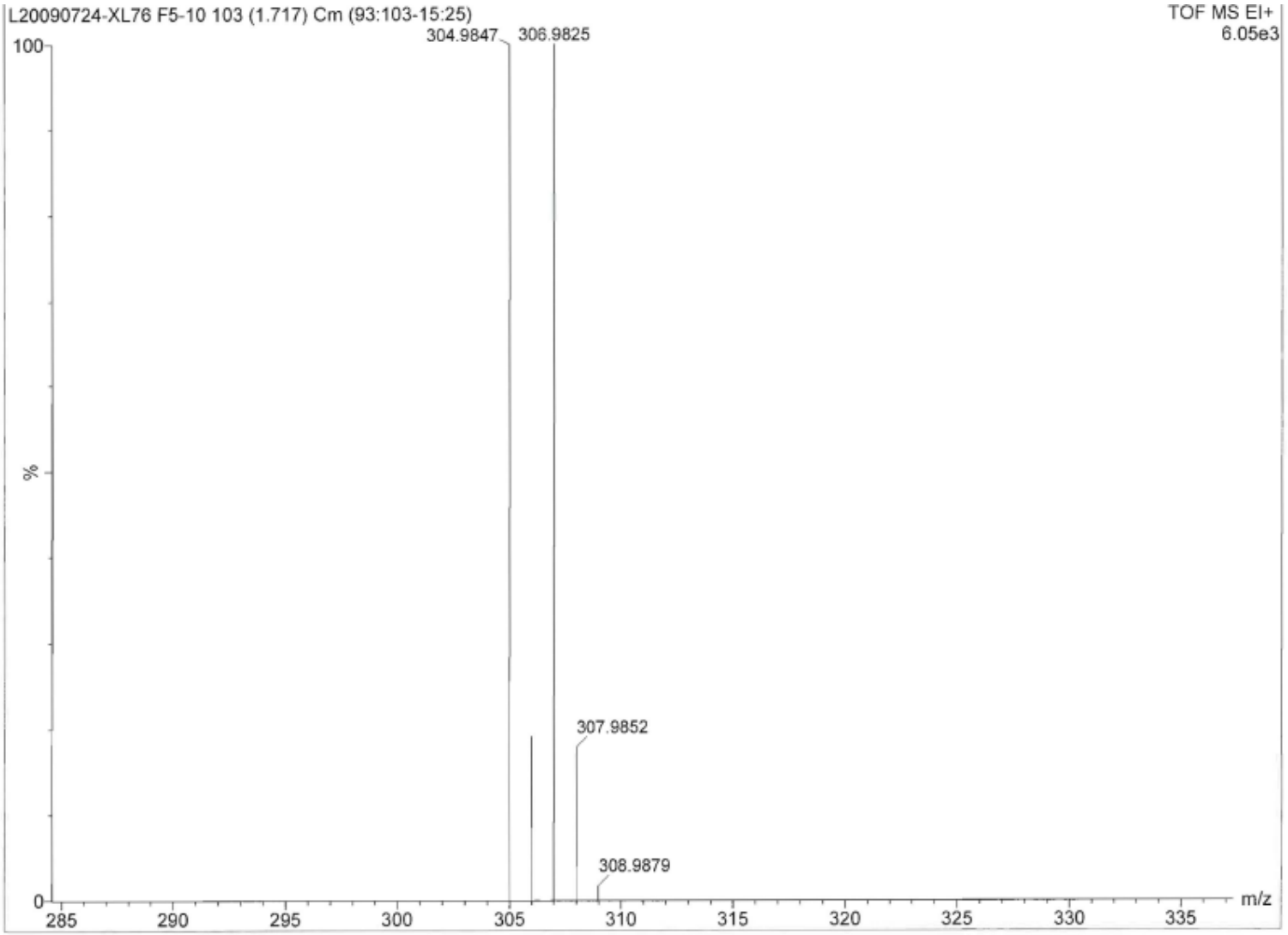


6-Bromopyrene-1-carbonitrile (**3.12**)



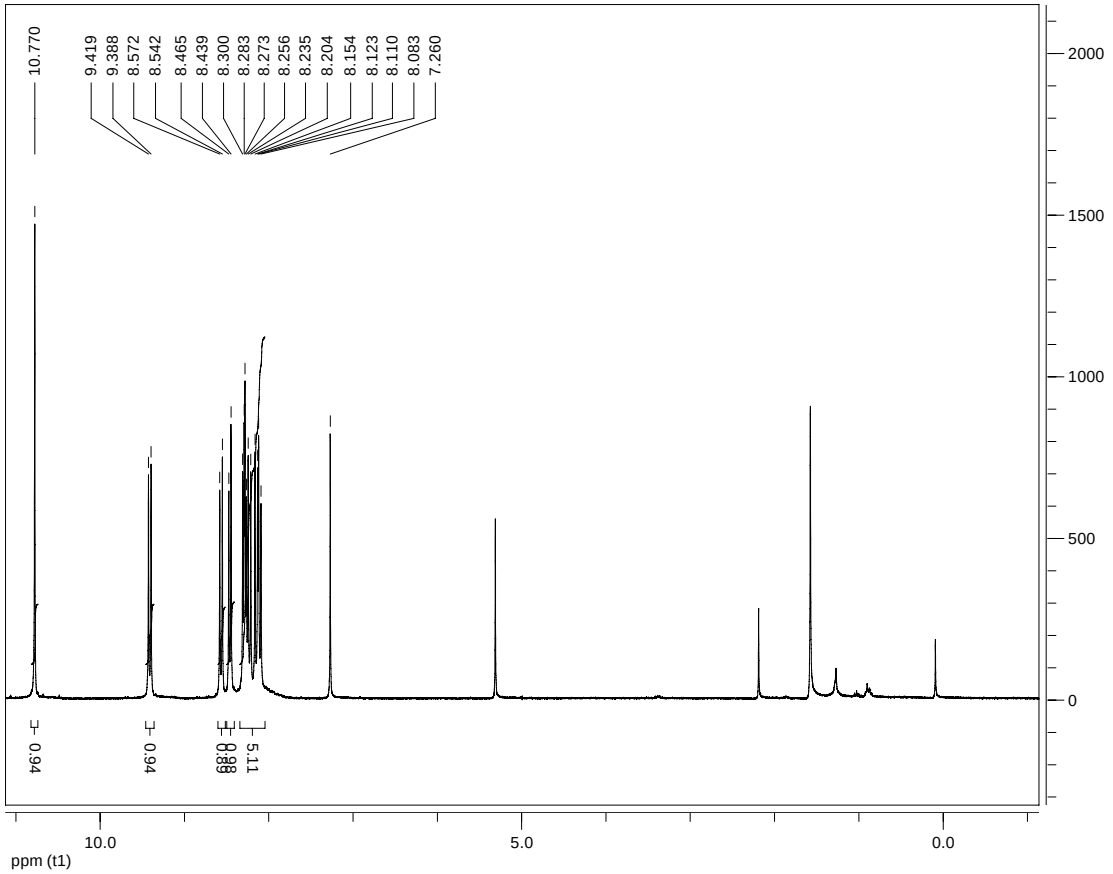
EI^+-MS

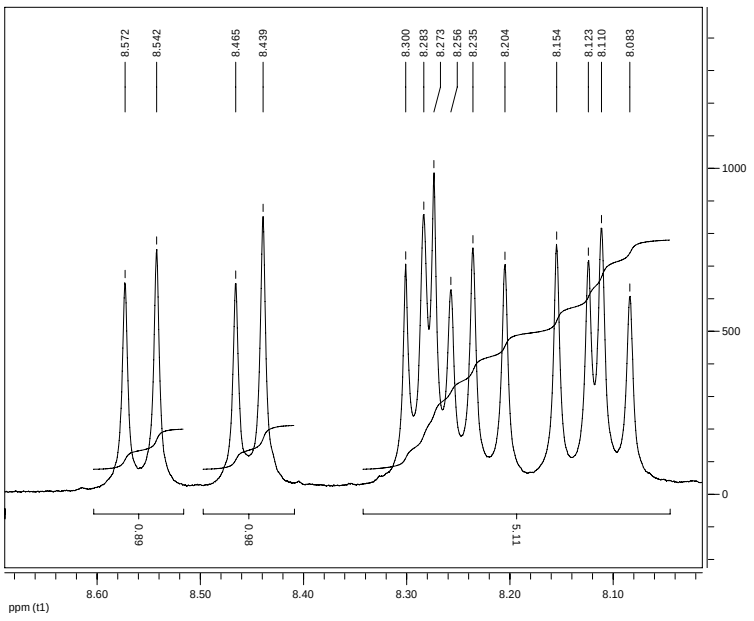



L20090724-XL76 F5-10 103 (1.717) Cm (93:103-15:25)
TOF MS EI+
6.05e3
304.9847
306.9825
307.9852
308.9879
100
%
0
285
290
295
300
305
310
315
320
325
330
335
m/z

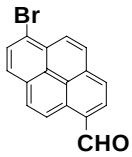
6-Bromopyrene-1-carbaldehyde (**3.13**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

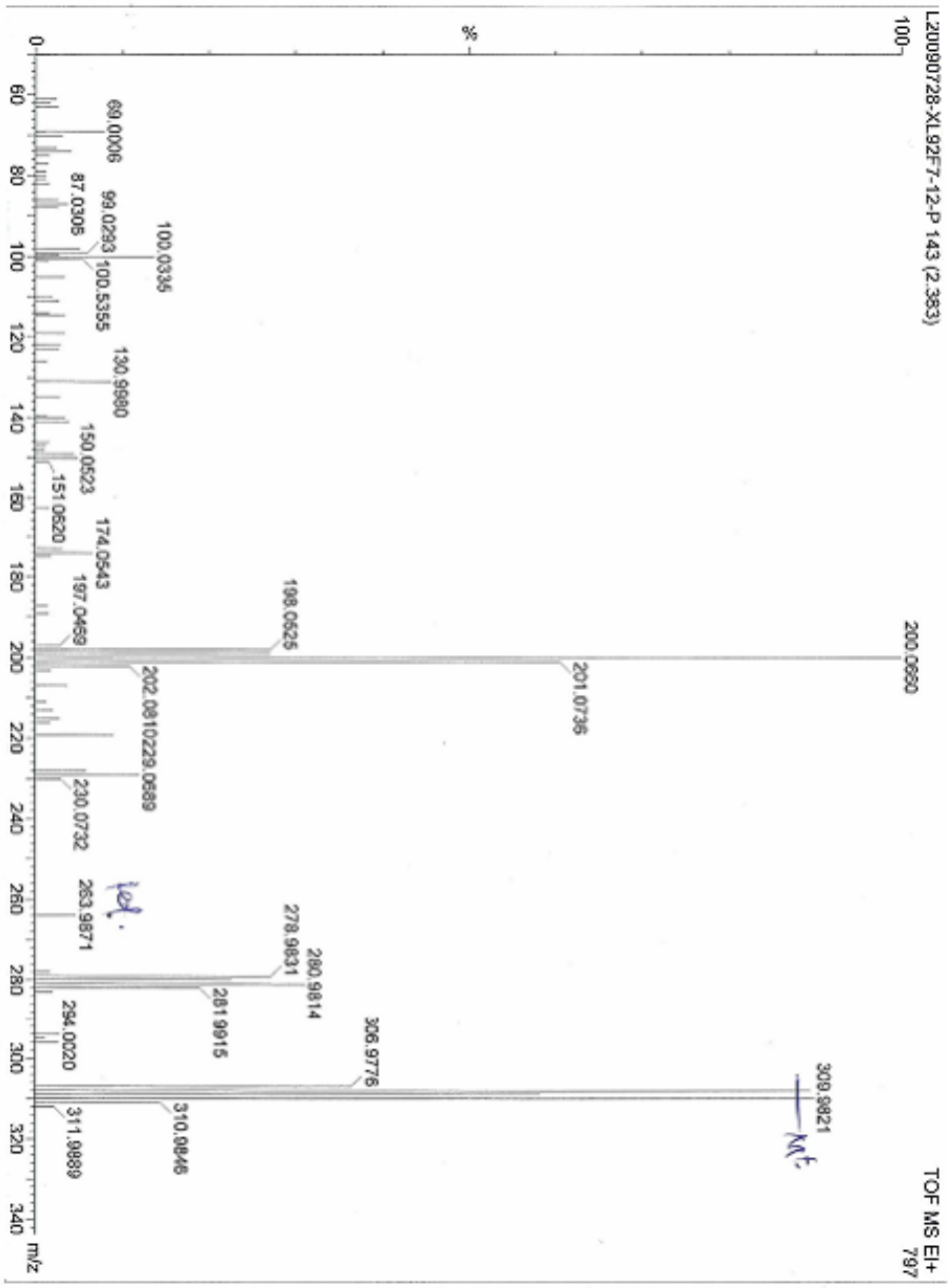


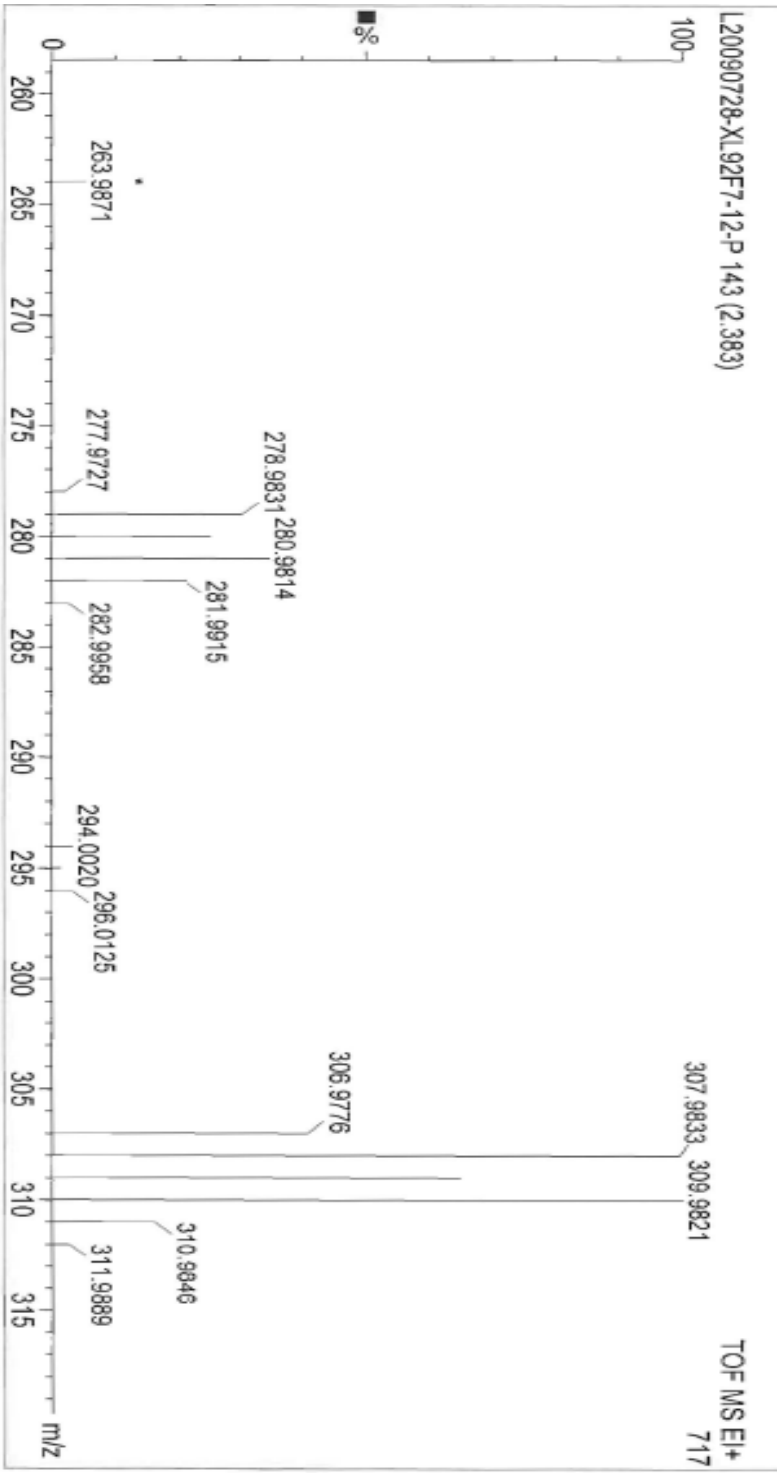


6-Bromopyrene-1-carbaldehyde (**3.13**)



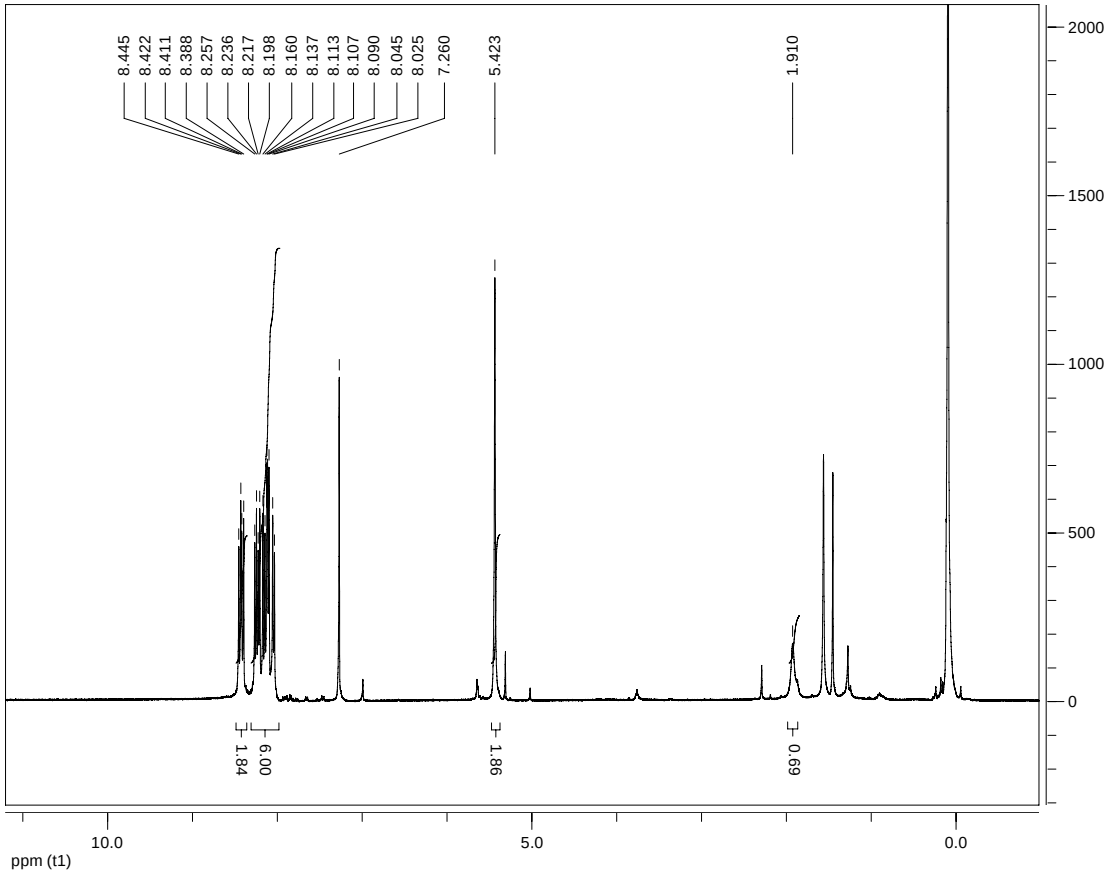
EI^+-MS

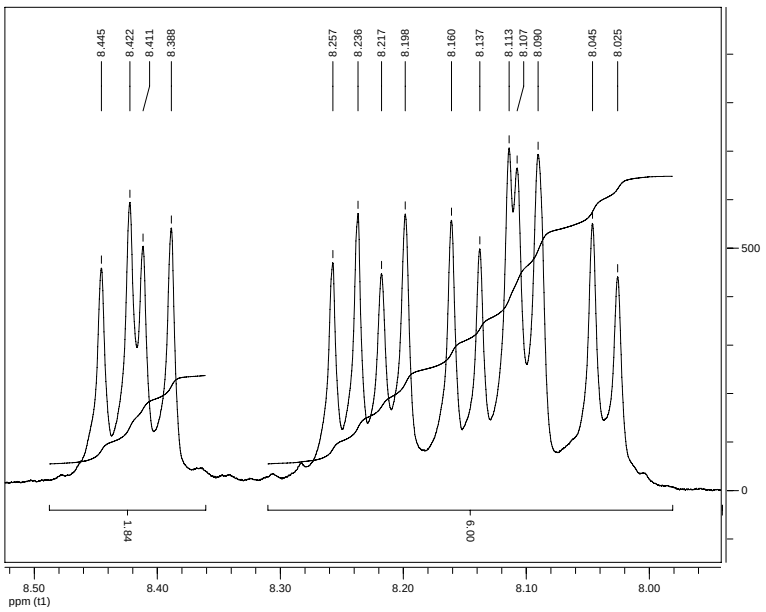



L20090728-XL92F7-12-P 143 (2.383)
TOF MS EI+
717
100
%
0
260
265
270
275
280
285
290
295
300
305
310
315
m/z
263.9871
277.9727
278.9831
280.9814
281.9915
282.9958
294.0020
296.0125
306.9776
307.9833
309.9821
310.9846
311.9889

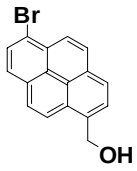
(6-Bromopyren-1-yl)methanol (**3.14**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

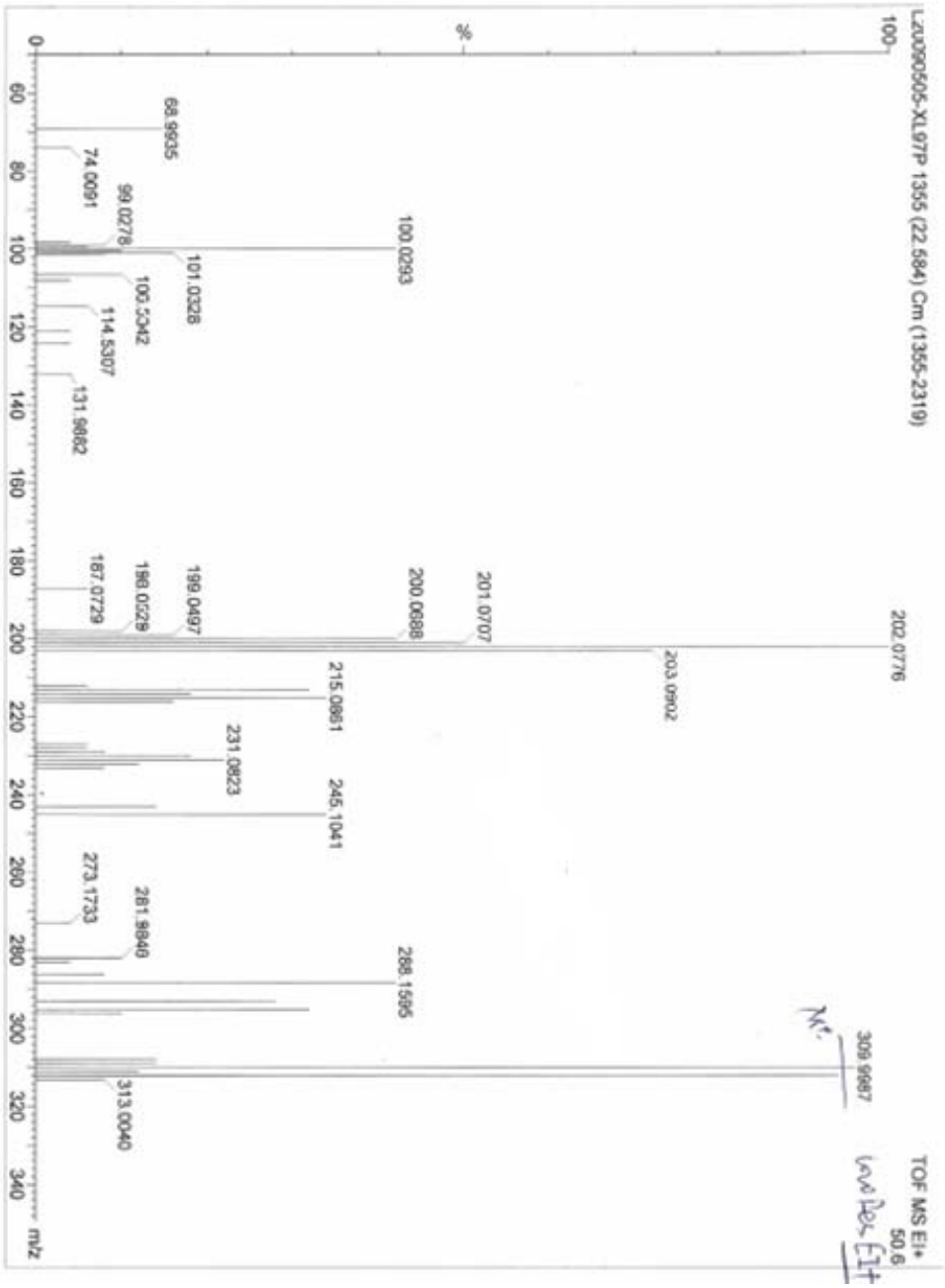


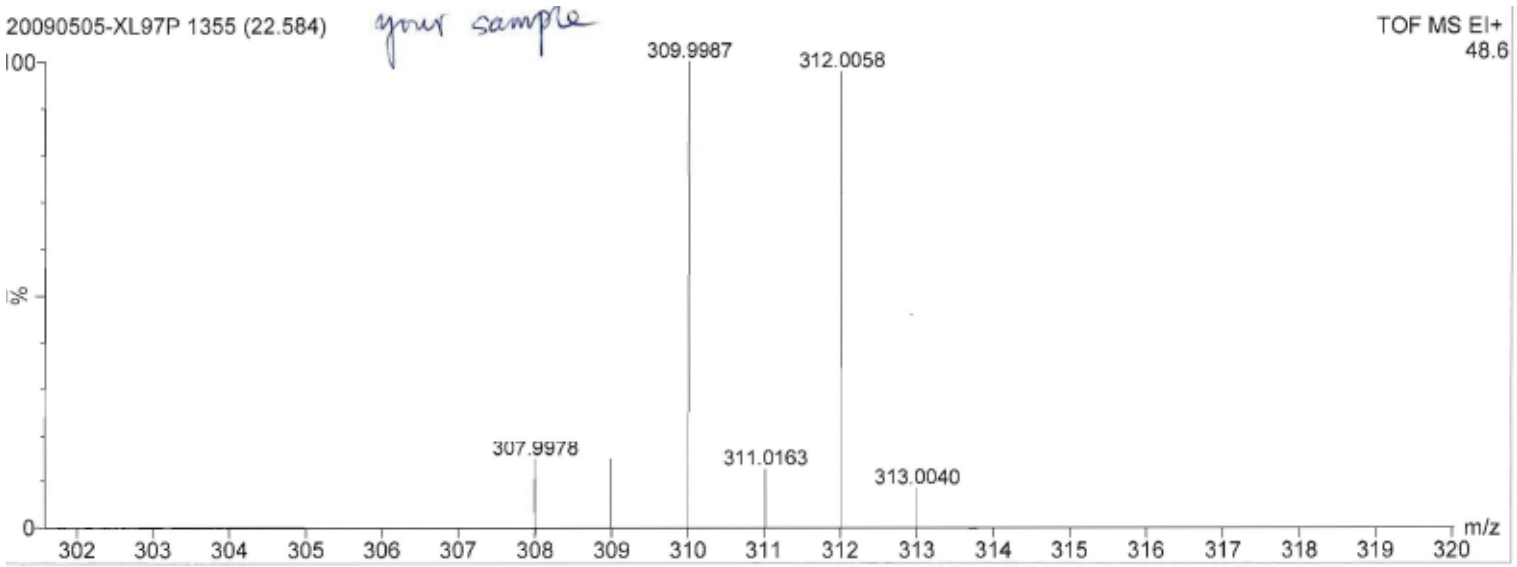


(6-Bromopyren-1-yl)methanol (**3.14**)



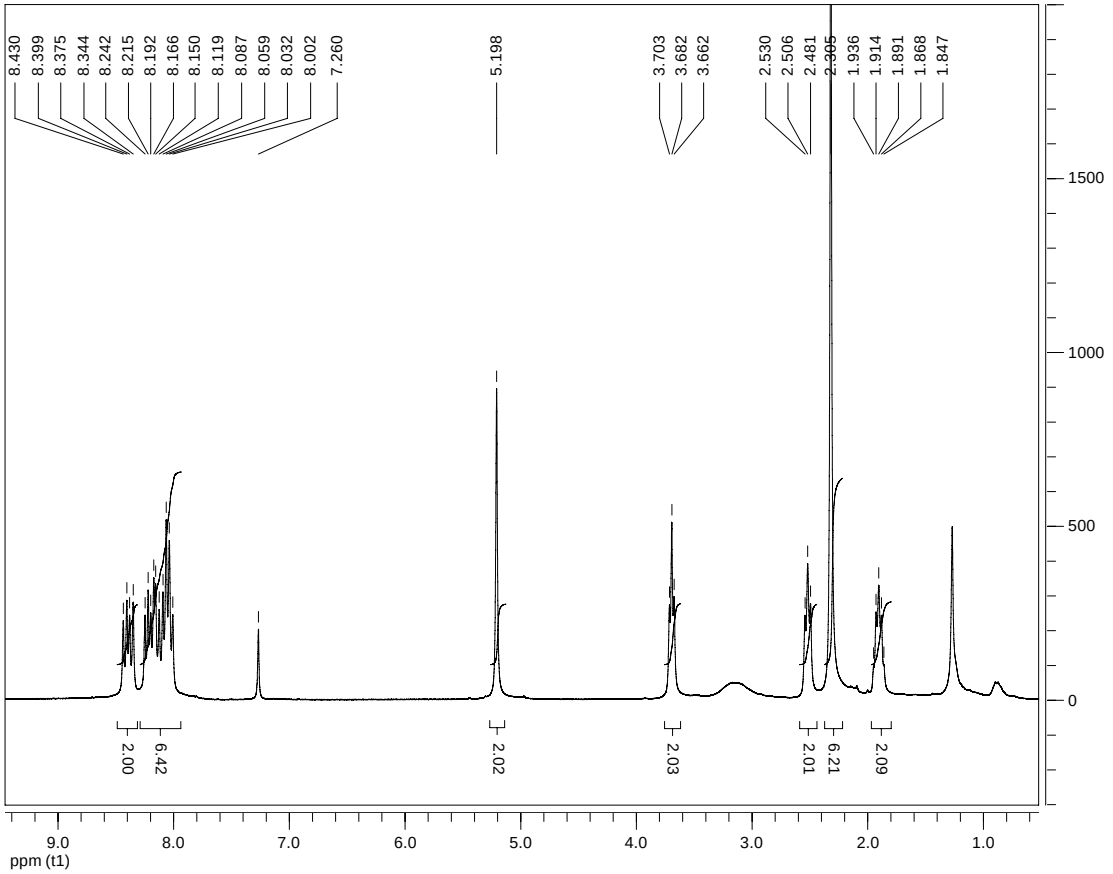
EI^+-MS

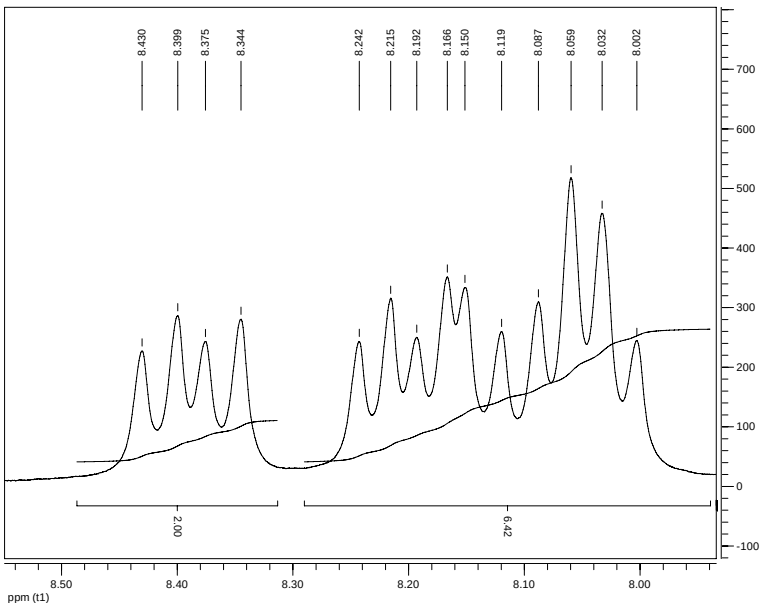



20090505-XL97P 1355 (22.584)
your sample
TOF MS EI+
48.6
100
%
0
309.9987
312.0058
307.9978
311.0163
313.0040
m/z
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320

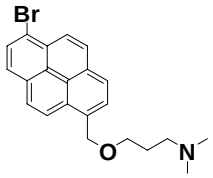
3-((6-Bromopyren-1-yl)methoxy)-N, N-dimethylpropan-1-amine (**3.15**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^oC)

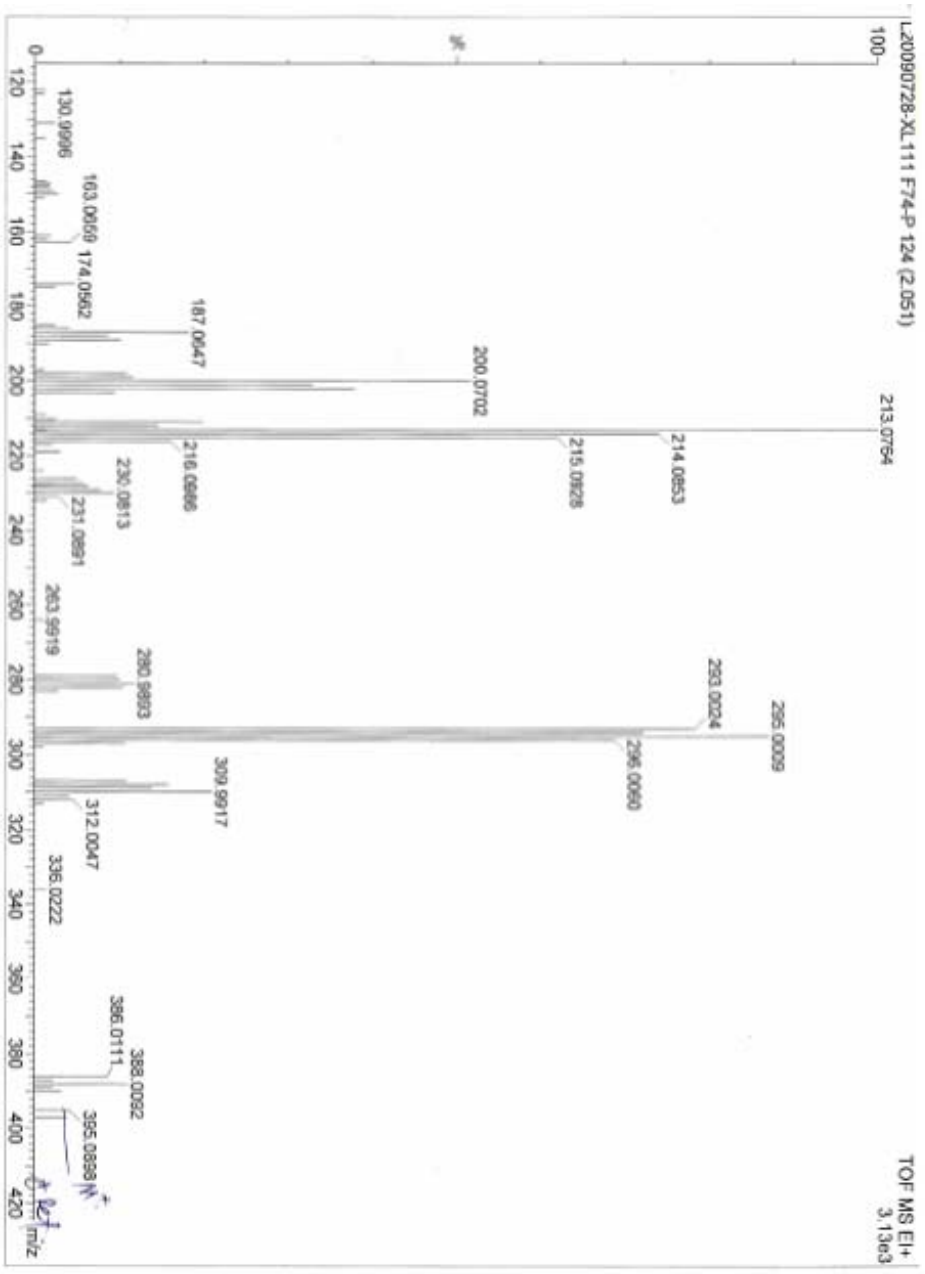


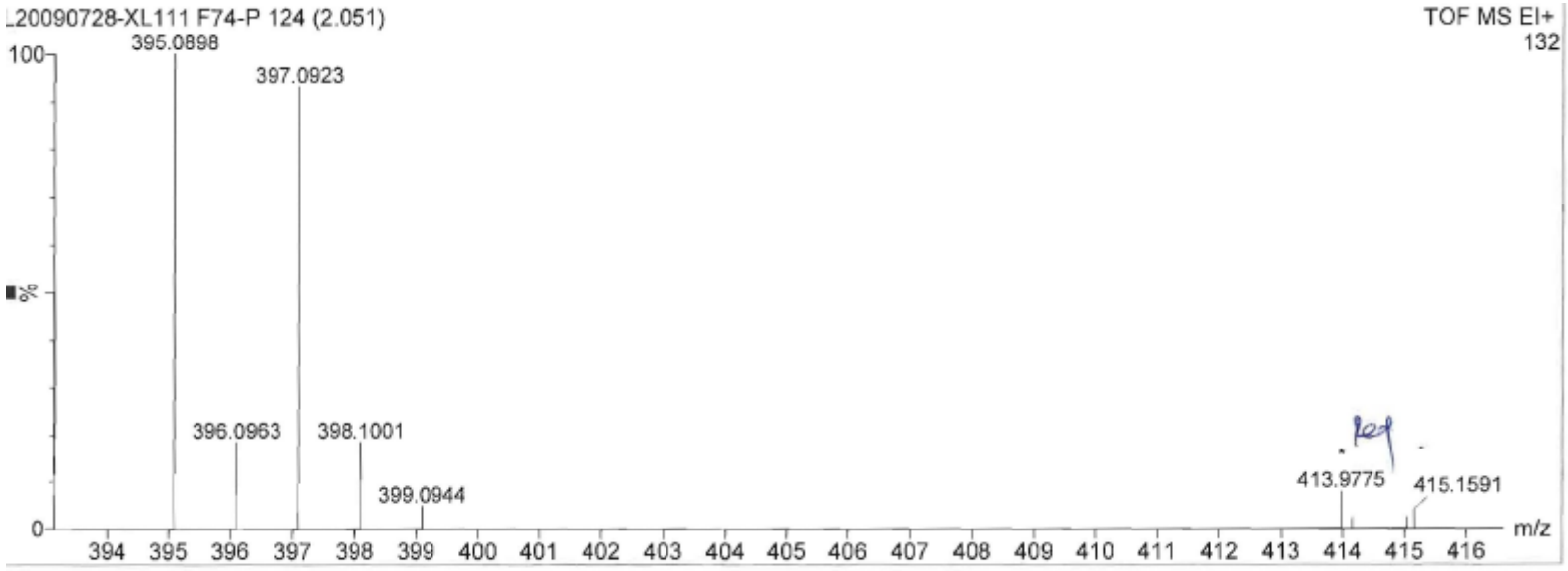


3-((6-Bromopyren-1-yl)methoxy)-N, N-dimethylpropan-1-amine (**3.15**)



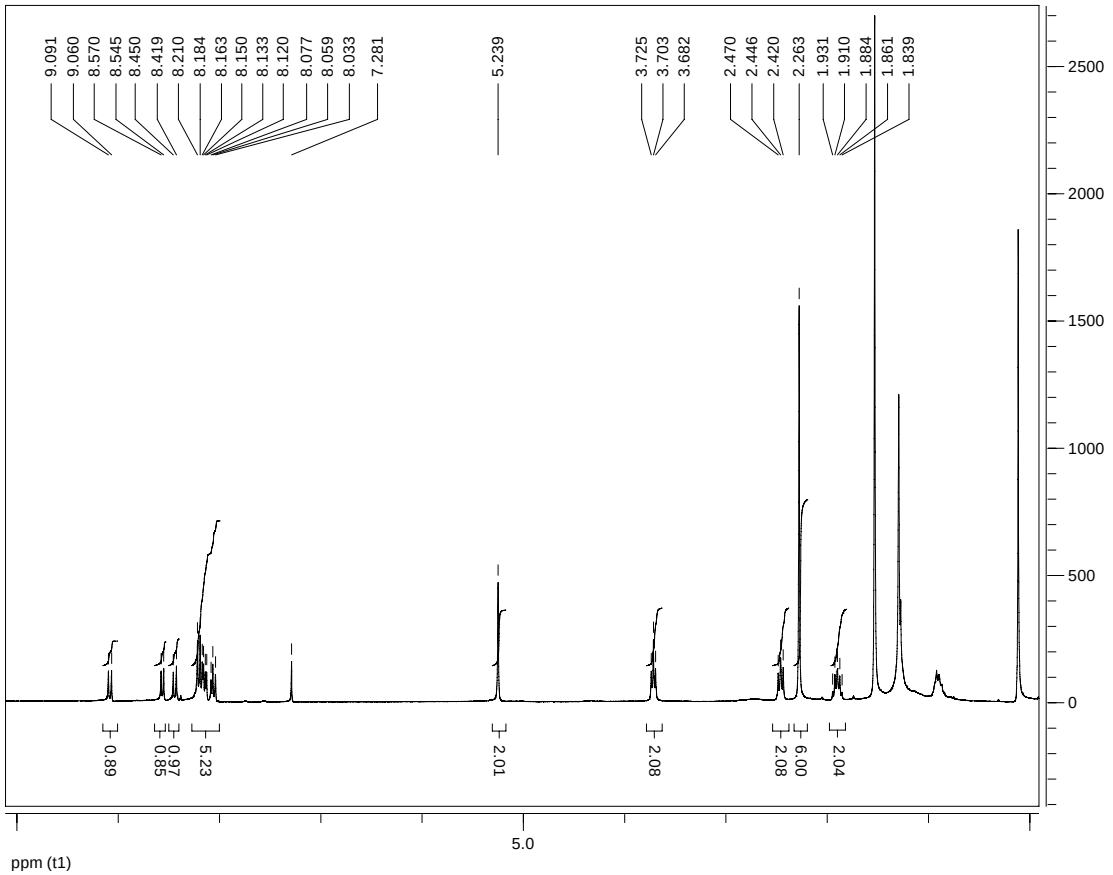
EI^+-HRMS

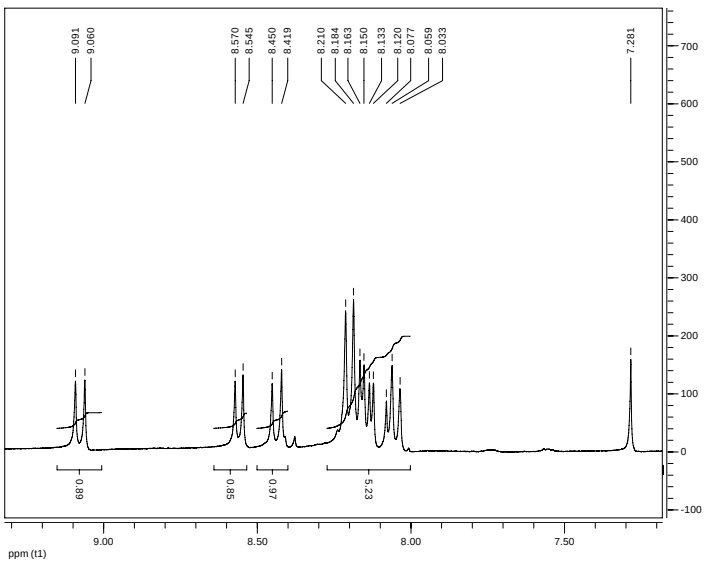



_20090728-XL111 F74-P 124 (2.051)
TOF MS EI+
132
100
%
0
395.0898
397.0923
396.0963
398.1001
399.0944
413.9775
415.1591
m/z
394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416

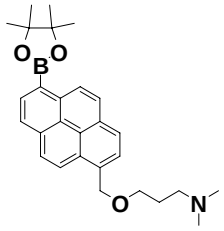
N,N-Dimethyl-3-((6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)methoxy)propan-1-amine (**4.4**)

^{1}**H NMR** (300 MHz, $CDCl_3$, 25 ^{o}C)

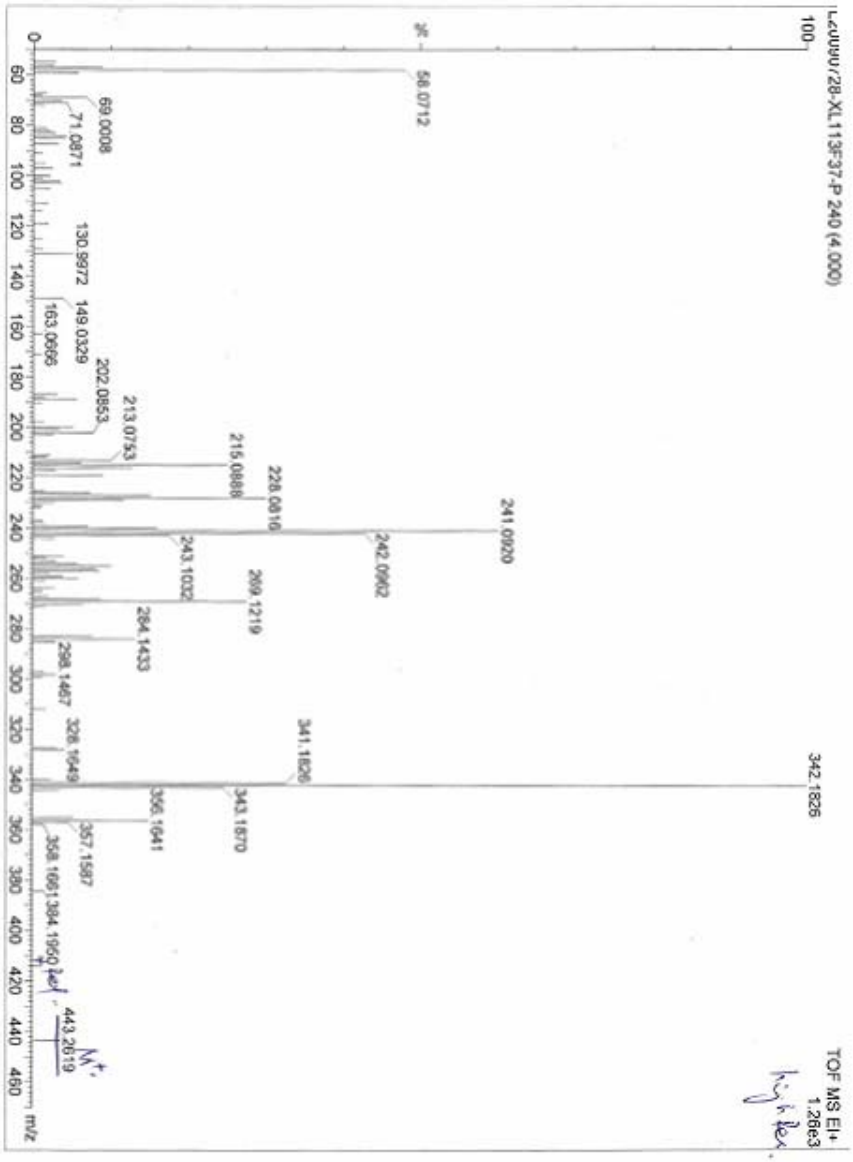




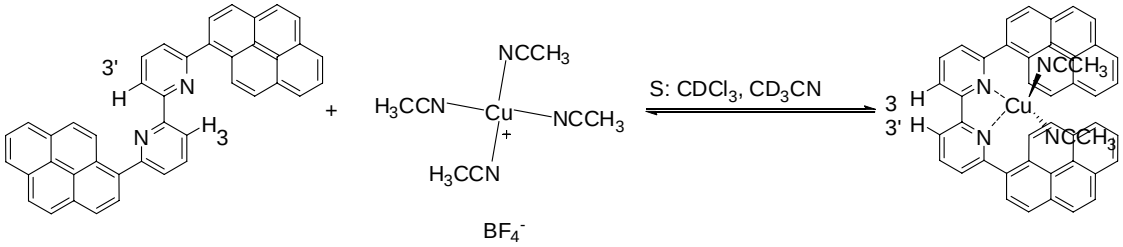
N,N-Dimethyl-3-((6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyren-1-yl)methoxy)propan-1-amine (**4.4**)



EI^+-HRMS



Model A + 0.5 equiv. Cu (I) $(CNCH_3)_4BF_4$



ESI^+-MS