



SCHOOL of  
GRADUATE STUDIES  
EAST TENNESSEE STATE UNIVERSITY

East Tennessee State University  
**Digital Commons @ East  
Tennessee State University**

---

Electronic Theses and Dissertations

Student Works

---

5-2008

# Toward the Synthesis of Nuclease Models.

Enni Nina Fomumbod  
*East Tennessee State University*

Follow this and additional works at: <https://dc.etsu.edu/etd>



Part of the [Enzymes and Coenzymes Commons](#)

---

## Recommended Citation

Fomumbod, Enni Nina, "Toward the Synthesis of Nuclease Models." (2008). *Electronic Theses and Dissertations*. Paper 1912.  
<https://dc.etsu.edu/etd/1912>

This Thesis - Open Access is brought to you for free and open access by the Student Works at Digital Commons @ East Tennessee State University. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons @ East Tennessee State University. For more information, please contact [digilib@etsu.edu](mailto:digilib@etsu.edu).

Toward the Synthesis of Nuclease Models

---

A thesis  
presented to  
the faculty of the Department of Chemistry  
East Tennessee State University

In partial fulfillment  
of the requirements for the degree  
Master of Science in Chemistry

---

by

Enni Nina Fomumbod

May 2008

---

Ismail O. Kady, Ph.D, Chair

David Young, Ph.D

Yu Lin Jiang, Ph.D

Keyword: Synthetic Nucleases, Synthetic Probes, Metallonucleases

## ABSTRACT

### Toward the Synthesis of Nuclease Models

by

Enni Nina Fomumbod

Nucleases are enzymes that can specifically recognize nucleic acids and hydrolyze their phosphodiester bonds effectively. As is the case with many hydrolases, nucleases often carry one or more metal centers. Cooperation between such metal centers and other interactions involving general acid-base activities are believed to be essential in multifunctional catalyses. Combination of such interactions in model compounds often resulted in larger than additive effects.

This work is aimed at synthesizing nuclease models that combine the ability to recognize phosphate groups and/or nitrogen bases of DNA together with the ability to catalyze phosphodiester hydrolysis. These models were designed to achieve optimum interaction between the recognition and the catalytic functionalities. Towards this goal, we chose phenonthiazonium ions (methylene blue analogues) and anthracene as spacers.

## DEDICATION

In loving memory of my dear uncle, John Tayoh; and to Mom and Dad and the  
Boys. Love you all so much

## ACKNOWLEDGEMENTS

Many thanks to East Tennessee State University for giving me the opportunity to come here to pursue my master's degree in chemistry. Studying here at ETSU has been an awesome experience; one for which I will remain grateful.

I would like to express my sincere gratitude to the faculty and staff of the Department of Chemistry for all their support and for imparting knowledge in me.

My appreciation goes to my thesis committee especially Dr. Ismail Kady, my supervisor. It has been a great experience working with him on this project. Many thanks to Dr. David Young and Dr. Yu Lin Jiang for helping me and providing me with material support and serving as members of my thesis committee.

Support from my friends and family has been the pillar of my success here at ETSU. On a special note, I want to thank my dear parents, Stella and Wilfred Fomumbod, for their ever-growing love and support. I would not have gone through this work without them. To my friends here at ETSU, I want to say thank you for all the home work we did together, especially my Cameroonian family of friends. To Auntie Grace, Kamah Bennah, Sharon Amsturtz, Samuel Siebo, Ryan and Katya Jackson, and Darnell Holt, all I can say is thank you for being there for me. And finally to my church family, the Covenant Presbyterian Church here in Johnson City, I want to extend my appreciation to everyone who took interest in me and contributed in one way or the other towards my success here in ETSU.

## TABLE OF CONTENTS

DEDICATION .....	3
ACKNOWLEDGEMENTS .....	4
LIST OF FIGURES .....	9
LIST OF SCHEMES.....	10
1. INTRODUCTION .....	11
Applications of Synthetic Probes.....	11
Related Features of DNA (and RNA).....	14
Related Features of Enzymes.....	17
Molecular Recognition and Complimentarity .....	17
Modes of Interaction.....	19
Hydrogen Bonding Interaction .....	20
$\pi$ -Stacking Interactions .....	20
Natural and Synthetic Metallonucleases.....	22
Natural and Synthetic Nonmetallonucleases .....	26
2. RESULTS AND DISCUSSION .....	32
Reaction of TBO with Maleic Anhydride.....	32
Reaction of Reduced TBO with Maleic Anhydride.....	32
Reaction of para-Nitroaniline with Maleic Anhydride .....	34
Reaction of para-Nitroaniline with 2-Bromoethanol .....	34
Synthesis of Toluidine Blue O .....	35
Synthesis of 3-Nitro-p-toluidine .....	36

Conversion of 3-Nitro-para-toluidine to 3-Nitro-para-toluenethiocyanate.....	37
Synthesis of 1,8-Bisbromomethylanthracene .....	37
Reaction of 1,8-Bisbromomethylanthracene with Thymine.....	39
Method 1 .....	39
Method 2 .....	40
Method 3 .....	40
Method 4 .....	40
Reaction of Benzyl Iodide with Thymine.....	41
Synthesis of Dithyminylmercury .....	41
Reaction of Dithyminylmercury with Benzyl Iodide.....	42
Reaction of Thymine with Benzyl Bromide.....	43
Protection of 1,8-Bishydroxymethylanthracene .....	43
 3. EXPERIMENTAL.....	 45
Materials and Methods.....	45
Reaction of TBO with Maleic Anhydride.....	46
Reaction of Reduced Toluidine Blue with Maleic Anhydride.....	46
Reaction of para-Nitroaniline with Maleic anhydride .....	47
Reaction of p-Nitroaniline with 2-Bromoethanol.....	47
Synthesis of Toluidine Blue O.....	48
Synthesis of 3-Nitro-p-toluidine .....	48
Conversion of 3-Nitro-para-toluidine to 3-Nitro-para-toluene .....	48
Reaction of Thymine with 1,8-Bisbromomethylanthracene.....	49
Method 1 .....	49

Method 2 .....	49
Method 3 .....	50
Method 4 .....	50
Synthesis of Dithyminylmercury .....	51
Reaction of Dithyminylmercury with Benzyl Iodide.....	51
Reaction of Thymine with Benzyl Bromide (and Benzyl Iodide) .....	52
Method 1 .....	52
Method 2 .....	52
Protection of 1,8-Bishydroxymethylanthracene .....	53
4. CONCLUSION.....	54
BIBLIOGRAPHY.....	55
APPENDICES .....	58
APPENDIX A: <sup>1</sup> H NMR Spectrum of Product Isolated from Reaction of TBO with Maleic Anhydride in Acetone.....	58
APPENDIX B: <sup>1</sup> H NMR Spectrum of 4 .....	59
APPENDIX C: <sup>1</sup> H NMR Spectrum of Compound 5 .....	60
APPENDIX D: <sup>1</sup> H NMR Spectrum of Compound 6.....	61
APPENDIX E: <sup>1</sup> H NMR Spectrum of Product Isolated from Reaction of Thymine with 1,8-Bisbromomethylanthracene in DMSO .....	62
APPENDIX F1: <sup>1</sup> H NMR Spectrum of Product Obtained from First Fraction of Reaction of Benzyl Iodide with Dithyminylmercury.....	63



APPENDIX F2: $^1\text{H}$ NMR Spectrum Product Obtained from Second Fraction of Reaction of Benzyl Iodide with Dithymylmercury.....	64
APPENDIX G1: $^1\text{H}$ NMR Spectrum of Compound 12a.....	65
APPENDIX G2: $^1\text{H}$ NMR Spectrum of Compound 12b.....	66
APPENDIX H: $^1\text{H}$ NMR Spectrum Product Obtained from Protection of 1,8- Bishydroxymethylanthracene with TBDMS .....	67
APPENDIX I: $^1\text{H}$ NMR Spectrum of Benzyl Iodide.....	68
APPENDIX J: $^1\text{H}$ NMR Spectrum of 1,8-Bisbromomethylanthracene.....	69
VITA.....	70

## LIST OF FIGURES

Figure	Page
1. Double Helix of DNA; Phosphate Backbone.....	14
2. Forms of DNA.....	15
3. Intercalation of a Metal Complexes in DNA.....	16
4. Rigid Structure of a Hypothetical Binding Site.....	20
5. Schematic Presentation of Two-Site Approach to Nucleotide Base Recognition.....	21
6. Modes of Aromatic-Aromatic Interactions.....	21
7. Copper(II) Phenanthrolines.....	23
8. Interaction of BAG with Phosphodiester.....	27
9. Methylene Blue Homologs.....	28
10. Nuclease Models Based on Toluidine Blue O.....	30
11. Nuclease Models Based on 1,8-Disubstituted Anthracene.....	31

## LIST OF SCHEMES

Scheme	Page
1. Mechanism of Action of Alkaline Phosphatase.....	22
2. The Binding of Copper(II) Phenanthroline to DNA.....	24
3. Phosphodiester Transesterification by a Dinuclear Metal Complex.....	24
4. Zn(II) Diaminopyridine Complexes.....	25
5. Proposed Mechanism of Model A.....	25
6. RNase-Catalyzed Hydrolysis of CpA.....	26
7. Mechanism of Hydrolysis of Phosphodiester.....	27
8. Illustration of Alkylation (or Acylation) of a 1° Amine.....	28
9. Proposed Mechanism of Phosphoester Hydrolysis by TBO-Based Models.....	30
10. Reaction of TBO with Maleic Anhydride.....	32
11. Reduction of TBO.....	33
12. Reaction p-Nitroaniline with Maleic anhydride.....	34
13. Reaction of p-Nitroaniline with 2-Bromoethanol.....	35
14. Outline of the Synthesis of Toluidine Blue O Derivative.....	36
15. Outline of Synthesis of 1,8-Bisbromomethylanthracene.....	38
16. Reaction of 1,8-Bisbromomethylanthracene with Thymine.....	39
17. Synthesis of Dithyminylmercury and its Reaction with Benzyl Iodide.....	41
18. Protection of 1,8-Bishydroxymethylanthracene with TBDMS.....	43

## 1. INTRODUCTION

### Applications of Synthetic Probes

The use of synthetic probes in molecular recognition and sequence-specific cleavage of DNA was the subject of intense research for the past 2 decades. These synthetic probes were designed as an alternative to relying on natural enzymes. Chemists had done enormous work in designing reagents that are complementary to the various segments of DNA; they could specifically tailor these probes to obtain useful structural information. Most often, metal complexes were incorporated in these experiments [1, 2, 3]. This is because the geometry of a metal complex and the structure of the ligand can be readily manipulated to acquire certain recognition features combined with catalytic activity toward cleaving DNA [4].

One important application of these reagents is in molecular biology, where they are used in DNA manipulation [5]. For example, the restriction endonucleases that are essential tools in molecular biology are used in the “cutting and pasting” of DNA sequences in recombinant DNA. Natural endonucleases are limited in number and in their DNA recognition. Four or six base pairs are the usual recognition sites for these enzymes. They are unable to recognize longer pieces, let alone an entire genome. Meanwhile, mapping and sequencing of the human genome requires enzymes or reagents that can recognize longer sequences. While it is unlikely to find a natural nuclease for a 15-base pair sequence, chemists had begun engineering metal-based complexes that could do the job. Moser and Dervan [6] found that a 15 nucleotide-long oligo(pyridine) strand, with an attached EDTA group, was able to recognize and cleave a specific 15-base pair long

oligo(purine)•oligo(pyridine) sequence within a 628 base pair long restriction fragment of plasmid DNA. In summary, research in the 1990s and before was focused on restriction enzyme analogues and their applications such as studying DNA structure and sequence determination, recombinant DNA manipulations, and gene isolation and analysis [5, 6]

This focus has intensified greatly in recent years, as more aggressive research is being done to address some of the most plaguing health problems of our century. Many of the terminal diseases nowadays are either directly or indirectly related to genetics. Two examples include the HIV/AIDS pandemic and cancer that are affecting the lives of many people all over the world today. The invention of new antiretroviral drugs requires good understanding of how the virus genetic information is being transcribed and how this process could be inhibited or interrupted. There is still need for better understanding of drug mechanisms in order to develop more potent drugs. Cancer on the other hand is ranked second in killer diseases after heart disease in the U.S today, claiming more than 556,902 lives in 2006, with an estimated 1.3 million cases for that year [7]. It is caused by factors identified mainly as either inherited or acquired (exposure to harmful radiation, toxins, etc). Irrespective of the cause, the illness is as a direct result of gene mutation. Enormous research has been done on cancer so it no longer is a mystery. However, its treatment and cure is what still baffles a lot of researchers. Several approaches to the treatment have been established and are relatively successful, depending on the age of the patient, type and stage of cancer, amongst many other factors. Many cancer patients have resorted to chemotherapy and radiation therapy for treatment. Although the national average for cancer deaths decreased by 2.1 % from 2002-2004, the number of people diagnosed with cancer increases each year in addition to the number whose cancers have

returned [8]. Moreover, most treatment processes are usually long, very intense, and physically, psychologically, and financially demanding on patients, family, friends, and even doctors. The questions here are: What happens when the cancer comes back? What do patients do if their cancers are in a late stage or terminal? The frustration and ache associated with this disease is driving some patients to opt for unapproved cancer drugs, (example, DCA, Dichloroacetate). Cancer patients opt for unapproved drugs because they no longer have the luxury of waiting for clinical trials before approval by the FDA. Moreover, it is estimated that 95% of cancer drugs that enter clinical trials do not get approved either because they are ineffective or unsafe [9]. Patients often try various means and risk their lives to make their last days less uncomfortable. Many of the cancer patients are desperate and are willing to try new therapies even before approval by the FDA. This presents a very troubling scenario for the FDA, ethicists, scientists, as well as the patients. Although current treatment options are limited to chemo or radiation therapies, other approaches are being sought. One of the promising therapies involves “Gene Correction” in which the repugnant DNA or the specific area of damage on the DNA strand is identified and either removed or repaired [3, 10].

Gene correction is now of great importance in biotechnology and medicine, where the hydrolysis of DNA or RNA by biomimetics is being exploited. The ability to cleave nucleic acids efficiently in a non-degradative manner with high levels of selectivity for site or structure has offered many applications for the manipulation of genes, the design of structural probes, and the development of novel therapeutics.

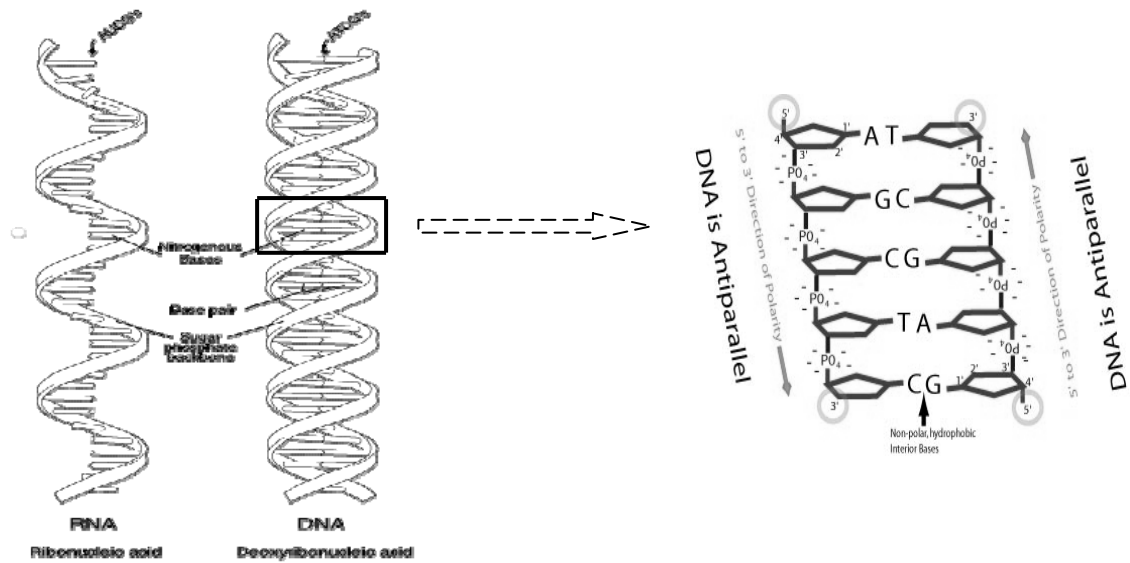


Figure 1. Double Helix of DNA; Phosphate Backbone

(Adapted from RNA Ribonucleic Acid by Darryl Leja, NHGRI [11])

### Related Features of DNA (and RNA)

DNA and RNA are polymeric macromolecules, the building blocks of which are nucleotides that are linked via phosphodiester bonds as illustrated in Figure 1. Watson and Crick won the Noble Prize for their work on the double helical structure of the DNA in 1953 [12], and since then it has been evident that the double-stranded polynucleotide adopts a wide family of conformations. These forms are the A, B, C, D, Z, super coiled circular-DNA, bent-DNA, the triple-stranded, quadruple-stranded, and the cruciform DNA. The latter five forms are referred to as the unusual DNA [13]. The A, B, and Z families are shown in Figure 2. The A form is the most common, while the C and D are considered as modified versions of the A and B DNA respectively. The intricacy of DNA

formation, DNA structure and conformations are of a different subject that will not be dealt with here. However, we are concerned with that fact that drugs that bind to DNA can also induce local variations in its conformation, and in targeting sites along the DNA strand with new

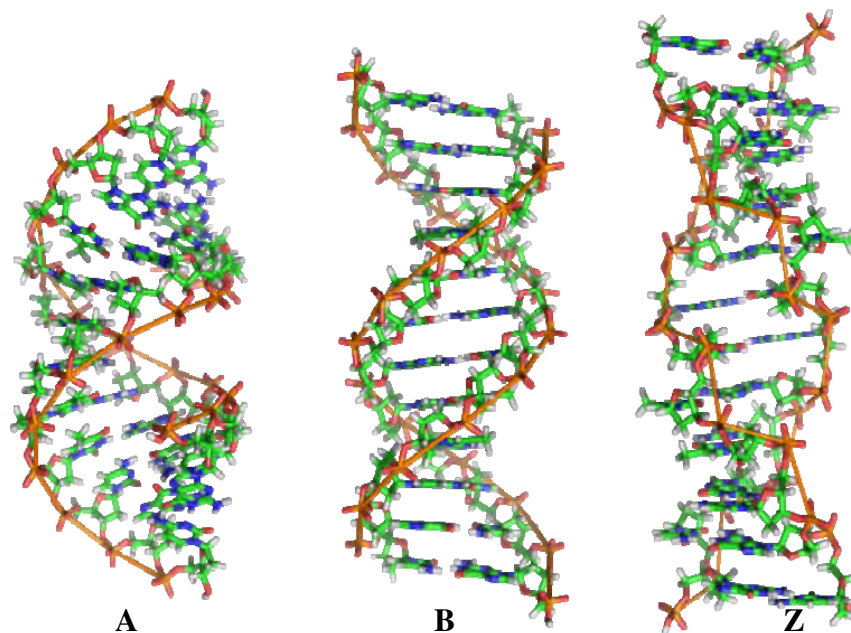
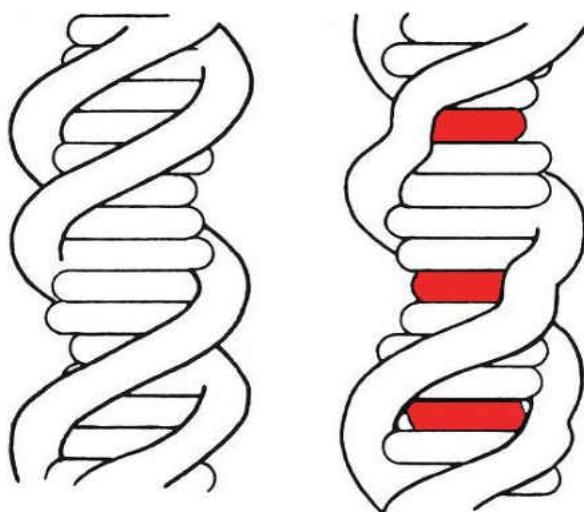


Figure 2. Forms of DNA by Wikipedia [14]

chemotherapeutic agents (or synthetic probes), the susceptibility of different sequences to such conformational changes must be considered [15]. Barton, in her report, cited that the simplest of such interference was probably intercalation, first described by Lerman at the University of Colorado, where flat aromatic, heterocyclic moieties insert and stack between the base pairs of the DNA helix, Figure 3. These interactions are described as topochemical, in the sense that the covalent bonds within both the guest and host are preserved.





Unchanged DNA Strand

Intercalation at Three Locations (Red)

Figure 3. Intercalation of a Metal Complex in DNA by Wikipedia [14]

DNA and RNA have a very remarkable hydrolytic stability that necessitates the use of enzymes (nucleases) to mediate the hydrolysis of their phosphodiester backbones under physiological conditions. It is estimated that the half-lives of DNA and RNA phosphodiester backbones when extrapolated to physiological conditions are approximately  $10^6$  and  $10^3$  years, respectively. That is why DNA of species that lived before the first Ice Age are still being isolated [3, 16]. Hydrolysis of the phosphodiester backbones is hindered mainly by the large negative charge around the poly-anionic backbone that inhibits attack of nucleophiles, and so charge neutralization by bound metal cofactors is one of the several mechanisms encountered in nucleases. Most of enzymes that catalyze the hydrolysis of phosphate esters or phosphoryl-group transfer reactions require divalent metal ions as cofactors [17]. Most of the metalloproteases contain  $Zn^{2+}$  ion in their active site. An

example is carboxypeptidase A, a 307-amino acid exopeptidase, from bovine pancreas [16]. More on metalloproteases will be discussed later in this chapter.

### Related Features of Enzymes

Enzymes are macromolecules that catalyze biochemical reactions. They have a highly complex three-dimensional structure formed by the spontaneous folding of the polypeptide chain. The fact that biological processes are mediated by enzymes has heralded the growth of biochemistry as an independent subject. Each reaction occurring in a biochemical pathway is catalyzed by a specific enzyme. Without enzymes, these reactions would be too slow to sustain life, as they are involved in processes such as metabolism, detoxification, excretion, etc. Each living cell contains thousands of enzymes. That is why these cells are capable of carrying out a huge repertoire of enzyme-catalyzed chemical reactions. Enzymes, as proteins, are affected by certain changes in their immediate environment like pH, temperature, and inhibitors. Two popular theories of enzymes mechanisms are widely considered: the lock-and-key and the induced-fit theories. Understanding of these two theories is the basis of molecular recognition in enzymatic reactions.

### Molecular Recognition and Complimentarity

Molecular recognition is an important subject in bioorganic chemistry, as it is the key to designing synthetic molecules that mimic various aspects of enzyme chemistry.

Adequate understanding of molecular recognition, based on model compounds, not only help to decipher enzyme activity, but also create a foundation for making new reagents that have some of the intriguing aspects of enzymes such as catalytic activity and specificity. The degree of success in this field is based on proper understanding of *molecular architecture*, where different regions or functional groups are positioned in a well-defined array to provide a specific chemical microenvironment [18]. This leads to another important aspect, complementarity, where the following features are considered:

- The enzyme or host must be able to recognize the substrate,
- The host must provide a cavity (active site) that matches the size and shape of the substrate for a perfect fit,
- This cavity must be lined with groups capable of interacting with complementary regions or functional groups on the substrate, forming weak attractive forces such as hydrogen bonds that can easily break off at the end of the catalytic cycle, liberating the product.

When the sugar-binding site of D-glucose is examined, 13 hydrogen bonds are formed between the peptide residues and the hydroxyl groups or pyranose oxygens of glucose to give a very high affinity for the sugar. In addition, two aromatic residues, phenylalanine-16 and tryptophan-183, are positioned above and below the mesh, forming a hydrophobic boundary to a strongly hydrophilic substrate. This gives the enzyme-substrate complex a chance to react without the interference of surrounding water. In molecular architecture, therefore, a microenvironment with similar controlled binding groups must be developed in order to achieve effective molecular recognition [18].

It is important to indicate here that our focus is on DNA (or RNA) as a substrate and implicit in these studies is the neglect of the underlying three-dimensional structure of DNA. It is clear that unlike proteins, DNA contributes to enzyme specificity by its ability to exist in alternate conformations or its ability to deform its structure to accommodate protein binding [19]. And so the synthetic probes are designed to target primarily particular functional groups in the substrate. Three classes of macromolecules have formed the bases of the artificial molecular recognition models - the cyclodextrins, the cyclophanes, and crown ethers. Work shows that these macrocyclic derivatives can form discrete complexes with substrates. The naturally occurring cyclodextrins and their synthetic counterpart cyclophanes, are cylindrical in shape and have a hydrophobic interior that bind hydrophobic substrates. On the other hand, the hydrophilic crown ethers, particularly the 18-crown-6 derivatives, form stable complexes with primary ammonium ions and metal ions.

### Modes of Interaction

In his work, Andrew Hamilton [18] used the barbiturate family of compounds as substrates for his research. These compounds are attractive because they are widely used as sedatives and anticonvulsants, so it would be very interesting to understand their modes of interaction. In discussing the modes of interactions involved in these complexes he explored hydrogen bonding interactions and  $\pi$ -stacking along with its influence on mode of approach. These interactions apply to all enzymes and must be taken into account when designing enzyme models:

### Hydrogen Bonding Interaction

An important design issue concerns not only positioning the hydrogen bonding groups but also considering the rigidity of the supporting framework. If the receptor (binding site) is too flexible, it becomes possible for intramolecular hydrogen bonding to occur between the N-H and C=O groups, resulting in collapse of the binding cavity. A plausible suggestion would require positioning rigid groups called spacers, which would provide the right spacing and rigidity in the complex as illustrated in Figure 6.

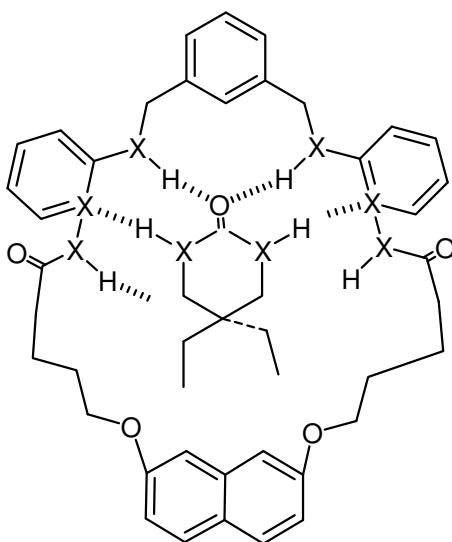


Figure 4. Rigid Structure of a Hypothetical Binding Site

### $\pi$ -Stacking Interactions

$\pi$ -Stacking describes where one plane of the molecule slips over the other. When  $\pi$ - $\pi$ -interactions are combined with hydrogen bonding, the recognition of planar heterocyclic substrates such as nucleotide bases is strongly enhanced, Figure 7.

Moreover, these receptors (and the substrates) contain many rings where  $\pi$ -orbitals are

very prominent, and electrostatic interactions between regions of complementary charge distribution on the rings play an important role in this  $\pi$ -interaction.

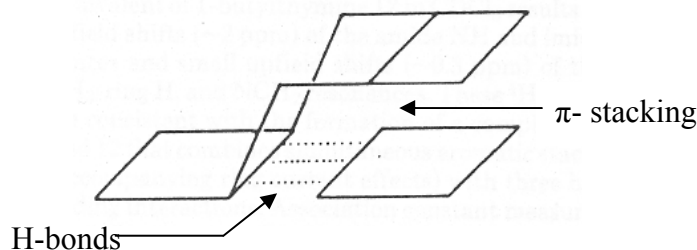


Figure 5. Schematic Presentation of Two-site Approach to Nucleotide Base Recognition

The mode of approach is based on the analysis that electronic interactions in the rings influence  $\pi$ -stacking and with this, the geometry of aromatic-aromatic interactions can be changed by varying their electronic properties. Two modes of approach include the face-to-face approach and the edge-to-face approach, Figure 9.

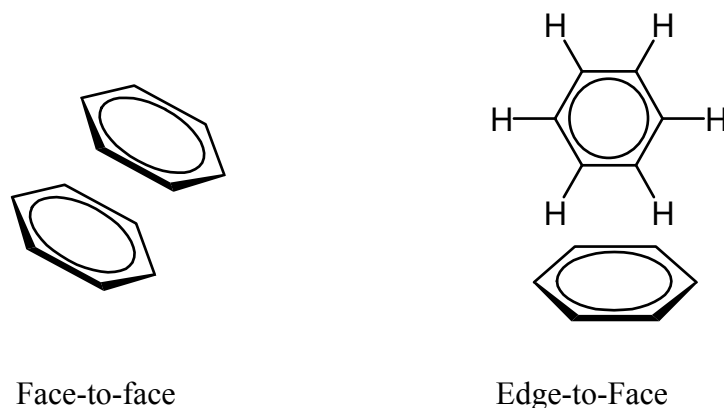
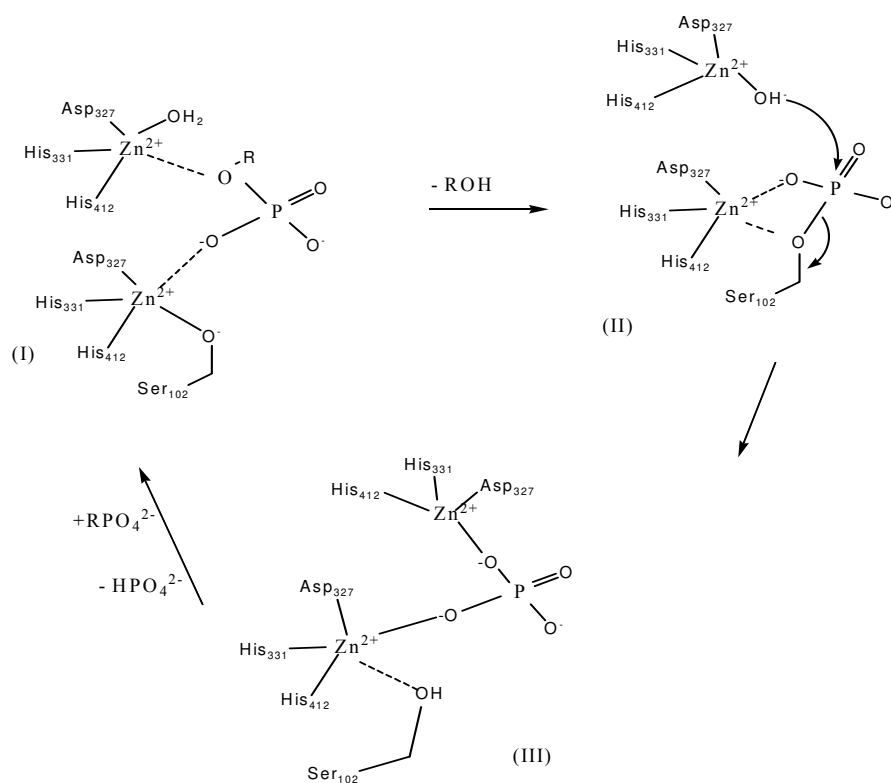


Figure 6. Modes of Aromatic-aromatic Interactions

The factors mentioned above have been widely considered in the design of synthetic “enzyme” models, particularly nuclease models, which are of great interest to us.

## Natural and Synthetic Metallonucleases

Natural metallonucleases in biological systems use metal ions cofactors such as zinc (Zn), Iron (Fe), Magnesium (Mg), and others. It is understood that the presence of these metal ions in the enzymes greatly enhances their catalytic reaction by providing extra enzyme-substrate binding via electrostatic interactions. An example is the alkaline phosphatase (AP), a Zn(II)-containing phosphomonoesterase that hydrolyzes phosphate monoesters ( $\text{ROPO}_3^{2-}$ ) at alkaline pH. Kimura and his co-workers [20] showed that the mechanism of action of this enzyme involves nucleophilic attack by the deprotonated serine(102) to yield a transient phosphoseryl intermediate. This is then attacked intramolecularly by the adjacent Zn(II)-bound hydroxide to complete the hydrolysis and reproduce the free form of serine(102) to reinitiate the catalytic cycle (Scheme 1).



Scheme 1. Mechanism of Action of Alkaline Phosphatase

The shape and structure of DNA provide a number of opportunities for the interaction with metal complexes. The negative charges of the phosphates that are regularly spaced along the DNA backbone mediate electrostatic interaction with positively charged metal centers. DNA has two grooves, the major and minor (Figure 2), in which covalent, hydrophobic, and hydrogen bonding interactions can occur. The DNA base pairs, stacked perpendicular to the axis of the double helix, offer sites of intercalation of flat aromatic groups through  $\pi$ -bond stacking.

The first example of artificial nucleases was bis(1,10-phenanthroline)copper(I), discovered by Sigman and his co-workers [21]. Copper phenanthroline binds in the minor groove of DNA and cleaves its backbone by copper mediated oxygen radical chemistry. Studies on two Cu(II) phenanthrolines, dmp (2,9-dimethyl-1,10-phenanthroline) and bcp (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline), Figure 10, indicated that hydrogen peroxide was an intermediate in such “nuclease” reaction.

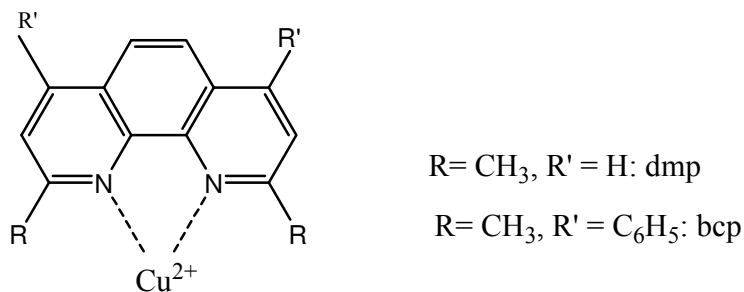
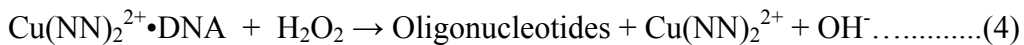
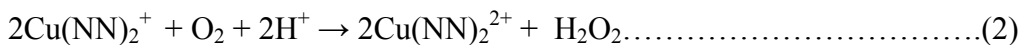
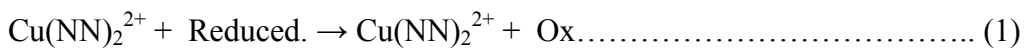


Figure 7. Copper(II) Phenanthrolines



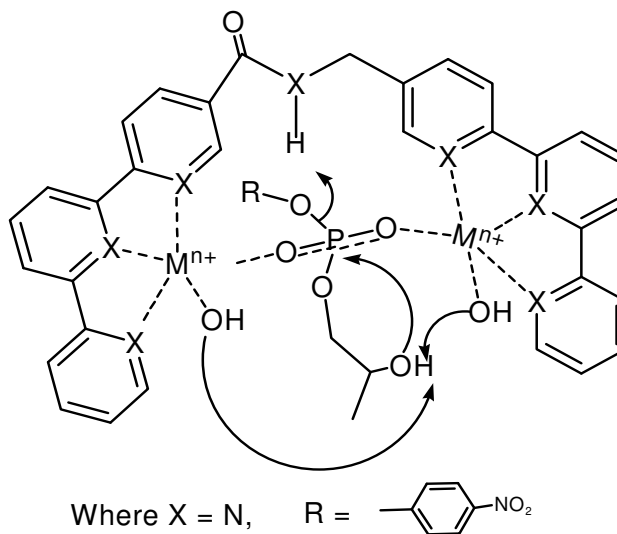
The proposed mechanism of action is shown in Scheme 2.



Scheme 2. Reaction of Copper(II) Phenanthroline with DNA

The work of Liu and Hamilton [23, 24] on 2-hydroxypropyl-p-nitrophenylphosphate, HPNPP, a widely used model compound for RNA, showed that the rate of transesterification of the substrate by such complexes is pH dependent. Based on such results, the authors suggested that the second Cu(II) ion in the structure provides not just general base catalysis through its coordinated hydroxyl group, but also Lewis acid activation of the P=O bond for nucleophilic attack,

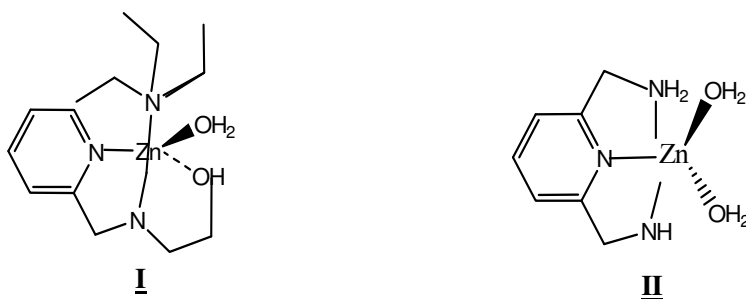
Scheme 3.



Scheme 3. Phosphodiester Transesterification by a Dinuclear Metal Complex

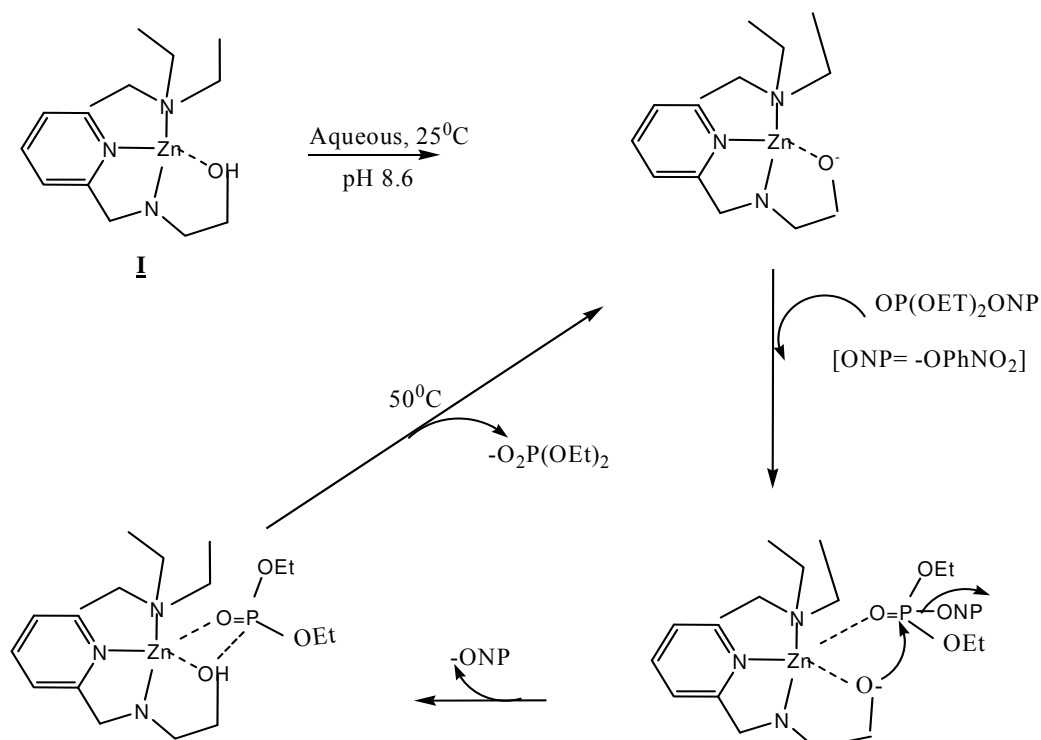
Another nuclease model that showed a remarkable catalytic activity toward hydrolysis of phosphate esters involved two polyamine-Zn(II) complexes, **I** and **II**

(Scheme 4).



Scheme 4. Zn(II) Diaminopyridine Complexes

Studies of these models showed that **I** is more active than **II** in promoting the hydrolysis of diethyl(4-nitrophenyl)phosphate through transesterification involving the alcohol pendant of the complex.

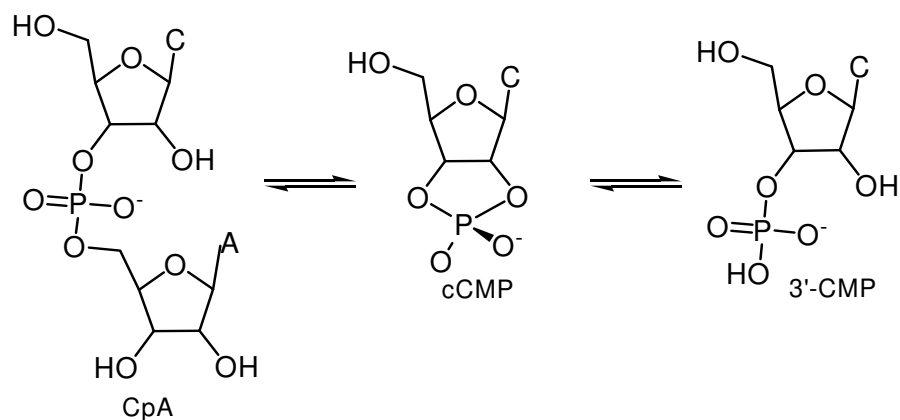


Scheme 5. Proposed Mechanism of Model I

Model I did not only hydrolyze neutral phosphotriesters but also facilitated their transesterification by the alcohol group of the ligand (Scheme 5), thus, it represents a good model for phosphotransferase enzymes [25].

### Natural and Synthetic Nonmetallonucleases

Although metallonucleases are very effective in their action and seem to be indispensable in DNA hydrolysis, there are metal-free nucleases that are just as efficient. Endoribonucleases, for example, constitute a class of nucleases that are found in animals, plants, and some microorganisms. This type of nuclease facilitates hydrolysis of various types of RNA (RNases) or DNA (DNases). They catalyze the hydrolysis of phosphodiester bonds and are highly specific in their action, cleaving at the 5'-O-P bonds [26]. One of the most studied members of this family is the Pancreatic RNase. Its mechanism of action is summarized in Scheme 6.



Scheme 6. RNase-catalyzed Hydrolysis of CpA

[Where: CpA = Cytidyl(3'-5') adenosine, cCMP= Cytidine cyclic 2', 3'-monophosphate,

3'- CMP= 3'-Cytidine monophosphate]

Another example is a DNase, called staphylococcal nuclease (SN). It hydrolyzes DNA several folds faster than some restriction nucleases [2]. Jubian, Dixon, and Hamilton designed a synthetic receptor that could mimic SN called bis-acylguanidinium (BAG), formed by a one-step reaction of dimethyl isophthalate and guanidinium hydrochloride. It was reported that (I) forms strong trigonal-bipyramidal complexes with phosphodiester in acetonitrile with a binding constant,  $K = 5 \times 10^{-4} \text{ M}^{-1}$ , Figure 12.

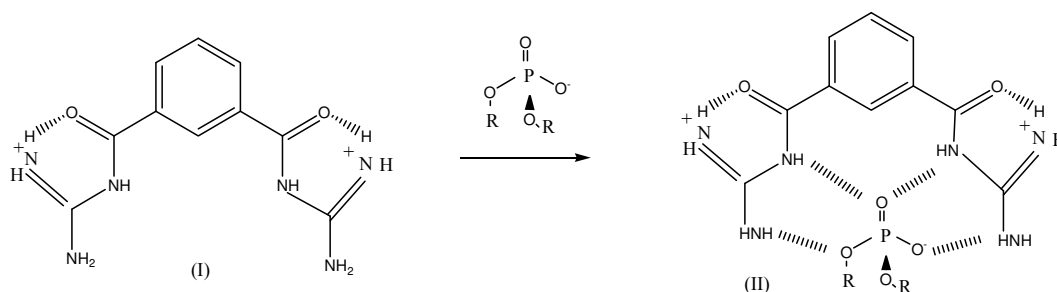
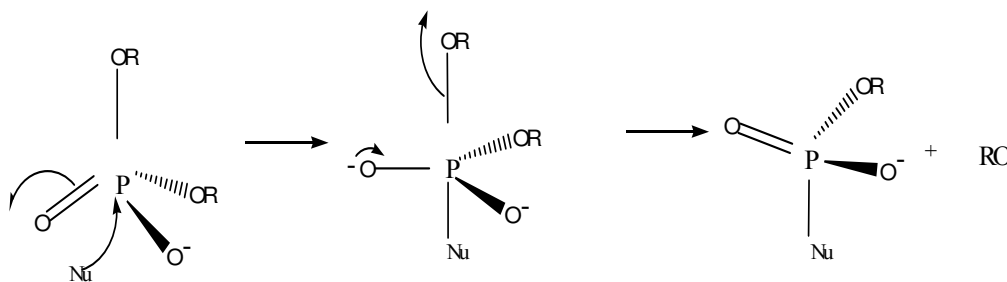


Figure 8. Interaction of BAG with Phosphodiester

The proposed mechanism of the hydrolysis of phosphodiester by such models proceeds as shown in Scheme 7. It involves nucleophilic attack on the phosphorous followed by elimination of the alkoxy group- (OR). The authors were able to show that this complex substantially enhanced the rate of phosphodiester cleavage reactions by binding the substrate via both hydrogen bonding and electrostatic complementarity in the trigonal-bipyramidal intermediate.



Scheme 7. Mechanism of Hydrolysis of Phosphodiester

## Design of Our Nuclease Models

Based on such great work and success, we decided to design and synthesize some novel compounds and to test their “nuclease” activities. In the first class of our “nuclease” models, toluidine blue-O was used as a DNA/RNA recognition group that is equipped with catalytic groups that can hydrolyze phosphodiester bonds. In the second class, we used anthracene as a spacer linking both DNA-recognizing groups and catalytic groups. As the first step geared toward designing our synthetic probes, we chose to synthesize compounds with specific features that qualify them to be good models for synthetic nucleases. These features include both DNA and phosphate-binding groups in conjunction with catalytic groups that can hydrolyze phosphodiester bonds.

Our first candidate involved toluidine blue O (TBO), a methylene blue homolog Figure 13. TBO is widely used as metachromatic nuclear counterstain and for staining mast cells. It is known to be friendly to cells and so it is preferred over ethidium bromide dye that is highly mutagenic. Toluidine blue is a deep blue solid that is sold in the form of

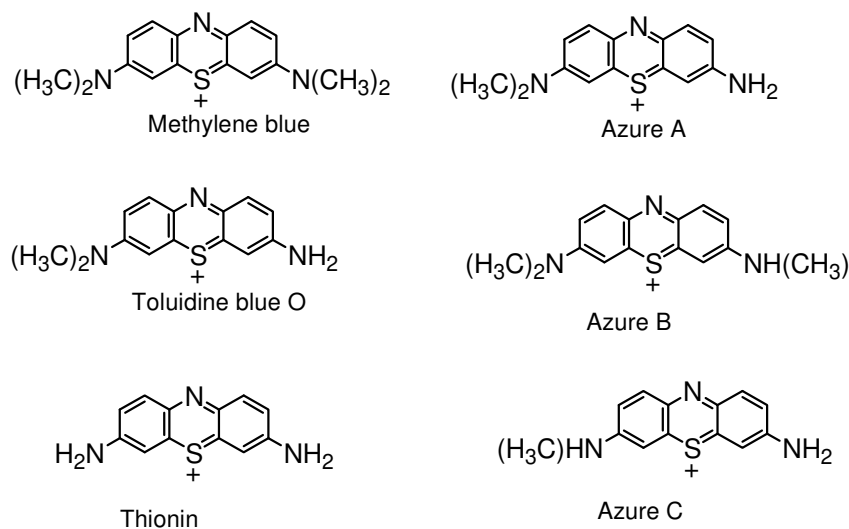
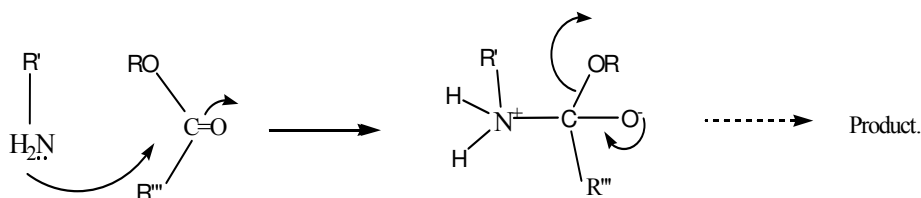


Figure 9. Methylene Blue Homologs

its chloride salt, tolonium chloride. It has been shown that TBO has a high specificity for the parathyroid, pancreas, and heart tissues, and, thus, it is used during surgery to trace parathyroid tissue during operations [27]. The mode of interaction of TBO is not very clear but it is believed that it binds DNA through either intercalation or charge neutralization of the phosphate making it possible to examine DNA under the microscope.

Amines can undergo acid-base reactions and can react as nucleophiles. TBO has three amine groups, two tertiary and one primary. Tertiary amines are generally weak nucleophiles, so these would not be our target for modification. On the other hand, the primary amine group would be alkylated or acylated to give derivatives that can be used as nuclease models after modification, Scheme 8.



Scheme 8. Illustration of Alkylation (or Acylation) of a 1° Amine

Our target nuclease models are of two categories. The first involves modifying TBO with covalently attached metal binding ligands; the other involves modification with guanidine or imidazole groups, to act as acid-base catalysts. As such, the anticipated models would include both the DNA-binding moiety (the phenothiazonium ring) and the catalytic groups, Figure 10.

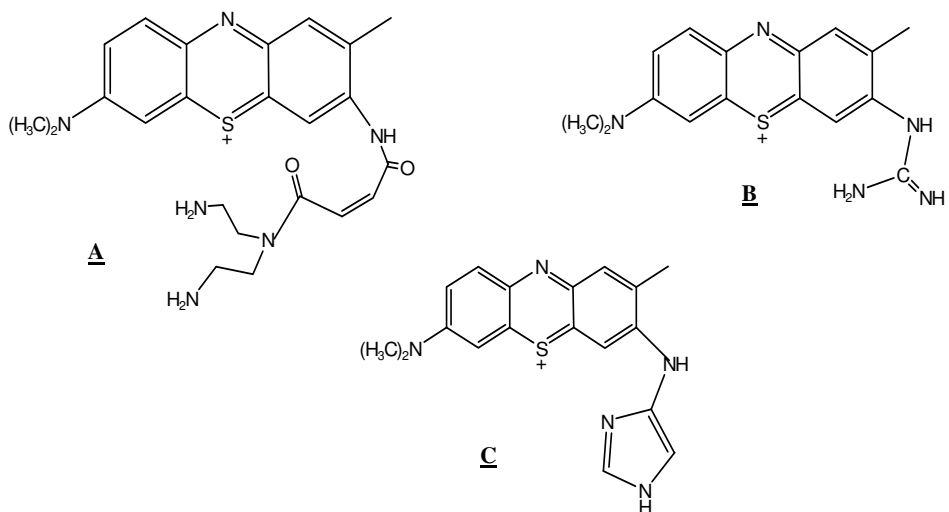
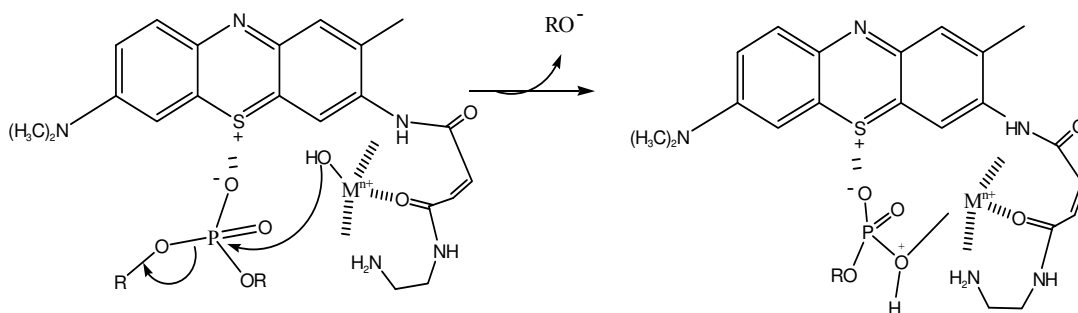


Figure 10. Nuclease Models Based on Toluidne Blue O



Scheme 9. Proposed Mechanism of Phosphoester Hydrolysis by TBO-based Models

One important feature in these models is their ability to bind metal cations by coordination to the polyamine ligand that is in close proximity to the phosphate-binding site (the positive sulfur), Scheme 9. This scenario would enhance cooperation between

the catalytic and the binding groups. Another important feature in these models is the incorporation of guanidine or imidazole group. Such groups are expected to catalyze phosphodiester hydrolysis presumably by general acid-base mechanism, Figure 15.

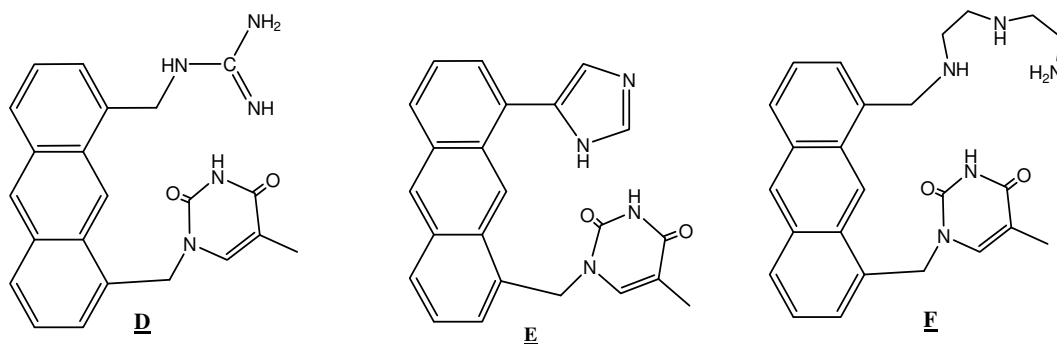


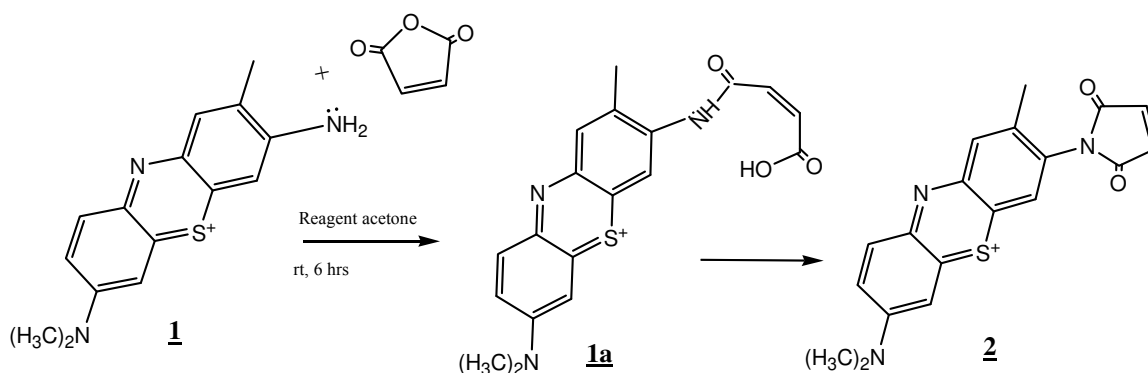
Figure 11. Nuclease Models Based on 1,8- Disubstituted Anthracene



## 2. RESULTS AND DISCUSSION

### Reaction of TBO with Maleic Anhydride

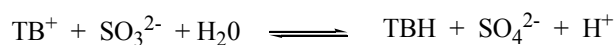
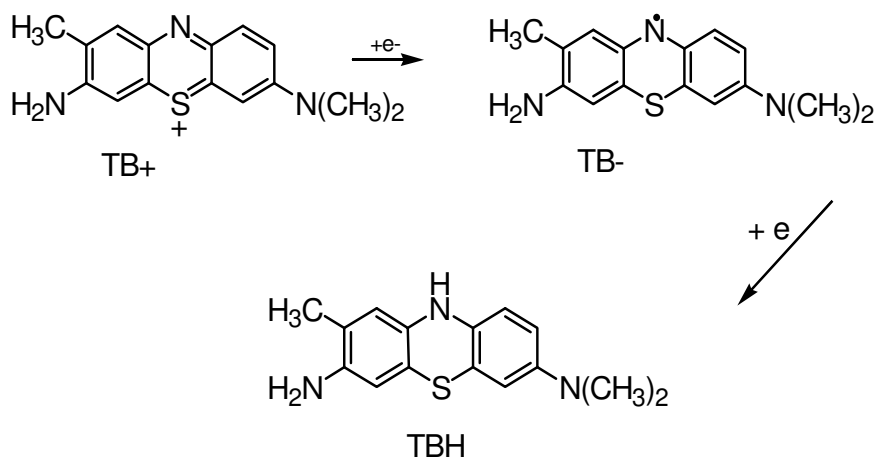
Acylation of the 1° amine group of TBO by maleic anhydride was expected to give the amide derivative **2**. <sup>1</sup>HNMR spectrum of the isolated product showed one doublet at 6.2 ppm instead of the expected two doublets for the two vinylic hydrogens in **1a**. This is consistent with the formation of the imide **2**, Scheme 10. Attempts to hydrolyze the imide were unsuccessful.



Scheme 10. Reaction of TBO with Maleic anhydride

### Reaction of Reduced TBO with Maleic Anhydride

The primary amine group on TBO is expected to be highly deactivated due to the electron withdrawing effect of both neighboring ring and positive sulfur. So we first attempted to minimize the deactivating effect of the positive sulfur by reducing it with sodium sulfite [29]. The reduction reaction is illustrated in Scheme 11.

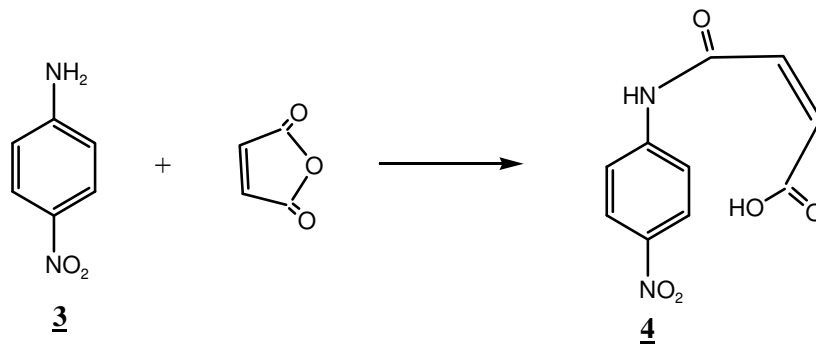


Scheme 11. Reduction of TBO

When the reduction was complete, as indicated by total disappearance of the dark blue color, maleic anhydride was added. After oxidation by air, TLC of the reaction mixture showed a blue spot that with a higher  $R_f$  than TBO starting material.  $^1\text{H NMR}$  of the isolated spot showed peaks that resembled those of TBO, and there was no evidence that maleic anhydride was part of the product. It was clear that the reduction proceeded well but, somehow, the reduced TBO did not react with maleic anhydride. This result indicates that the effect of the positive sulfur on the reactivity of the amine group of TBO may not be the only cause of its low reactivity. We decided to explore the reaction of maleic anhydride with other relatively deactivated amines such as *p*-nitroaniline to gain more insight about such reactions.

### Reaction of para-Nitroaniline with Maleic Anhydride

When para-nitroaniline was refluxed with maleic anhydride in anhydrous acetonitrile, Scheme 12, it provided **4** as yellow crystals in 72% yield as confirmed by <sup>1</sup>H NMR analysis.

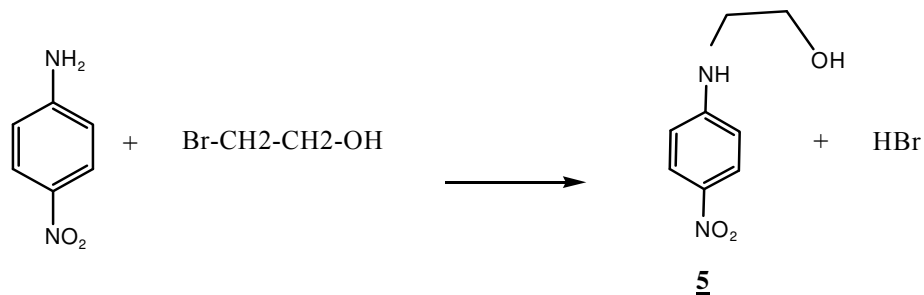


Scheme 12. Reaction p-Nitroaniline with Maleic anhydride

The results indicate that a deactivated amine such as para-nitroaniline could be acylated via this method in good yield. To further confirm the reactivity of this amine, another reaction involving 2-bromoethanol was carried out.

### Reaction of para-Nitroaniline with 2-Bromoethanol

When 2-bromoethanol was refluxed with p-nitroaniline in DMF, Scheme 13, a yellow crude solid was obtained. This product was purified by column chromatography giving pure **5** in 36% yield. The structure was confirmed by <sup>1</sup>H NMR (Scheme 13).

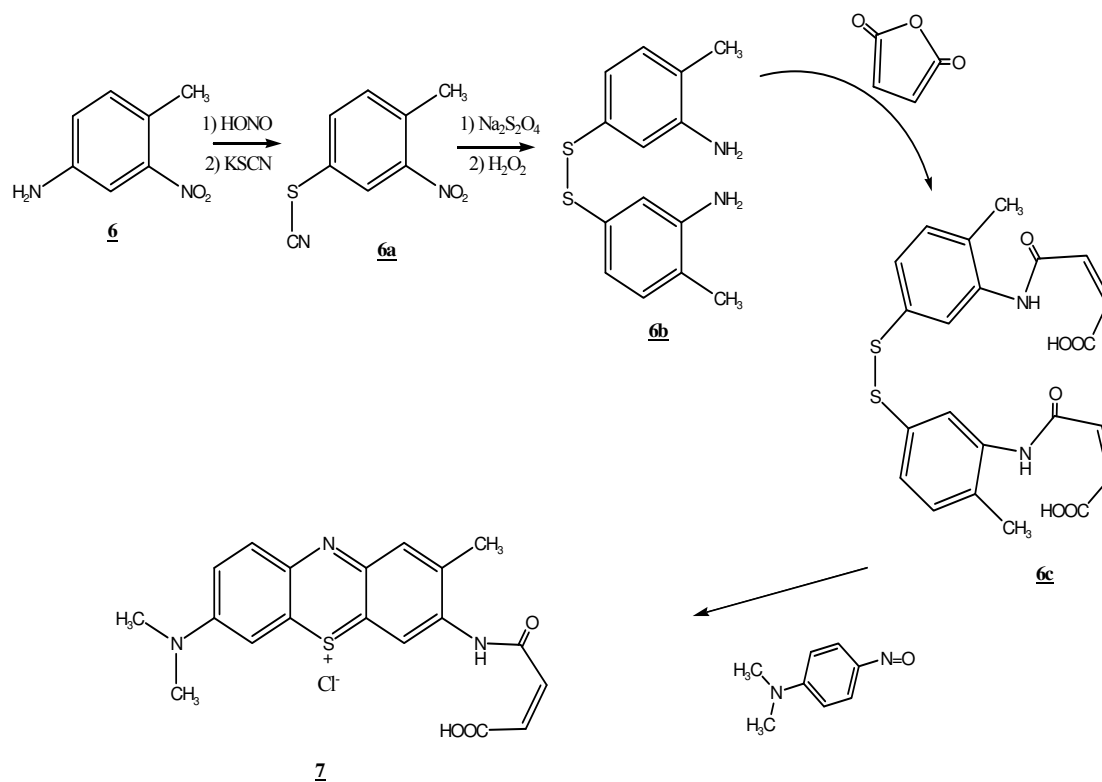


Scheme 13. Reaction of p-Nitroaniline with 2-Bromoethanol

This reaction also showed that the deactivated amine of p-nitroaniline was a nucleophile strong enough to displace a halogen.

### Synthesis of Toluidine Blue O

The two methods described in sections 2.3 and 2.4 were repeated with TBO, but the reactions were still unsuccessful. It was evident that TBO is very unreactive and other ways had to be sought to construct our target compounds. This required that we synthesize the toluidine blue molecule entirely, but in the course of this synthesis, acylate the amine group prior to ring closure, while it is more reactive. This would result in the desired TBO derivatives. The outline of the modified synthesis strategy is shown in Scheme14 [27].



Scheme 14. Outline of the Synthesis of Toluidine Blue O Derivative

### Synthesis of 3-Nitro-p-toluidine

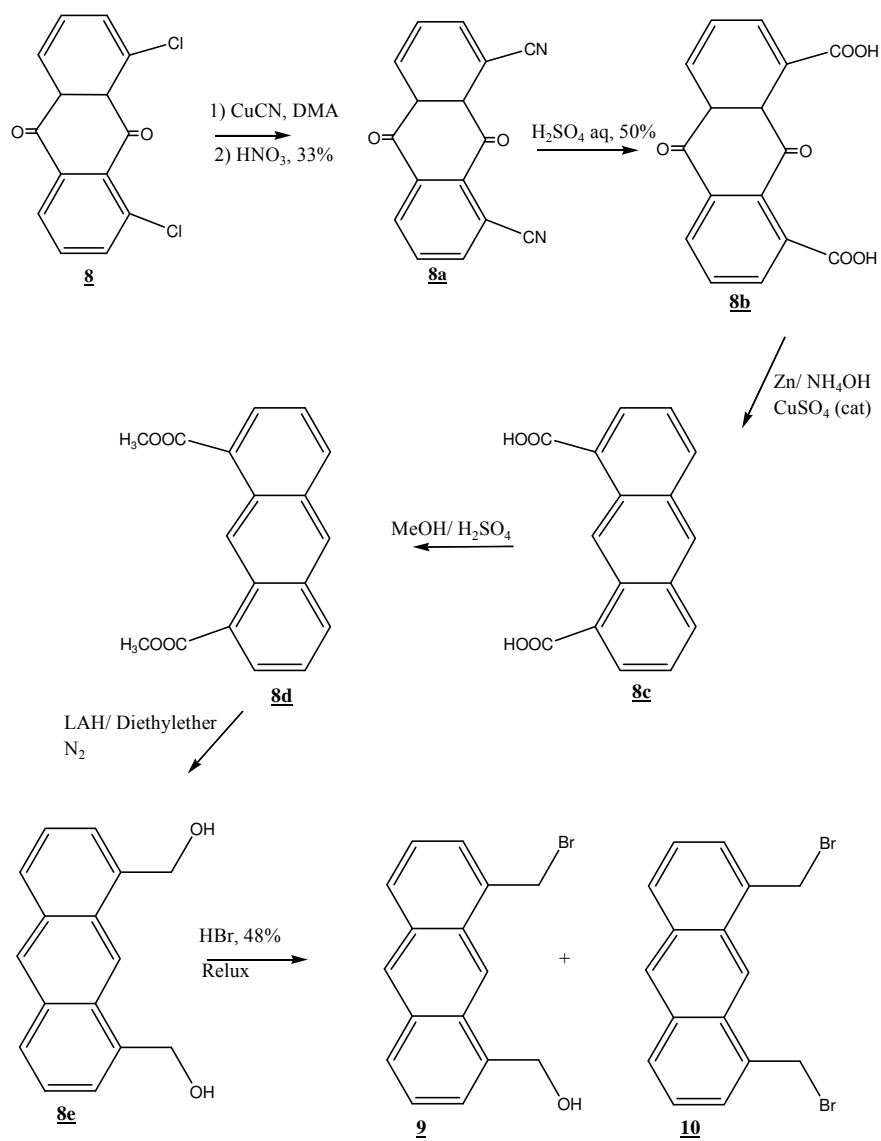
The first step involved the synthesis of 3-nitro-p-toluidine from p-toluidine, using a nitrating mixture that was prepared in situ. After neutralization of the reaction mixture and recrystallization, yellow crystals were obtained in 87% yield, mp 77-79°C. <sup>1</sup>H NMR confirmed the structure of **6** [30].

### Conversion of 3-Nitro-para-toluidine to 3-Nitro-para-toluenethiocyanate

Compound **6** was first treated with nitrous acid that was prepared in situ, to convert the 1° amine to diazonium salt. When potassium thiocyanide was added to the reaction mixture, a dark brown precipitate was obtained that was isolated in more than 90% yield [27]. Insolubility of this product hindered its NMR analysis, and further steps using it were abandoned. Due to time constraint, this approach to the synthesis of TBO derivatives was put on hold, while an alternative model was pursued that is based on the use of 1,8-disubstituted anthracene.

### Synthesis of 1,8-Bisbromomethylantracene

The synthesis of 1,8-bisbromomethylantracene involved multi-step synthesis that began with the use of 1,8-dichloroanthraquinone as starting material, Scheme 15. The final product was successfully synthesized following exact procedures published in literature [31, 32], to obtain yields in environs of 70%.

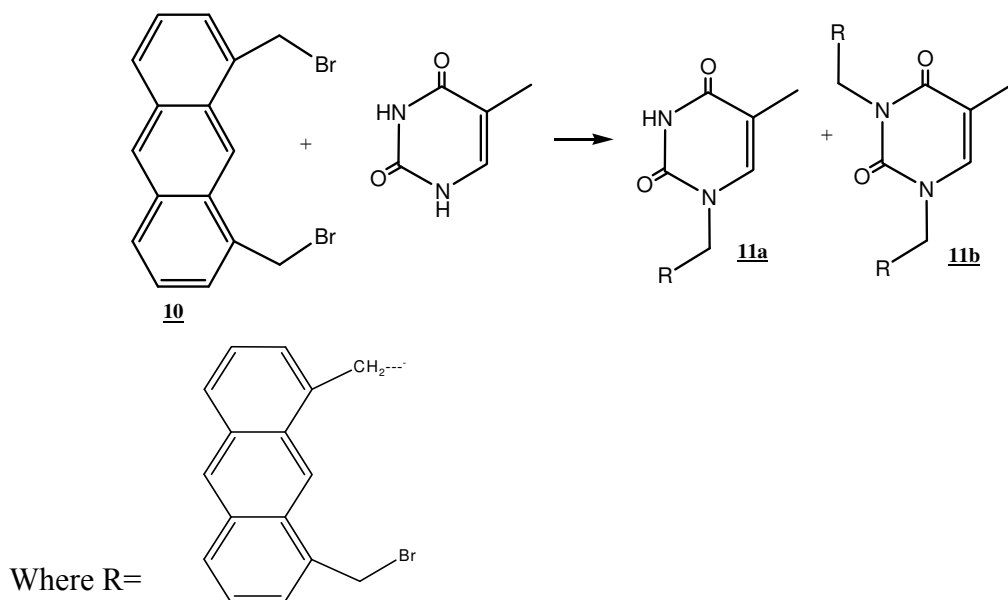


Scheme 15. Outline of Synthesis of 1,8-Bisbromomethylantracene

## Reaction of 1,8-Bisbromomethylanthracene with Thymine

### Method 1

Thymine and 1,8-bisbromomethyl were refluxed in acetonitrile containing diaza(1,3)bicyclo[5.4.0]undecane, DBU, as a base [33], Scheme 16. The reaction mixture was worked up and a cream-white product was isolated in about 20% yield. Proton NMR analysis indicated absence of the anthracene ring. Furthermore, the NMR spectrum resembled that of thymine. It was concluded therefore that the two compounds did not react and while the 1,8-bisbromomethylanthracene was lost during the work-up, thymine was recovered. Another possible explanation of the reaction failure could be due to the low solubility of thymine in acetonitrile. However, repetition of the reaction at elevated temperature, and using larger volume of solvent gave the same results.



Scheme 16. Reaction of 1,8-Bisbromomethylanthracene with Thymine



## Method 2

In the second method, the solvent was changed to DMSO to ensure complete dissolution of thymine and sodium carbonate was used instead of DBU [33]. <sup>1</sup>HNMR analysis of the product showed the same results as in the previous method, where thymine was recovered unreacted.

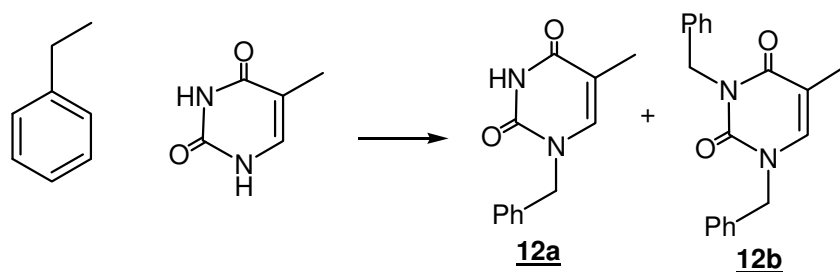
## Method 3

In this method, thymine was set to react with the bisbromomethylantracene, employing DMSO as solvent, with cesium carbonate as a base [33]. <sup>1</sup>HNMR analysis showed that the product was definitely not the expected monoalkylated **11a** or the dialkylated thymine, **11b**.

## Method 4

Thymine and 1,8-bisbromomethylantracene were refluxed in DMF containing NaH. The expectation was to have both the monoalkylated and dialkylated products so that the mono product could be isolated and used to synthesize the anthracene-based models. Unfortunately, the three different fractions obtained from the reaction showed NMR peaks corresponding to aliphatic protons only. This implied that neither of the fractions has expected product or any of the reactants. These peaks must have been impurities most likely picked up from solvents either during the reaction or separation. This reaction was repeated two times and the results were the same, with the NMR showing only aliphatic peaks. These results were very disturbing because such methods using other alkylhalides had been reported in literature to give high yield. To verify this method, we decided to test the reaction between other alkyl halides and thymine.

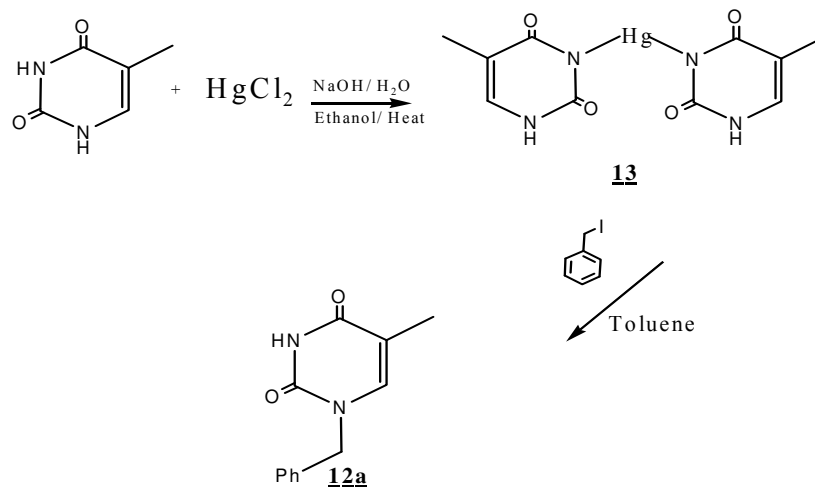
### Reaction of Benzyl Iodide with Thymine



The reaction was set up as in method 1, with benzyl iodide and thymine refluxed in acetonitrile with DBU. The crude product was isolated and separated by column chromatography to give three main fractions. These were dried and analyzed by <sup>1</sup>HNMR, which showed the first and second fractions to be the dialkylated and monoalkylated product **12b** and **12a**, respectively. The third fraction showed no thymine protons.

### Synthesis of Dithyminylmercury

The amine group of thymine is a relatively weak nucleophile and as such it is slow to react especially with weak electrophiles. Converting thymine into its metal amide salt is expected to make the amine group more nucleophilic. Thymine was dissolved in sodium hydroxide and treated with a solution of mercuric chloride in ethanol, Scheme 17.



Scheme 17. Synthesis of Dithyminylmercury and its Reaction with Benzyl Iodide

The white precipitate was filtered and thoroughly washed and dried to give over 90% yield [34]. The melting point was over 300°C, and due to its insolubility, its structure could not be confirmed by <sup>1</sup>HNMR analysis.

#### Reaction of Dithyminymercury with Benzyl Iodide

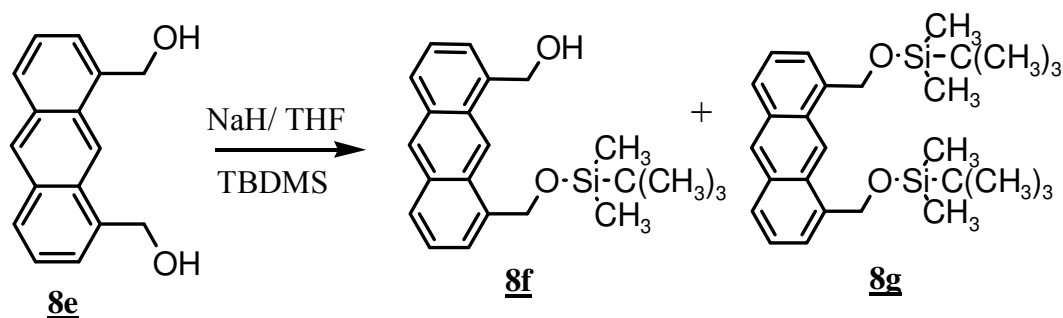
This reaction was carried out as a means to confirm the structure of dithyminymercury and to test the feasibility of its reaction with bisbromomethylantracene. Benzyl iodide and HgT<sub>2</sub> (**13**) were refluxed in toluene, Scheme 17, to give crude product that was dissolved in methylene chloride and purified by column chromatography [34]. Two major fractions were isolated and analyzed by NMR. The first fraction matched benzyl iodide on TLC and its <sup>1</sup>HNMR confirmed that it is unreacted benzyl iodide. The second fraction, however, was found to be the expected benzyl thymine product 12a, but the yield was very low (< 10%). These results are indicative of the fact that HgT<sub>2</sub> (**13**) has the correct structure, but the low yield of its reaction with benzyl iodide discouraged carrying out the reaction with 1,8-bisbromomethylantracene. Several attempts to improve the reaction yield were unsuccessful.

### Reaction of Thymine with Benzyl Bromide

One of the attempts to optimize the yield of the alkylation of thymine by bisbromomethylantracene involved reacting thymine with benzyl bromide in DMF in the presence of equivalent amount of sodium hydride. The reaction mixture gave two major products that were separated by column chromatography.  $^1\text{H}$ NMR of the first fraction showed two distinct peaks for the two methylene groups presumably of the dialkylated product **12b**, while the second fraction was clearly the monoalkylated product **12a**. Because the yield was in excess of 33%, this method was preferred over the use of DBU; therefore, it was adopted for reacting thymine with 1,8-bisbromomethylantracene.

### Protection of 1,8-Bishydroxymethylantracene

Tert-butyltrimethylsilylchloride, TBDMS, is reported in the literature [35] to be the reagent of choice for mono protection of dialcohols. One equivalent of TBDMS was allowed to react with 1,8-bishydroxymethylantracene in THF, Scheme 18.



Scheme 18. Protection of 1,8-Bishydroxymethylantracene with TBDMS

The product was expected to be the mono-protected product only or a mixture of the mono- and di-protected alcohols. However, the proton NMR spectrum of the isolated product showed peaks whose integration did not match either of the expected products. This reaction was run several times following the exact procedure in literature, but the mono-protected alcohol was never obtained. It is possible that the high reactivity of benzylic alcohol groups require milder reaction conditions for monoprotection.

### 3. EXPERIMENTAL

#### Materials and Methods

All commercial reagents were used without further purification unless indicated otherwise. The following chemicals were purchased from the Fisher Scientific Company: toluidine blue O (electrophoresis grade), para-nitroaniline, sodium cyanoborohydride, cupric sulfate, tert-butanol, potassium tert-butoxide, acetonitrile, acetone, chloroform, hexane, methanol, methylene chloride, DMF, THF, hydrochloric acid, nitric acid, and sulfuric acid. The following reagents were purchased from Aldrich Chemicals: 2-pyridine carboxaldehyde, 2-bromoethanol, 1,8-dichloroanthraquinone, thymine, thiamine, para-toluidine, and chloroform-d (1% v/v TMS). Benzyl bromide, benzyl iodide, and t-Butyldimethylsilylchloride (TBDSiCl) were purchased from Acros Organics.

All NMR spectra were recorded on the JEOL-NMR Eclipse spectrometer at 400 MHz in CDCl<sub>3</sub> unless stated otherwise. Chemical shifts were recorded as delta values in parts per million (ppm) relative to TMS. The multiplicity of signals is reported as follows: s, singlet; d, doublet; dd, double doublet; dt, doublet of triplet; t, triplet; tt, triplet of triplet; q, quartet; m, multiplet.

Column chromatography separation was done on silica gel purchased from TSI Chemical Company. Thin layer chromatography (TLC) was done using silica gel plates with fluorescent indicator UV<sub>254</sub>, bought from Aldrich. Melting points were recorded on Cambridge MEL-TEMP instrument and were not corrected.

### Reaction of TBO with Maleic Anhydride

TBO, 0.05g (0.16mmol) and 0.15g (1.5mmol) of maleic anhydride, were dissolved in 5mL of reagent grade acetone in a 25mL flask and stirred at room temperature for 6 hours, after which a thick mixture was obtained. The solid was filtered out and recrystallized from methanol to give **2**: yield 0.04g, mp was above 310°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>/5% CD<sub>3</sub>OD), Appendix A: δ 7.81-7.20 (m, 5H), 6.17 (s, 2H), 4.00 (bs, 6H), 2.21 (s, 3H).

### Reaction of Reduced Toluidine Blue with Maleic Anhydride

TBO, 0.05g (0.16mmol) was dissolved in 5mL of distilled water. To the deep blue solution was added Na<sub>2</sub>SO<sub>3</sub> (20mg) and the resulting solution was stirred for 30 minutes under nitrogen gas. During this period, the blue color faded to just a tint of blue. Maleic anhydride (30mg, 0.3mmol) dissolved in 1mL of CH<sub>3</sub>CN was quickly added to the reaction mixture and stirring continued for 30 more minutes. The reaction mixture was then exposed to air (where it is quickly oxidized into deep blue). The solution was allowed to evaporate to dryness. The residue was dissolved in 2% methanol/ chloroform solution and spotted on TLC. Only one broad spot was observed and it moved just slightly faster than TBO starting material. This fraction was separated by column chromatography using a gradient of 1%- 10% methanol/chloroform solution and 30g of silica gel, to give the unreacted TBO.

### Reaction of para-Nitroaniline with Maleic anhydride

p-Nitroaniline, 0.03g (0.22mmol) was dissolved in 5mL of anhydrous acetonitrile and stirred for 5 minutes, forming a yellow solution. Maleic anhydride, 21mg (0.21mmol) of was added and the reaction mixture was refluxed for 90 minutes in a sand bath that was maintained at 98- 102°C. By then, all reactants dissolved into a clear yellow solution that was cooled in ice to give yellow crystals. The crystals were filtered and washed with chilled acetonitrile and dried to obtain **4**: yield 0.022g, mp 198- 200°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>), Appendix B: δ 9.14 (d, 1H), 8.46 (2H), 8.03 (d, 2H) 4.89 (s, 1H), 4.02 (s, 1H).

### Reaction of p-Nitroaniline with 2-Bromoethanol

p-Nitroaniline, 3.0g (228mmol) was dissolved in 10mL of anhydrous DMF and stirred until dissolved. 2-Bromoethanol, 1.674mL ( 23.6mmol) was then added to the solution and refluxed for 2 hours in a sand bath maintained between 158-165°C. The reaction mixture was monitored by TLC until all 2-bromoethanol reacted. The solvent was evaporated and the crude product was purified by column chromatography eluting with 5% methanol/chloroform solution and 85g of silica gel to give **5**: yield 1.08g, mp 87- 90°C. <sup>1</sup>H NMR (CDCl<sub>3</sub>), Appendix C: δ 8.03 (d, 2H), 6.53 (d, 2H), 5.20 (s, 1H), 3.84 (t, 2H), 3.34 (t, 2H), 1.80 (s, 1H).



## Synthesis of Toluidine Blue O

### Synthesis of 3-Nitro-p-toluidine

p-Toluidine, 107mg (1.0mmol) was dissolved in 1mL of concentrated sulfuric acid in a 20mL beaker and cooled in ice. A nitrating mixture was prepared in a separate test tube by adding drop-by-drop 1mL of ice cold sulfuric acid to 2mL of ice cold concentrated nitric acid maintaining the temperature below 5°C. This nitrating mixture was added gradually to the beaker containing p-toluidine with continuous stirring and cooling in ice. After the addition was complete, the solution was stirred at room temperature for 15 minutes and then neutralized with 40% sodium hydroxide to precipitate a yellow solid. The solid was filtered, washed repeatedly with cold water, and dried. Recrystallization from 98% ethanol provided **6**: yield 85.6mg, mp 77- 79°C (lit 79°C [29]). <sup>1</sup>H NMR (CDCl<sub>3</sub>), Appendix D: δ 7.28-6.80 (m, 3H), 3.81 (s, 2H), 2.53 (s, 3H).

### Conversion of 3-Nitro-para-toluidine to 3-Nitro-para-toluenethiocyanate

3-Nitro-para-toluidine, 150mg (2.83mmol) was dissolved in 0.5mL of concentrated hydrochloric acid and cooled in ice. Sodium nitrite (40% aqueous solution), 1mL, was cooled and added drop wise to the ice-cold HCl solution, while stirring and maintaining temperature below 5°C. After 10 minutes, potassium thiocyanide (saturated solution in 1mL of water), was added to the reaction mixture, and a dark brown precipitate formed immediately. The solid was filtered out and washed with cold water to provide 120mg of product. Due to its insolubility, it was not analyzed by NMR.

## Reaction of Thymine with 1,8-Bisbromomethylanthracene

### Method 1

1,8-Bisbromomethylanthracene, 20mg (0.55mmol), synthesized from 1,8-dichloroanthraquinone using literature procedure [30, 31] and 27.6mg (2.2mmol) of thymine were dissolved in 10mL of freshly distilled anhydrous acetonitrile and stirred for 10 minutes. The thymine remained mostly insoluble. DBU, 50mg (0.33mmol) was then added to the reaction mixture and refluxed for 24 hrs. The solvent was evaporated under reduced pressure to obtain a residue that was dissolved in methylene chloride and filtered. The methylene chloride solution was separated by column chromatography using 2% methylene chloride/ methanol solution and 15g of silica gel. The product was analyzed by <sup>1</sup>HNMR (CDCl<sub>3</sub>), Appendix E: δ 2.16 (s, H), 1.59 (s, H), 1.24 (s), 0.79 (m, H).

### Method 2

1,8-Bisbromomethylanthracene, 20mg (0.055mmol), and thymine, 8.3mg (0.066mmol) were dissolved in 10mL of acetonitrile containing 5mL of DMSO and stirred for 10 minutes. Most of thymine dissolved. Sodium carbonate, 23mg, was added to the reaction mixture which was then refluxed for 24h. The reaction was quenched by adding ice-cold water until a cloudy precipitate was formed. The solid was filtered, rinsed with water, and dried to give 12mg of crude product. This was purified by column chromatography using methanol/methylene chloride solution (1-5% gradient) and 15g of silica gel to give 3.6mg of product. <sup>1</sup>HNMR spectrum showed aliphatic peaks only, Appendix F.

### Method 3

Thymine, 8.3mg (0.066mmol), was dissolved in 1mL DMSO upon heating. The solution was then cooled to room temperature and cesium carbonate, 107.3mg (0.32mmol), was added and stirred for 2 h before adding 1,8-bisbromomethylanthracene, 20mg (0.055mmol). The solution was refluxed for 24 h under nitrogen. The reaction was quenched by adding 3mL of cold water until the solution turned cloudy. The white precipitate was filtered out and the filtrate was extracted with 3mL of t-butyl methyl ether. The ether layer was spotted on TLC and showed no organic compounds. The aqueous layer was extracted with an equal volume of ethyl acetate. TLC showed no extracted compounds. The filtered solid was dissolved in methanol and TLC (10% methanol/methylene chloride) showed a spot with an R<sub>f</sub> value between that of thymine and bisbromomethylanthracene. The fraction was separated by flash column chromatography using methanol/ methylene chloride solution (1-12% gradient) and 20g of silica gel. <sup>1</sup>HNMR (DMSO, D<sub>6</sub>), Appendix E: δ 8.74 (s, H), 8.60 (s, H), 8.00 (d, H), 7.49 (m, H), 5.43 (d, H), 5.13 (d, H), 3.44-3.28 (d), 2.67-2.33 (t).

### Method 4

Sodium hydride, 12.6mg (0.525mmol), was washed with anhydrous hexane and then suspended in 3mL of anhydrous DMF. Thymine, 12.6mg (0.1mmol), was added to this suspension and heated to 100°C until the thymine dissolved. The reaction mixture cooled to room temperature before bisbromomethylanthracene, 36.4mg (1mmol), was

added. The solution was stirred at room temperature for 2 h until TLC of reaction showed very little bisbromomethylantracene left. Two spots were observed by TLC, with the major one slightly faster than thymine. Separation was carried out on preparative-TLC plate using methanol/ methylene chloride (5%). Among the several bands observed, two bands were major which were isolated and products in them were recovered.  $^1\text{H}$ NMR (DMSO,  $\text{D}_6$ ), Appendix H: Fraction 1:  $\delta$  7.27-7.25 (s), 4.20 (t), 1.69 (s), 1.26-1.24 (d), 0.92-0.84 (q), 0.05-0.01 (s).  
Fraction 2:  $\delta$  7.25 (s), 1.68 (s), 1.24 (s).

### Synthesis of Dithyminylmercury

Thymine, 1.26g (10mmol), was dissolved in 40mL of hot water containing 0.40g (10mmol) of sodium hydroxide. To the clear solution was added a saturated solution of 13.5g (50mmol) of mercury chloride dissolved in methanol. A heavy white precipitate was formed immediately. The reaction mixture was cooled and the white solid **13** was filtered and washed successively with cold water, ethanol, and ether to give 16g (35.5mmol) of dry product. This product was used in the next step without further purification.

### Reaction of Dithyminylmercury with Benzyl Iodide

Dithyminylmercury, 0.5g (1.11mmol), was pulverized and suspended in 25mL of toluene in a round-bottom flask equipped with Dean-Stark azeotrope distillation head. The cloudy solution was refluxed until approximately one-third of the toluene was

distilled out. Then benzyl iodide, 0.484g (2.22mmol), was added to the solution and refluxed for 2 h at room temperature. The warm reaction mixture was filtered to remove unreacted dithyminymercury. An equal volume of petroleum ether was added to the cold filtrate, but no precipitate was obtained so the solvent was evaporated and the residue was redissolved in methylene chloride. Separation was done by column chromatography using 2% methanol/ methylene chloride solution and 100g of silica gel. <sup>1</sup>HNMR (CDCl<sub>3</sub>), Appendix F: Fraction 1: δ 7.39-7.25 (m, 5H), 4.50-4.46 (s, 2H). Fraction 2: δ 8.55 (s, 1H), 7.36-7.29 (m, 5H), 7.26-7.25 (s, 1H), 4.48 (s, 2H), 1.87 (s, 3H).

#### Reaction of Thymine with Benzyl Bromide (and Benzyl Iodide)

##### Method 1

Thymine, 1g (7.94mmol), was dissolved by heating in 50mL of tert-butyl alcohol. Potassium t-butoxide, 0.865g (9.4mmol), was added to the solution and stirred for 20 minutes before adding 1.356g (7.92mmol) of benzyl bromide. The solution was refluxed until all the benzyl bromide reacted (2h). The solvent was evaporated completely and the residue was dissolved with methylene chloride. TLC showed one major spot that was separated by column chromatography using a methanol/methylene chloride solution (1-5% gradient) and 30g of silica gel. <sup>1</sup>HNMR (CDCl<sub>3</sub>), Appendix A0: δ 1.64 (s, H), 1.24 (s, H) 0.88 (s, H).

##### Method 2

Sodium hydride, 0.12g (3mmol), was washed with anhydrous hexane under nitrogen and then suspended in 3mL of freshly distilled DMF. Thymine, 0.2522 g

(2mmol), was added followed by 0.342g (2mmol) of benzylbromide. The reaction mixture was refluxed for 20 h under nitrogen and then quenched by adding ice to the mixture. The white precipitate formed was extracted with 10mL of methylene chloride. The methylene chloride layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. TLC of the solution showed two spots that were separated by column chromatography eluting with 1% methanol/ methylene chloride and 40g of silica gel. <sup>1</sup>HNMR (CDCl<sub>3</sub>), Appendix G: Fraction 1, 12b: δ 7.51-7.27 (m, 10H), 6.97 (s, 1H), 5.17 (s, 2H), 4.89 (s, 2H), 1.89 (s, 3H). Fraction 2, 12a: 9.96 (s, 1H), 7.35-7.28 (m, 5H), 6.75 (s, 1H), 4.89-4.84 (s, 2H), 1.86-1.81 (s, 3H).

#### Protection of 1,8-Bishydroxymethylanthracene

Sodium hydride, 4mg (0.17mmol), was washed with anhydrous hexane under nitrogen then suspended in 2mL of freshly distilled THF. 1,8-Bishydroxymethyl anthracene, 25mg, was added and the solution was stirred under nitrogen for 40 minutes. Tert-butyldimethylsilyl chloride (TBDMSCl), 15mg dissolved in 1mL of THF, was added drop-wise over a period of 20 minutes then stirred for an additional 20 minutes. The reaction was quenched with 5mL of t-butylmethylether, followed by an equal volume of 40% sodium carbonate solution saturated with NaCl. The ether layer was separated and dried over calcium chloride and evaporated to give a faint yellow oily residue that was dissolved in methylene chloride. TLC of solution showed one major spot. It was separated by column chromatography, eluting with 2% methanol/methylene chloride. <sup>1</sup>HNMR (CDCl<sub>3</sub>), Appendix H: δ 7.27-7.14 (m, 8H), 5.06-5.03 (s, 4H), 4.12-3.84 (s, 15H), 2.29 (s, 4H), 0.95 (s, 5H), 0.30 (s, 2H).

## 4. CONCLUSION

Understanding of the activity of naturally occurring metallo- and non-metallonucleases is essential in designing synthetic nucleases. Many examples of both natural and synthetic nucleases have been discussed and shown to be very effective in their ability to hydrolyze phosphodiester bonds or catalyze transesterification.

The characteristics of toluidine blue O make it a suitable candidate for the design of our “nucleases” models. Although the synthesis of the final models was not realized in this project, the models fit the criteria discussed in molecular recognition, with TBO being both a spacer and a binding group for the substrate. Also, other models were designed based on anthracene derivatives with the incorporation of imidazole and guanidine groups that are expected to hydrolyze phosphodiesters by general acid-base mechanism.

Many reactions have been conducted in the course of this research aimed at synthesizing such model compounds. The experience was very helpful in understanding the chemical nature of TBO and the anthracene derivatives, as well as many other areas of synthetic organic chemistry. We hope that future work will build on the experience gained from this project.

## BIBLIOGRAPHY

1. Barton, J.K. *Science* 1986, 233, 727-734.
2. Jubian, V; Dixon, R; Hamilton, D. A, *J. Am. Chem. Soc.* 1992, 114, 1120-1121.
3. Cowan, J. A, *Current Opinions in Chemical Biology*, December 2007, volume 5, Issue 6, 634-642.
4. Tullius, T.D, *Metals and Molecular Biology*, Metal-DNA Chemistry, 1989,1-21, ACS Symposium Series, 0097-6156, 402.
5. Dervan, P.B. *Science*, 1986, 232, 464-467.
6. Moser, H.E; Dervan, P.B. *Science* 1987, 238, 645-650.
7. American Cancer Society, *Cancer Facts & Figures*, 2006.
8. <http://www.ghchealth.com/chemotherapy-quotes.html>, *The Truth about Chemotherapy Quotes*, November, 2006.
9. Cancer Patients Opt for Unapproved Cancer Drug, DCA. *Nature News*, March 28th, 2007.
10. <http://www.cancer.gov/cancertopics/When-Cancer>Returns/page2>, Nov 2006.
11. RNA Ribonucleic Acid by Darryl Leja, National Human Genome Research Institute (NHGRI). <http://www.genome.gov>, Feb 2008.
12. Watson, J.D; Crick, F.H.C. *Nature*, 1953, 171, 737-738.
13. Wells, R.D.J. *Biol.Chem*, 1988, 263, 1095-1098.
14. Wikipedia, the Free Encyclopedia, [www.wikipedia.org/wiki/DNA](http://www.wikipedia.org/wiki/DNA), [www.wikipedia.org/wiki/DNAintercalation](http://www.wikipedia.org/wiki/DNAintercalation).
15. Barton, J.K, Recognizing DNA, *Chem.Eng.News*, Sep 26th, 1988.



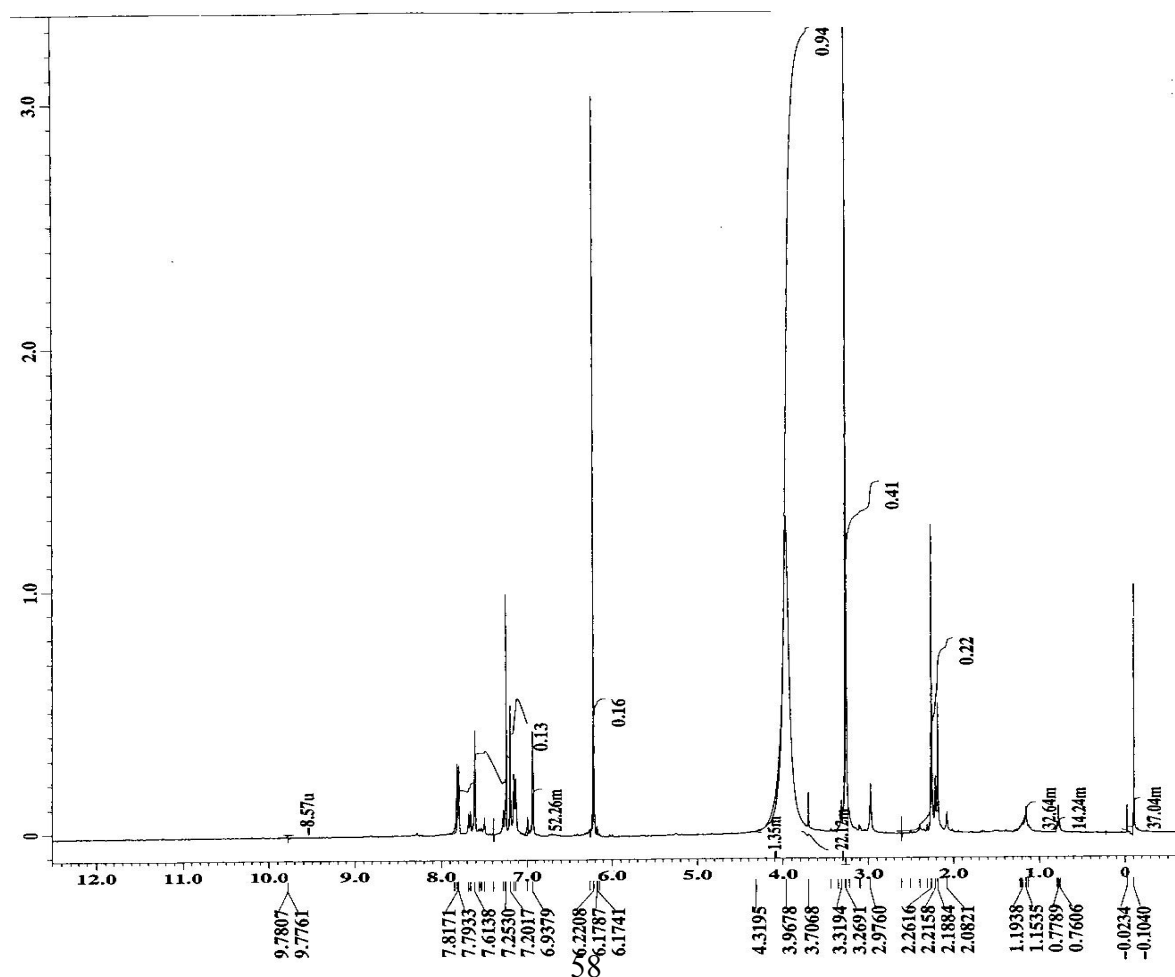
16. Bugg, T. An Introduction to Enzyme and Coenzyme Chemistry, Blackwell Publishing, 1997, 7-25.
17. <http://www.nature.com/nature/journal/v435/n7042/abs/nature03556.html>. *Highly Efficient endogenous Human Gene Correction using designed Zinc-finger Nucleases*, March 2007.
18. Hamilton, A.D; *J. Chem. Educ.* 1990, 67, 821.
19. Aggarwal, A.K; Rodgers, D.W, *Science* 1988, 242, 899-907.
20. Kimura, E; Nakamura, I; Koike, T; Shionoya, M; Kodama, Y; Ikeda, T, *J. Am. Chem. Soc.* 1994, 116, 4764-4771.
21. Sigman, D. A; Graham, D.R, *J. Biol. Chem.* 1979, 254, 12269-12271.
22. Tamilarasan, R, *Excited State Modalities for Studying the Binding of Copper Phenanthroline to DNA*, Metal-DNA Chemistry, 1989, 48-58, ACS Symposium Series, 0097-6156, 402.
23. Liu, S; Hamilton, A, *Bioorganic & Medicinal Chemistry Letters*, 1997, 7, 13, 1779-1784.
24. Liu, S; Hamilton, A, *Tetrahedron Letters*, 1997, 38, 1107.
25. Kady, I. O; Tan, B; Ho, Z; Scarborough, T, *J. Chem. Soc., Chem. Commun.*, 1995, 1137-1138.
26. Eftink, M. R; Biltonen, R. L, *Pancreatic Ribonuclease A: the most Studied Endoribonucleas*, Hydrolytic Enzymes, 333-334, © 1987 Elsevier Science Publishers B.V.
27. Groves, J. T, *J. Med. Chem.* 1974, 17, 902-904.

28. Benkovic, S.J.; Schray, K.J. In *The Enzymes*; Boyer, P.D., Ed.; Academic Press: New-York, 1973; Vol. 8, pp 201.
29. Jonnalagadda, S.B; Gollapalli, N. R, *J. Chem. Educ.* 2000, 77, 506.
30. Kady, I.O, *J. Chem. Educ.* Vol 72, Number 1, Jan, 1995, A9-A10.
31. Golden, Ronald; Stock, M. Leon, *J. Am. Chem. Soc.* 1972, 94, 3086-3087.
32. Akiyama, Shuzo; Misumi, Soichi; Nakagawa, Masazumi, *J. Am. Chem. Soc.* 1962, 1834.
33. Jiang, Yu Lin; *Facile One-pot Synthesis of Alkylated DNA Bases*; Poster presentation, Department of Chemistry, East Tennessee State University, 2007.
34. Fox, J; Yung, N; Davoll, J; Brown, G. B; *Org. and Biol. Chem.*, 1956, 78, 2117-2120.
35. McDougal, P; Rico, J; Oh, Y; Condon, B, *J. Org. Chem.* 1986, 51, 3388-3391.

APPENDICES

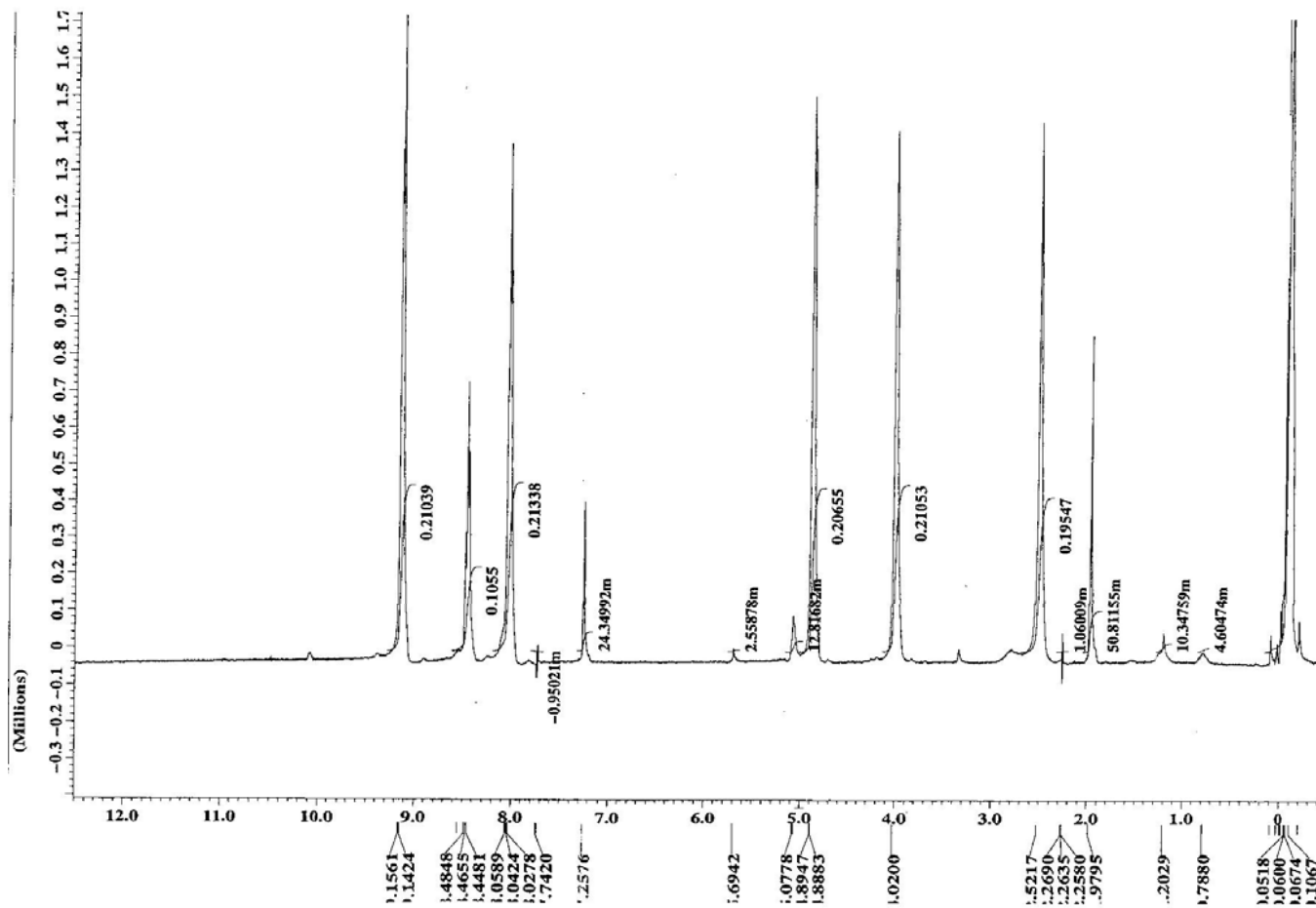
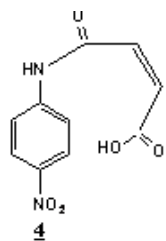
APPENDIX A

$^1\text{H}$  NMR Spectrum of Product Isolated from Reaction of TBO with Maleic Anhydride in Acetone Solvent d-  $\text{CDCl}_3$  (5%  $\text{CD}_3\text{OD}$ )/TMS.



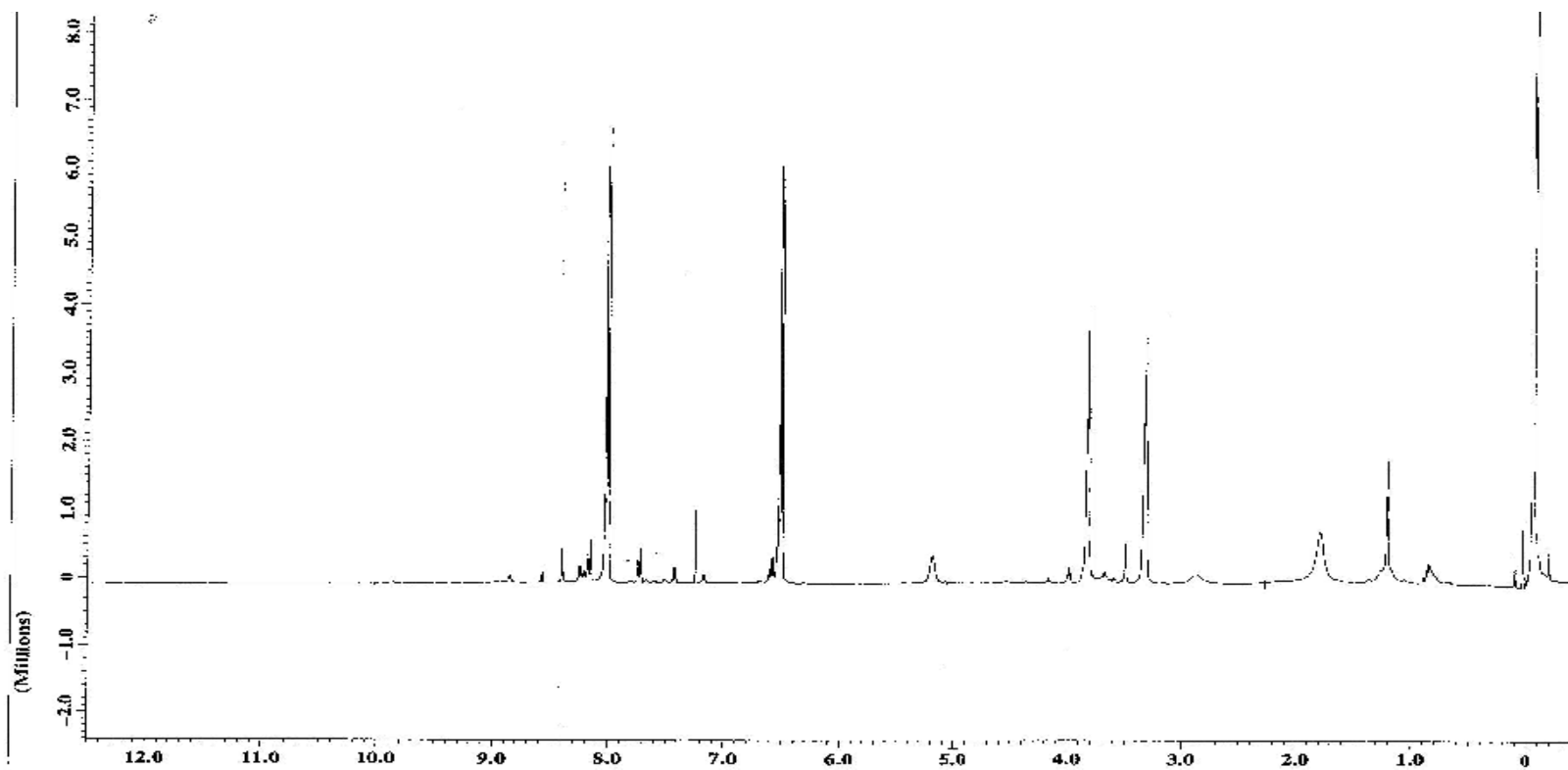
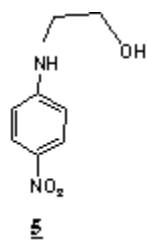
APPENDIX B

<sup>1</sup>H NMR Spectrum of **4**  
Solvent d-CDCl<sub>3</sub>/TMS



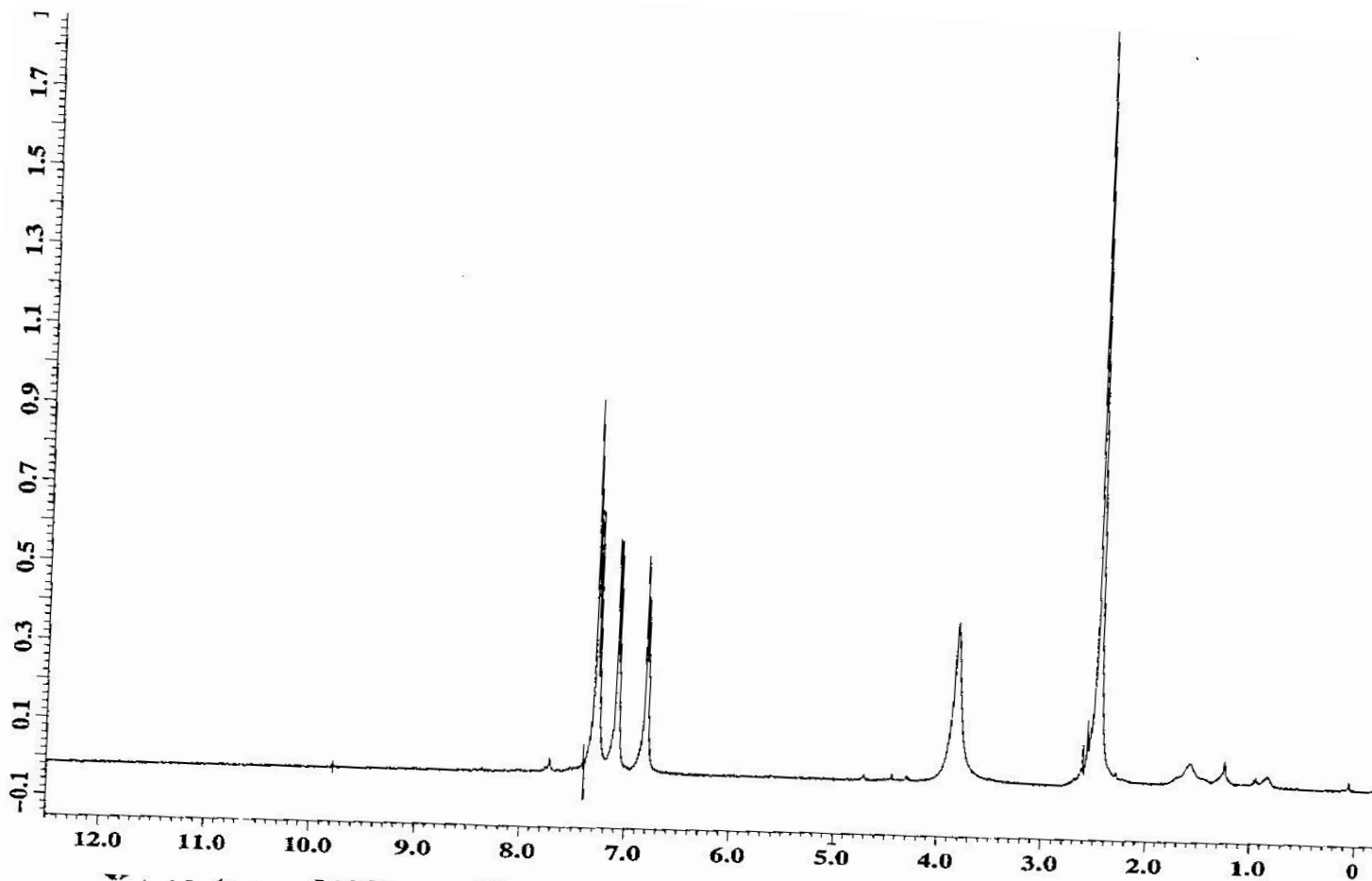
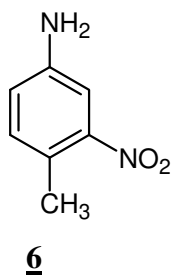
APPENDIX C

$^1\text{H}$  NMR Spectrum of Compound 5  
Solvent  $\text{d-CDCl}_3/\text{TMS}$



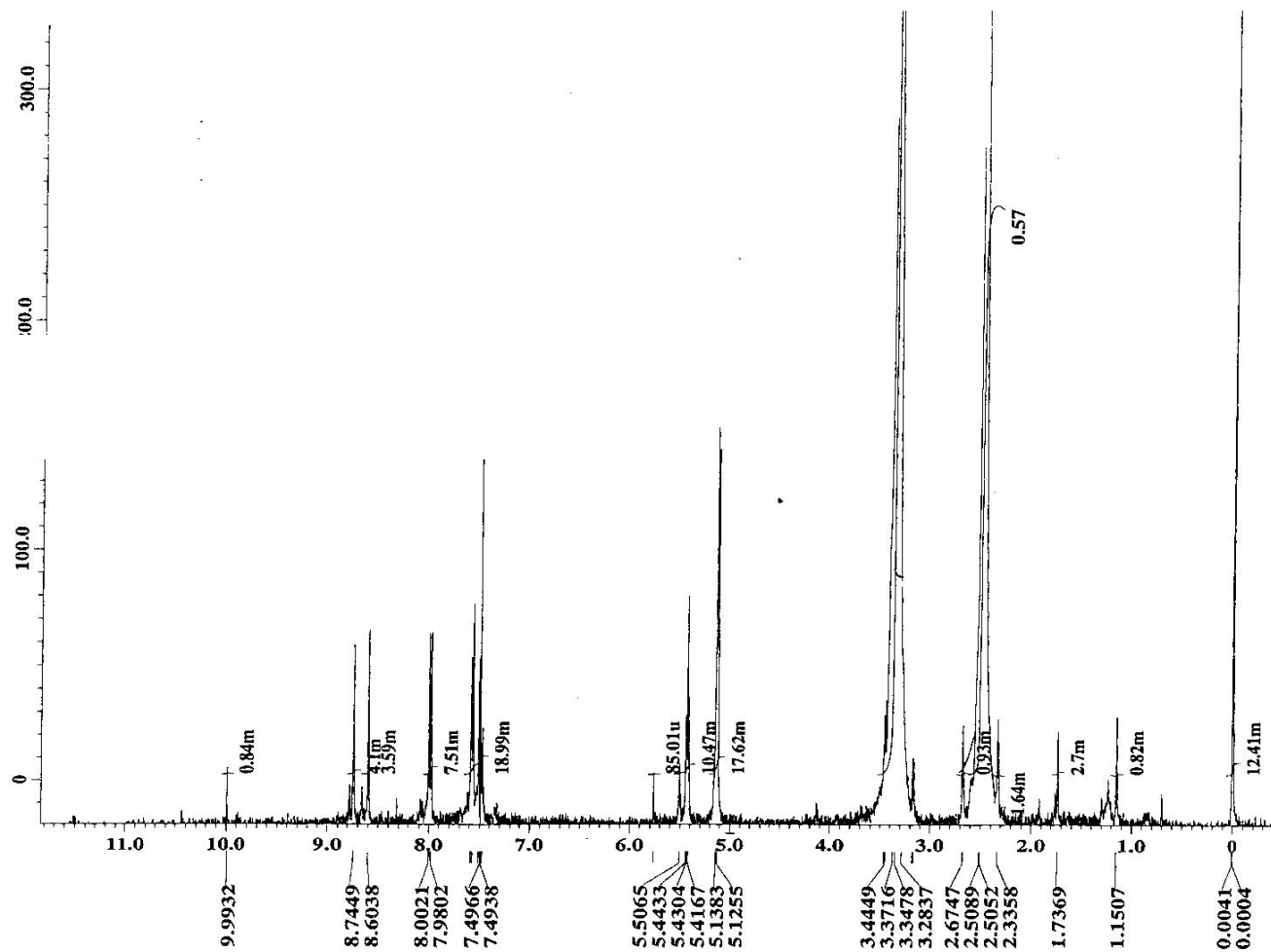
APPENDIX D

$^1\text{H}$  NMR Spectrum of Compound 6  
Solvent d-  $\text{CDCl}_3$ /TMS



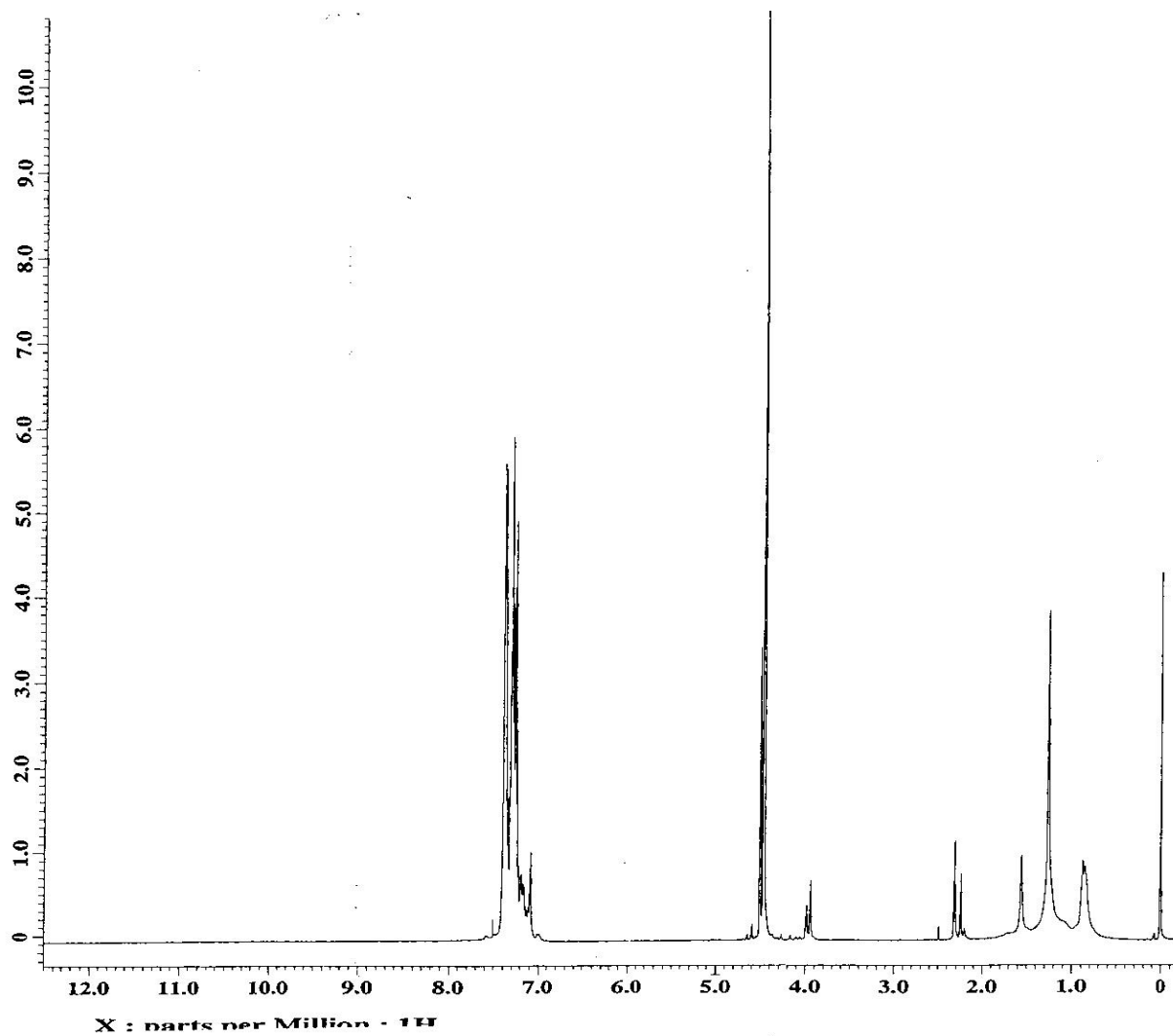
APPENDIX E

$^1\text{H}$  NMR Spectrum of Product Isolated from Reaction of Thymine with 1,8-Bisbromomethylantracene in DMSO  
Solvent  $\text{d}_6$ -DMSO/TMS



APPENDIX F1

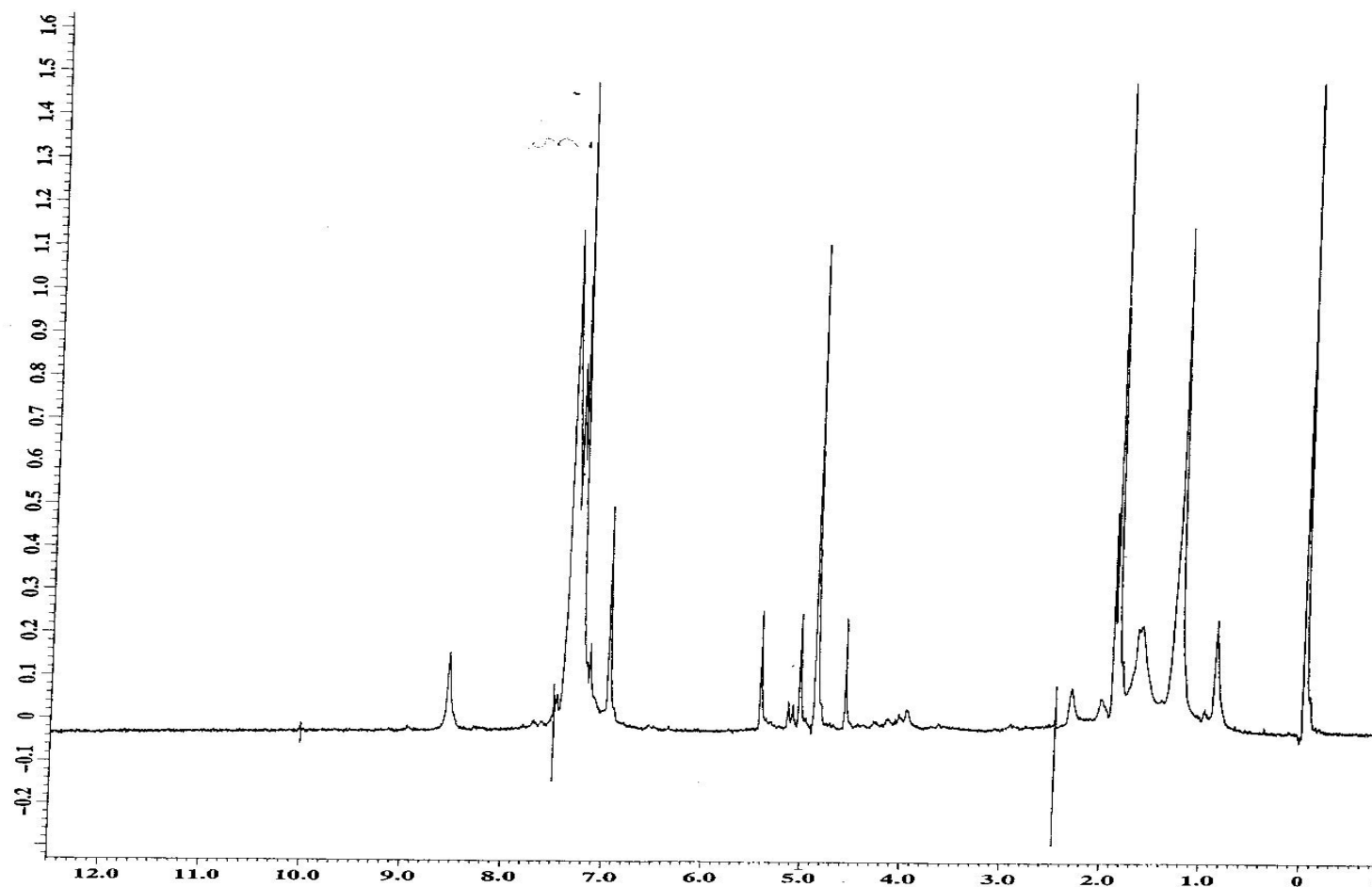
$^1\text{H}$  NMR Spectrum of Product Obtained from First Fraction of Reaction of Benzyl Iodide with Dithymylmercury.  
Sweep width 800Hz. Solvent d-  $\text{CDCl}_3$ /TMS.





APPENDIX F2

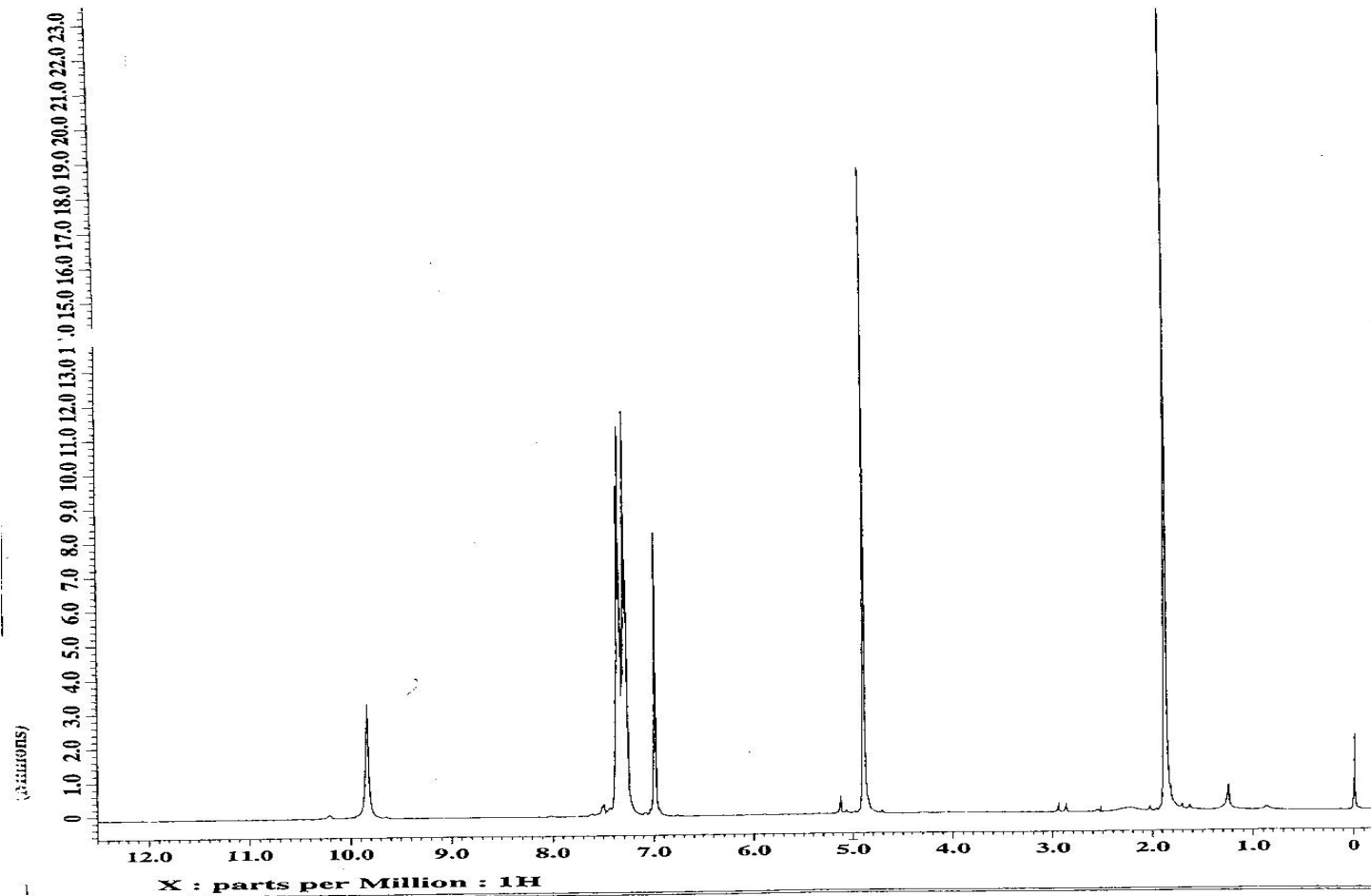
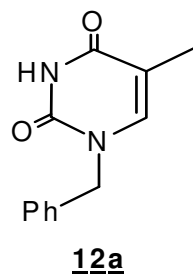
$^1\text{H}$  NMR Spectrum Product Obtained from Second Fraction of Reaction of Benzyl Iodide with Dithymylmercury.  
Solvent d-  $\text{CDCl}_3$ /TMS.



APPENDIX G1

$^1\text{H}$  NMR Spectrum of Compound **12a**.

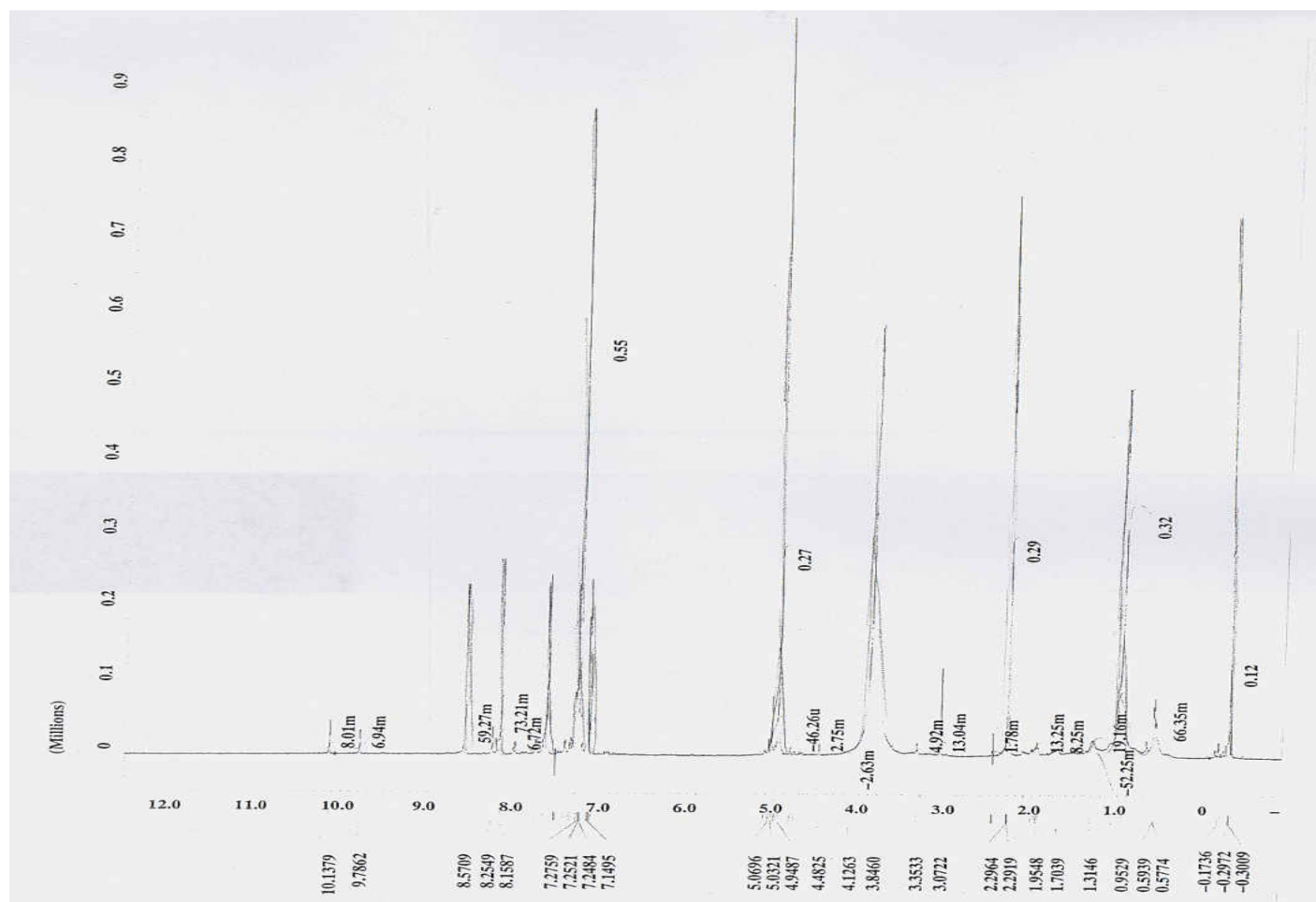
Solvent d-  $\text{CDCl}_3$  / TMS.





## APPENDIX H

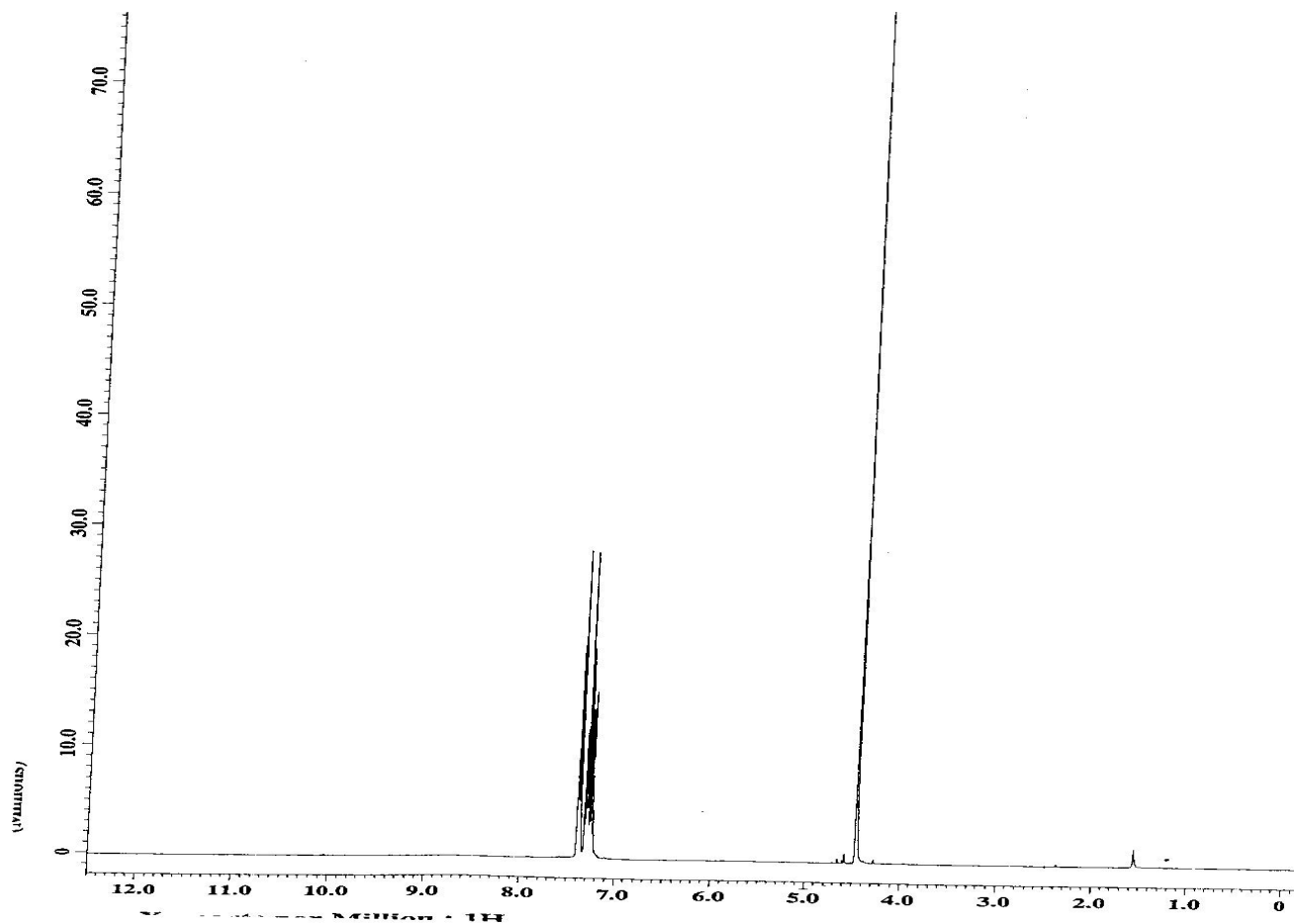
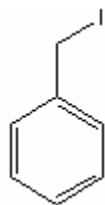
$^1\text{H}$  NMR Spectrum Product Obtained from Protection of 1,8-Bishydroxymethylanthracene with TBDMS  
Solvent  $\text{d-CDCl}_3/\text{TMS}$



APPENDIX I

$^1\text{H}$  NMR Spectrum of Benzyl Iodide.

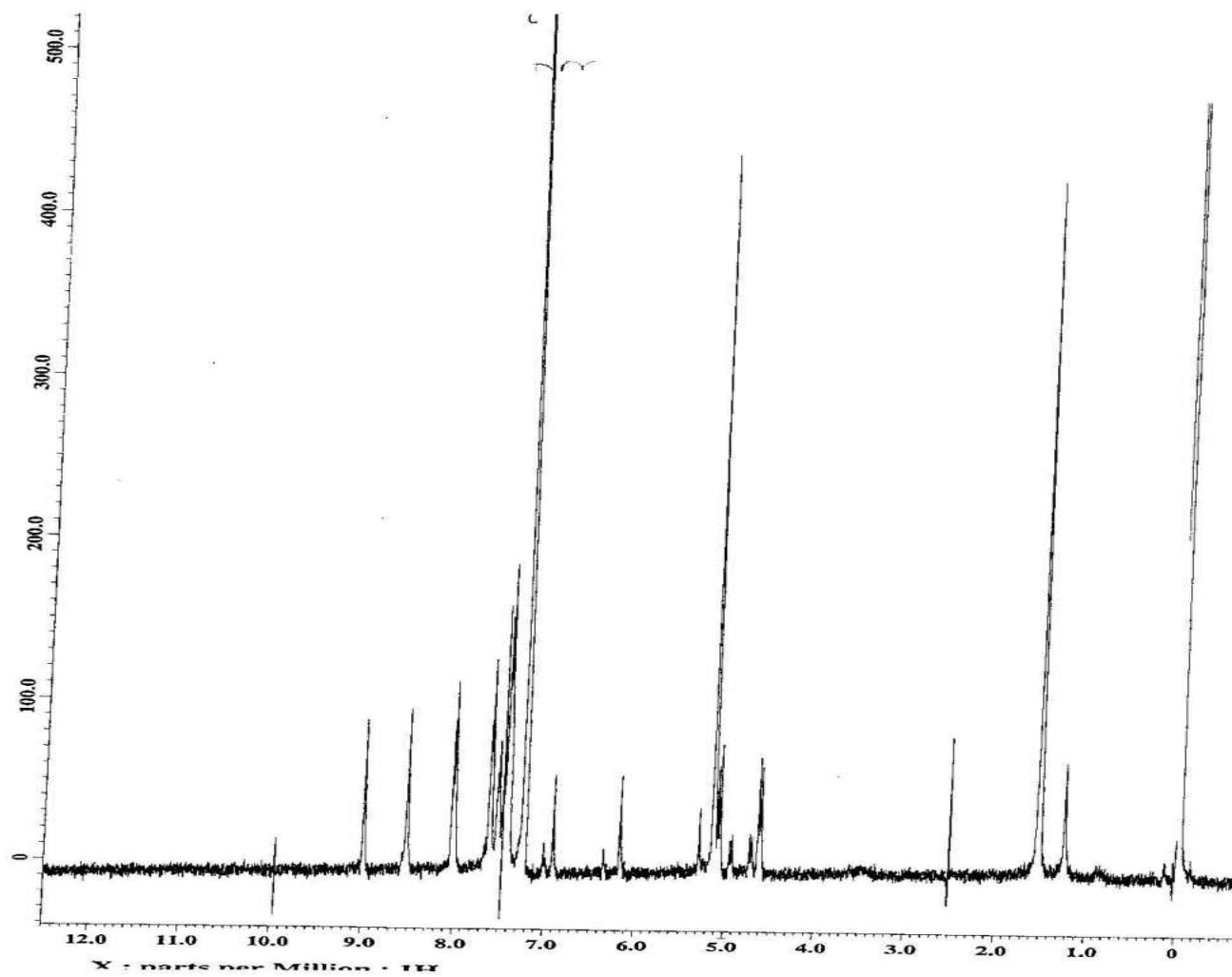
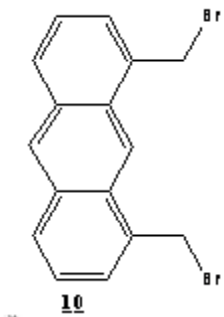
Solvent d-  $\text{CDCl}_3$  / TMS.



APPENDIX J

$^1\text{H}$  NMR Spectrum of 1,8-Bisbromomethylantracene.

Solvent  $d\text{-CDCl}_3$ /TMS.



## VITA

ENNI N. FOMUMBOD

Personal Data:                   Date of Birth: April 15, 1981  
  Place of Birth: Nyasoso, Cameroon  
  Marital Status: Single

Education:                        Public/ Private Schools, Bamenda, Cameroon  
  B.S. Chemistry and Material Science and Technology,  
  University of Buea, Cameroon 2004  
  M.S. Chemistry, East Tennessee State University, Johnson  
  City, Tennessee 2008

Professional Experience:        Social Worker, A.I-ChrisWOV, Bamenda,  
  Cameroon 2002-2005  
  Graduate/ Teaching Assistant, East Tennessee State  
  University 2005-2007  
  Research in Organic Chemistry, East Tennessee State  
  University 2006-2007

Honors and Awards:             Award of Academic Excellence, Ministry of Higher  
  Learning, Republic of Cameroon 2004

Extracurricular:                Member/ Liturgist/ Volunteer Sunday School teacher,  
  Covenant Presbyterian Church, Johnson City,  
  Tennessee 2005-2007  
  Member/ Volunteer, Presbyterian Campus Ministry  
  Four mission trips, Katrina Relief (Gulf Port)/ Care for the  
  Homeless (New York), 2005-2007