Studies on the Synthesis and Properties of Modified Pyrimidine Nucleobases, *Bis*-Pyrimidine Dimers and Peptide Nucleic Acids

A Dissertation Submitted to the Indian Institute of Technology Guwahati,

As Partial Fulfillment for the Degree of Doctor of Philosophy in Chemistry

Submitted by

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June 2014

Dedicated to

My parents

INDIAN INSTITUTE OF TECHNOLOGY

GUWAHATI

Department of Chemistry

STATEMENT

I do hereby declare that the matter embodied in this thesis is the result of investigations carried out by myself in the Department of Chemistry, Indian Institute of Technology Guwahati India, under the supervision of **Dr. Lal Mohan Kundu**

In keeping with the general practice of reporting scientific observations, due Acknowledgements have been made wherever the work described is based on the findings of other investigators.

June, 2014

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CERTIFICATE

This is to certify that Burgula. Laxminarayana has been working under my supervision since July, 2009 as a regular registered Ph. D. student. I am forwarding his thesis entitled "**Studies on the Synthesis and Properties of Modified Pyrimidine Nucleobases, *Bis*-Pyrimidine Dimers and Peptide Nucleic Acids**" being submitted for the Ph. D. (Science) degree of this Institute. I certify that he has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in his thesis and this work has not been submitted elsewhere for a degree.

Dr. Lal Mohan Kundu
Supervisor
Department of Chemistry
IIT Guwahati

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Finally, my Ph. D. endeavor could not have been completed without the endless love, unending support, tolerance and blessings from my family. I wish to express my sincere gratitude to my parents (Ramachandraiah and Rambai), brother (Srinivas), sister (Ilamma and Shoba rani), brother in law (Venkanna and Purna chander), nephew (Kiran and Charan) and niece (Dhanalaxmi and Meghna). I am also grateful to my cousins (Raju and Dayakar), and their family members for their affection and deep concern for career. They are the main soul and inspiration for each and every step that I achieve in my life.

Burgula Laxminarayana

Summary of my research:

The content of my thesis mainly includes synthesis and study the activities of modified bioactive molecules, primarily nucleic acids. A summary of my research is given below.

1. Synthesis modified pyrimidine and pyrimidinones nucleobases:

Modified pyrimidines, pyrimidinones and their nucleic acids derivatives have found many biological and pharmaceutical applications. Several of the C-5, C-6 and N-3 substituted compounds are shown selective antitumor, antiviral, antitubercular and antifungal activity due to their high bioactive nature. In last decade the synthesis usually carried out by using harsh conditions, expensive catalyst and metal coupling reactions. Here we have developed a new methodology for synthesis of various uracil, thiouracil, cytosine, and thiocytosine derivatives in microwave-directed reactions. We also demonstrated chemoselective synthesis of N-3 arylated and alkylated pyrimidinones with induced electronic and steric properties at C-5 and C-6 positions.

(The overall goal is to develop a variety of modified nucleobases in environment friendly methods)

2 Study of UV-induced DNA damages and mutations:

UV-induced DNA damage is the major cause of skin cancer and aging. Mostly pyrimidine nucleobases undergo lesion formation when exposed to UV-radiation, leading to mutations. Here, we have synthesized *bis*-pyrimidine that contained the modified pyrimidine nucleobases. We have thoroughly investigated the effect of substitution on the rate of UV lesion formation. Both the free nucleobase analogs as well as their homo- and hetero- *bis*-pyrimidine were examined. A systematic study, under controlled UV-radiation, show that many of the synthesized analogs are highly stable towards UV-light. Such nucleobases and their oligonucleotides, therefore, could have potential application to prevent damages and as sunscreen agents.

(The overall goal is to develop modified nucleotides leading to skin therapeutic agents)

3 Synthesis of modified nucleic acids for un-natural base-pair:

Modified oligonucleotides are important biomolecular probes for the detection of genomic mutations, damages, gene targeting and gene silencing. Modified nucleotides are widely used in many drugs, DNA repairing processes, gene mutations study and altering base pairing. The development of solid-phase, automated methods for the synthesis of DNA, RNA, and PNA in the last decade led to widespread use of nucleic acids and their analogues. Oligonucleotides and their analogues are also used as therapeutic agents targeted to human disease. To this end, we have successfully synthesized PNA oligomers containing modified nucleobases, which could potentially alter the base-pairing properties.

(This project leads abroader goal with the focus on gene silencing and as molecular probes

Abbreviations:

EtOH	Ethanol
MeOH	Mthanol
THF	Tetrahydrofuran
MeCN	Acetonitrile
DCM	Dichloromethane
NEt_3	Triethylamine
TFA	Trifluoroacetic acid
NMR	Nuclear Magnetic Resonance
MS	Mass Spectrometry
UV	Ultra-violet
Vol	Volume
Min	Minute
Hr	Hour
µM	Micro-molar
µL	Micro-liter
L	Liter
Oligo	Oligonucleotide
Ac_2O	Acetic anhydride
Boc	tert-butyloxycarbonyl
DCM	Dichloromethane
DIPEA	Diisopropylethyl amine
DMF	N, N dimethyl formamide
ESI-MS	Electrosprayionization mass spectrometry
Et_2O	Diethyl ether
Fmoc	9-Fluorenylmethoxycarbonyl
FT-IR	Fourier transformation infra red spectroscopy
HPLC	High pressure liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
mM	millimol

µM	micro mol
MW	Molecular weight
NMR	Nuclear magnetic resonance
RP	Reverse phase
SPPS	Solid phase peptide synthesis
TFA	Trifluoroacetic acid
UV	Ultraviolet
LiOH	Lithum hydrioxide
NaOH	Sodium hydrioxide
HCl	Hydrochloricacid
BSA	Bissilylacetamide
HBTU	*N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazolyl) uronium hexafluorophosphate
MW	Microwave
$BF_3.Et_2O$	Boron trifluoride ethyl etherate
TFMSA	Trifluoromethanesulfonic acid
K_2CO_3	Potassium carbonate
Pip	Piperidine
ORTEP	Oak Ridge Thermal Ellipsoid Plot
FESEM	Field Emission Scanning Electron Microscopy
PNA	Pepitide nucleicacid
SMAs	Supramolecular architectures
FA	Formicacid

Synopsis

The contents of this thesis entitled "**Studies on the Synthesis and Properties of Modified Pyrimidine Nucleobases, *Bis*-Pyrimidine Dimers and Peptide Nucleic Acids",** is divided into six chapters based on the results of experimental work performed during the research tenure. The introductory chapter of the thesis presents an overview of various synthetic routes of modified nucleic acids and their applications in biomolecular research. Chapter-II describes the synthesis of unnatural uracil, cytosine, thiouracils and thiocytosine derivatives using a microwave-assisted method. Chapter-III demonstrates an efficient chemo-selective, microwave-assisted methodology for direct synthesis of N-3 alkylated and arylated pyrimidinones from their beta-carbonylester precursors. Chapter-IV illustrates the design and synthesis of self-assembled, novel modified nucleobases for supra-molecular architectures. Chapter-V deals with the synthesis of C-5 and C-6 substituted modified pyrimidine homo- and hetero-dimers and the study of UV-induced photochemical damages, where, a number of synthesized pyrimidinones show remarkable stabilities under UV-radiation. Chapter-VI mainly focuses on synthesis of modified peptide nucleic acid (PNA) which forms stable duplex with DNA. Each chapter consists of four sub sections: introduction, present work, experimental methods and spectral data.

1. Introduction

In this chapter recent literature related to synthesis of modified nucleobases, model dinucleotides, peptide nucleic acids, modified oligonucleotides and their structural features are described. Mechanism of action and the recent development of non-natural nucleobases and nucleic acids in biological and pharmaceuticals applications have been discussed. This chapter also demonstrates the effect of UV-radiation in inducing DNA damages and lesions, leading to mutations in cellular DNA.

2. Synthesis of modified uracil, thiouracil, cytosine and thiouracil derivatives in a microwave-directed method

In this chapter we have demonstrated development of a new methodology to synthesize various uracil, thiouracil, cytosine, and thiocytosine derivatives in microwave-directed reactions. Such classes of compounds have been reported to be pharmaceutically active or could be potential unnatural nucleobase. In the past decade the microwave-directed methods were also used to synthesize non-nucleobase pyrimidines. Here we found a environmentally-benign, efficient synthesis of pyrimidine nucleobases with altered steric and electronic properties at C-5 and C-6 positions. We found that the use of microwave-assisted synthesis under solvent-free conditions led to high yields of uracil, thiouracil, cytosine and thiocytosine derivatives. The yield of the reactions as well as the rate of conversions were further enhanced, significantly, by use of $BF_3.Et_2O$ as Lewis acid.

Scheme 1

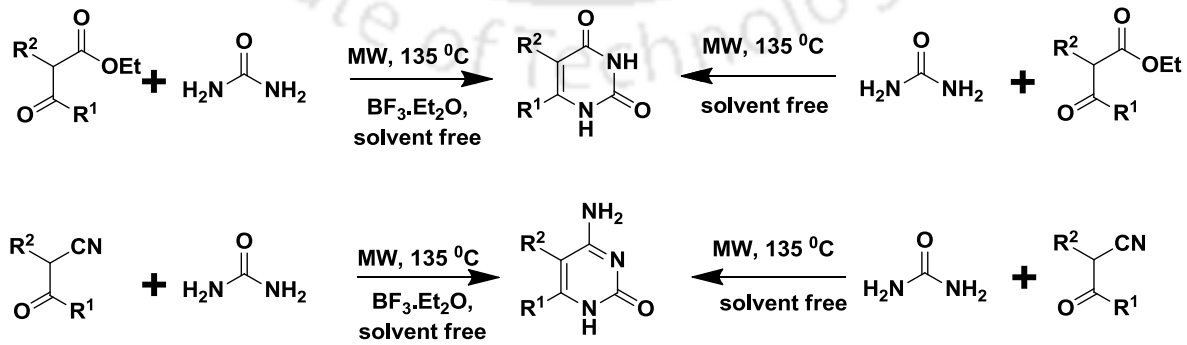


***Figure 1**: Schematic representation for the synthesis of uracil and cytosine derivatives*

Scheme 2

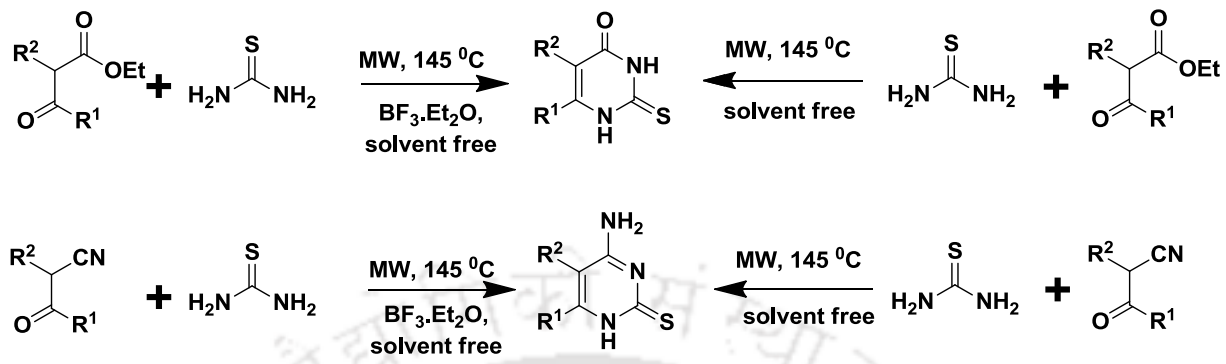


Figure 2: *Schematic presentation for synthesis of thiouracil and thiocytosine derivatives*

Advantage of the present method

1. Shorter reaction time and easy handling.
2. Environmentally benign as no toxic reagent is used
3. Use of modern techniques
4. A one –pot synthesis
5. Cheaper method and free from solvents

3. An efficient chemo-selective, microwave-assisted methodology for direct synthesis of N-3 alkylated and arylated pyrimidinones from their beta-carbonylester precursors

Modified nucleobases, their N3-derivatives and nucleic acids have found many biological and pharmaceutical applications. Several of the C-5, C-6 and N-3 substituted pyrimidine nucleobase derivatives showed selective antitumor, antiviral, antitubercular and antifungal activity. They were usually synthesized by using harsh conditions, expensive catalyst, metal coupling reactions or Blaise reaction intermediate. We wish to report here direct, chemoselective, one-pot method for the synthesis of a variety of modified alkylated/benzylated or arylated derivatives selectively at N-3 position of the nucleobases with high yields under solvent-free conditions. An array of compounds with varied substitution at C-5 and C-6 were synthesized. The reaction yields were further improved

by addition of Lewis acid as a catalyst. Apart from ^{1}H NMR, ^{13}C NMR and HRMS, the compounds were also characterized by NOESY and rare crystal structures.

Scheme 3

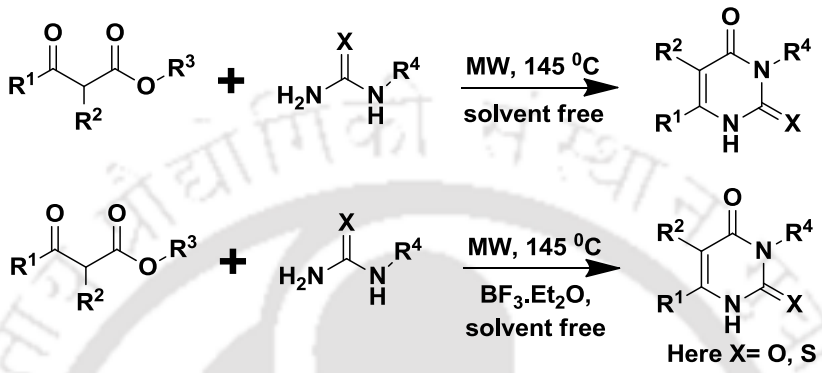


Figure 3: *Schematic presentation for synthesis of N3-derivatives of uracil and thiouracil*

Some representative examples are shown in below:

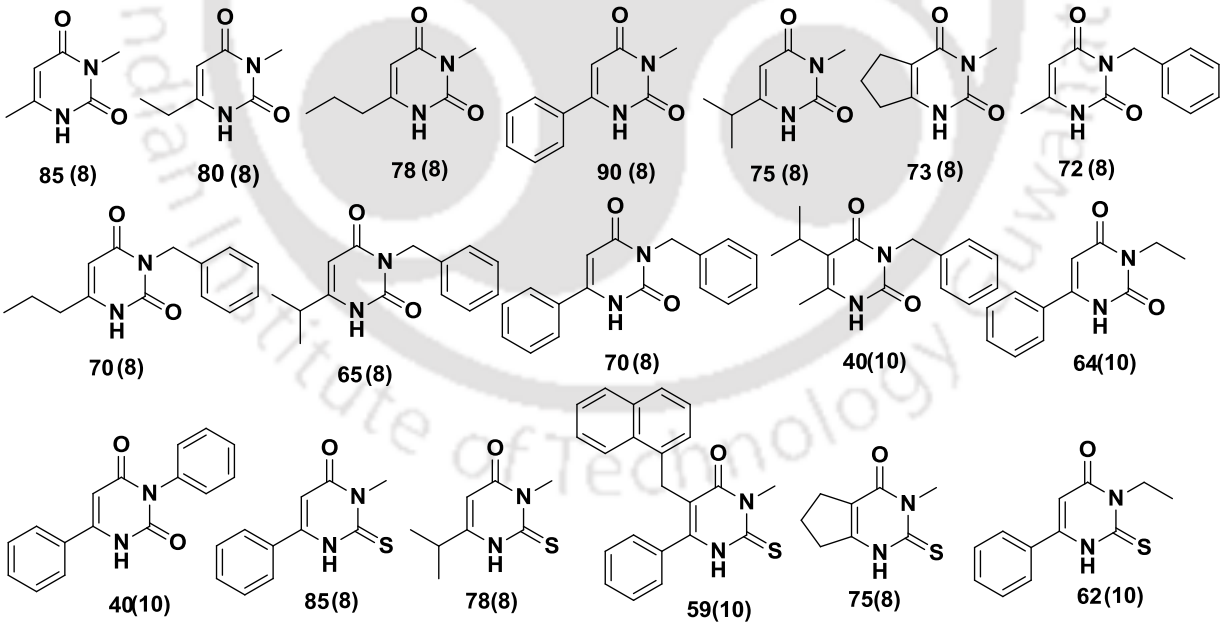


Figure 4*: Synthesis of a series of N3-substituted nucleobase derivatives*

Conclusion

In summary, we have devised a simple and efficient protocol for direct, chemoselective synthesis of N3- derivatives of uracil and thiouracil by using $BF_3.Et_2O$ as catalyst, under solvent free microwave condition. The main advantage of the present protocol is chemoselectivity, mild reaction condition, shorter reaction time, easy to handle and compatible with a wide range of substrates.

4. Design and synthesis of self-assembled novel modified nucleobases for supra-molecular architectures

The concept of self aggregation is widely used in heterocyclic chemistry, drug delivery, protein chemistry, peptide chemistry, material chemistry and DNA chemistry. Such self aggregation is mainly due to H-bonding and pi-stacking forces in between the molecules. The double helical structure of naturally occurring DNA is largely attributed to Watson–Crick base pairing interactions (H-bonding), along with pi-stacking forces. We have synthesized self-assembled novel nucleobases which act as molecular architectures. The shape of the supramolecular architectures could be controlled by substitution and chain length. These nucleobases have shown remarkable molecular orientation and different kind of H-bonding as well as pi-stacking.

Scheme 4

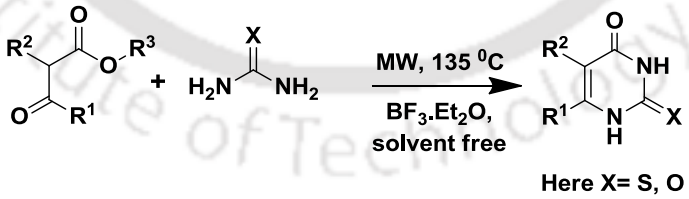


Figure 5: *Schematic presentation for synthesis of novel modified nucleobases*

Some representative examples are shown in table 1:

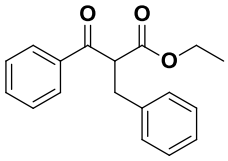
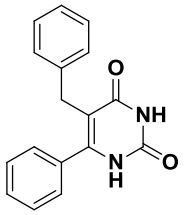
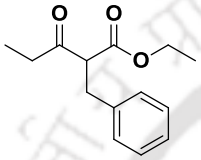
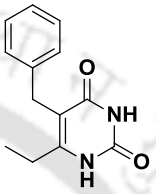
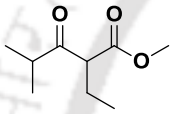
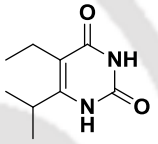
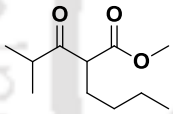
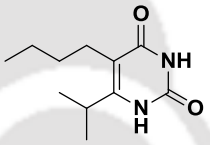
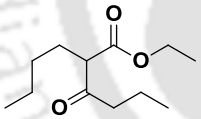
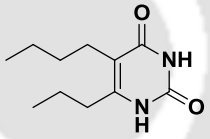
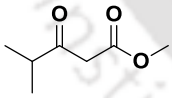
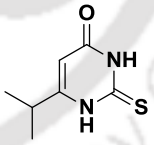
S.No	Substrate 1a-6a	Microwave irradiation(min)	%Yield	Product 1-6	%Yield with $BF_3.Et_2O$(min)
1		6	80		85 (5)
2		6	78		80 (5)
3		7	75		78 (6)
4		6	80		90(5)
5		8	68		75 (6)
6		7	75		78 (6)

***Table 1**: Synthesis of a series of novel nucleobase derivatives for supramolecular architechture*

Conclusion

In this chapter we described synthesis of tailor-made pyrimidinone nucleobases which act as molecular architecture. These molecules are new and were synthesized in a microwave-directed reaction in single step and without using any expensive reagents. We have also demonstrated the first crystal structures of all the above molecules.

5. Synthesis of C-5 and C-6 substituted *bis*-pyrimidine dimers and their photochemical evaluations show remarkable stabilities under UVC radiation

UV-induced DNA damage is the major cause of skin cancer and aging. Mostly pyrimidine nucleobases undergo lesion formation when exposed to UV-radiation, leading to mutations. Here, we have synthesized model *bis*-pyrimidine dimers that contained the modified pyrimidine nucleobases. We have thoroughly investigated the effect of substitution on the rate of UV lesion formation. Both the free nucleobase analogs as well as their homo- and hetero- dimers were examined. A systematic study, under controlled UV-radiation, show that many of the synthesized analogs are highly stable towards UV-light. Such nucleobases and their oligonucleotides, therefore, could have potential application to prevent damages and as sunscreen agents.

Scheme 5

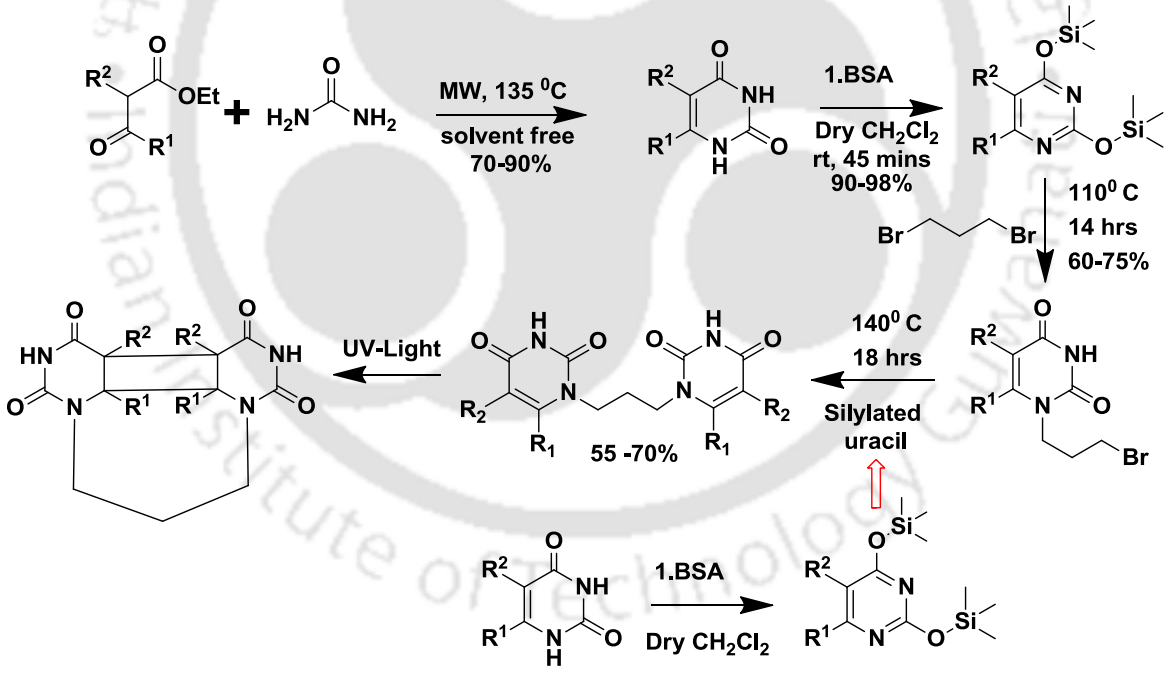


Figure 6: *Schematic presentation Synthesis of modified dinucleotide pyrimidine derivatives. BSA: Bis(trimethylsilyl)acetamide*

UV-irradiation result of free nucleobases and *bis*-pyrimidine dimers:

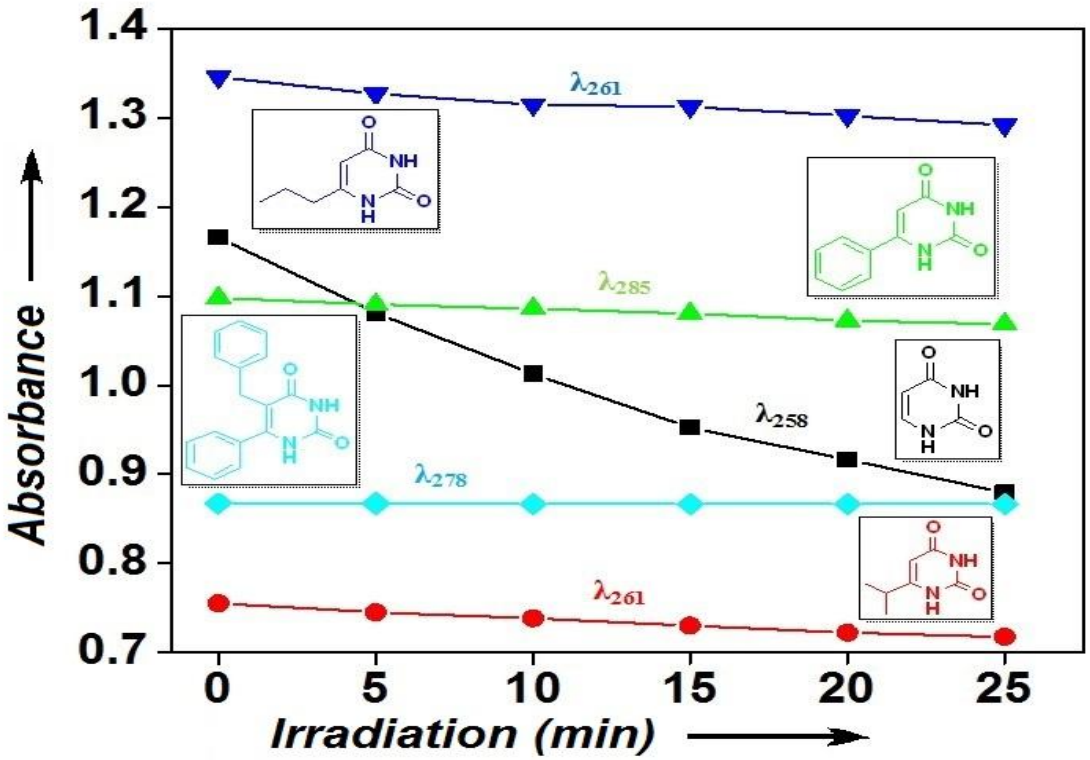


Figure 7: *UV-study of nucleobase*

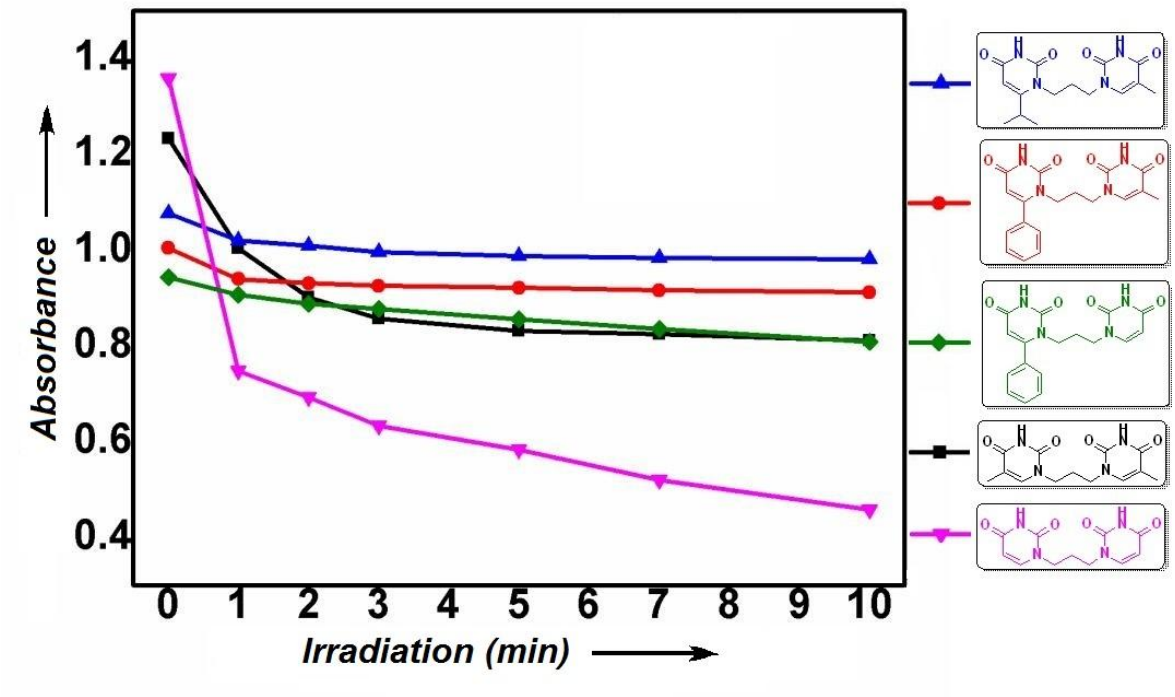


Figure 8: *UV-study of bis-pyrimidine dimers*

Conclusion

A few model compounds containing homo- and hetero-dimer of the highly substituted pyrimidinones were synthesized. Time dependent photochemical damages, under UVC-light were carried out systematically. Some of the *bis*-pyrimidine dimers were found to be very stable under UV dose, compared to the natural uracil or thymine. Thus, such variety of nucleobases could be potential sunscreen agents in preventing DNA damage under UV-exposure.

6. Synthesis of peptide nucleic acid which forms stable base-pair with DNA

The synthesized nucleobases with altered steric and electronic properties are expected to change the base-pairing properties and could be useful as probe oligonucleotides for detection of point mutation and disease diagnosis. The aim of the project is to develop oligonucleotides with above mentioned nucleobases in a peptide backbone (PNA).

Scheme 6

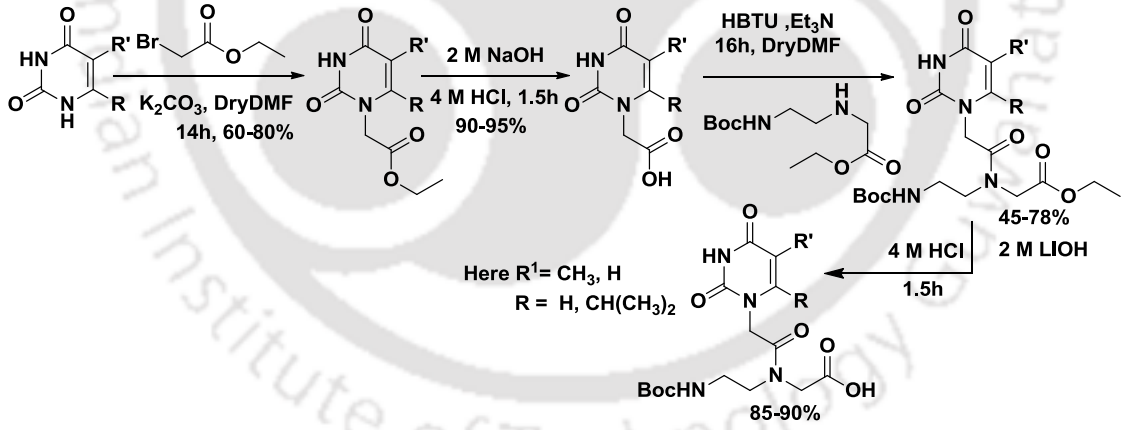


Figure 9: *Schematic representation for synthesis of modified PNA monomers*

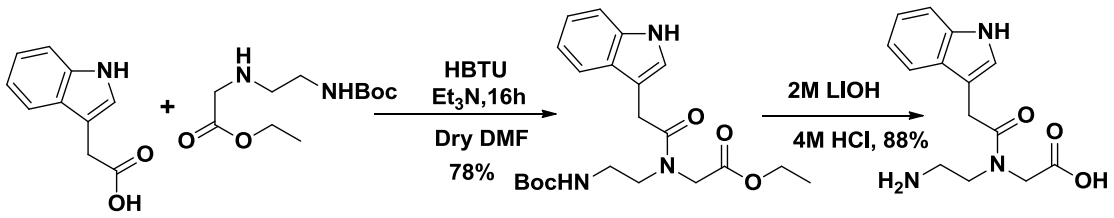


Figure 10: *Schematic representation for synthesis of modified PNA monomers*

Peptide nucleic acid synthesis by using solid phase:

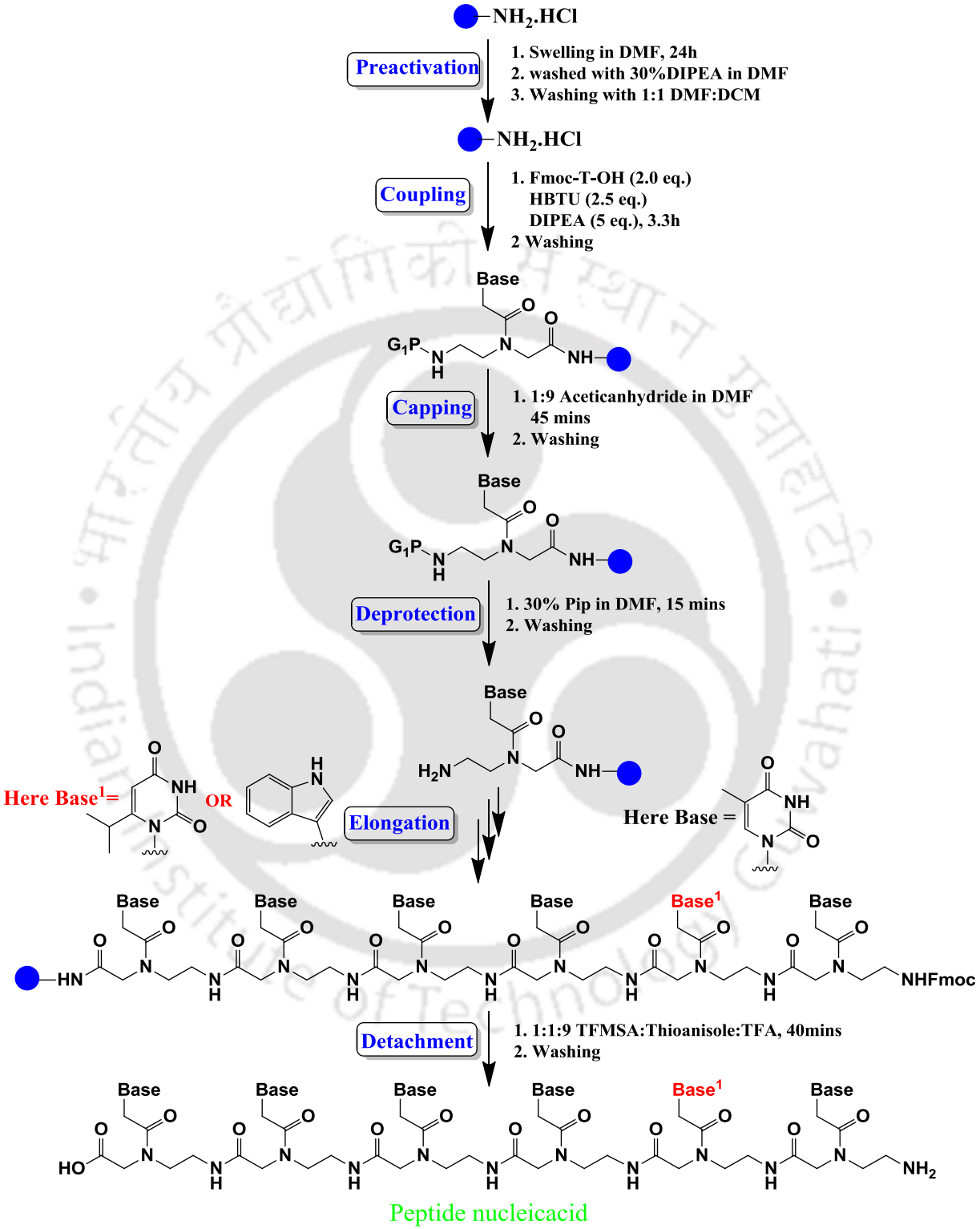


Figure 11: *Schematic representation for synthesis of peptide nucleic acid*

Conclusion:

We have synthesized PNA monomers containing C-6 substituted uracil nucleobase. Solid phase peptide synthesis protocol was used to synthesize peptide nucleic acids. We have successfully synthesized peptide nucleic acids containing modified uracil, in order to incorporate artificial base-pairing.

Graphical abstract:

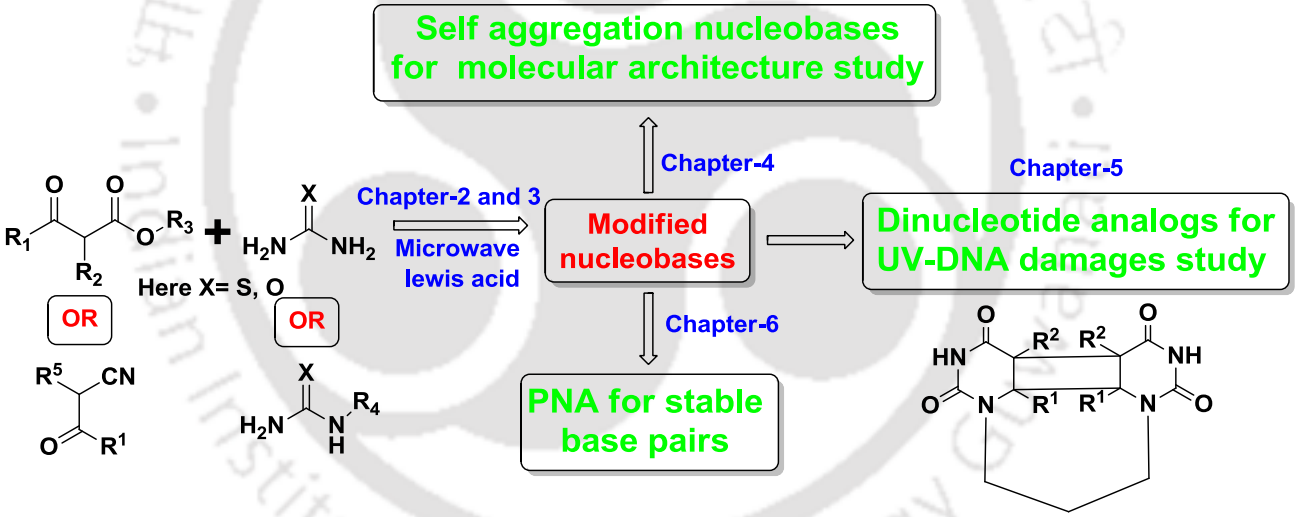


Table of content:

CHAPTER-I

Introduction

I.1. Structure and properties of nucleic acids

The naturally available nucleobases are thymine, cytosine, adenine, guanine and uracil. The canonical form of pyrimidine nucleobases are thymine, cytosine and uracil, where as adenine and guanine are known as purine nucleobases ***(Figure I.1.1)***.

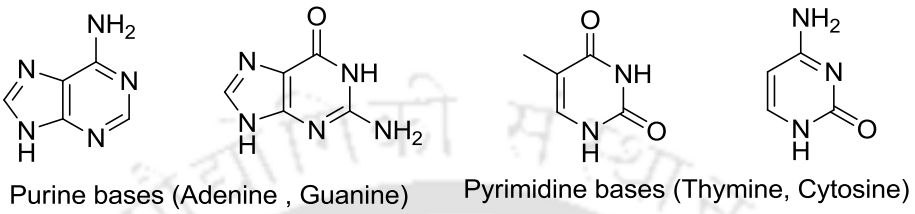


Figure I.1.1: *DNA nucleobases*

Specific combination of the purine and pyrimidine nucleobases form naturally abundant DNA which preferably form a double helical structure, generated mainly due to base pairing between the purine and pyrimidine nucleobases as well as π-stacking. The base pairings, first introduced by Watson and Crick in 1953, is very important supramolecular interactions in DNA ***(Figure I.1.2***. [1] In nature DNA exits B-type double helical structure.

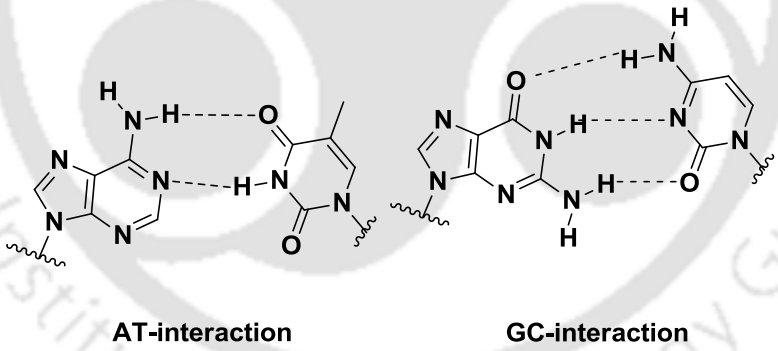


Figure I.1.2: *DNA base pairing*

DNA is a storage unit and carrier of genetic information in many living organisms. The unique double helical structure of DNA enables it to amplify, thereby re-generating itself. The outer surface of the double helix is occupied by the negatively charged phosphates, making it highly water soluble. In the interior of the duplex four aromatic nucleobases, attached to the deoxyribose sugar moiety, are stacked and paired with each other through hydrogen bonding. The base pair distance of 3.4 Å and helical pitch of 36° per base pair results in a complete helix turn approximately every 10 base pairs for the predominant B-type conformation of DNA. The

stability of the double helix is resulted from strong H-bonding as well as π-stacking interaction between the neighboring nucleobases. DNA is a highly stable biomolecule in a wide pH range and temperature even up to 100°C.

In the cell, DNA is organized into long structure unit called chromosome. The information is transmitted to the off-springs through the replication process of DNA. The sequence of the bases present in the DNA defines many features of the organism.

I.2. Non covalent Interactions Which Stabilize DNA and RNA

The stability of the nucleic acid depends upon the hydrogen bonding and/or base stacking interactions in between the nucleobases. Natural DNA usually requires complementary hydrogen-bonding partners to form H-bonds within the helix. Such H- bonding is an electrostatic interaction between a good electron donor and an acidic proton. In order to increase the strength of H-bonding interactions in DNA/RNA, the nucleobase needs to be modified such that the basic nature of the acceptors or the acidity of the H-bond donors are improved ***(Figure I.2.1).***[2]

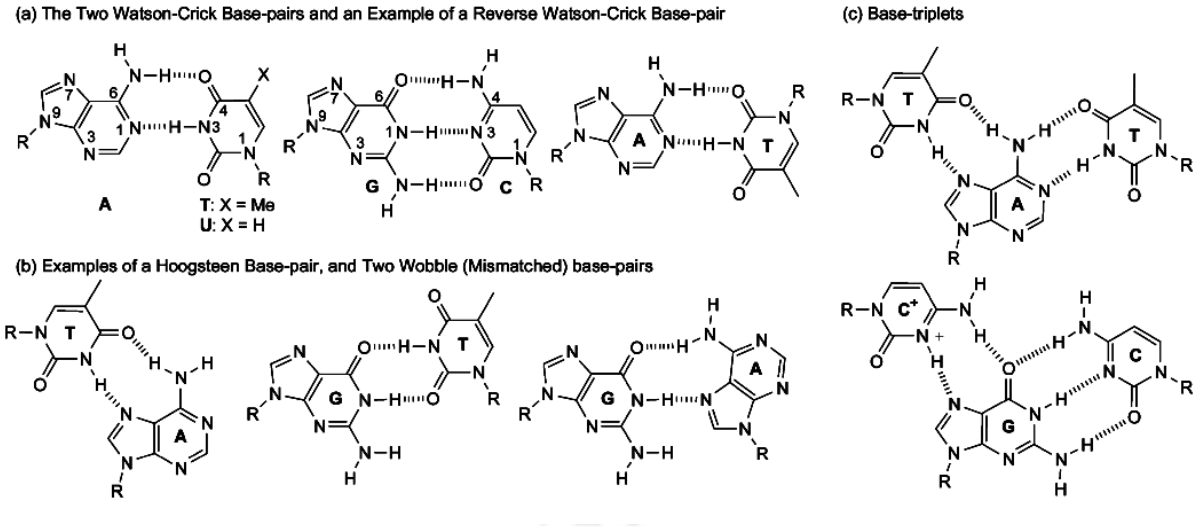


Figure I.2.1: *complementary base-pairing with different kind of H-bondings.*

Additionally base stacking between the neighboring nucleobases also plays very important role in stabilizing DNA helices. A third important factor in DNA duplex is presence of negatively charged phosphate groups. The charge repulsions between the phosphate groups make the helix

unstable. Therefore helix stabilization could also be improved by altering the charges in the backbone. However, this may lead to lesser solubility in water ***(Figure I.2.2).***[3a-b]

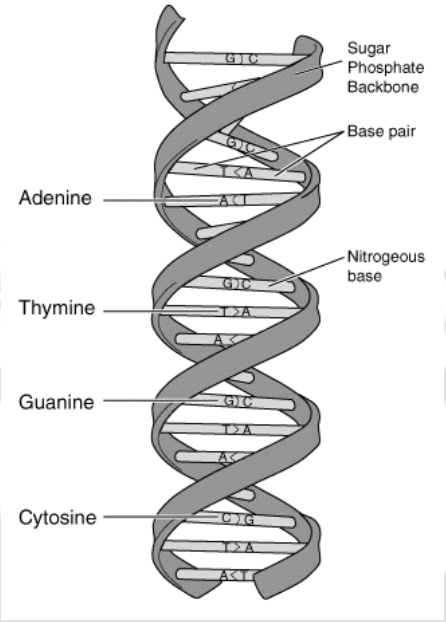


Figure I.2.2: *Watson-Crick structure of DNA*

I.3. Modified nucleic acids

Development of modified nucleic acids or nucleobases is an important aspect in nucleic acids research. Synthetic oligonucleotides are usually designed either to improve the helix stability or to impose new properties, [3c] such as fluorescence, cell viability etc, into the oligonucleotides. Evolution of modified nucleic acids have enabled researchers to study various aspects and problems such as detection of point mutations (SNP), targeting specific gene sequence, protein DNA interactions using oligonucleotide-based fluorescence markers, mechanisms and kinetics of DNA damages and repair processes, to outline a few. Artificial oligonuclotides have also been developed to expand the genetic alphabets. Moreover, plenty of nucleobases and nucleotide analogues have shown remarkable potential as inhibitors, antitumor, anti-bacterial and anti-HIV agents. [3c-h]

The naturally abundant DNA/RNA contains three specific units in its skeleton. These are ***1)*** *Nucleobase*, ***2)*** *Ribose/deoxyribose sugar* and ***3)*** *Phosphodiester backbone*. Modified nucleic acids are generated by altering these units through chemical transformations. (***Figure I.3.1).***

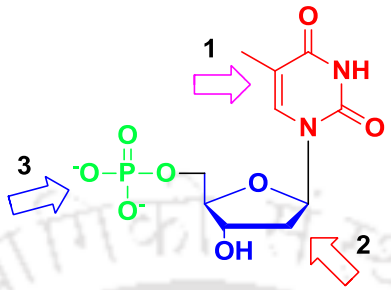


Figure I.3.1: *Diagrammatic representation of modified nucleobases.*

I.3.1 Modification of the nucleobases:

C-5, C-6 and N-3 positions of the pyrimidine nucleobases are favored for the chemical reactions to get modified nucleobases, shown in ***Figure I.3.1.1***

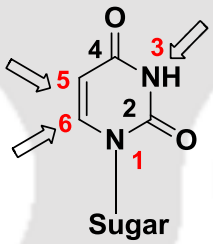


Figure I.3.1.1: *Diagrammatically representation of modified nucleobases (uracil)*

I.3.2. Addition of simple substituent's to DNA bases

C-5 substituted pyrimidines have been found to stabilize the nucleic acid helices than the natural pyrimidine nucleobases. Halogen substitutions (fluorine, chlorine, bromine) have been found to enhance the *van der Waals* interactions with neighboring bases **(2)** and pyrimidine C-5 propyne groups **(6)** also found to stabilize the double helices ***(Figure I.3.2.1).*** [4a]

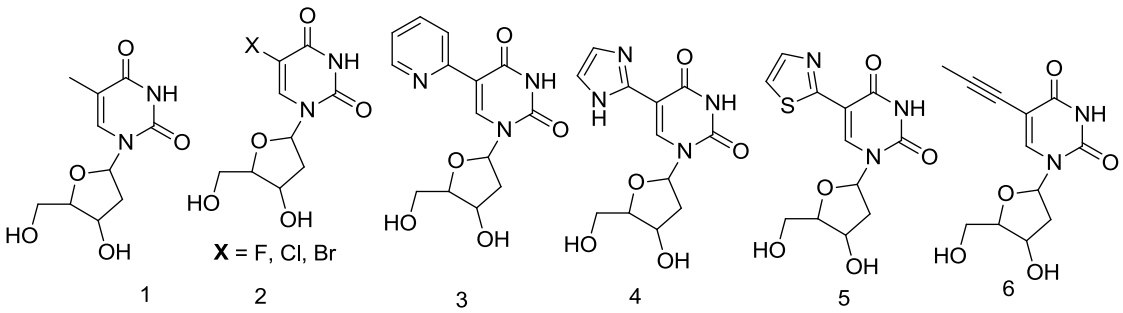


Figure I.3.2.1: *Addition of simple substitutes to bases.*

Some of the chromophores substituted at C-5, C-6 and N-3 positions of nucleobases have shown highly stable duplex properties. (***Figure I.3.2.2***). [9a]

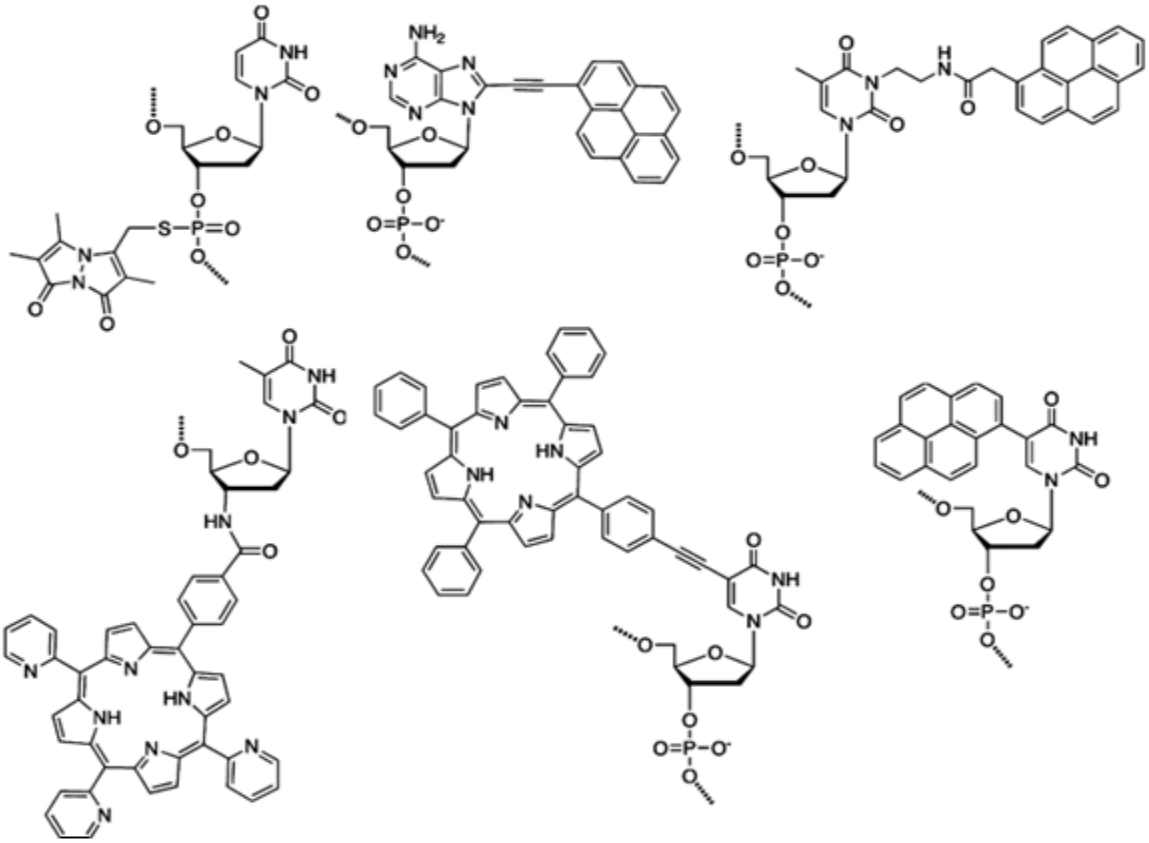


Figure I.3.2.2: *Examples of chromophores linked as side chains of nucleosides*

Modification of the nucleobases was implemented to generate a new form of genetic alphabets, as in x-DNA and y-DNA, shown in ***Figure I.3.2.3***. It was established that interactions in a

double helix composed of x-DNA are more potent compared to typical interactions in native DNA. The high stabilities of such oligonucleotides do not come from H-bonds, which are nearly identical in x-DNA and y-DNA compared to the native DNA. The base stacking interaction is the source to induce stability and such changes were evident from the melting temperature studies. They were further modified for additional hydrogen bonding offered by cytosine and 2-aminoadenine analogue as shown in ***Figure I.3.2.3***. [4b, 4c]

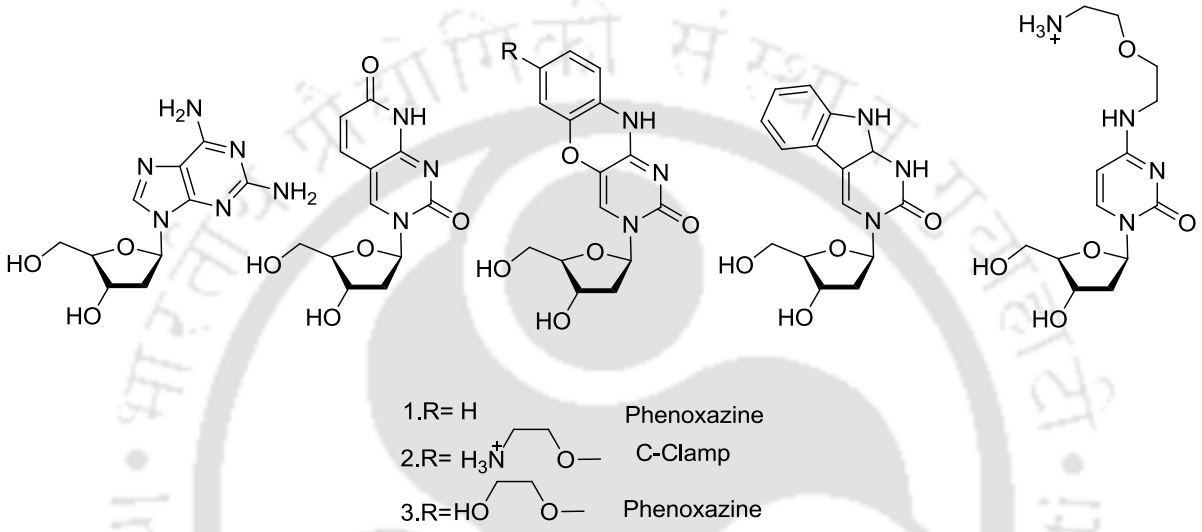


Figure I.3.2.3*: Modified nucleobases showing additional bonding interaction*

Eric Kool et al. demonstrated **C-5** modified uridine nucleosides **(3, 4 and 5)** to stabilize the nucleic acid helices by enhancing base stacking (***Figure I.3.2.1)***. This development has proved that helices could be stabilizes by the addition of strongly π-stacking bases at the end of the helix, without requirement of H-bonds. These are shown in ***Figure I.3.2.4*** [4d, 4e]

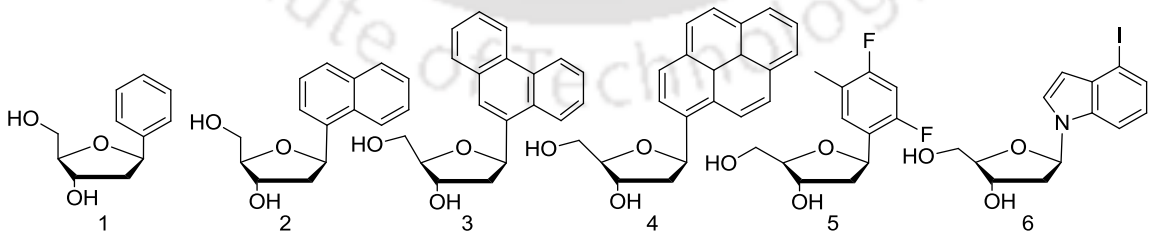


Figure I.3.2.4: *Some of well known non polar aromatic hydrocarbons as DNA bases analogs.*

From the literature we have found that non-nucleoside linked chromophores were also used in the new genetic alphabets. Multi-chromophoric systems are very interesting in many bio-

applications due to their novel properties. In last decade these novel properties were widely used in the preparation of conjugated polymers, photonic wires, dendrimers, oligo-amides, functionalized polypeptides, viral-nanoparticles as well as multichromophores in DNA (***Figure I.3.2.5)*** [4f]

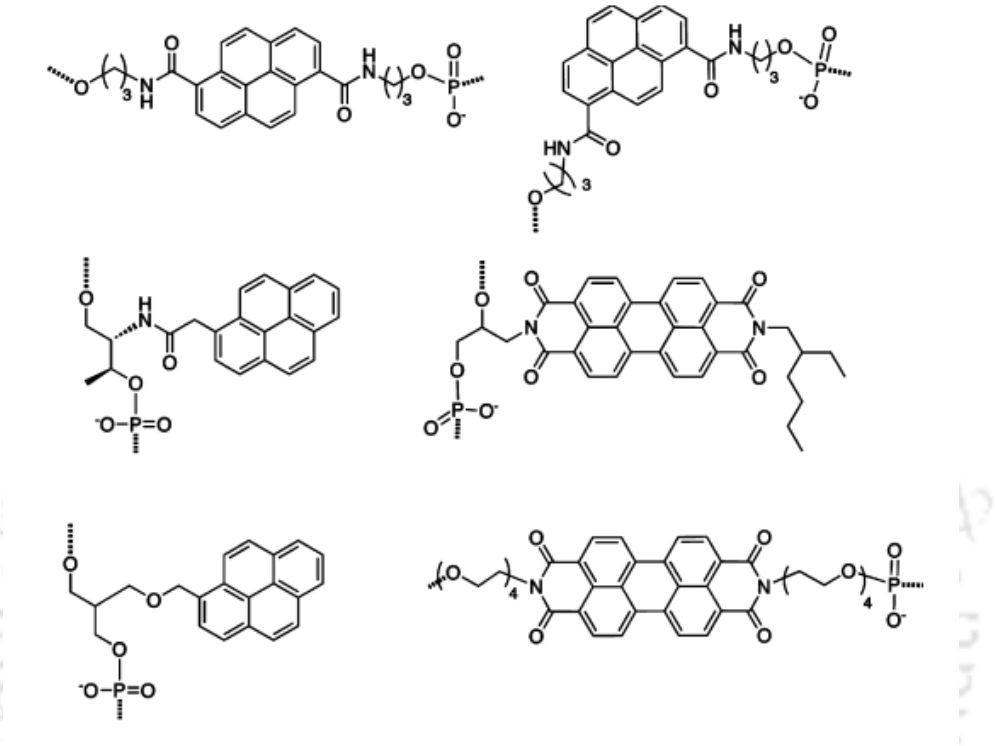


Figure I.3.2.5: *some of non-nucleosidic linked chromophores incorporated into DNAs.*

I.3.3. Size-Expanded Ribonucleosides

The biological processing and recognition of nucleotides must require specific efficient target and selective binding of nucleobases. A set of size-expanded nucleobases and oligonucleotides (xDNA) have been reported with unexpectable biophysical and biochemical properties with high selectivity in RNA ***(Figure I.3.3.1).*** [5a-g]

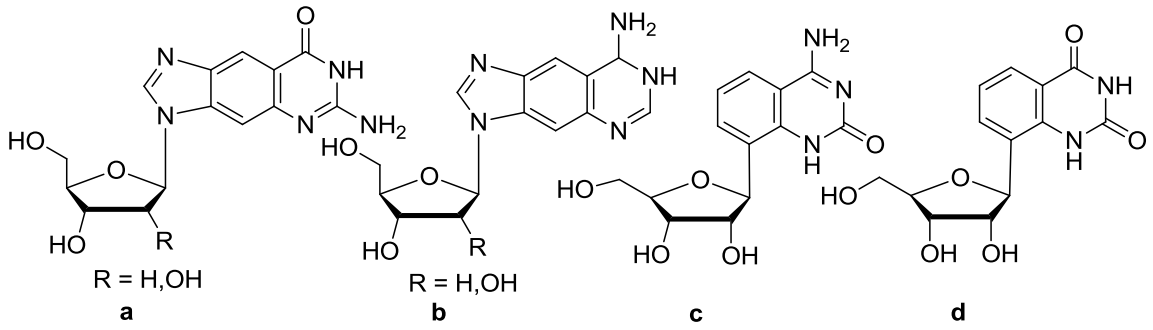


Figure I.3.3.1: *well known size expanded RNA bases.*

I.3.4. Modified nucleobases as pharmaceutical drugs

Nucleoside derivatives of 5-substituted uracil have been explored for their potential application as antiviral agents **(2)** and in the treatment of tumors **(4)**. Numerous pyrimidines have been prepared and their pharmacological activities have been evaluated. [6, 7a-c] ***Figure I.3.4.1*** demonstrates some of the pyrimidine nucleobase derivatives as various pharmaceutical drugs. [8a-c]

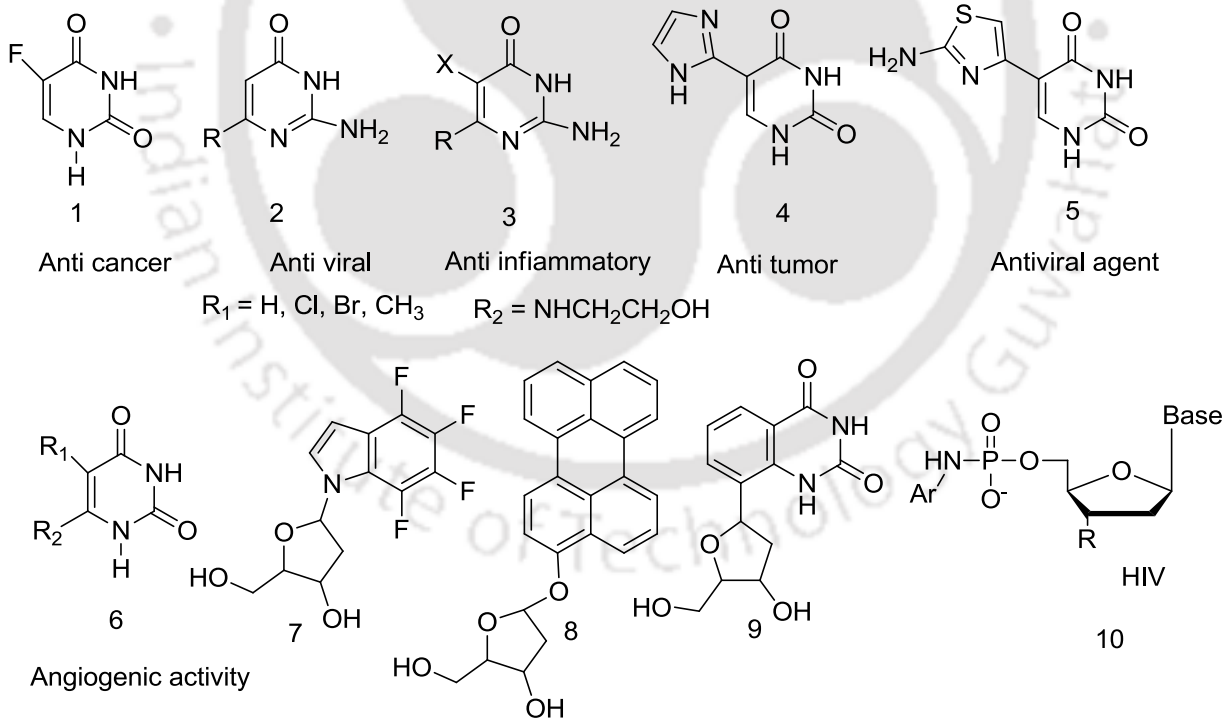
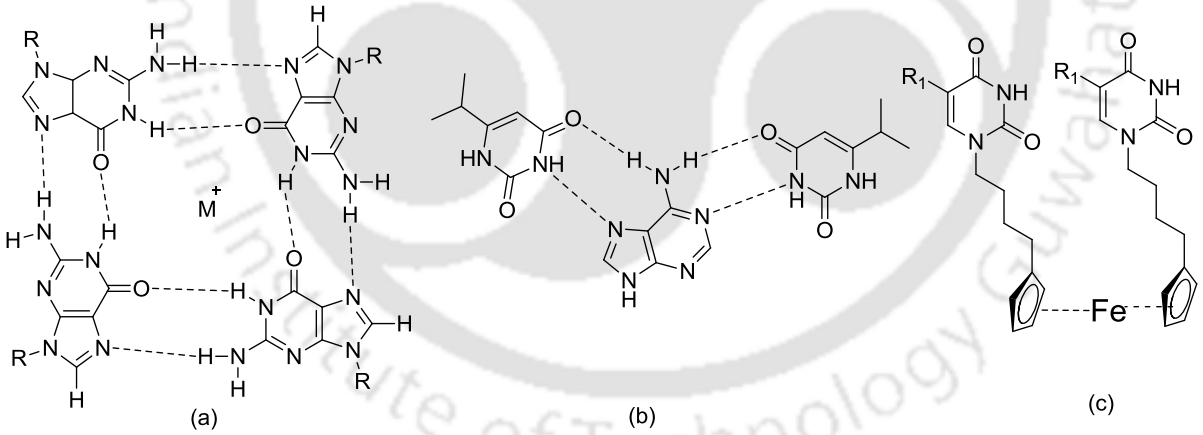


Figure I.3.4.1: *some of well known drugs as nucleobases analogs*

I.3.5. Modified nucleobases as a supramolecular architecture

Supramolecular architecture has a broad usage in biomedical and therapeutic applications. Especially, the molecular architecture constricted by using modified nucleobases and small heterocyclic compounds were given more attention due their bioactive nature. They are also responsible for the formation of G-quadruplex and metal mediated DNAs. Such modified nucleobases not only stabilize the duplex structure but also follow the Watson-crick and Hoogsteen base pairing properties which are present in nature and which are more favorable for triple helix formation **(b, c)**. A number of crystal structures, NMR and melting temperature studies of the short oligonucleotide were reported to elucidate various pairing interactions including metal mediated DNA/RNA protein complexes and G-quadruplex (**a)**. Recent our group have reported a rare co-crystal structure of tri-base pairing from nucleobases; an equimolar mixture of 6-isopropyluracil and adenine forms of a tri-base pairing. We have noticed that one molecule of free adenine is adhered to two molecules of the free pyrimidine nucleobases involving both Watson–Crick and Hoogsteen base-pairing interactions (***Figure I.3.5.1)*** [9a-d]



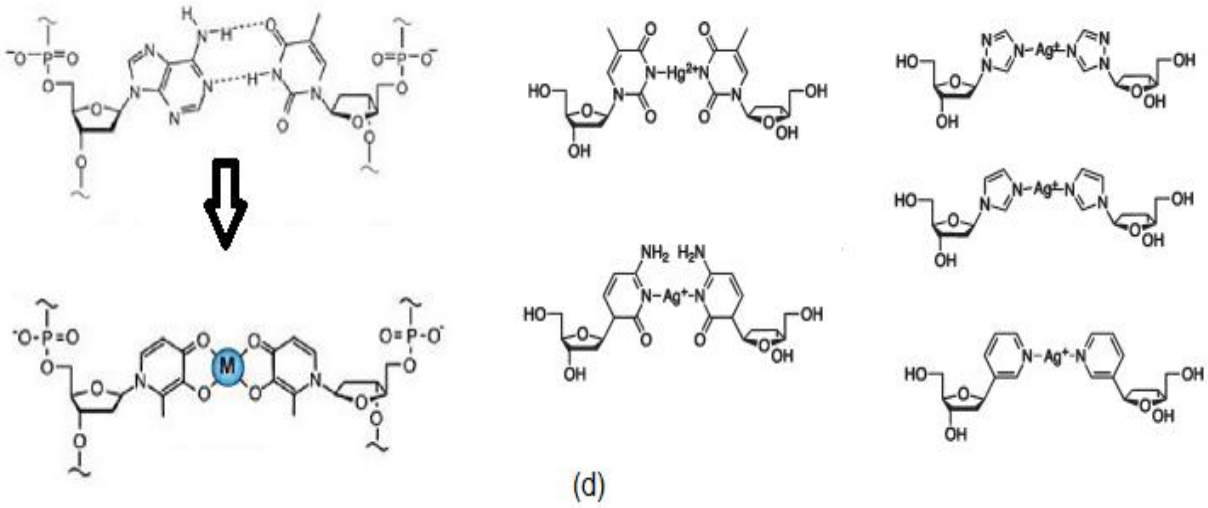
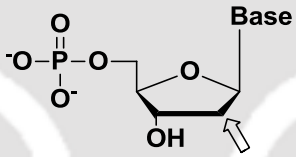


Figure I.3.5.1: *Nucleobases involved in supramolecular architure*

I.3.6. Modification of the sugar moiety



Many different chemically modified nucleosides are available and their wide range of properties have been explored in drug development, medical diagnosis and clinical therapy.

Locked nucleic acid (LNA):

Locked nucleic acid (LNA), developed by *Jesper wengel*, is a modification at the deoxyribose sugar moiety to introduce conformational restriction into the oligonucleotides. Such modified oligonucleotides have been used as potential therapeutic agents and as gene silencer.[10a] Apart from LNA, a number of various oligonucleotides have been reported with modifications at the sugar part in order to improve target selectivities.[10b-c]

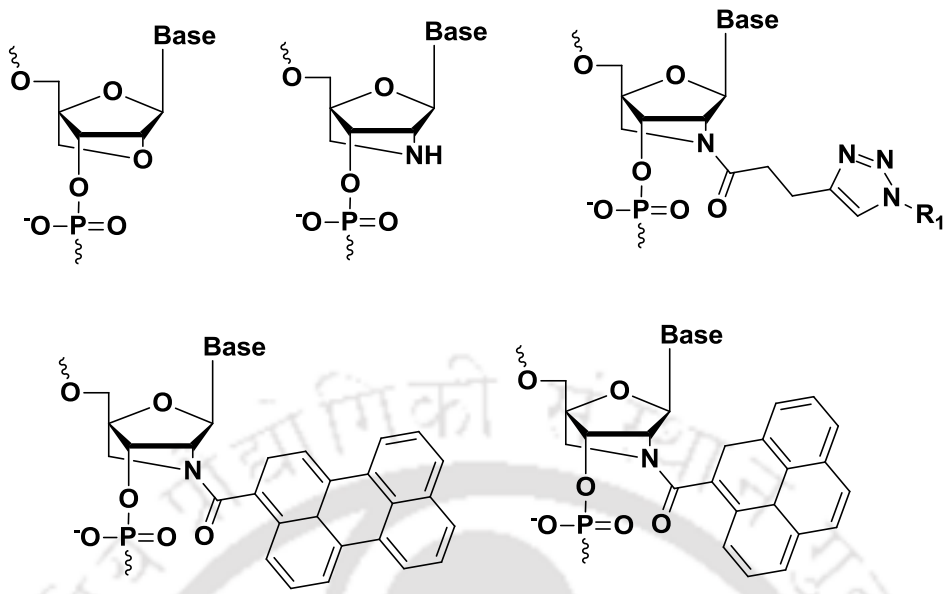


Figure I.3.4.1: *Schematic presentation of novel LNAs.*

Unlocked nucleic acid (UNA): In contrast to the increased rigidity of LNA nucleosides, unlocked nucleic acid (UNA) substitutions were introduced. Very recently, researchers have explored decreased rigidity as a variable for improving the function of oligonucleotides. UNA nucleosides are acyclic and the connection between the C2′and C3′atoms is locked away. These systems could be useful in improvement of nanoscale devices based on DNA hybridization (**b**).[10d-i]

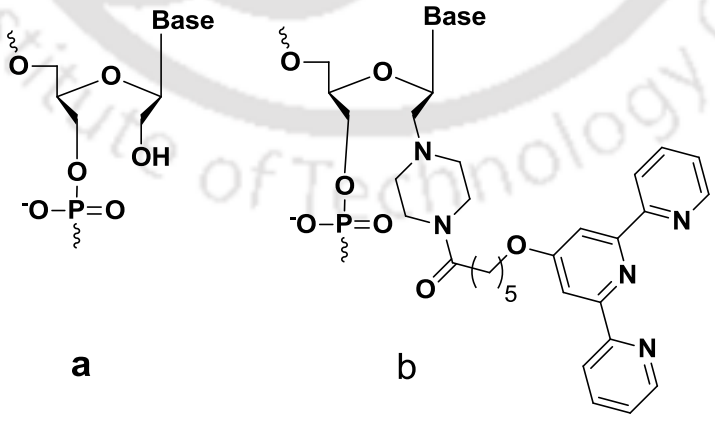


Figure I.3.4.2: *Schematic presentation of novel UNAs*

I.3.7. Modification of the backbone

A variety of nucleic acid analogues containing natural nucleobases and/or non natural nucleobases have been synthesized by changing the backbone portion. In this modifications, most important one is peptide nucleic acid (PNA) introduced by *Nielsen and co-workers* in 1991. PNA is a neutral polyamide and composed of N-(2-aminoethyl)glycine units to which the bases are attached through a carboxy-methyl linkers. PNA, owing to its neutral backbone, is highly target specific and capable of forming triplex and quadruplex with target DNA. Moreover, due to its high stability towards nucleobases, PNA oligonucleotides have gained an enormous range of applications.[11, 12]

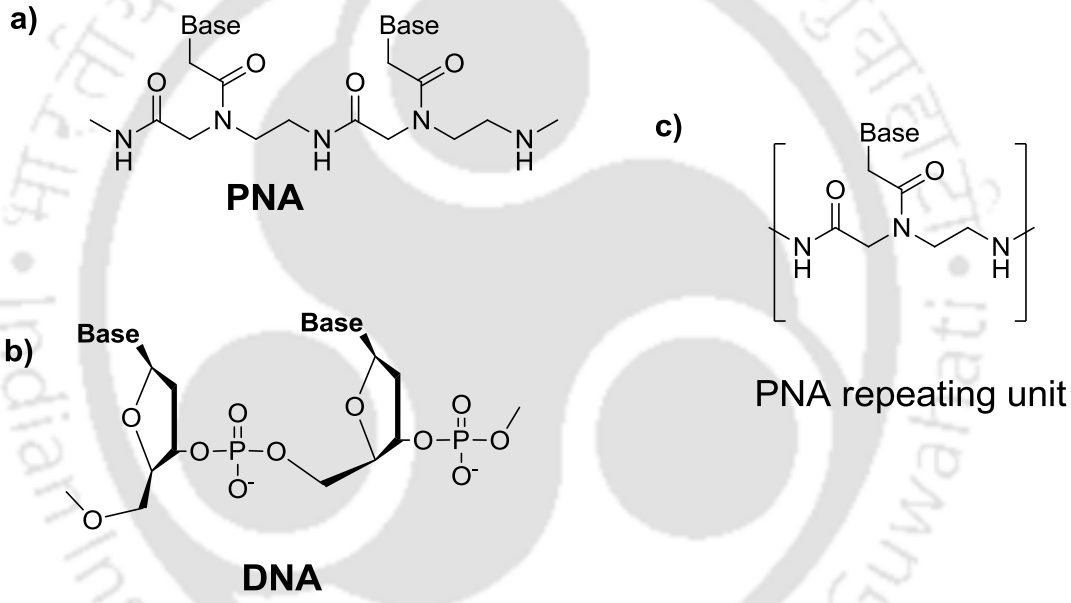


Figure I.3.5.1*: Peptide nucleic acid (PNA).*

A variety of conformational constrained PNA analogues have been developed by *K. N. Ganesh, et al.* and *his co-workers* ***(Figure I.3.5.2)*** [13a-c]

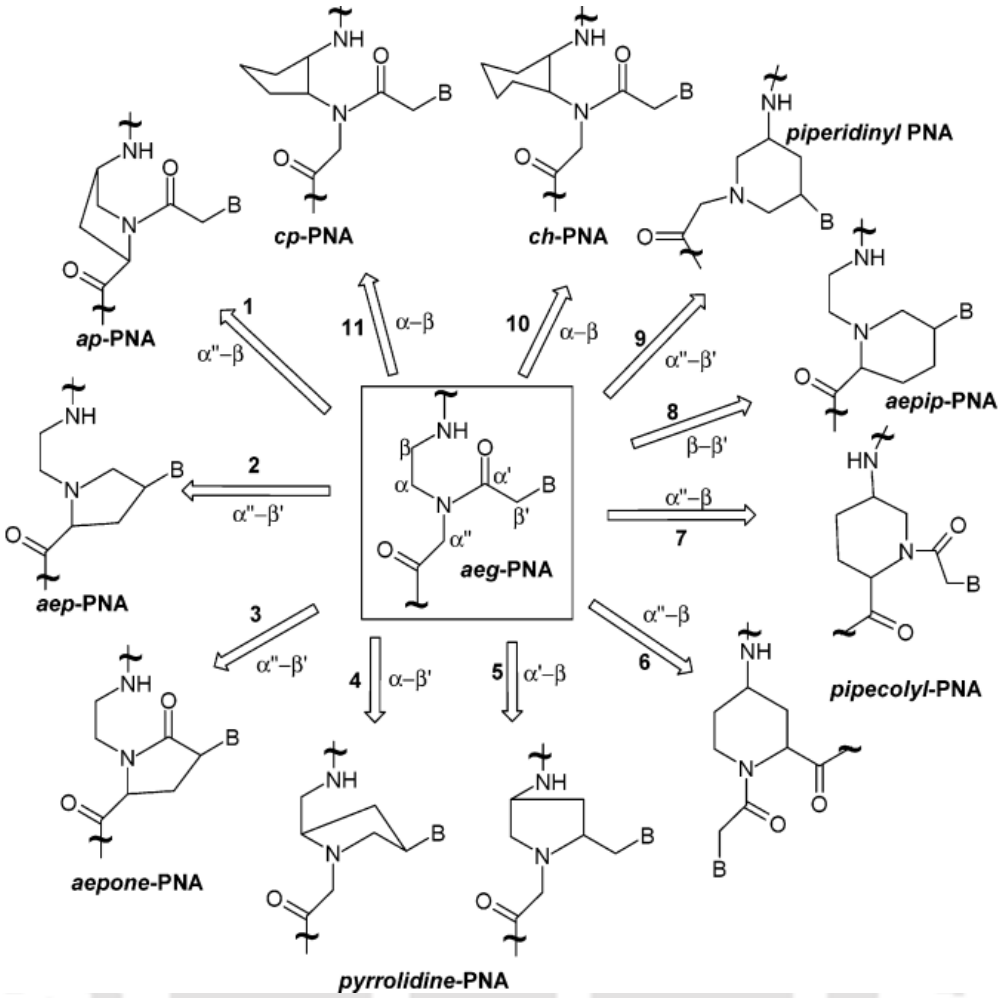


Figure I.3.5.2*: Conformational constrained PNA analogues.*

Some of the examples for PNAs synthesized to yield high affinity binding with complementary DNA, in a regio- and stereo-preferred conformations are shown in ***(Figure 1.3.5.3)***.[13d-f]

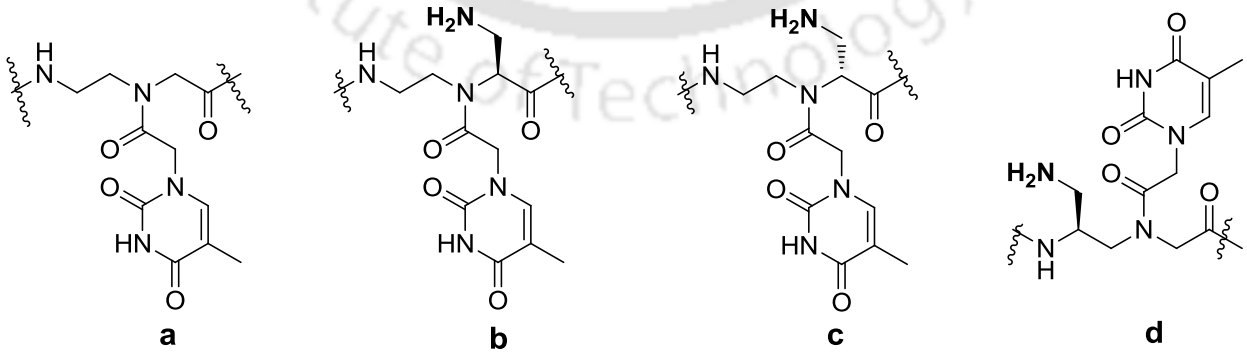


Figure I.3.5.3*: Regio and stereo controlled PNAs.*

I.4. DNA damage and mutations

Cellular DNA when exposed to chemicals, reactive oxygen species (formed during metabolism) or UV-radiation generates various types of DNA damages. Most common damages are: oxidative lesions, UV-induced photolesions and DNA methylated products.

I.4.1. Oxidative DNA lesions

DNA is sensitive to many kinds of damage and one of these is oxidative damage. The damages are mainly formed due free radical formation in the human bodies and can produce oxidative damages. The source of endogenous reactive oxygen species, such as the hydroxyl radical and H_2O_2, superoxide radical, pose a significant threat to cellular integrity in terms of damage to DNA. Oxidative DNA lesions lead to mutations causing cancer and aging. Effected products by the reactive species are shown in ***Figure I.4.1.1*** and ***Figure I.4.1.2*** [14a-d]

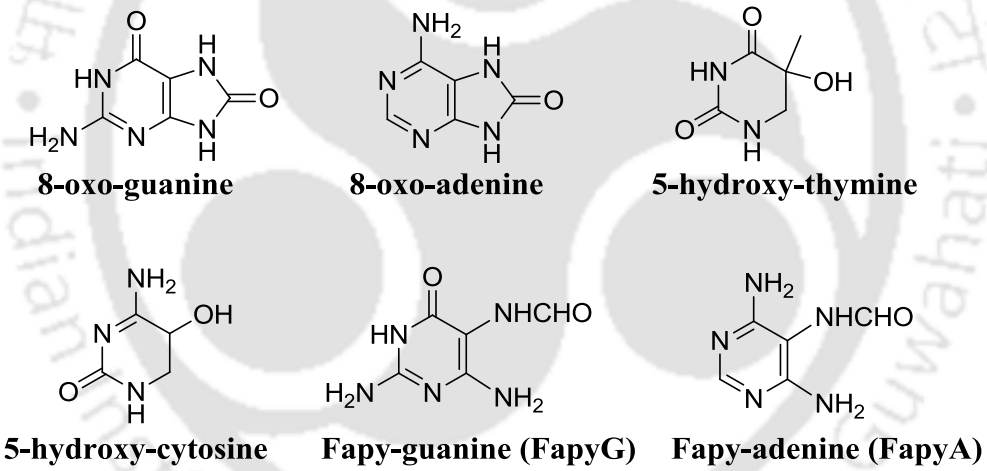


Figure I.4.1.1: *Oxidative DNA lesions (More possible forming product)*

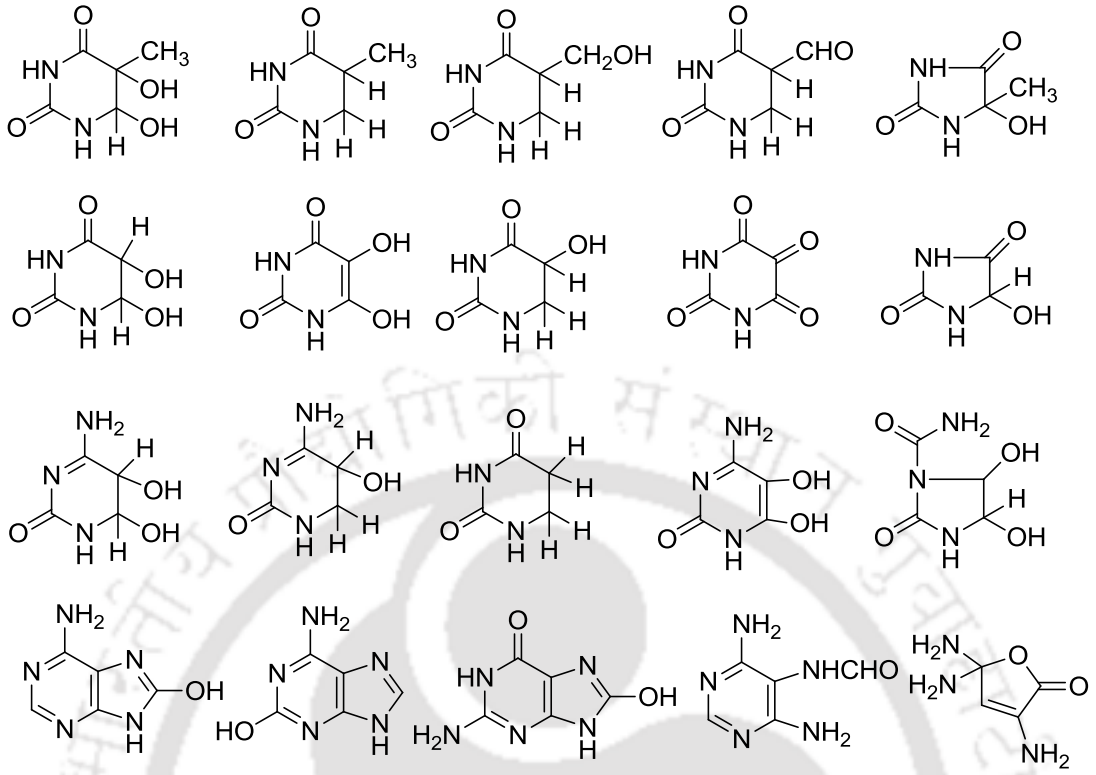


Figure I.4.1.2: *Oxidative DNA lesions (Less possible forming product).*

I.4.2. UV-induced DNA lesions

Gradual depletion of ozone layer and consequently increased ultraviolet (UV) radiation on the Earth's surface induces DNA-lesions (oxidative and dimeric photolesions) inside the genome. It has been proposed that about 10^4-10^6 lesions occur in each human cell per day. Many of these damages, if not repaired by the repair systems, may lead to cell death, aging and tumor formation. (***Figure I.4.2.1).*** [15-23]

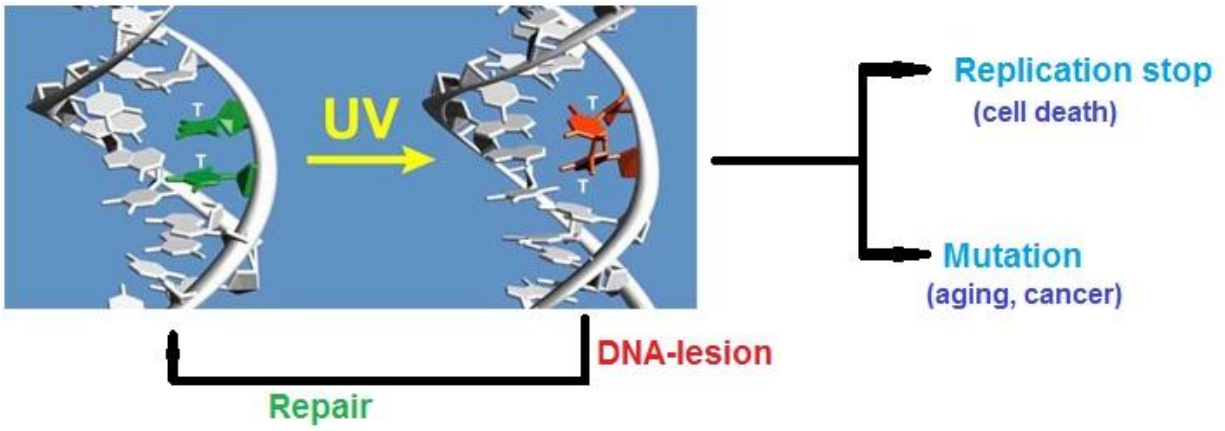


Figure I.4.2.1: *Schematic representation of UV effect on DNA.*

The heterocyclic bases in DNA/RNA exhibit UV absorption in the range of 260 to 280 nm. Radiation in this wavelength region raises the bases to their excited singlet or triplet state which then undergo various photochemical reactions. [24, 25] Generally, the nucleobases react with their immediate neighboring counterparts present in the same DNA/RNA strand, [26, 27] although the formation of interstrand photoproducts have also been reported.[28-32] Intra-strand dimers are favorable then the inter-strand crosslink photoproducts due to the well organized B-duplex structure of DNA and partially due to intra-strand stacking between the neighboring bases. Pyrimidine nucleobases were found to be the most vulnerable sites for such photochemical reactions when exposed to UVB/C light, leading to formation of photolesions. [33, 34] As DNA nucleobases absorb light between 260 nm and 280 nm, cells are most vulnerable to UVC (230-290 nm) radiation. The commonly occurring photo products from UVC radiation are: TT-dimers, TC/CT-dimers and CC-dimers. [34-35] The best-known photoproducts are cyclobutane pyrimidine dimers (CPD), (6-4) pyrimidine-pyrimidone dimers {(6-4) PP} and their Dewar valence isomeric lesions. The CPD-lesions are formed by *[2π+2π]* cycloaddition reaction between the adjacent pyrimidines. The (6-4) PP photoproducts are formed due to *Paternó Büchi* reaction which proceeds viaan oxetane or azetidine intermediate. These intermediate products are unstable above -80 °C and undergo rapid ring opening to the (6-4) PP photolesions. (***Figure I.4.2.2***). The structures of well-known photoproducts are given in ***Figure I.4.2.3***. The (6-4) PP photolesions undergo photo-isomerization to their Dewar valence counterparts when irradiated with UVB light (around 320nm). [22, 35-39]

Mechanism of the UV-damages in DNA

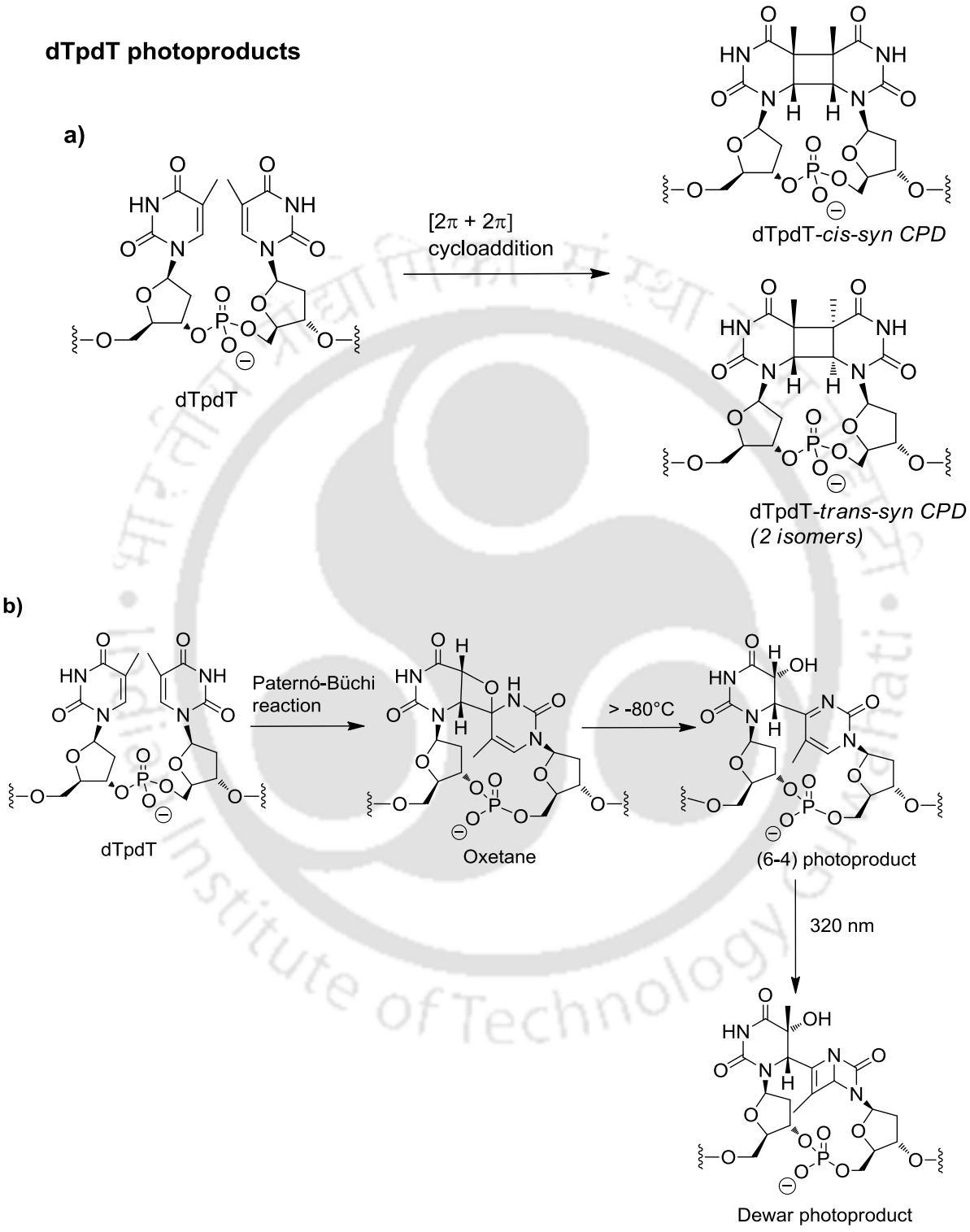


Figure I.4.2.2: *UV-induced DNA lesions (In between the thymines).*

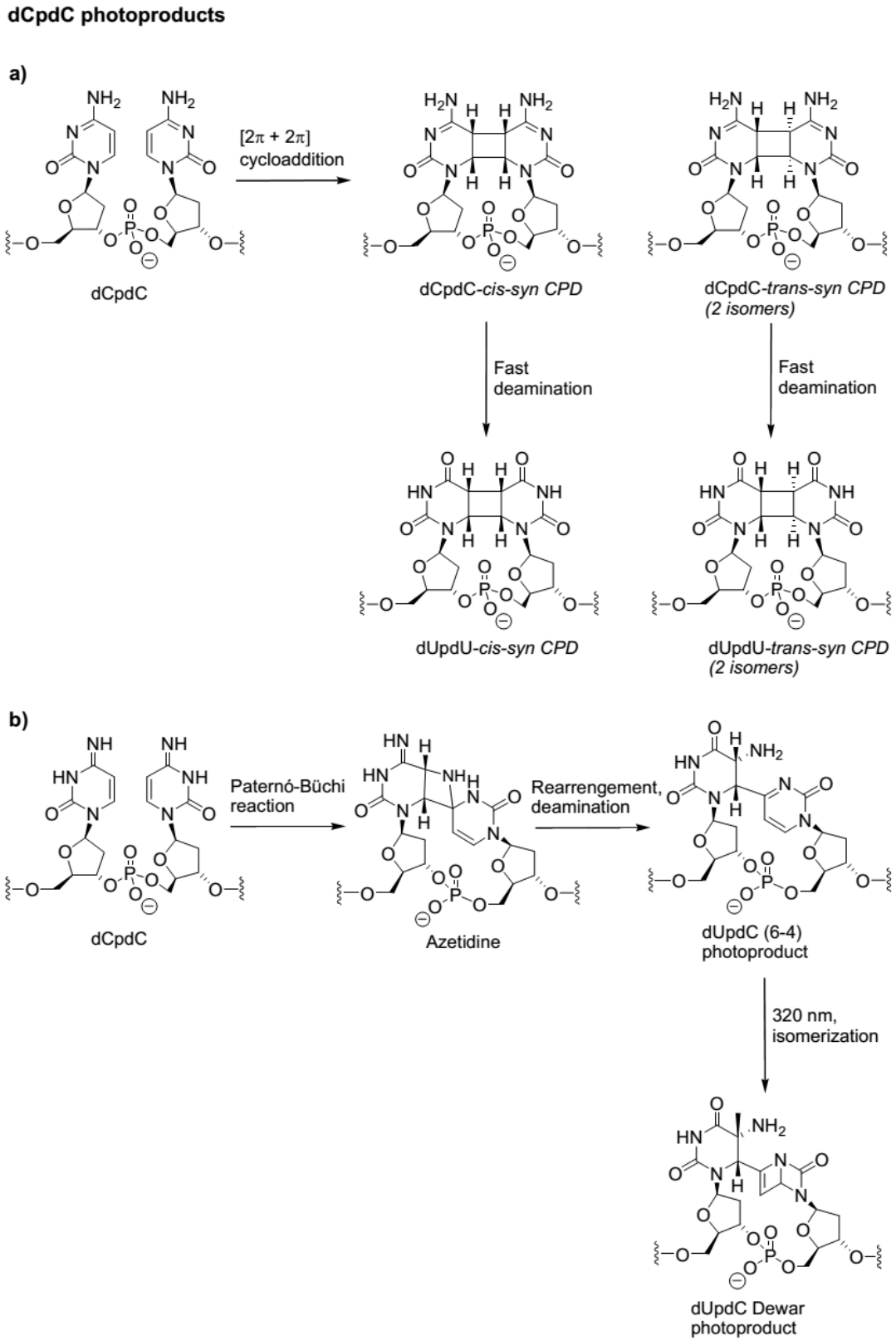


Figure I.4.2.3: *UV-induced DNA lesions (In between the cytosines).*

I.4.3. Photolesions and mutagenesis

In the cell division process, DNA makes a copy of itself for the new cell. This procedure, called replication, is carried out by a set of specialized enzymes known as polymerases. The single-stranded DNA, after denaturation of the double helix, serves as a template for replication. The presence of any damage inside DNA is usually detected by the polymerase before replication and is immediately being repaired/ replaced with correct nucleotides by repair enzymes. However, the lesions that is not detected by the polymerase and is bypassed during replication. During bypass, the lesions, due to different base-pairing properties than their undamaged precursors, lead to mutations of the genomic DNA. [40-43]

Cyclobutane pyrimidine dimer lesions (CPD) induced mutations

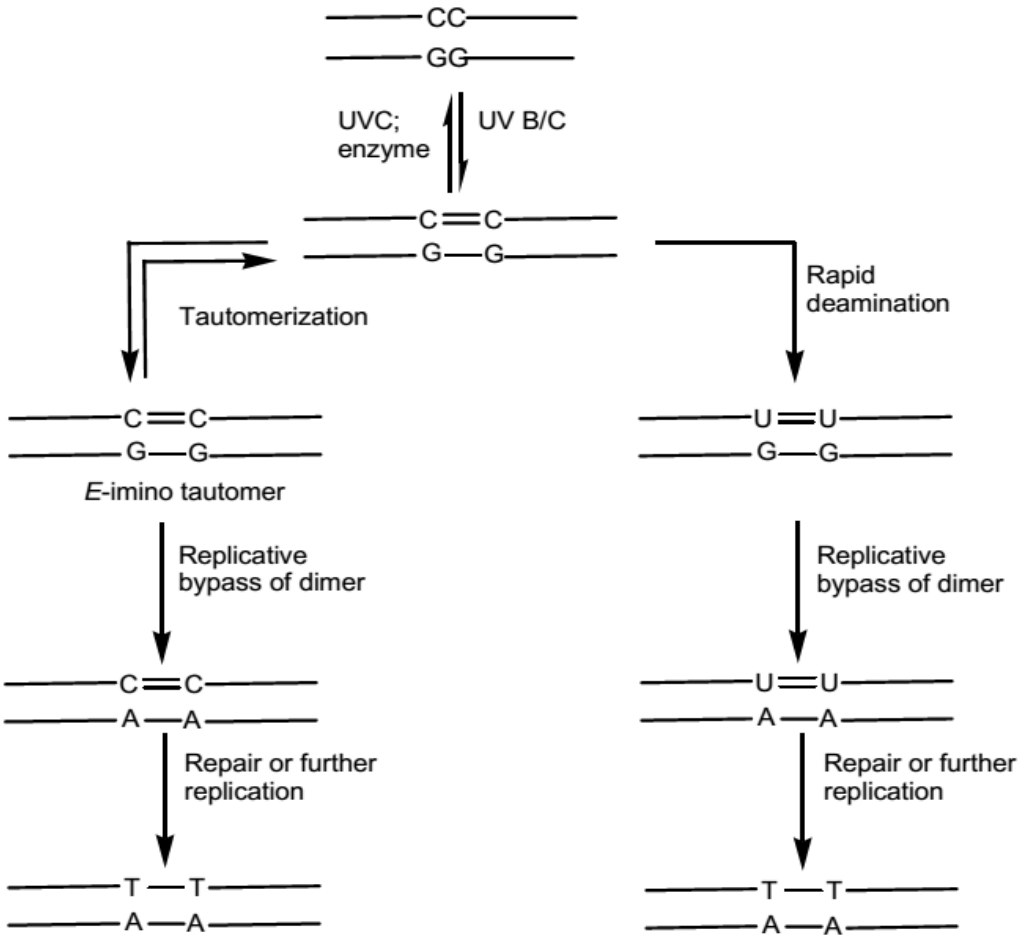
The cytosine-derived CPD-lesion, formed upon UVB/C exposure, undergoes T→C point mutation or CC→TT double point mutations via fast deamination into UU-CPD-lesion, described in **Figure I.4.3.1.** The deamination of the dCpdC CPD-lesion is a fast process, with a half-life of only about 5-6 hours, in vivo. The resulting dUpdU dinucleotide serves as a DNA template for polymerase and directs incorporation of 2-deoxyadenosines in the counter-strand, which on further replication yields C→T transformation. The most common types of skin cancers have been found to possess C→T mutations including CC→TT double mutations. [44-48]

The dTpdT cyclobutane pyrimidine dimer (CPD) lesions are in general not mutagenic. Although dT[c, s]pdT lesion is efficiently bypassed by the polymerase, it is not mutagenic due its inability to alter the Watson-Crick base pairing properties compared to the undamaged dTpdT dinucleotide. The 5-dT of 5-dT[t, s]pdT-3 lesion act as a mismatch as the methyl group (syn conformation) stays at the interface of Watson-Crick base pairing.[48-50]

Mutation of (6-4) PP lesions

The (6-4) PP photoproducts of dTpdT, dTpdC/dCpdT and dCpdC are highly mutagenic. The dTpdT-(6-4) PP lesion induces a T→C mutation in the genome. Studies showed that the 3-dT of this lesion preferentially pairs with a 2-deoxyguanosine while the 5-dT remains well-paired with a 2-deoxyadenosine in the opposite strand as shown in ***Figure I.4.3.1***. The (6-4) PP lesions of dCpdC and dTpdC photoproducts probably induce a C→T mutation at the cytosine site due to its fast deamination into uracil.

a)



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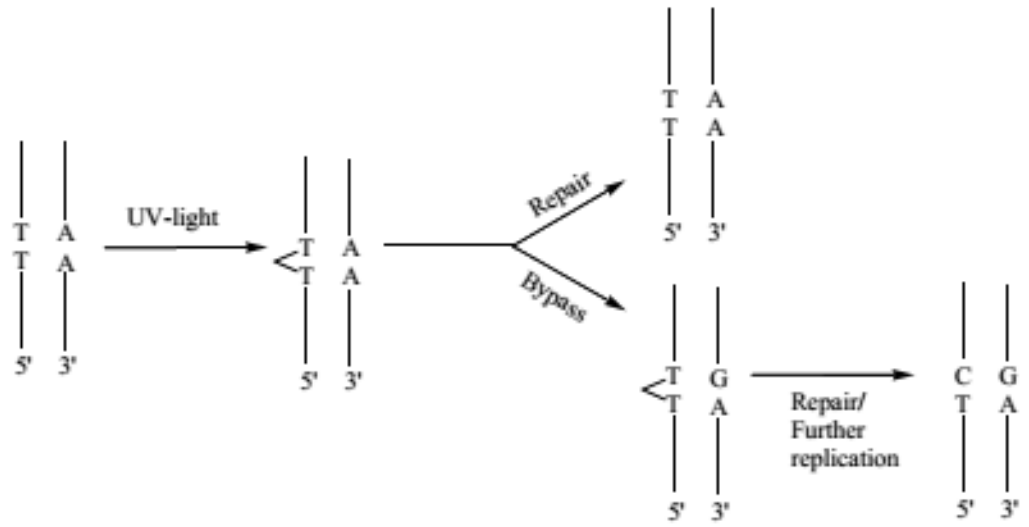

Figure I.4.3.1: *CPD formation in pyrimidine nucleobases by the effect of UV radiation.*

Mutagenicity of Dewar valence lesions

Dewar valence photoproduct of a dTpdT-dinucleotide is also highly mutagenic. The exact kind of mutation by Dewar lesion is not very well-understood. As like (6-4) PP lesion, the 3-T of the Dewar-dTpdT lesion was also found to induce T→C mutation, although occurrence of double point mutation may not be ruled out. [51]

I.5. References

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CHAPTER-II

Synthesis of modified uracil, cytosine, thiouracil and thiocytosine derivatives in a microwave-directed method

II.1. Structure and Nomenclature

The naturally abundant DNA preferably show a B-type double helical structure, generated mainly due to base pairing and π-stacking between the purine and pyrimidine nucleobases. These are cytosine, guanine, adenine, thymine and uracil, abbreviated as C, G, A, T, and U ***(Figure II.1.1)***.

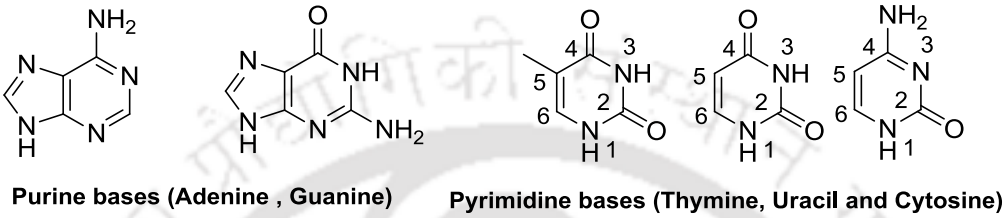


Figure II.1.1: *DNA/RNA nucleobases*

II.2. Biomolecular applications

Modified pyrimidine nucleobases, oligonucleotides and their analogs have been developed for many bio-molecular studies ranging from detection of genomic mutations, [1-4] disease diagnosis, [5] gene silencing [6, 7] and as other molecular probes.[8-11]. A variety of modified synthetic pyrimidine nucleobase analogs are widely used in the treatment of many diseases, e.g.: 5-fluorouracil, anilinouracils and cyclopentenyl cytosines are well-known antitumor and antibacterial drugs.[12-14] (***Figure II.2.1)***.

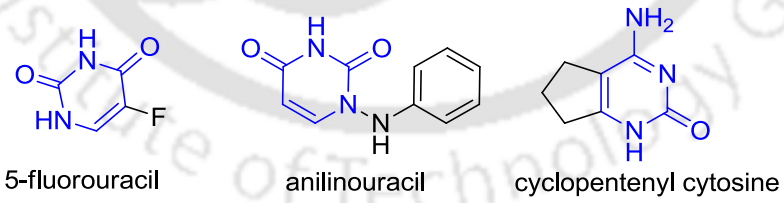


Figure II.2.1: *Nucleobases act as drugs*

In the past decade a spectrum of non-natural nucleobases was also developed to induce altered base-pairing properties into the DNA/RNA duplex as well as for the expansion of genetic alphabets [15, 16.] In other hand, synthesis of pyrimidine nucleobases is of particular interest due to their propensity to undergo rapid photochemical lesion formations under ultraviolet radiation,

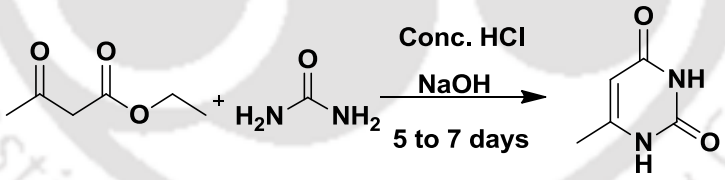
causing genomic damages.[17] Therefore, synthesis of modified nucleobases has drawn much interest in bio-molecular studies as well as in pharmaceutical industries.

II.3. Existing methods

The synthesis of uracil derivatives from ethyl acetoacetate and urea was described first by Behrend in 1885. These substances were also synthesized by the action of lead hydroxide on methylthiouracil in an alkaline medium and diketene with urea.[19a-d]

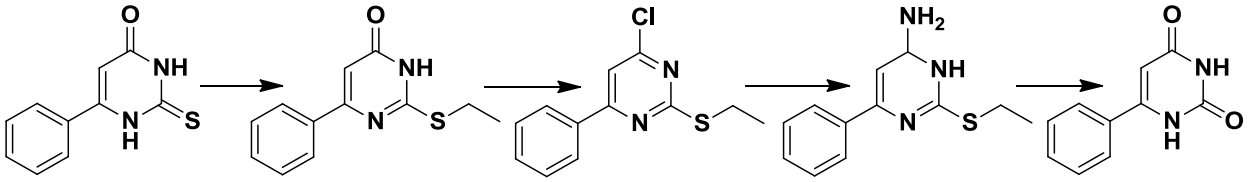
Uracil and its derivatives are best synthesized by *in situ* oxidative decarboxylation of the reaction product of malic acid and urea with modest yields. The reaction involves two components condensation reaction with low yields, especially, in case of substituted uracils. Another method for the preparation of uracil is the reaction of β-ketoester with urea and subsequent ring closure of the intermediate on treatment with sodium ethoxide. Substituted malonic ester derivatives were reported to react with urea or thiourea to yield uracil and thiouracil derivatives in two steps.[20a-c]

Due to very poor in yield, lack of lead hydroxide and bases, Donleavy and Kise *et al.* introduced a new method for the synthesis of uracil derivatives by using ethyl acetoacetate and urea as a starting materials in 1943 ***(Scheme II.3.1).***[19f.]



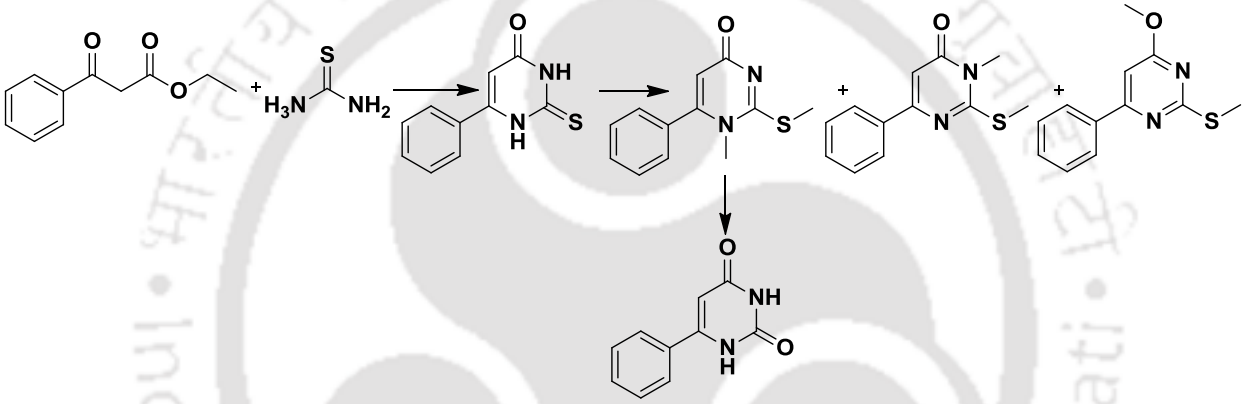
Scheme II.3.1

Johnson and his co-works described another method for synthesis modified pyrimidine from the hydrolysis thiopyrimidine in 1915 (***Scheme II.3.2).*** [21]



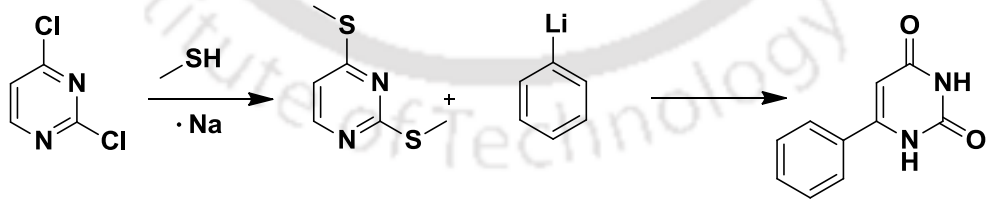
Scheme II.3.2

Skulnick *et al.* have used thiourea for the preparation of modified pyrimidines which are synthetically and biological important. In this approach they obtained mixture of compounds ***(Scheme II.3.3)***. [22]



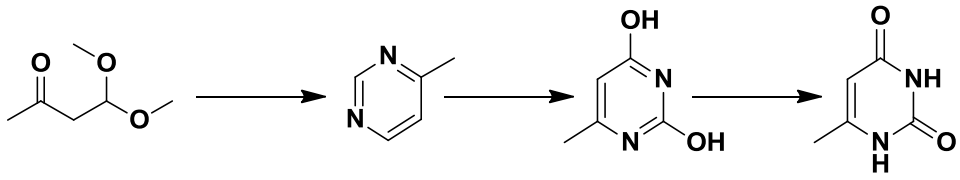
Scheme II.3.3

In 1988, Strekowshi *et al.* group have developed a synthetic route for pyrimidine nucleobases by using heterocyclic moiety as a starting material reacted with aryl lithium (***Scheme II.3.4).*** [23]



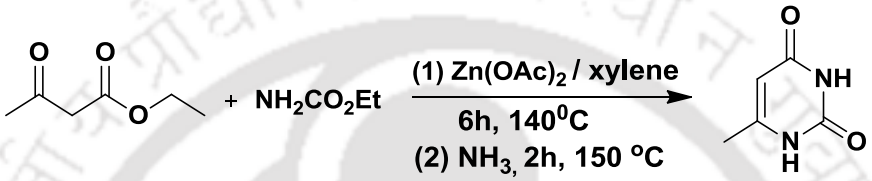
Scheme II.3.4

Gotor, V. *et al*. have also synthesized modified pyrimidines by using heterocyclic compound as a starting material in 1997 (***Scheme II.3.5).*** [20c]



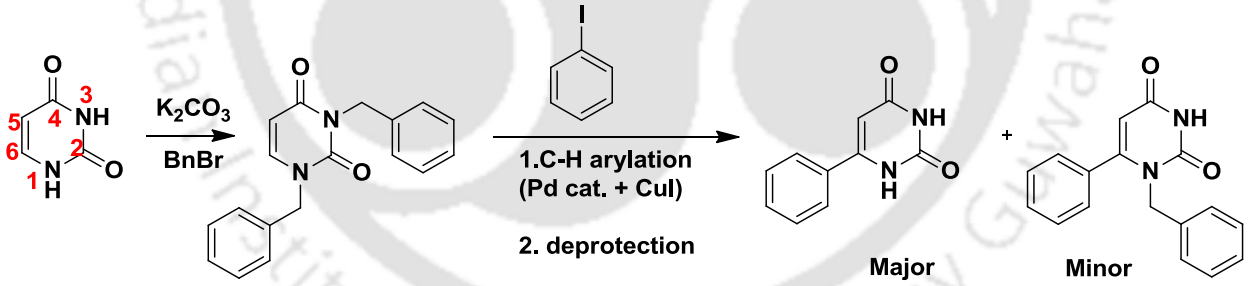
Scheme II.3.5

Further in 2009, Daxue *et al*. have developed a method to synthesize pyrimidine derivatives by using $Zn(OAc)_2$ and ammonia , and the reactions were complete within 2h (***Scheme II.3.6)*** [20e]



Scheme II.3.6

Very recently, Cernova, M. *et al.* have developed a new method for the synthesis of modified pyrimidine nucleobases through coupling reaction of pyrimidine moiety with aryl halides in 2011 ***(Scheme II.3.7*)**. [24]

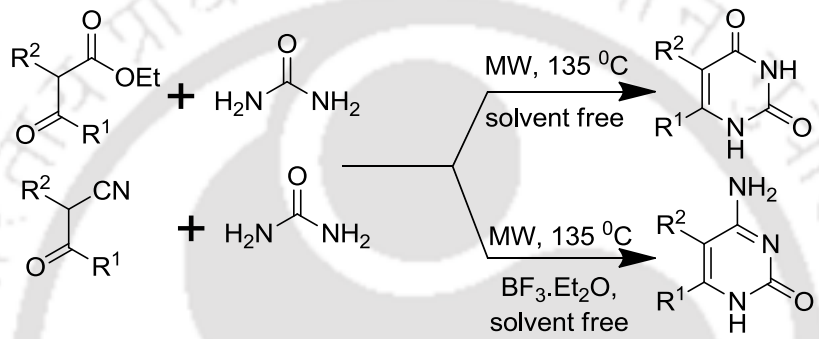


Scheme II.3.7

However, these conventional methods for the synthesis of pyrimidine nucleobases require either multi-step reactions or harsh experimental conditions and long reflux time.[25, 26] Several methods have been developed, in the past, for efficient synthesis of natural and modified nucleobases, including metal catalyzed cross coupling reactions.[27] Microwave-directed methods were also used to synthesize non-nucleobase pyrimidines, using N-vinyl and N-aryl amides as a starting substrates.[28, 29]

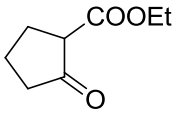
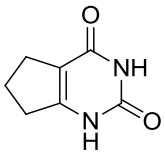
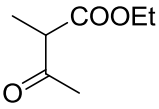
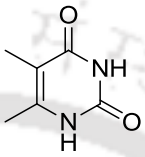
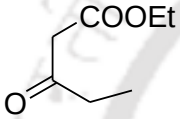
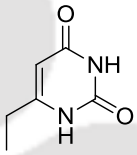
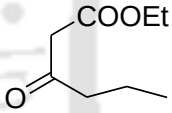
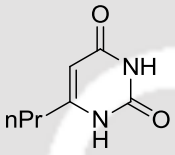
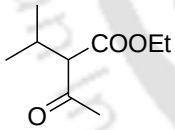
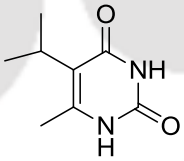
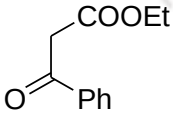
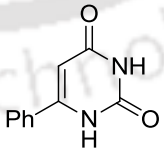
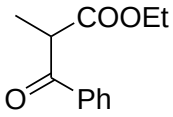
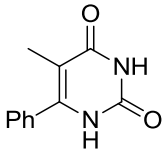
II.4. Present work

We developed a new synthetic method to synthesize various uracil, cytosine and thymine derivatives in microwave condition. Here we demonstrated a one-pot, solvent-free and very efficient synthesis of pyrimidine nucleobases with altered steric and electronic properties at C-5 and C-6 positions. We found that the use of microwave-assisted synthesis under solvent-free conditions led to high yields of uracil and cytosine derivatives (***Scheme II.4.1)***



Scheme II.4.1

We observed some of the reactions, especially highly substituted β-ketoesters proceeded with moderate to low yield. Therefore, the yield of the reactions as well as the rate of conversions was further enhanced, significantly, by use of $BF_3.Et_2O$ as Lewis acid (***Scheme II.4.1).*** A series of uracil and cytosine nucleobase derivatives were synthesized in a single step, using this method. All reactions were carried out in a *Anton Paar Synthos 3000 or CEM Discover Labmate* closed vessel microwave reactor at about 135°C-145°C for variable durations.[33] Uracil nucleobases (**1-10**) were synthesized by treatment of the respective β-ketoesters or β-aldehydo ester with urea, whereas the cytosine derivatives (**11, 12**) were obtained from benzoylacetonitrile or *N,N,*-diethylamide precursors. The substrate β-ketoesters (**1a-8a, 10a**), β-aldehydo ester (**10a**), benzoylacetonitrile and *N,N*-diethylamide (**11a, 12a**) were either purchased or synthesized following published procedures.[30-32] ***Table II.4.1*** demonstrates the synthesis of the nucleobases under microwave conditions.[33]

Entry No.	Substrate (1a-12a)	Microwave irradiation (Min)	Product (1-12)	%Yield	% Yield with $BF_3.Et_2O$ (Min)
1		9		72	83 (5 min)
2		8		70	85 (5 min)
3		8		72	86 (5 min)
4		10		70	83 (5 min)
5		12		48	60 (7 min)
6		8		80	92 (4 min)
7		9		67	76 (7 min)

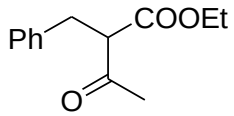
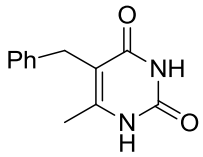
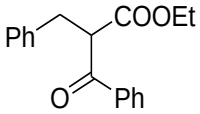
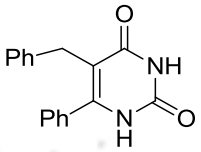
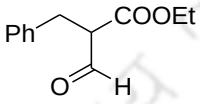
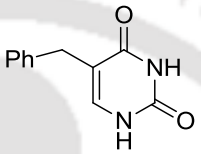
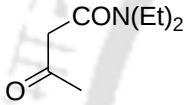
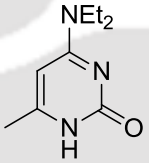
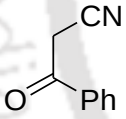
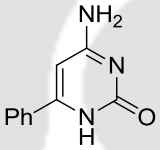
8		12		55	75 (9 min)
9		15		25	55 (9 min)
10		9		75	87 (7 min)
11		9		70	80 (7 min)
12		6		65	78 (4 min)

Table II.4.1 *synthesis of pyrimidine nucleobase derivatives*

Here, the substrates were designed so that the product nucleobases were expected to have different stereo-electronic properties at C-5 and C-6 positions and hopefully which is use in the unnatural base-pairing in DNA/RNA. In order to test the robustness of the method, we have used β-aldehydo ester as substrate instead of a β-ketoester, which also shows rapid conversion (Compound **10**). The versatility of the method was further demonstrated by the synthesis of cytosine derivatives, **11** and **12**. The optimized irradiation times in ***Table II.4.1*** reflect completion of reactions, monitored by checking TLC (Thin Layer Chromatography).

Mechanism of the reaction:

The mechanism of the reaction is proposed in ***Figure II.4.1*** where, in the first step, the carbonyl carbon of the substrate β-ketoester (**4a, 10a**) is activated by the Lewis acid. The following reaction with urea gives an intermediate, characterized by **HRMS** and **NMR**. Finally, the intermediate undergoes ring closer to yield the cyclized product (**4, 10**).

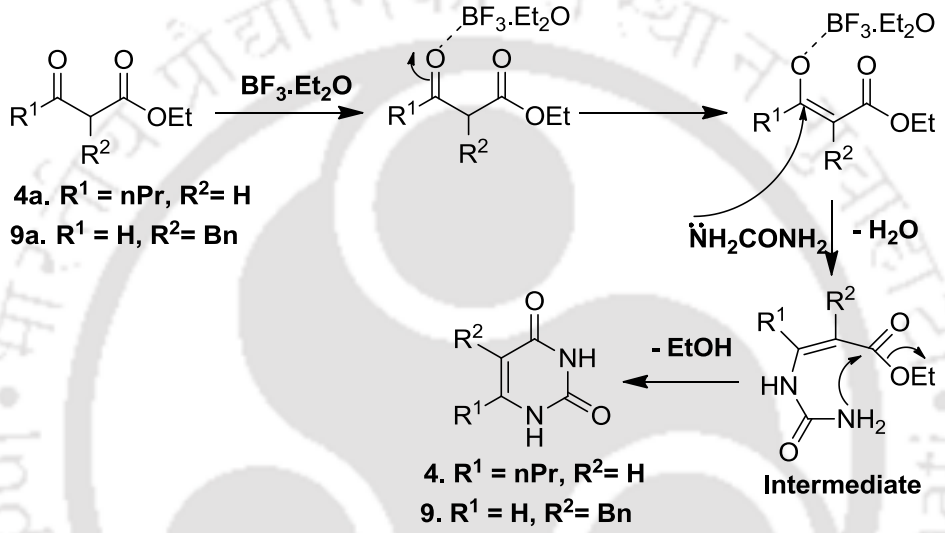
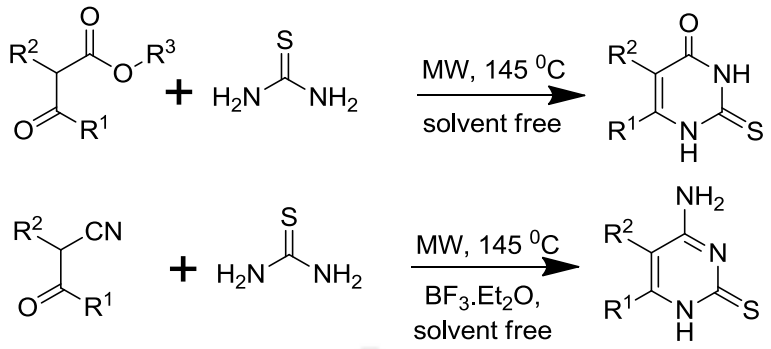


Figure II.4.1*: Schematic presentation of the reaction mechanism*

Another aim of this chapter is to establish an efficient method to synthesize thiouracil and thiocytosine derivatives. Thiouracils and thiocytosines are important class of compounds which are widely used due to their high bioactivity and inhibitory effects such as antitumor, anti-inflammatory and virucidal activities.[34] The derivatives are reportedly synthesized under harsh conditions in presence of strong acid, organic solvent and required long refluxing hours.[35-37] Moreover, the procedures are limited to variations at C-5 and C-6 substitution of the pyrimidinone analogs.[38, 39]

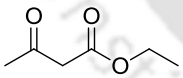
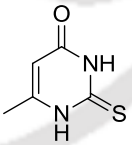
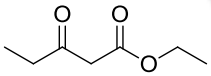
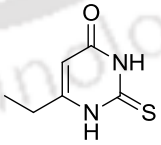
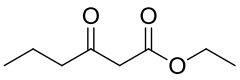
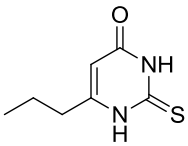
We described a new method to syntheses modified thiouracils from β-ketoester or β-aldehydo esters precursors under microwave condition at 145^0C by using $BF_3.Et_2O$ as a lewis acid. ***(Scheme II.4.2)***.



Scheme II.4.2

Further, this method was extended to synthesis of thiocytosines (**22**, **23**) from their respective starting materials (benzoylacetonitrile or *N*, *N*,-diethylamide). The substrates were either purchased or synthesized following published procedures.[30-32]

Herein, a wide range of compounds (**13-24**) were synthesized with high yield in a one-pot, microwave-directed method. Many of the molecules presented here are new and not reported (Compound **17, 20, 22** and **23**). Again, all reactions were carried out in absence of any solvent ***(Table II.4.2)***. The reaction yields as well as the rate of conversions were further increased by using $BF_3.Et_2O$ as Lewis acid.

S.No.	Substrate 20a-29a	Microwave irradiation (Min)	%Yield	Product 20-29	%Yield with $BF_3.Et_2O$(Min)
13		12	78		90(8)
14		12	75		90(8)
15		12	74		88(8)

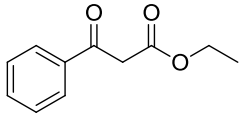
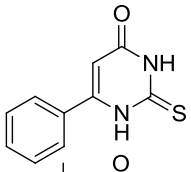
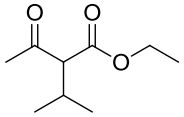
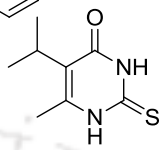
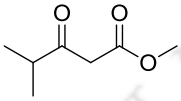
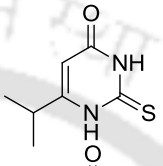
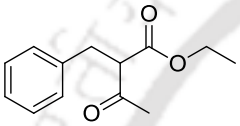
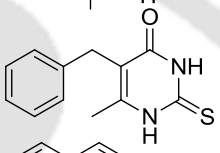
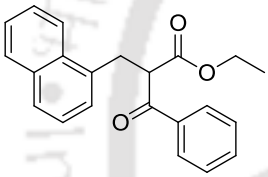
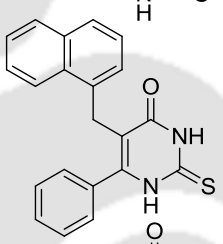
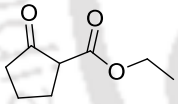
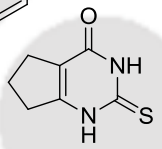
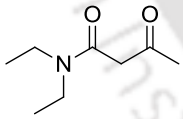
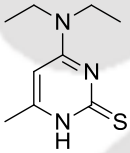
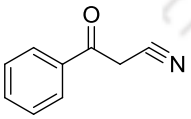
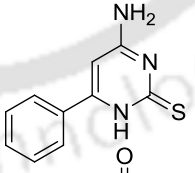
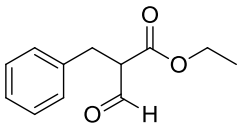
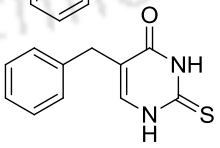
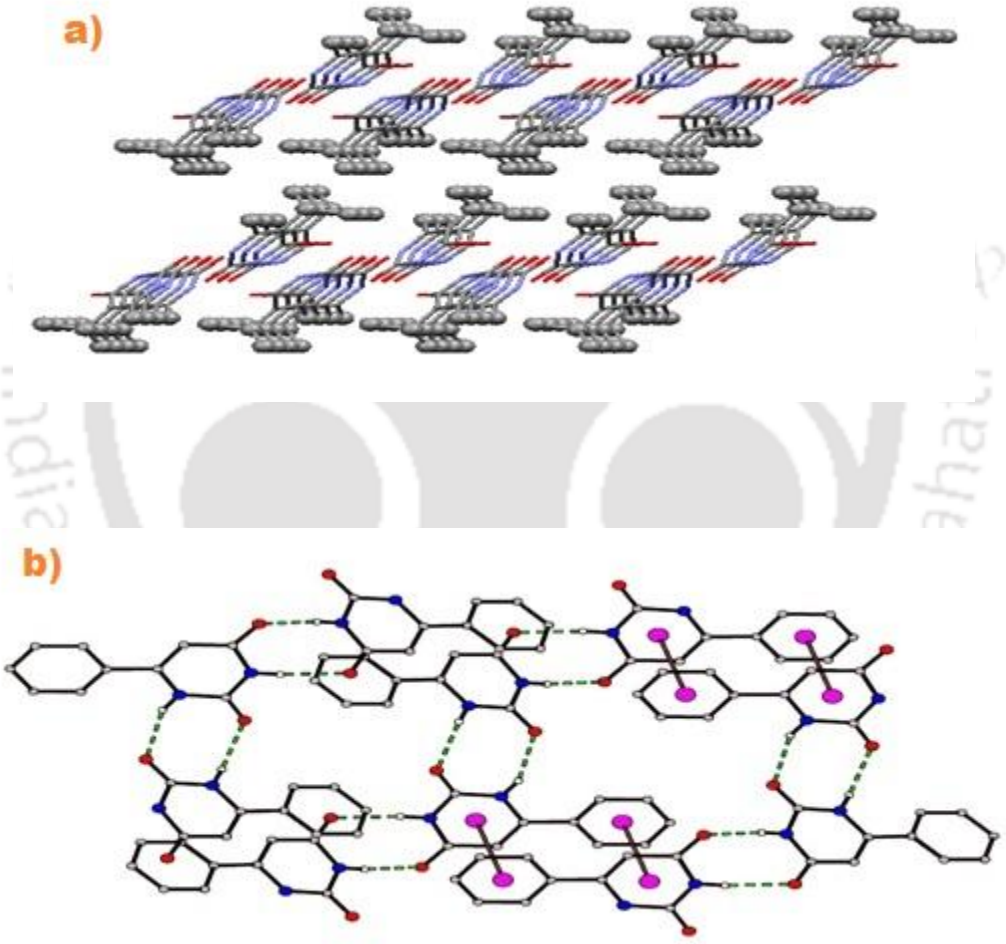
S.No.	Substrate 20a-29a	Microwave irradiation (Min)	%Yield	Product 20-29	%Yield with $BF_3.Et_2O$(Min)
16		12	80		92(8)
17		15	65		75(10)
18		12	71		80(8)
19		15	62		73(10)
20		15	42		58(10)
21		12	64		75(10)
22		12	54		65(8)
23		12	65		78(8)
24		12	48		60(8)

Table II.4.2: *synthesis of thiopyrimidine nucleobases derivatives*

We also demonstrate the first crystal structure of 5-isopropyl-6-methyluracil (**5**), 6-phenyluracil (**6**) and 5-isopropyl-6-methylthiouracil **(17)** which clearly show different packing and altered

hydrogen bonding with good pi-stacking abilities, shown in ***Figure II.4.2***. The crystal structure of **5** and **17** shows a highly organized pattern with alternative hydrophobic and hydrophilic layers. The branched alkane chain forms the hydrophobic layer whereas, the polar C(4)=O(2) accounts for the hydrophilic interactions. The crystal structure of **6** also shows self-assembled pattern through H-bonding (2.03 and 2.17 Å) as well as strong π-π interactions (3.65 Å) between the phenyl and the heterocyclic ring. [29] The details of the crystal data are given in the characterization section. Based on the above results we strongly hope that these can be useful for better pairing in DNA/RNA duplexes.



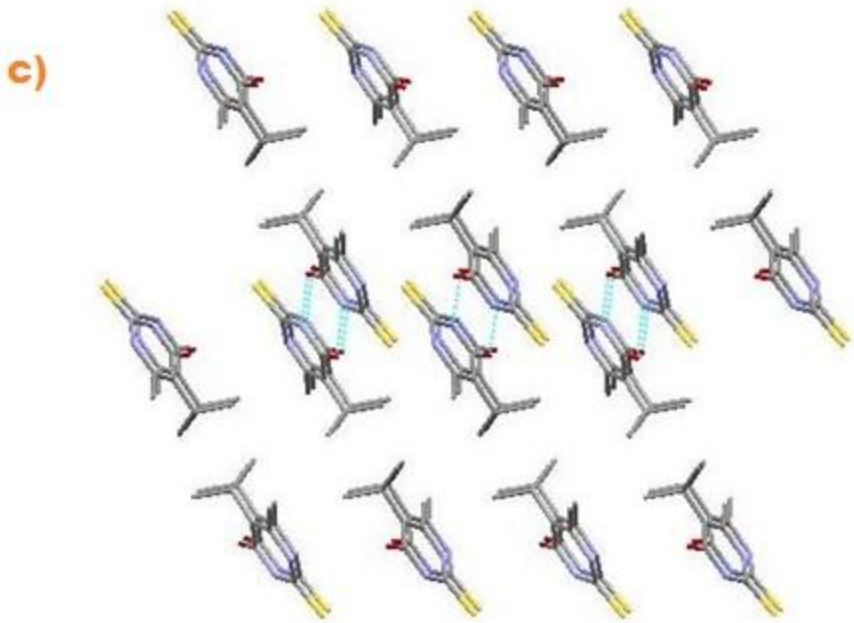


Figure II.4.2: ***a)*** *packing diagram of* ***5*** *showing organized layered structure.* ***b)*** *Packing diagram of* ***6*** *showing H-bondings (N-H·····O=C, green dash) and π-stacking (purple colored dummy atoms) interactions. Blue and red represents 'N' and 'O' atoms respectively.* ***c****). Compound* ***(17)*** *supramolecular architecture diagram*

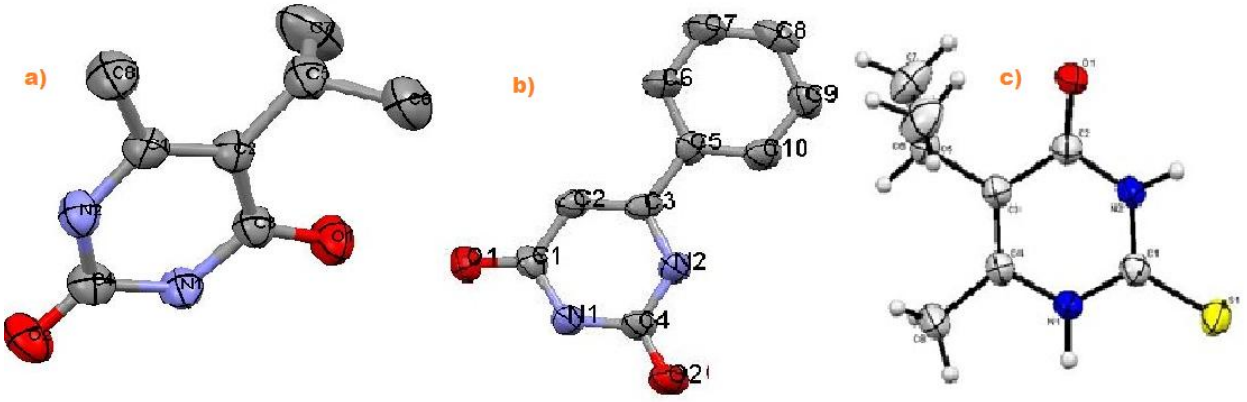


Figure II.4.3: a) *ORTEP diagram compound* ***5****.* ***b)****. ORTEP diagram compound* ***6****.* ***c).*** *ORTEP diagram compound* ***17.***

In conclusions, we demonstrated a new synthetic method to synthesize modified uracil, thiouracil, cytosine and thiocytosine nucleobases by using microwave assisted method. The main advantages this method is shorter reaction time, easy handling, environmentally benign as no

toxic reagent is used, usage of modern techniques, free from solvents, cheaper method and one - pot synthesis.

II.5. Experimental section

II.5.1. General Information: All chemicals were purchased from SRL, Merck, and Sigma Aldrich and were used without further purification. All microwave-directed reactions were carried out in an *Anton Paar Synthos 3000* and *CEM Discover Labmate* closed vessel microwave reactor at 600W at about 135°C -145°C for variable durations. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were all recorded from a *DRX-400 Varian spectrometer* using $CDCl_3$, D_2O or DMSO-D_6 as solvents. Chemical shifts are reported in parts per million (ppm). Melting points were determined using *Büchi B-545* apparatus and are uncorrected. High resolution mass spectrometry was analyzed from *Agilent Q-TOF 6500 LC/MS* system and *Micromass Q-TOF ESI-MS* instrument (model *HAB 273*). X-Ray data were collected from a *Bruker SMART APEX* equipped with a CCD area detector using *Mo*. The structures were solved by direct method using *SHELLX-97* (Göttingen, Germany).

II.5.2. General Procedure (compound 1-12): A β-ketoester (**1a-12a**, 2 mmol), taken in a reactor vessel was mixed thoroughly for 1 min with urea (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at about 135°C. The compound (**1-12**) was further purified by column chromatography. **Yield:** as shown in ***table II.4.1***.

Synthesis using Lewis acid (1-12): A β-ketoester (**1a-12a**, 2 mmol), taken in a reactor vessel with $BF_3\,Et_2O$ (2.4 mmol) was mixed thoroughly for 1 min with urea (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at 135°C. The compound (**1-12**) was further purified by column chromatography. **Yield:** as shown in ***table II.4.1***.

II.5.3. General Procedure (compound 13-24): substrate (**13a-24a**, 2 mmol) taken in a reactor vessel, was mixed thoroughly for 1 min with thiourea (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 12-15 min at about 145°C. The compound (**13-24**) was further purified by column chromatography. The exact duration of microwave exposure and percentage yield are as shown in ***table II.4.2***.

Synthesis using Lewis acid (13-24): 2 mmol of substrate (**20a-31a**), taken in a reactor vessel with $BF_3.Et_2O$ (2.4 mmol) was mixed thoroughly for 1 min with thiourea derivatives (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 8-10 min at 145°C. The compound (**13-24**) was further purified by column chromatography. The observed yield and time of irradiation are given in ***table II.4.2***.

A). Representative procedure for compound 4: Ethyl 3-oxohexanoate (**4a**, 2 mmol), taken in a reactor vessel, was mixed thoroughly for 1 min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for 10 min at about 135^0C. The completion of reaction was monitored by checking TLC at regular time intervals. Compound (**4**) was further purified by column chromatography (Silica gel 60–120 mesh, 75% ethyl acetate in hexane).

Synthesis using Lewis acid: Ethyl 3-oxohexanoate (**4a**, 2 mmol), taken in a reactor vessel with $BF_3.Et_2O$ (339 mg, 2.4 mmol) was mixed thoroughly for 1 min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at 135^0C for about 5 min. Reaction was complete within 5 min irradiation, which was verified by TLC. Compound (**4**) was further purified by column chromatography (Silica gel 60–120 mesh, 75% ethyl acetate in hexane).

B) Representative procedure for compound (10): Ethyl 2-benzyl-3-oxopropanoate (402 mg, 2 mmol) taken in a reactor vessel was mixed thoroughly for 1 min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 9 min at about 145°C. The compound (**10**) was further purified by column chromatography. **Yield:** 75%. We followed same procedure to produce compound **24.**

Synthesis using Lewis acid (10): Ethyl 2-benzyl-3-oxopropanoate (402 mg, 2 mmol) taken in a reactor vessel with $BF_3.Et_2O$ (339 mg, 2.4 mmol) was mixed thoroughly for 1 min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 7 min at about 145°C. The compound (**10**) was further purified by column chromatography. **Yield:** 87%. We followed same procedure to produce compound **24**

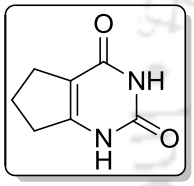
C) Representative procedure for compound (12): 3-oxo-3-phenylpropanenitrile(290 mg, 2 mmol) taken in a reactor vessel with $BF_3.Et_2O$ (339 mg, 2.4 mmol) was mixed thoroughly for 1

min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 6 min at about 135°C. The compound (**12**) was further purified by column chromatography. **Yield:** 65%. We followed same procedure to produce compound **23**

Synthesis using Lewis acid (12): 3-oxo-3-phenylpropanenitrile (290 mg, 2 mmol) taken in a reactor vessel with $BF_3.Et_2O$ (339 mg, 2.4 mmol) was mixed thoroughly for 1 min with urea (156 mg, 2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation for about 4 min at about 135°C. The compound (**12**) was further purified by column chromatography. **Yield:** 78%. We followed same procedure to produce compound **23**

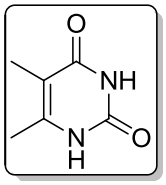
II.6. Characterization Data:

6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione (1)



Yield: 83%, white solid, m.p: 298°C, ^{1}H NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ 10.71 (s, 1H), 9.58 (s, 1H), 2.49 (t, 2H, *J*= 7.8 Hz), 2.40 (t, 2H, *J*= 7.4 Hz), 1.84 (m, 2H), ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 163.0, 157.3, 153.1, 110.4, 31.5, 26.7, 21.4. UV-262 nm. LRMS (ESI) m/z $[M+H]^+$ calcd for $C_7H_9N_2O_2$: 153.06; observed: 153.13.

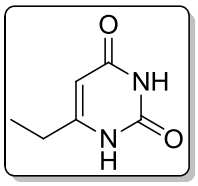
5,6-dimethylpyrimidine-2,4(1H,3H)-dione (2)



Yield: 85%, White solid, m.p 295°C, ^{1}H NMR (400 MHz, D_2O) δ 2.25 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, D_2O+DMSO-d_6) δ 163.3, 158.9, 157.4, 107.0, 18.6, 13.0. FT-IR (KBr ν/cm^{-1})

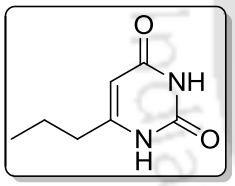
3409, 3203, 2926, 2854, 1719, 1706, 1416, 1072, 544. UV λ_{max} 260 nm. HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_6H_9N_2O_2Na$: 163.0478; observed: 163.0468.

6-ethylpyrimidine-2,4(1H,3H)-dione (3)



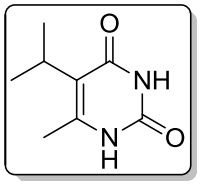
Yield: 86%, White solid, m.p 302°C, ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ.10.64 (s, 1H), 10.19 (s, 1H), 5.24 (s, 1H), 1.63 (q, 2H, *J*= 7.7 Hz), 0.95 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 164.8, 156.5, 152.1, 98.2, 20.0, 13.1. FT-IR (KBr ν/cm^{-1}) 3411, 3208, 2924, 2854, 1718, 1714, 1588, 1414, 1361, 1072. UV λ_{max} 260 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_6H_9N_2O_2$: 141.0659; observed: 141.0667.

6-propylpyrimidine-2,4(1H,3H)-dione (4)



Yield: 83%, White solid, m.p 270°C, ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 10.67 (s, 1H), 10.24 (s, 1H) 5.32 (s, 1H), 2.27 (t, 2H, *J*= 7.4 Hz), 1.62 (m, 2H), 0.97 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 164.7, 156.4, 152.1, 98.4, 34.1, 20.2, 13.0. FT-IR **(**KBr ν/cm^{-1}) 3426, 3342, 2959, 2926, 2854, 1727, 1698, 1508, 1609, 1426, 1354, 1084, 541. UV λ_{max} 262 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_7H_{11}N_2O_2$: 155.0776; observed: 155.0780.

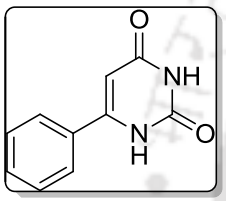
5-isopropyl-6-methylpyrimidine-2,4(1H,3H)-dione (5)



Yield: 60%, White solid, m.p 285°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 10.49 (s, 1H), 2.83-2.76 (m, 1H), 2.10 (s, 3H), 1.15 (d, 3H, *J*= 6.8 Hz), 0.92 (d, 3H, *J*= 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d6) δ 164.5, 151.6, 148.0, 118.7, 27.7, 20.9, 20.8, 19.4. FT-IR (KBr ν/cm^{-1}) 3383, 3164, 2957, 2928, 2873, 1725, 1659, 1427, 1083, 774. UV λ_{max} 266 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_8H_{13}N_2O_2$: 169.0972; observed: 169.0967.

Crystal data for **5**: CCDC # 860110; C8 H12 N2 O2; M = 168.2, m.p. = 285-287°C, monoclinic; P21/c, a = 11.0751(18) Å; b = 7.0759(12) Å, c = 11.598(2) Å, α = 90°, β = 105.156(12)°, γ = 90°, V = 877.3(3) Å, Z = 4, μ = 0.093 mm^{-1}, ρ = 1.273 g.cm^{-3}, Mo-K_α radiation, R1 = 0.0489, wR2 = 0.0883, S = 1.102.

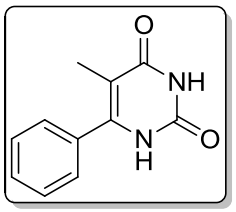
6-phenylpyrimidine-2,4(1H,3H)-dione (6)



Yield: 92%, White powder, m.p 266°C, ^{1}H NMR (400 MHz,$CDCl_3$+DMSO-d_6) δ 10.64 (s, 2H), 7.40-7.20 (m, 5H), 5.52.(s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 164.1, 152.3, 151.6, 131.1, 130.3, 128.1, 126.0, 97.7. FT-IR (KBr ν/cm^{-1}) 3407, 3166, 3001, 2924, 1717, 1651, 1487, 1451, 1236, 1037. UV λ_{max} 285 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_9N_2O_2$: 189.0659; observed: 189.0662.

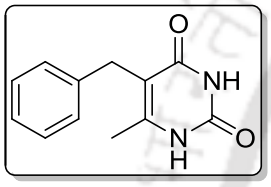
Crystal data for **6**: CCDC # 844500; C10 H8 N2 O2; M = 188.18, m.p. = 260-265°C, monoclinic; C-2/c, a = 9.5412(4) Å; b = 11.0300(4) Å, c = 16.1903(7) Å, α = 90°, β = 91.564(3)°, γ = 90°, V = 1703.22(12) Å, Z = 8, μ = 0.105 mm^{-1}, ρ = 1.468 g.cm^{-3}, Mo-K_α radiation, R1 = 0.0453, wR2 = 0.0834, S = 1.355.

5-methyl-6-phenylpyrimidine-2,4(1H,3H)-dione (7)



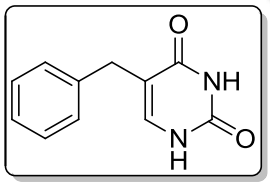
Yield: 76%, White solid, m.p 276°C, ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 10.54 (s, 2H), 7.66-7.46 (m, 5H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6), δ 167.7, 154.1, 152.3, 131.1, 130.3, 128.0, 126.2, 107.7, 28.7. FT-IR (KBr ν/cm^{-1}) 3432, 2979, 1644, 1629, 1025. UV λ_{max} 270 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{11}N_2O_2$: 203.0815; observed: 203.817.

5-benzyl-6-methylpyrimidine-2,4(1H,3H)-dione (8)



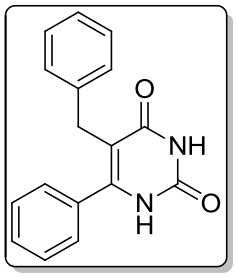
Yield: 75%, White solid, m.p 215°C, ^{1}H NMR (400 MHz, D_2O+DMSO-d_6) δ 7.27-7-19 (m, 5H), 2.68 (s, 2H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 152.7, 149.7, 139.6, 128.6, 127.8, 125.8, 110.3, 29.8, 20.0. FT-IR (KBr ν/cm^{-1}) 3385, 2980, 2934, 1657, 1587, 1084, 530. UV λ_{max} 275 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{12}H_{13}N_2O_2$: 217.0972; observed: 217.0980.

5-benzylpyrimidine-2,4(1H,3H)-dione(9)



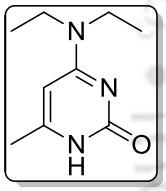
Yield: 87%, White solid, m.p 282°C. ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 9.97 (s, 1H), 9.94 (s, 1H), 7.66 (s, 1H), 7.27-7.13 (m, 5H), 3.60 (s, 2H). ^{13}C NMR (100 MHz, CDCl3+DMSO-d_6) δ.167.5, 154.3, 138.9, 135.7, 127.5, 127.2, 125.2, 104.8, 29.9. FT-IR (KBr ν/cm^{-1}). 3436, 3032, 1721, 1680, 1205, 736, 534. UV λ_{max} 265 nm. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{11}N_2O_2$: 203.0815; observed:203.0825.

5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione (10)



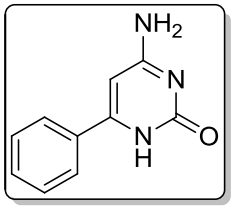
Yield: 55%, White solid, m.p: 252°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 11.17 (s, 1H), 7.98 (d, 2H, *J*= 8.4 Hz), 7.71 (d, 2H, *J*= 7.2 Hz), 7.53-7.49 (m, 3H), 7.36-724 (m, 3H), 3.80 (s, 2H). FT-IR (KBr ν/cm^{-1}). 3436, 3219, 2914, 2853, 1741, 1724, 1469, 1053.UV λ_{max} 285 nm**.** HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_2$: 279.1128; observed: 279.1135.

4-(diethylamino)-6-methylpyrimidin-2(1H)-one (11)



Yield: 80%, White solid, m.p: 220°C. ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 8.72.(s, 1H), 4.88 (s, 1H), 2.91 (q, 4H, *J*= 7.2 Hz), 1.89.(s, 3H), 0.78 (t, 6H, *J*= 7.0 Hz) ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 168.4, 157.9, 155.8, 98.7, 40.4, 17.9, 13.0. FT-IR (KBr ν/cm^{-1}).3439, 2963, 2926, 2855, 1633, 1096, 802. UV λ_{max} 260 nm**.** HRMS (ESI) m/z $[M+H]^+$ calcd for $C_9H_{16}N_3O$: 182.1288; observed: 182.1285.

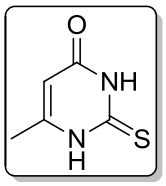
4-amino-6-phenylpyridin-2(1H)-one(12)



Yield: 78%, White solid, m.p: 242°C, ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 11.08.(s, 1H), 7.57-7.41 (m, 5H), 6.50 (s, 1H), 6.44 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 163.2,

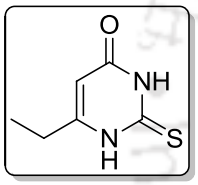
159.8, 154.7, 138.2, 129.4, 128.7, 128.2, 94.5. FT-IR (KBr ν/cm^{-1}).3417, 3192, 2924, 1671, 1421, 1026. UV λ_{max} 278 nm. LRMS (ESI) $[M-H]^-$ calcd for $C_{10}H_{10}N_3O$: 186.0673; observed: 186.6389.

2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1H)-one (13)



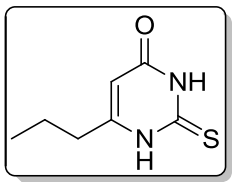
Yield: 90%, white solid, m.p: 220-225°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 11.92 (s, 1H), 5.38 (s, 1H), 1.94 (s, 3H).

6-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (14)



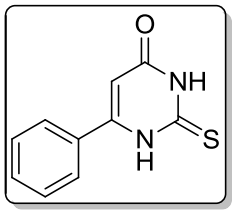
Yield: 90%, white solid, m.p: 230-232°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 12.20 (s, 1H), 5.65 (s, 1H), 2.38 (t, 2H, 7.2 Hz), 1.10 (t, 3H, *J*= 7.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.0, 161.2, 158.2, 102.0, 24.7, 11.6

2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1H)-one (15)



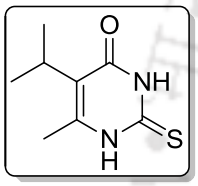
Yield: 88%, white solid, m.p: 206-210°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 12.21 (s, 1H), 5.67 (s, 1H), 2.33 (t, 2H, *J*= 7.4 Hz), 1.56-1.52 (m, 2H), 0.88 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.3, 161.7, 157.2, 103.2, 33.5, 20.8, 13.4. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_7H_{10}N_2OS$: 171.0587; observed: 171.0590.

2,3-dihydro-6-phenyl-2-thioxopyrimidin-4(1H)-one (16)



Yield: 92%, white solid, m.p: 263-265°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 2H), 7.97 (d, 2H, *J*= 7.2 Hz), 7.62-7.48 (m, 3H), 6.07 (s, 1H), ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.2, 162.9, 154.7, 134.8, 129.9, 129.5, 128.1, 103.4. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_8N_2OS$: 205.0436; observed: 205.0427

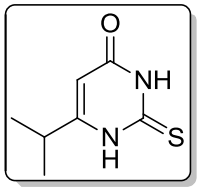
2,3-dihydro-5-isopropyl-6-methyl-2-thioxopyrimidin-4(1H)-one (17)



Yield: 75%, white solid, m.p: 257-258°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.75 (s, 2H), 2.82 (m, 1H), 2.10 (s, 3H), 0.99 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3, 160.2, 146.5, 118.9, 25.7, 19.1, 15.4. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_8H_{12}N_2OS$: 185.0743; observed: 185.0745.

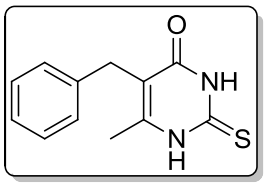
Crystal data for **17**: CCDC # 991091; C8 H12 N2 O S; M = 184.26, m.p. = 285-287°C, monoclinic; P21/c, a = 10.8054(4) Å; b = 7.7058(3) Å, c = 11.6627(4) Å, α = 90°, β = 103.968(2)°, γ = 90°, V = 942.37(6) Å, Z = 4, μ = 0.298 mm^{-1}, ρ = 1.299 $g.cm^{-3}$, Mo-K_α radiation, R1 = 0.0457, wR2 = 0.0997, S = 0.975.

2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one (18)



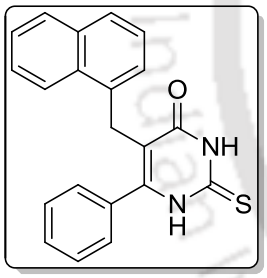
Yield: 80%, white solid, m.p: 176-180°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.18 (s, 1H), 5.66 (s, 1H), 2.69-2.62 (m, 1H), 1.13 (d, 6H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.5, 163.4, 162.6, 100.8, 31.0, 20.9. HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_7H_{10}N_2OSNa$: 193.0406; observed:193.0408.

5-benzyl-2,3-dihydro-6-methyl-2-thioxopyrimidin-4(1H)-one (19)



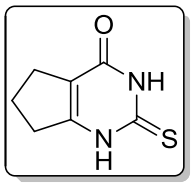
Yield: 73%, white solid, m.p: 256-257°, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.13 (s, 1H), 11.31 (s, 1H), 7.22-7.18 (m, 5H), 3.72 (s, 2H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 174.3, 161.8, 149.5, 138.7, 128.7, 128.0, 125.9, 114.4, 29.3, 16.2

2,3-dihydro-5-((naphthalen-1-yl)methyl)-6-phenyl-2-thioxopyrimidin-4(1H)-one (20)



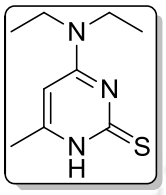
Yield: 58%, white solid, m.p: 298°C, ^{1}H NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ 11.43 (s, 1H), 11.92 (s, 1H), 7.84 (d, 2H, *J*= 7.6 Hz), 7.70 (d, 2H), 7.45-7.10 (m, 6H), 5.38 (s, 1H), 1.94 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 174.6, 161.6, 151.0, 134.6, 133.0, 133.0, 129.7, 127.9, 127.4, 126.1, 125.0, 124.9, 123.8, 122.7, 113.1, 27.5. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{21}H_{16}N_2OS$: 345.1056; observed: 345.1061.

2,3,6,7-tetrahydro-2-thioxo-1H-cyclopenta[d]pyrimidin-4(5H)-one (21)



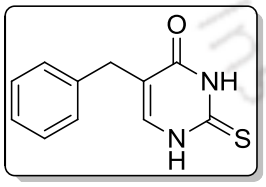
Yield: 75%, white solid, m.p: 276-278°C, ^{1}H NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ 10.35 (s, 1H), 10.32 (s, 1H), 2.36 (t, 2H, *J*= 7.2 Hz), 1.61 (m, 2H), 0.98 (t, 2H, *J*= 7.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.5, 160.5, 157.5, 116.5, 32.8, 27.6, 21.8.

4-(diethylamino)-6-methylpyrimidine-2(1H)-thione (22)



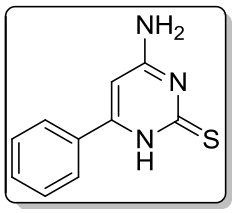
Yield: 65%, white solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.25 (s, 1H), 5.67 (s, 1H), 3.26 (t, 2H, *J*= 6.8 Hz), 2.03 (s, 3H), 1.07 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 175.5, 160.9, 152.2, 103.2, 52.2, 17.7, 15.2. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_9H_{16}N_3S$: 198.1059; observed: 198.1059.

4-amino-6-phenylpyrimidine-2(1H)-thione (23)



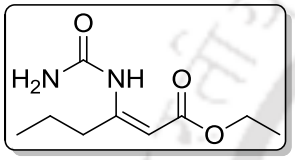
Yield: 78%, White solid, m.p: 220-223°C, ^{1}H NMR (400 MHz, DMSO-d_6+$CDCl_3$) δ 11.42 (s, 1H), 7.78-7.65 (m, 5H), 6.35 (s, 2H). HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_{10}N_3S$: 204.0590; observed: 204.0590.

5-benzyl-2,3-dihydro-2-thioxopyrimidin-4(1H)-one (24)



Yield: 60%, White solid, m.p: 210-215°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 9.94 (s, 1H), 7.72 (s, 1H), 7.33-7.25 (m, 5H), 3.65 (s, 2H). ^{13}C NMR (100 MHz, CDCl3) δ.167.5, 154.3, 138.9, 135.7, 127.5, 127.2, 125.2, 104.8, 29.9.

(Z)-ethyl 3-ureidohex-2-enoate (intermidiate for 6-propylpyrimidine-2,4(1H,3H)-dione)



White solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.51 (s, 1H), 4.96 (s, 2H), 4.79 (s, 1H), 4.09 (q, 2H, *J*= 7.2 Hz), 2.68 (t, 2H, *J*= 7.6 Hz), 1.57 (m, 2H), 1.20 (t, 3H, *J*= 7.6 Hz), 0.90 (t, 3H, *J*= 7.4 Hz). FT-IR (KBr ν/cm^{-1}). 3427, 3263, 3186, 2962, 2924, 2872, 2851, 1757, 1692, 1665, 1635, 1545, 1459, 1411, 1316, 1260, 1218, 1097. LRMS (ESI) $[M+Na]^+$ calcd for $C_9H_{16}N_2O_3Na$: 223.1059; found: 223.1070.

II.7. References

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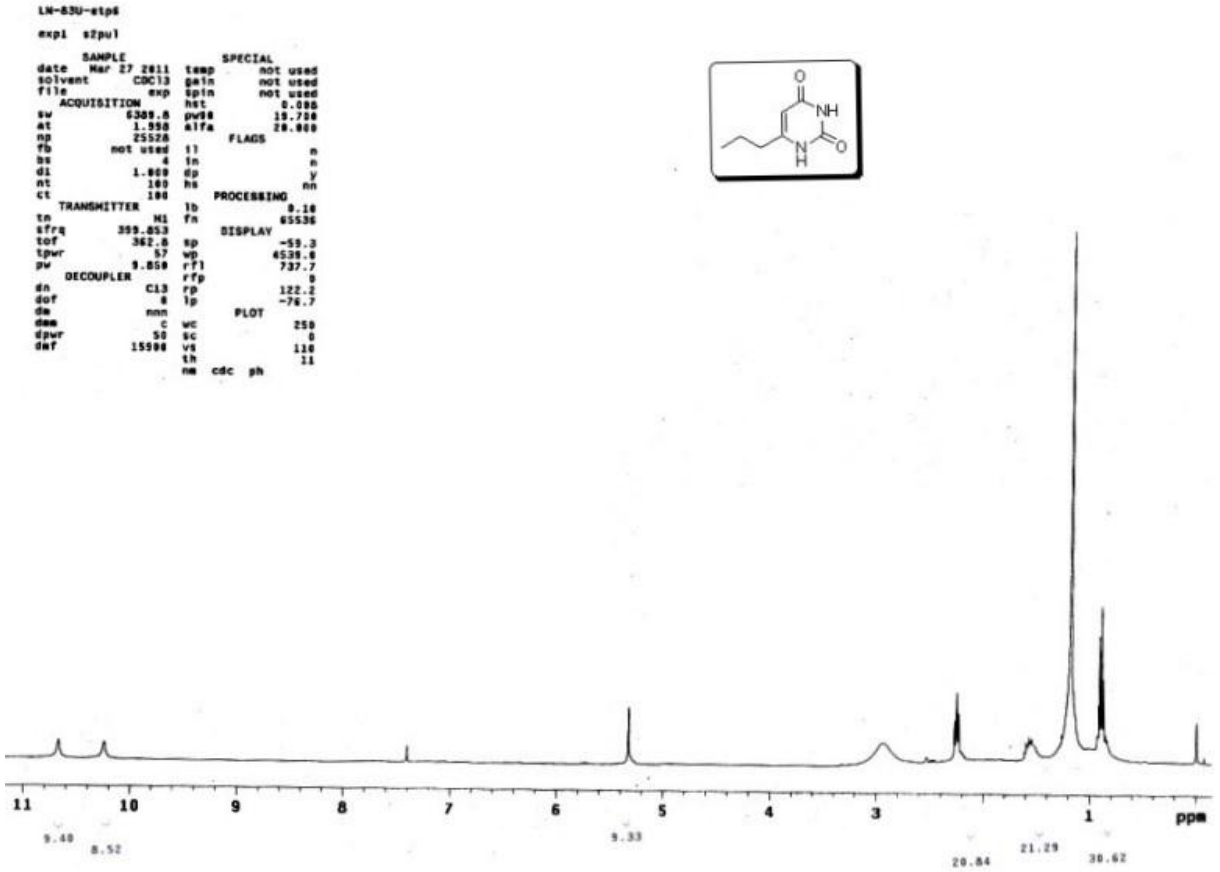
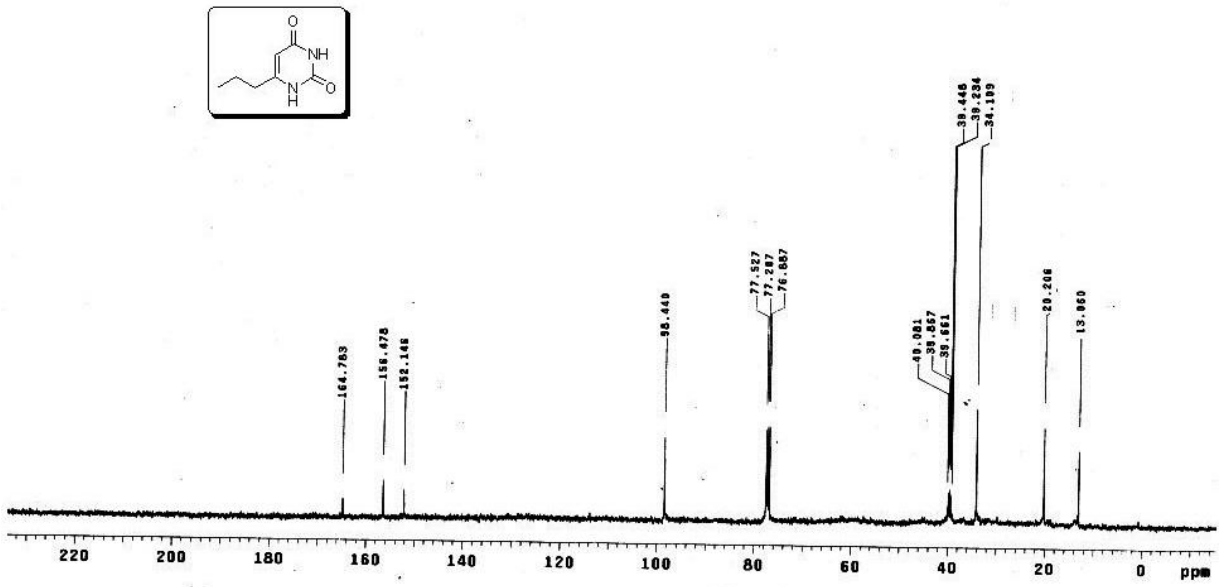
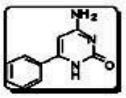
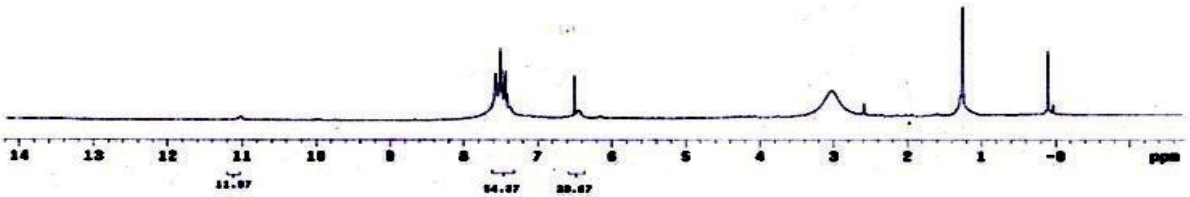
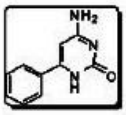
II.8. Selected spectra

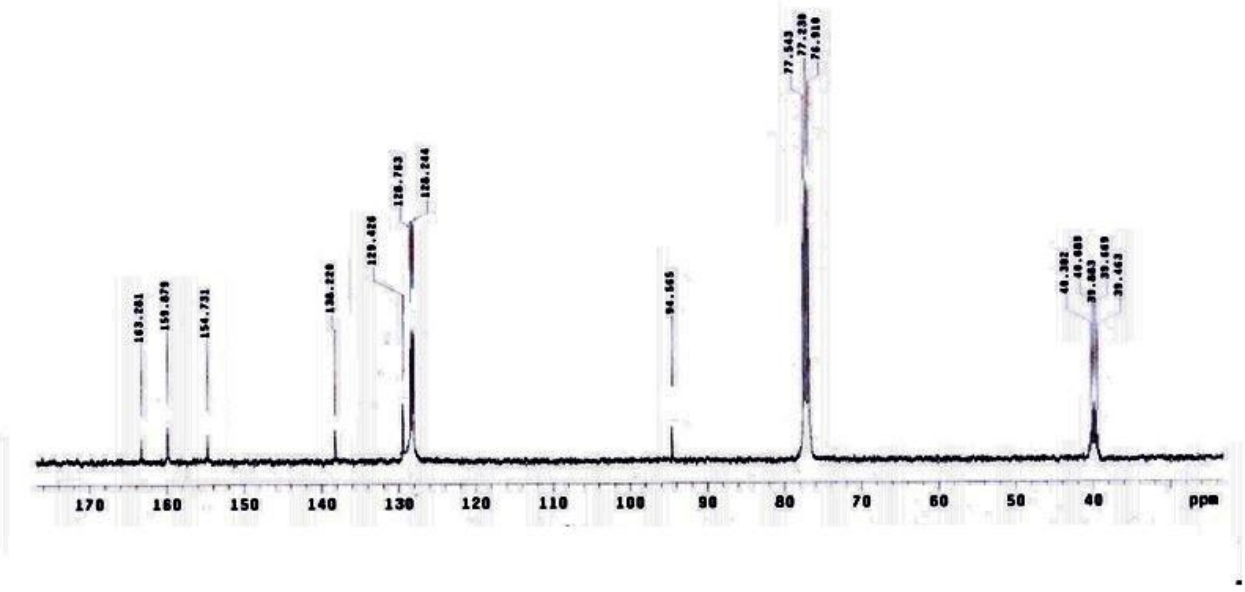


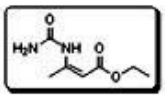


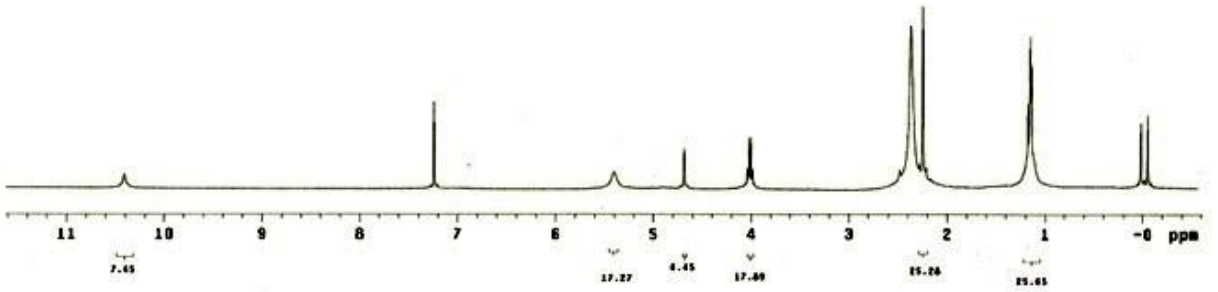


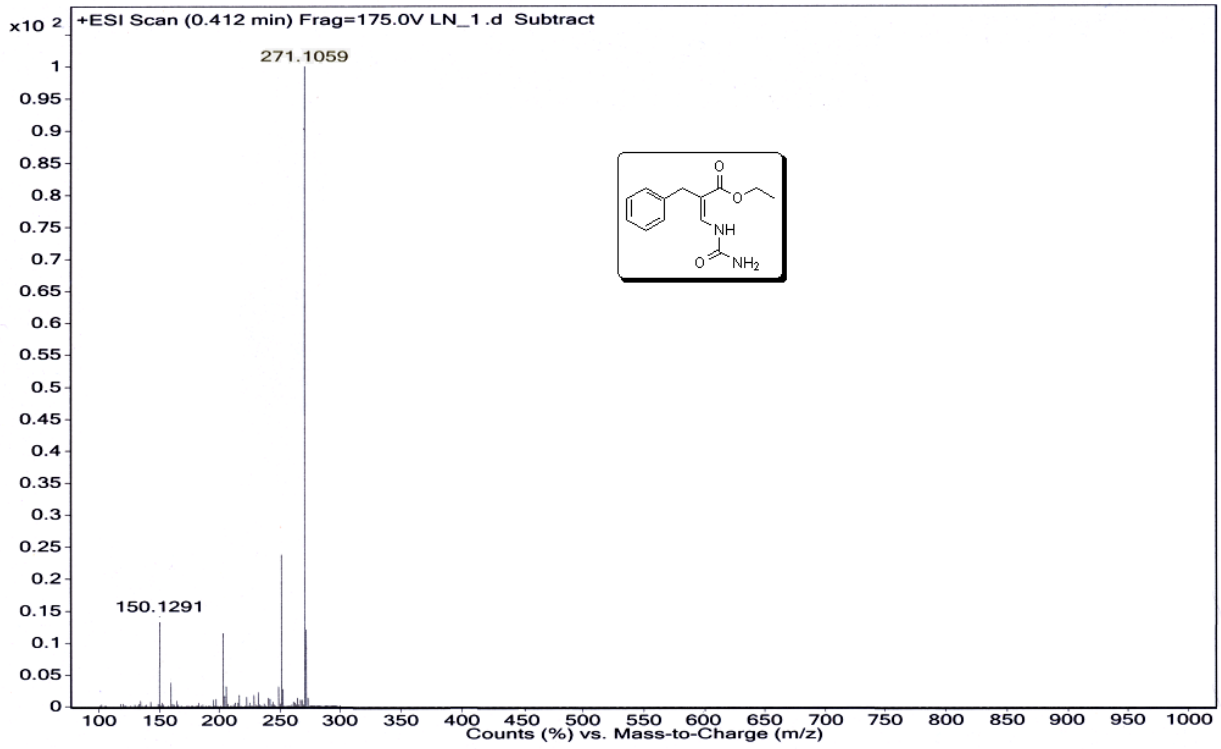


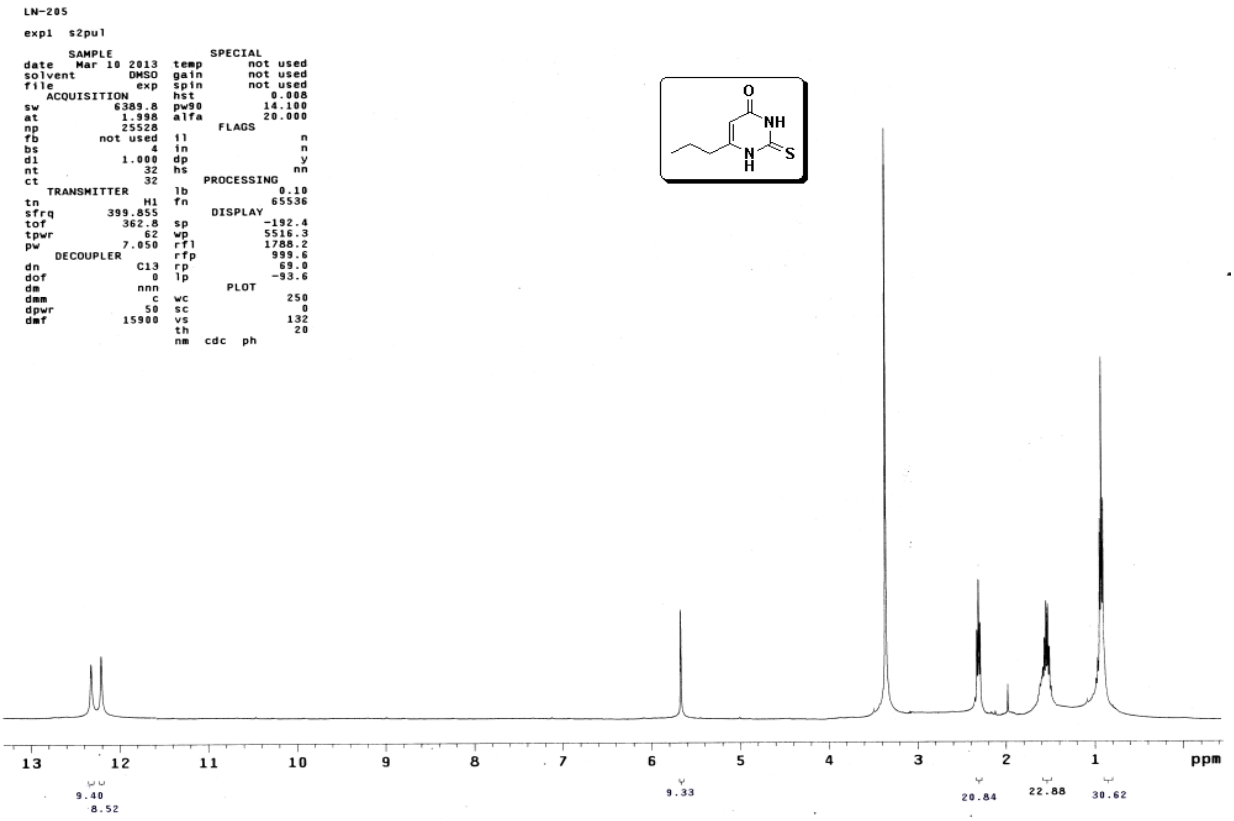


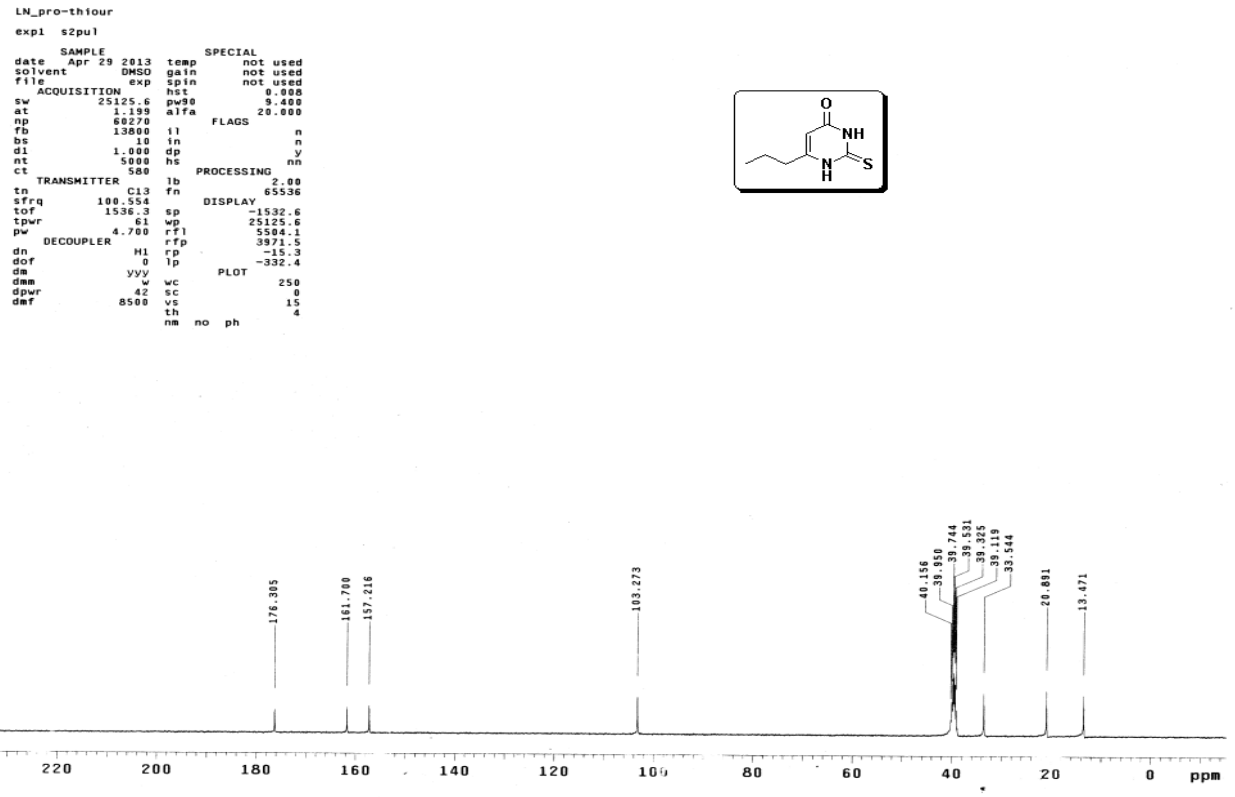


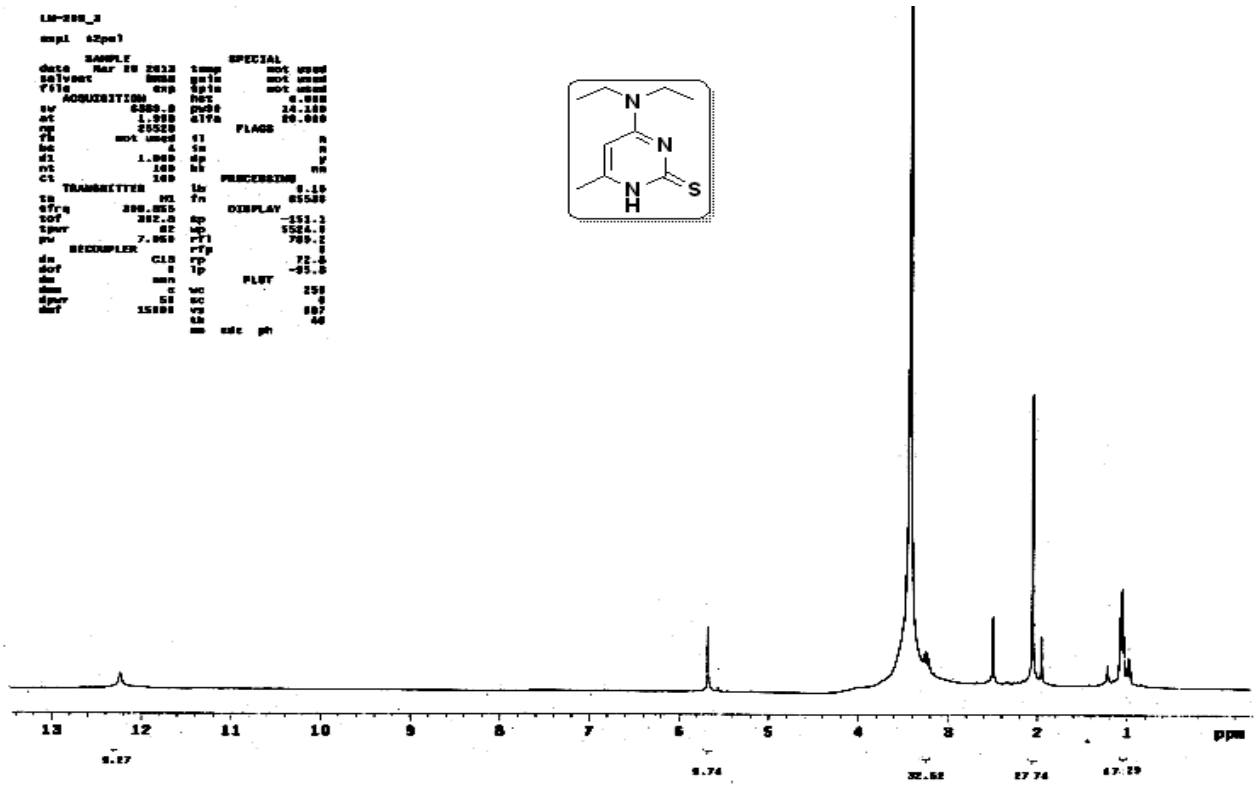


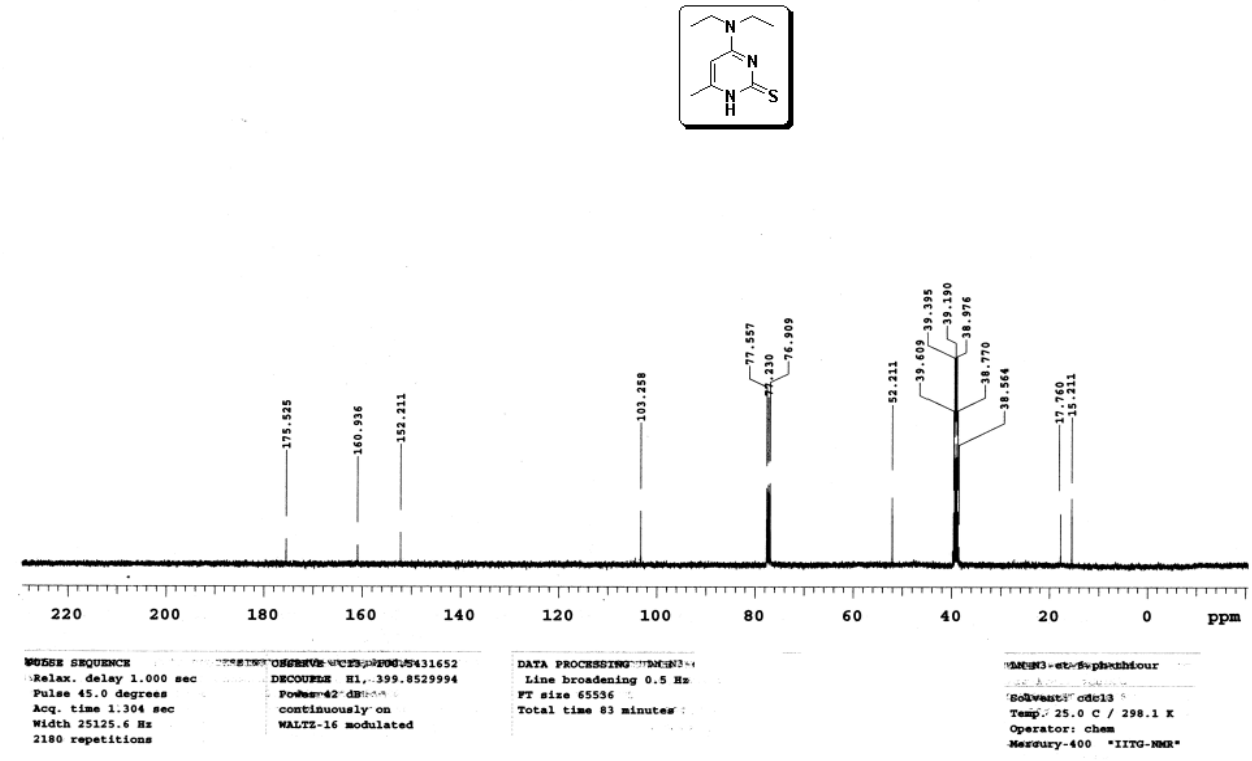












CHAPTER-III

Direct chemoselective synthesis of N-3 substituted pyrimidinones in a microwave-assisted method.

III.1. Structure and Nomenclature

Pyrimidinone is a heterocyclic organic compound. It has two nitrogens at positions **1** and **3** in the ring. In the case of nucleic acids, three types of nucleobases are pyrimidinones derivatives: cytosine (C), thymine (T), and uracil (U). These bases form hydrogen bonds with their complementary purines in DNA and RNA analogues ***(Figure III.1.1).*** [1]

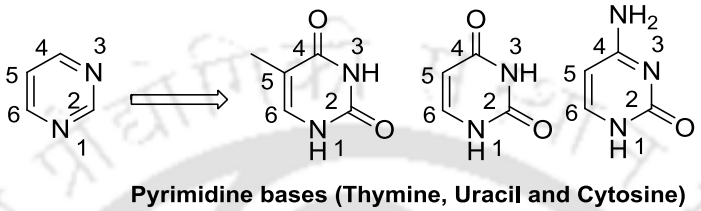


Figure III.1.1: *pyrimidine nucleobases*

III.2. Applications

Development of modified nucleobases is important for many biomolecular applications such as, chemically modified DNA, RNA or PNA analogues.[2-5] A number of nucleic acid derivatives and oligonucleotides have been synthesized to act as therapeutic agents as well as for drug delivery systems.[9-11] Especially, modified nucleobases and oligonucleotides have gained popularity for the development of biomolecular probes,[2-7] therapeutic agents,[8-11] fluorescent nucleic acids[12-14] and self-assembled DNA hybrid materials.[15-18] Pyrimidine analogs, especially the C-5, C-6 and N-3 substituted pyrimidinones showed selective anticancer, antitumor, antiviral, antitubercular and antifungal activity.[19-22] Followed by well known antitumor agent like 5-fluorouracil, several derivatives of uracil and thiouracil have been synthesized to show antitumor proliferating activities.[23, 24] Reports are available where pyrimidine derivatives have also been applied for anti-HIV activities. For example, 6-substituted uracil derivative HEPT and DABO's showed a strong and selective activity against HIV-1 ***(Figure III.2.1)***. [25a-e] Apart from their pharmaceutical potentials, pyrimidinones in general, have been widely studied for alternative non Watson-Crick base pairing properties for the development of biomolecular probes.[4, 26]

Protected N-3 derivatives of the pyrimidine nucleobases are also important for sugar nucleobase coupling. These coupling often leads to formation of a mixture of N-1 and N-3 products, especially problem is more when pyrimidine is crowded. This could be avoided through selective protection of N-3 that would allow the synthesis of the desired nucleosides.

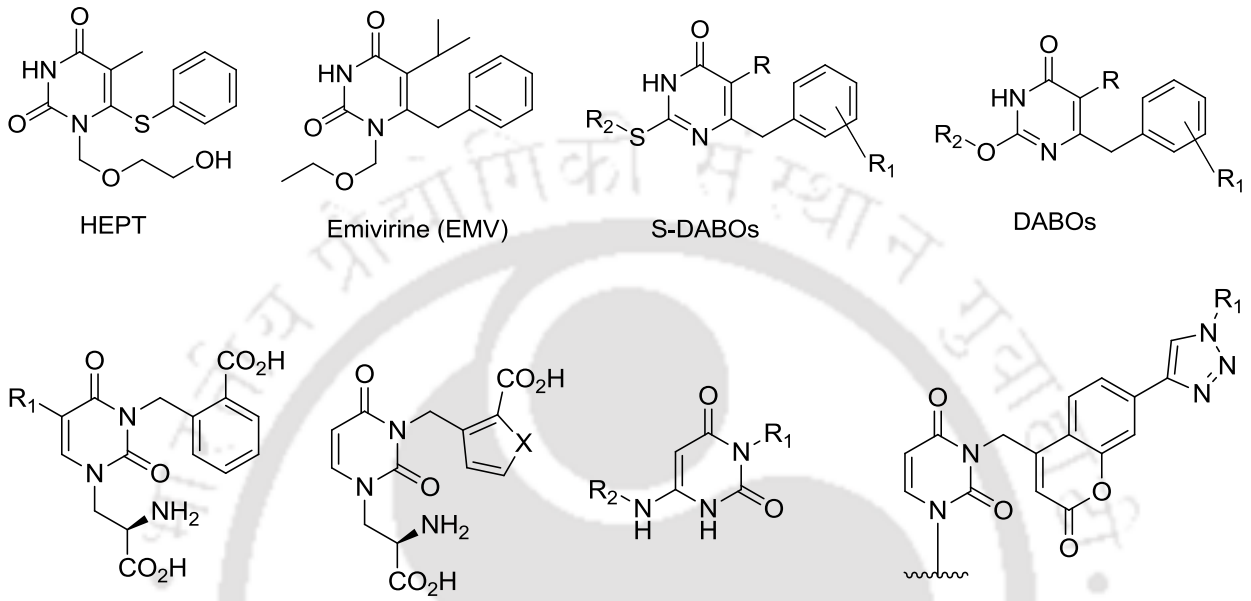


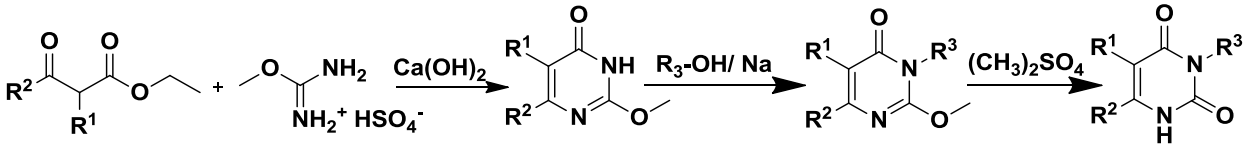
Figure III.2.1: *5,6-Disubstituted uracil derivatives shown potent drug activities*

Synthesis of modified nucleobases especially the family of uracils, thiouracil, thiocytosines and their N-1 or N-3 derivatives are very interesting because of their wide natural occurrence and high bioactivity.[27-29] Biological importance of such synthesized pyrimidinones is primarily attributed to their structural similarities to that of the natural nucleobases and coenzymes. This enables the modified pyrimidinones to act as inhibitors.

III.3. Existing methods

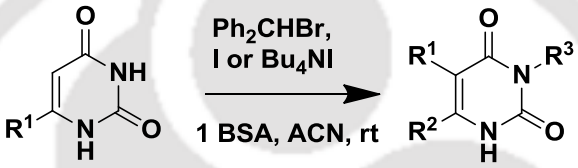
The conventional methods for synthesis of N-1 or N-3 protected pyrimidinones are usually performed through multistep protection and deprotection chemistry.[30] However, chemo- or regio-selective substitution at either N-1 or N-3 position of nucleobases is difficult to achieve because of poor reactivity difference between the two heteroatoms, especially, where C-5 and C-6 substitutions are present. As a result the reaction of the pyrimidinones with alkyl or aryl halides often leads to mixture of mono-substituted as well as di-substituted derivatives.[31-33]

Gambocotra *et al.* succeeded chemoselective alkylation of uracils by using HSAB in 1999 ***(Scheme III.3.1)***. [34]



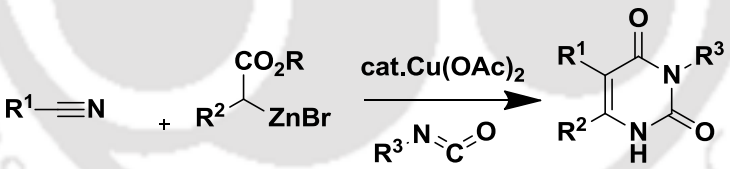
Scheme III.3.1

Weaver and co-workers have developed regio-selective N-3 protection of uracils by using tetrabutylammonium iodide as a catalyst in 2004 (***Scheme III.3.2).*** Later on Willis *et al.* have reported palladium catalyzed regioselective synthesis of N-alkylated uracil analogs. [35a-b]



Scheme III.3.2

Recently, Lee *et al.* have synthesized N-3 arylated derivatives using blaise reaction intermediate in 2013 ***(Scheme III.3.3).*** [38]



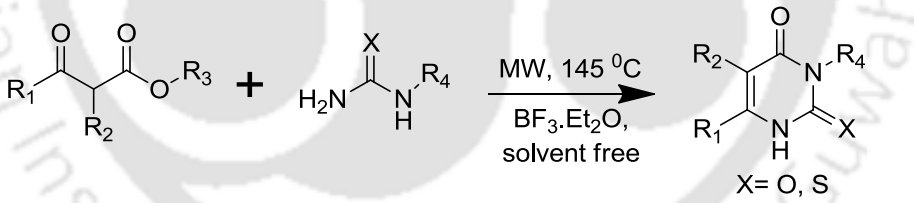
Scheme III.3.3

However, most of these conventional methods are post-synthetic modifications of the pyrimidinones and are multistep processes which required long reaction time, multiple reagents, catalysts and high temperature.[36, 37] Recently, advances in synthesis of aryl-substituted pyrimidindiones were achieved using transition metal catalyzed cross-couplings reaction of the halogenated pyrimidindiones via direct C-H activation.[41] Synthetic procedures of selective N-substituted pyrimidinones from readily available precursors are very limited.[39-41]

III.4. Present work

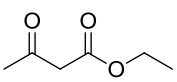
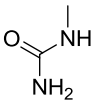
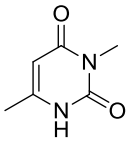
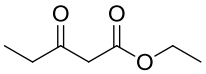
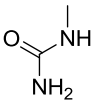
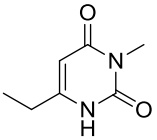
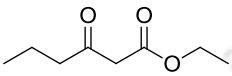
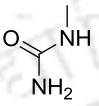
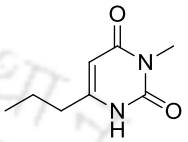
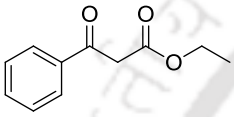
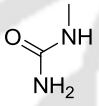
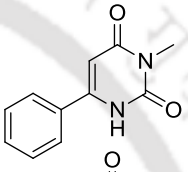
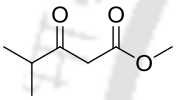
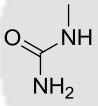
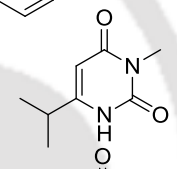
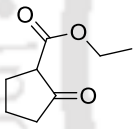
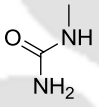
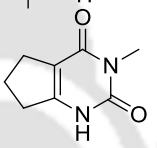
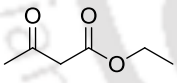
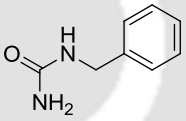
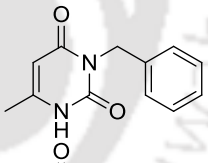
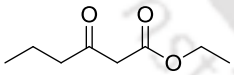
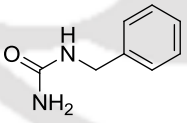
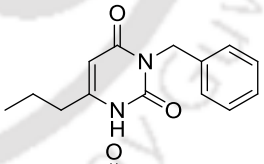
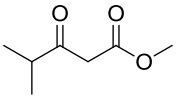
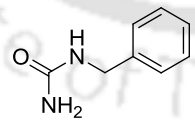
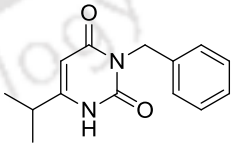
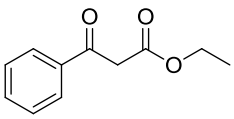
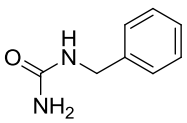
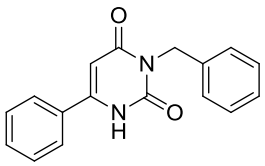
Alkylation or arylation of the pyrimidinones invariably yields mixture of N-1 and N-3 substitution. Although N-1 nitrogen is significantly more reactive compared to N-3, the reactivity was found to be drastically reduced when C-6 substitution is present. This is attributed to steric hindrance offered by the substituent at the C-6 position of the pyrimidinones. As a result selective substitution at N-1 is difficult to achieve, especially for C-6 modified pyrimidine nucleobases. On the other hand, selective substitution at N-3 position is extremely difficult without prior protection of the N-1 position.[42]

We have successfully developed a single-step, chemoselective methodology for *de novo* synthesis of N-3 substituted pyrimidinones from their β-carbonylester precursors, using substituted urea and microwave-assisted method. In this chapter we reported the synthesis of alkyl, benzyl and aryl substituted pyrimidinone nucleobases selectively at N-3 position ***(Scheme III.4.1).*** The methodology was further extended for synthesis of a variety of C-5, C-6 and N-3 substituted thiouracil. All reactions were carried out without the requirement of any solvent and were completed in a very short period of time. The reaction yields were further improved by using BF_3.ether as Lewis acid.



Scheme III.4.1: *schematic representation of the chemoselective N-3 substituted uracil and thiouracil pyrimidines.*

We have utilized a chemoselective approach to synthesize the N-3 protected pyrimidinones directly from their β-carbonylester precursors. ***Scheme III.4.1*** demonstrates a one-pot, microwave-directed approach for N-3 protection of the pyrimidines. This is a relatively greener protocol as no solvent was required and all reactions were complete within few minutes of irradiation, avoiding long hours of reflux. All the experimental conditions are listed in ***Table III.4.1***

S.No.	Substrate 1a-10a	Urea	Microwave irradiation(min)	%Yield	Product 1-10	%Yield with $BF_3.Et_2O$(min)
1			12	76		85 (8)
2			12	68		80 (8)
3			12	67		78 (8)
4			12	78		90 (8)
5			12	66		75 (8)
6			12	65		73 (8)
7			12	61		72 (8)
8			12	60		70 (8)
9			12	56		65 (8)
10			15	58		70 (8)

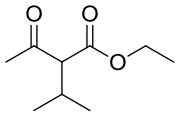
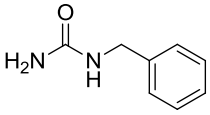
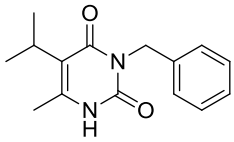
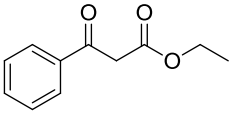
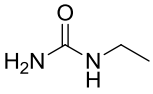
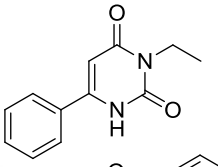
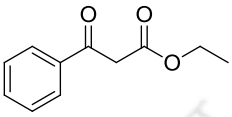
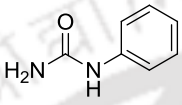
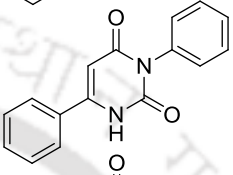
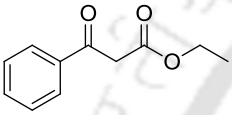
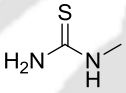
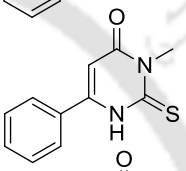
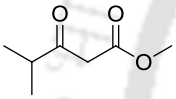
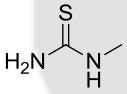
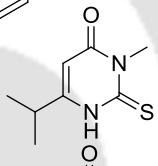
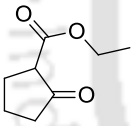
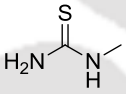
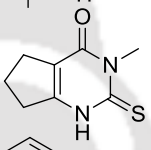
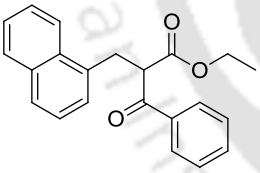
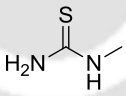
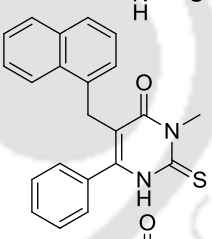
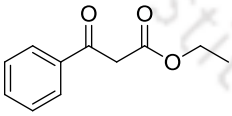
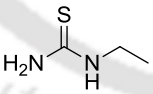
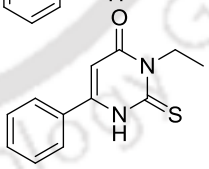
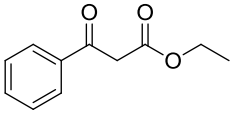
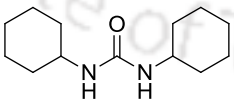
S.No.	Substrate 11a-19a	Urea	Microwave irradiation(min)	%Yield	Product 11-19	%Yield with $BF_3.Et_2O$ (min)
11			15	32		40(10)
12			15	55		64(10)
13			15	25		40(10)
14			12	72		85(8)
15			12	65		78(8)
16			12	64		75(8)
17			15	38		59(10)
18			15	48		62(10)
19			15		No reaction	

Table III.4.1: *Synthesis of chemoselective N-3 substituted uracil and thiouracil derivatives (1-19) in a solvent free, single step reaction using microwave-assisted method*

Reaction occurs when the substrate β-carbonylester reacts with the urea derivative at elevated temperature in a closed vessel *CEM Discover LabMate* microwave reactor for short period of irradiation. As can be evident, a library of compounds (**1-19**) with varying substitution at C-5 and C-6 of uracil and thiouracils were synthesized. A number of urea derivates have been used to show diversity of the method. Although the reactions could proceed moderately well without presence of additional reagents, use of Lewis acid, such as BF_3 in ether, was found to enhance the yields to a significant extent. The substrate β-ketoesters and the β-aldehydoesters were either purchased or were synthesized following published protocols. All the compounds **(1-19)** were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. We also have obtained three crystal structures of compound **3**, **4** and **15**, which confirm that isolated compounds are indeed N-3 derivative ***(Figure III.4.1)*** shown in ***III.8.***

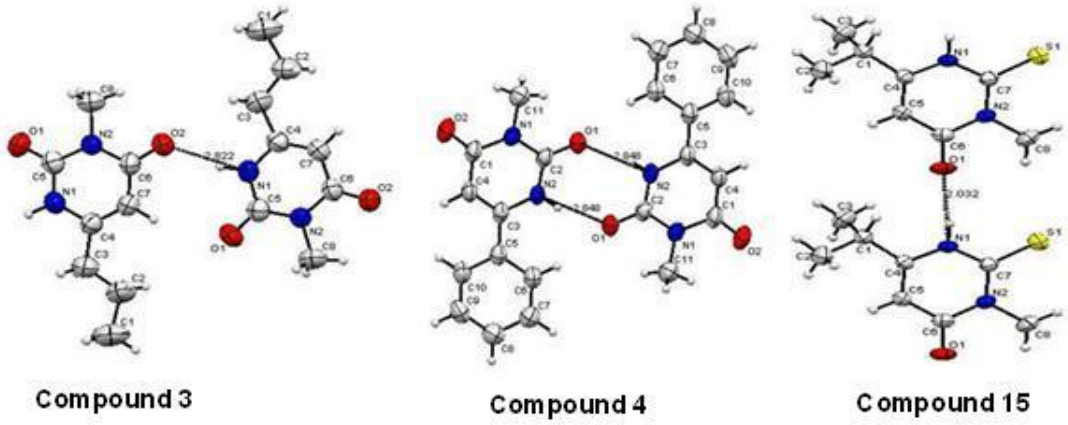


Figure III.4.1*: ORTEP diagram of compound* ***3, 4*** *and* ***15,*** *respectively.*

We have performed a number of NOESY analyses to authenticate that the selective alkylation and benzylation were taking place indeed at N-3 and not at N-1 of the pyrimidines (***Figure III.4.2 and III.8***). Compounds having C-6 substitution clearly shows proton-proton interaction with the free N-1 proton.

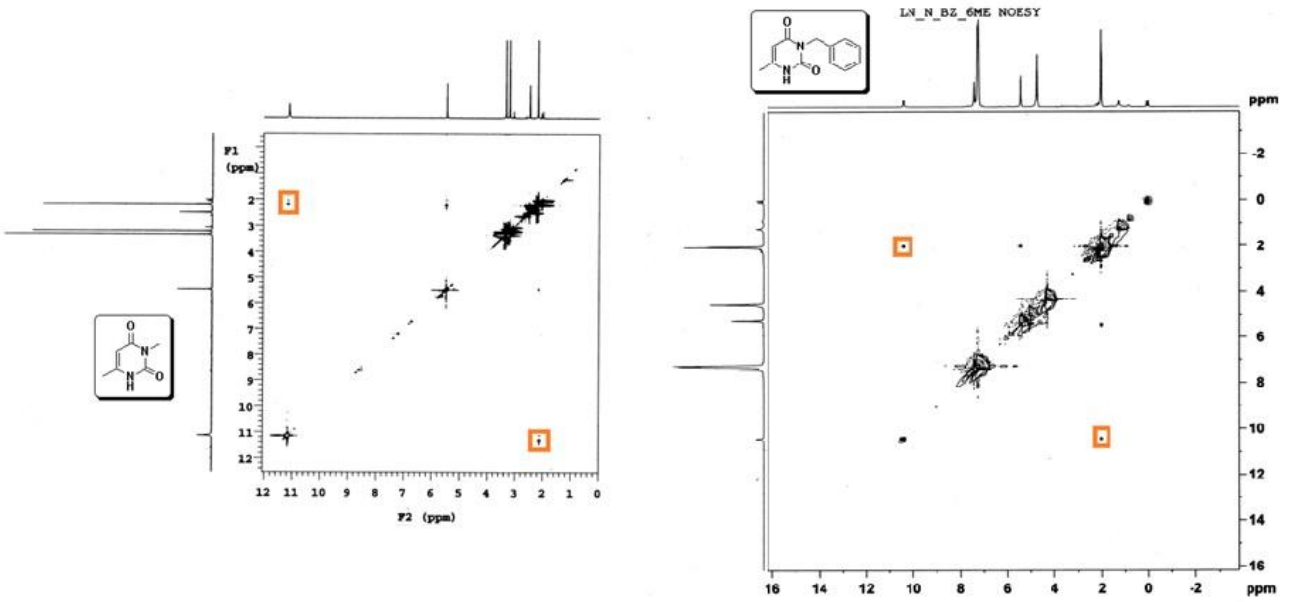


***Figure III.4.2**: Representative NOESY spectra of compound **1** and **7,** respectively.*

Mechanism of the reaction:

The proposed mechanism for chemoselective reaction is shown in ***Figure III.4.3***. In **chapter II** we have established that the activated β-carbonyl carbon first reacts with urea followed by dehydration, forming the first intermediate.[43] This intermediate was trapped and characterized. In the present case, where unsymmetrical urea is used, preferentially the free $-NH_2$ group of the urea reacts with the β-carbonyl carbon, compared to that of the secondary amine. This is presumably to avoid a large amount of steric strain that would be created from reaction of the substituted nitrogen (-NHR) of urea and R^1 of the β-carbonylester, making the intermediate highly unfavorable. A similar mechanism had been reported by *Kappe* for a 3-component Biginelli reaction.[44]

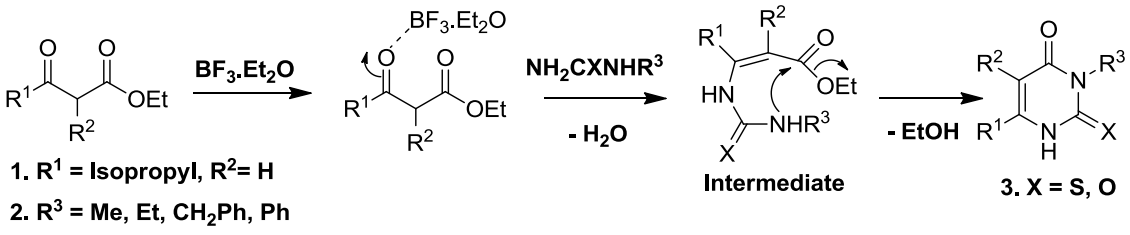


Figure III.4.3: *Proposed mechanism of formation of selective N-3 protected nucleobases, using Lewis acid.*

In conclusions, we have successfully demonstrated a robust chemoselective method for direct synthesis of N-3 alkylated and arylated pyrimidinones with varying substitution at C-5 and C-6 position. The method involves short microwave irradiation of the β-carbonylester precursor and urea derivative in a solvent-free reaction. Such selective N-3 derivatization otherwise require multistep reactions in harsh condition. Addition of Lewis acid was found to accelerate the reactions significantly.

III.5. Experimental section

III.5.1. General Information: All chemicals were purchased from reputed pharmaceuticals and were used without further purification. All microwave-directed reactions were carried out in a closed vessel *CEM Discover LabMate* microwave reactor at about 145°C for variable durations. The temperature of the reaction mixtures were all measured by an internal built-in IR sensor. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were all recorded from a *DRX-400 Varian spectrometer* using $CDCl_3$ and DMSO-D_6 as solvents. Chemical shifts are reported in parts per million (ppm). Melting points were determined using *Büchi B-545* apparatus and are uncorrected. High resolution mass spectrometry was analyzed from *Agilent Q-TOF 6500 LC/MS* system and *Micromass Q-TOF ESI-MS* instrument (model HAB 273). X-Ray data were collected from a *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structures were solved by direct method using *SHELLX-97* (Göttingen, Germany). The melting points, characterization and relevant literature of the reported compounds is given in the characterization data.

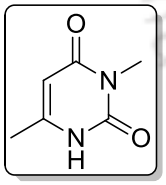
III.5.2. General Procedure (compound 1-19): A β-ketoester (2 mmol), taken in a reactor vessel was mixed thoroughly for 1 min with urea derivative (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at about 145°C. Reactions were also performed at 130°C and 140°C, however, best results were obtained at 145°C. The compound (**1-18**) was further purified by column chromatography (50-65% ethyl acetate in hexane). The time of irradiation and observed yield of the compounds are listed in ***Table III.4.1***.

Synthesis using $BF_3.Et_2O$ (1-19): A β-ketoester (2 mmol), taken in a reactor vessel with $BF_3.Et_2O$ (339 mg, 2.4 mmol) was mixed thoroughly for 1 min with urea derivatives (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at 145°C. The compound (**1-18**) was further purified by column chromatography. The time of irradiation and observed yield of the compounds are listed in ***Table III.4.1***.

III.5.3. Synthesis of 3-methyl-6-phenylpyrimidine-2,4(1H,3H)-dione (4): In a 10 ml microwave reaction vial containing a 3 mm magnetic stirring bar, 384 mg (2 mmol) of Ethyl 3-oxo-3-phenylpropanoate was taken along with methyl urea (193 mg, 2.6 mmol) and $BF_3.Et_2O$ (339 mg, 2.4 mmol). The vial was sealed immediately with a septum and allowed to mix for 1 min. The closed vessel was irradiated at 145°C for 8 min. Completion of the reaction was tested by Thin Layer Chromatography (50% ethyl acetate in hexane) and finally the compound **4** was purified by column chromatography (60% ethyl acetate in hexane). Isolated yield: 90%.

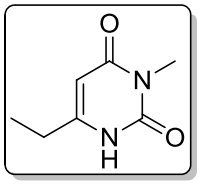
III.6. Characterization data

3,6-dimethylpyrimidine-2,4(1H,3H)-dione (1)



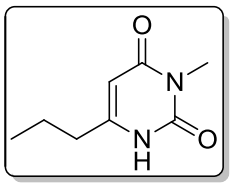
Yield: 85%, white solid, m.p: 260-265°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.13 (s, 1H), 5.47 (s, 1H), 3.22 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.8, 153.6, 152.3, 99.6, 27.8, 19.1. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_6H_8N_2O_2$): 141.0659; observed: 141.0659.

6-ethyl-3-methylpyrimidine-2,4(1H,3H)-dione (2)



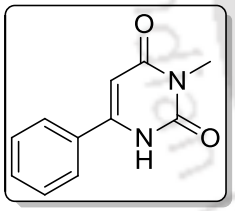
Yield: 80%, white solid, m.p: 240-243°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 5.44 (s, 1H), 3.08 (s, 3H), 2.33 (t, 2H, *J*= 7.8 Hz), 1.14 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.3, 152.5, 150.9, 97.2, 27.9, 25.5, 12.1. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_7H_{10}N_2O_2$): 155.0815; observed: 155.0811.

3-methyl-6-propylpyrimidine-2,4(1H,3H)-dione (3)



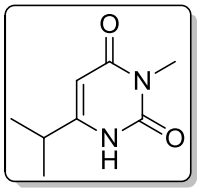
Yield: 78%, white solid, m.p: 245-248°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.07 (s, 1H), 5.45 (s, 1H), 3.08 (s, 3H), 2.28 (t, 2H, *J*= 7.2 Hz), 1.58-1.54 (m, 2H), 0.89 (t, 3H, *J*= 7.6 Hz).). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.0, 156.4, 153.0, 98.5, 31.1, 27.5, 21.3, 14.2. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_8H_{12}N_2O_2$): 169.0972; observed: 169.0969.

3-methyl-6-phenylpyrimidine-2,4(1H,3H)-dione (4)



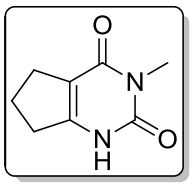
Yield: 90%, white solid, m.p: 230-232°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 7.74 (d, 2H, *J*= 6.8 Hz), 7.56-7.49 (m, 3H), 5.96 (s, 1H), 3.17 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.3, 152.6, 151.7, 132.0, 129.6, 128.4, 127.5, 97.5, 27.3. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{11}H_{10}N_2O_2$): 203.0815; observed: 203.0817.

6-isopropyl-3-methylpyrimidine-2,4(1H,3H)-dione (5)



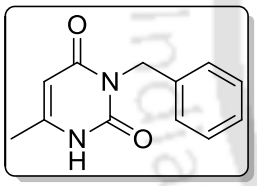
Yield: 75%, white solid, m.p: 235-238°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H), 5.53 (s, 1H), 3.24 (s, 3H), 2.59-2.55 (m, 1H), 1.19 (d, 2H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2 159.3, 153.9, 97.2, 31.9, 27.1, 20.4. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_8H_{12}N_2O_2$): 169.0972; observed: 169.0978.

6,7-dihydro-3-methyl-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione (6)



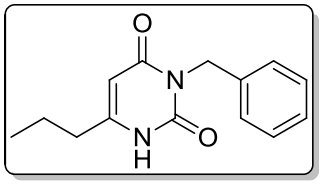
Yield: 73%, white solid, m.p: 225-228°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (s, 1H), 3.09 (s, 3H), 2.67 (t, 2H, *J*= 7.2 Hz), 2.50-2.47 (m, 2H), 1.97 (t, 2H, *J*= 7.2 Hz). HRMS (ESI) m/z $[M+H]^+$ calculated ($C_8H_{10}N_2O_2$): 167.0815; observed: 167.0814.

3-benzyl-6-methylpyrimidine-2,4(1H,3H)-dione (7)



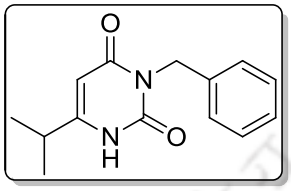
Yield: 72%, white solid, m.p: 194-198°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 7.40 (d, 2H, *J*= 6.8 Hz), 7.27-7.22 (m, 3H), 5.50 (s, 1H), 5.03 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5, 158.6, 152.0, 137.0, 128.2, 128.1, 127.1, 99.1, 42.9, 18.4. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{12}H_{12}N_2O_2$): 217.0972; observed: 217.0973.

3-benzyl-6-propylpyrimidine-2,4(1H,3H)-dione (8)



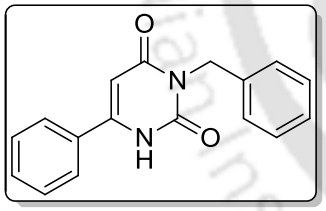
Yield: 70%, white solid, m.p: 200-202°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H), 7.42 (d, 2H, *J*= 6.4 Hz), 7.28-7.23 (m, 3H), 5.55 (s, 1H), 5.05 (s, 3H) 2.31 (t, 2H, *J*= 6.4 Hz), 1.66-1.60 (m, 2H), 0.98 (t, 3H, *J*= 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 158.1, 154.3, 139.6, 127.6, 126.6, 126.1, 97.5, 42.5, 34.4, 19.8, 12.7. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{14}H_{17}N_2O_2$): 245.1285; observed: 245.1286.

3-benzyl-6-isopropylpyrimidine-2,4(1H,3H)-dione (9)



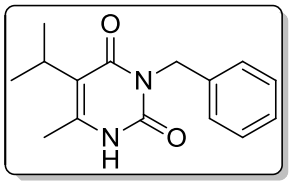
Yield: 65%, white solid, m.p: 215-220°C, NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H), 7.45 (d, 2H, *J*= 6.8 Hz), 7.30-7.26 (m, 3H), 5.59 (s, 1H), 5.06 (s, 2H) 2.59-2.55 (m, 1H), 1.23 (d, 6H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 159.6, 153.8, 136.9, 129.1, 128.4, 127.3. 97.5, 44.6, 31.9, 20.3. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{14}H_{17}N_2O_2$): 245.1285; observed: 245.1292.

3-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione (10)



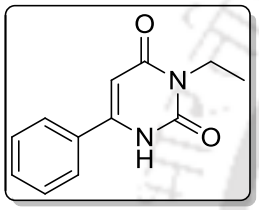
Yield: 70%, white solid, m.p: 195-198°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.62 (d, 2H, *J*= 8.0 Hz), 7.42-7.35 (m, 8H), 6.01 (s, 1H), 5.12 (s, 2H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 155.7, 153.3, 139.3, 138.5, 128.7, 128.6, 128.5, 127.4, 127.3, 126.2, 98.9, 44.5. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{17}H_{14}N_2O_2$): 279.1128; observed: 279.1128.

3-benzyl-5-isopropyl-6-methylpyrimidine-2,4(1H,3H)-dione (11)



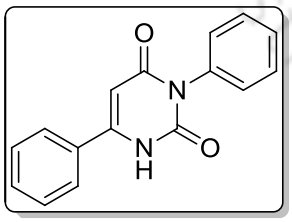
Yield: 40%, white solid, m.p: 180-183°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 7.27-7.20 (m, 5H), 4.96 (s, 2H), 2.45 (m, 1H), 1.15 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 159.4, 153.8, 139.2, 137.0, 129.1, 128.8, 127.5, 107.6, 43.7, 32.0, 20.4. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{15}H_{19}N_2O_2$): 259.1441; observed: 259.1444.

3-ethyl-6-phenylpyrimidine-2,4(1H,3H)-dione (12)



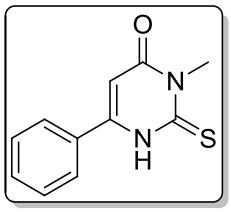
Yield: 64%, white solid, m.p: 220-223°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.62-7.45 (m, 5H), 5.84 (s, 1H), 3.24 (q, 2H, *J*= 6.0 Hz), 1.15 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 152.8, 150.5, 136.2, 131.4, 126.5, 125.6, 98.7, 35.6, 14.5. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{12}H_{13}N_2O_2$): 217.0972; observed: 217.0971.

3*,6-diphenylpyrimidine-2,4(1H,3H)-dione (13)*



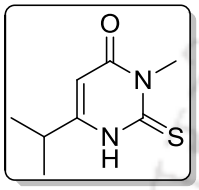
Yield: 40%, white solid, m.p: 286-290°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H), 7.82 (d, 2H, *J*= 8.0 Hz), 7.82-7.45 (m, 8H), 6.01 (s, 1H).

2,3-dihydro-3-methyl-6-phenyl-2-thioxopyrimidin-4(1H)-one (14)



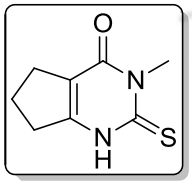
Yield: 85%, white solid, m.p: 240-245°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (s, 1H), 7.53 (d, 2H, *J*= 7.2 Hz), 7.40-7.33 (m, 3H), 5.91 (s, 1H), 3.54 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 159.7, 157.6, 133.5, 130.7, 129.4, 127.7, 108.4, 41.0

6-isopropyl-3-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one(15)



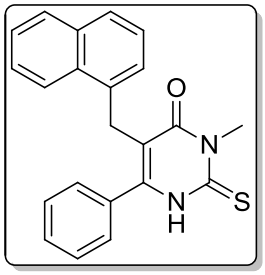
Yield: 78%, white solid, m.p: 260-263°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (s, 1H), 5.95 (s, 1H), 4.82 (s, 3H), 3.86 (m, 1H), 1.24 (d, 6H, *J*= 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 164.0, 103.7, 37.3, 31.0, 21.0

3-methyl-2-thioxo-2,3,6,7-tetrahydro-1H-cyclopenta[d]pyrimidin-4(5H)-one(16)



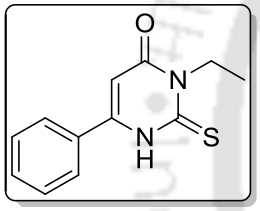
Yield: 75%, white solid, m.p: 296-300°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (s, 1H), 3.35 (s, 1H), 2.74 (t, *J*= 7.2 Hz), 2.55 (m, 2H), 1.98 (t, *J*= 7.2 Hz).

3-methyl-5-(naphthalen-1-ylmethyl)-6-phenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one(17)



Yield: 59%, white solid, m.p: 305-308°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.86-7.68 (m, 4H), 7.40-7.25 (m, 6H), 6.92-6.85 (m, 2H), 3.90 (s, 3H), 3.79 (s, 2H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 160.5, 155.0, 133.8, 132.3, 131.7, 131.1, 130.2, 129.6, 129.3, 128.8, 128.7, 127.6, 127.4, 125.4, 124.6, 124.4, 117.7, 41.2, 29.9. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{22}H_{19}N_2OS$): 359.1213; observed: 359.1213.

3-ethyl-2,3-dihydro-6-phenyl-2-thioxopyrimidin-4(1H)-one (18)



Yield: 62%, white solid, m.p: 206-208°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 7.82 (d, 2H, *J*= 7.4 Hz), 7.58 (t, 2H, *J*= 7.6 Hz), 5.97 (s, 1H), 3.35 (q, 2H, *J*= 6.8 Hz), 1.21 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.2, 161.5, 149.5, 139.4, 128.0, 127.6, 126.9, 113.8, 43.0, 16.0. HRMS (ESI) m/z $[M+H]^+$ calculated ($C_{12}H_{13}N_2OS$): 233.0743; observed: 233.0749.

III.7. References

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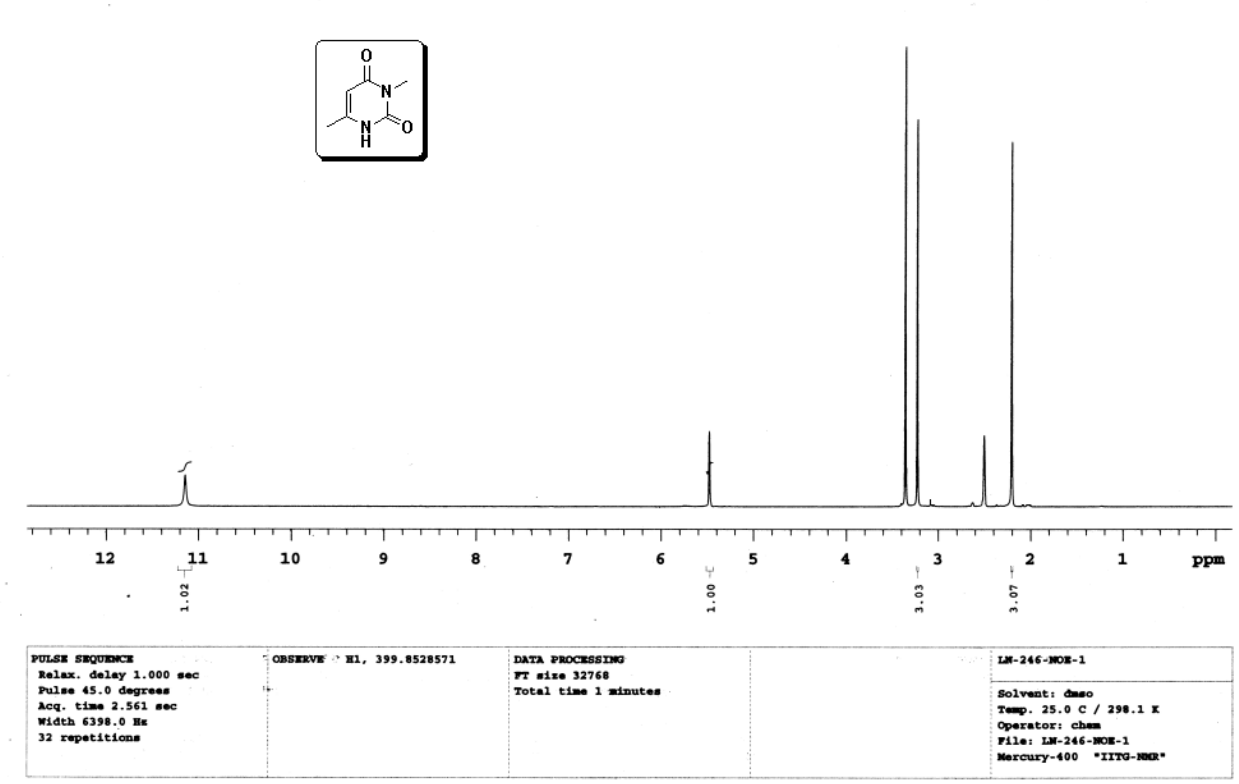
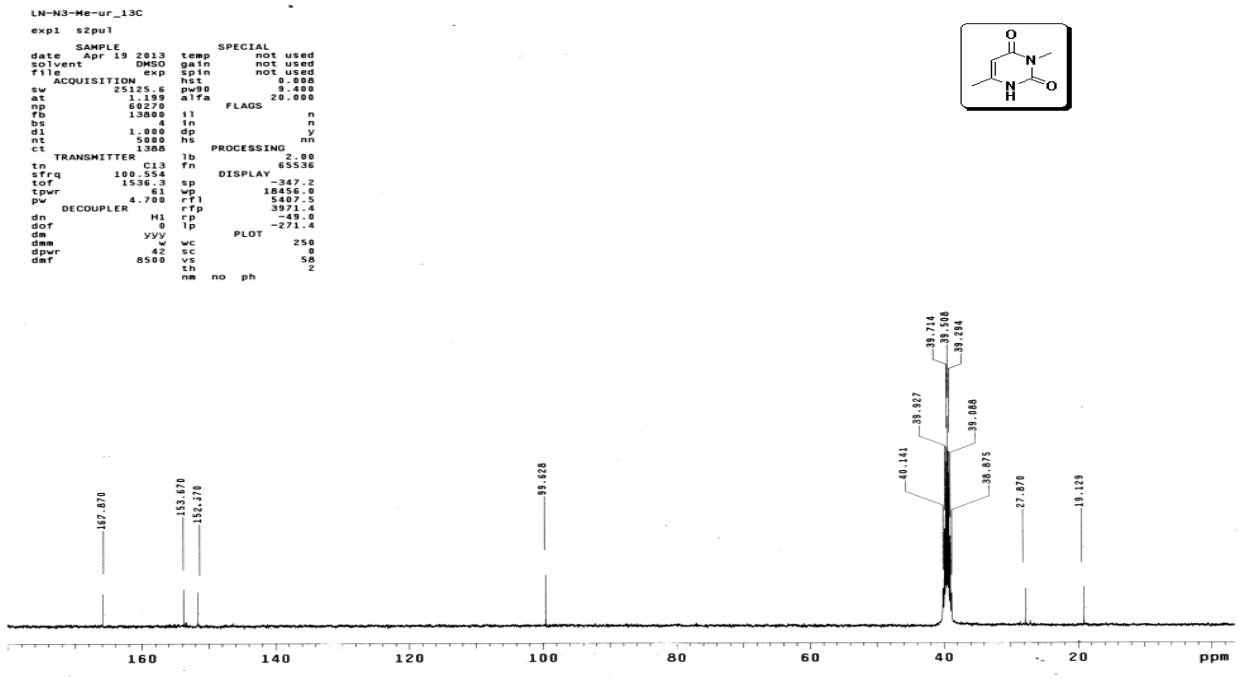
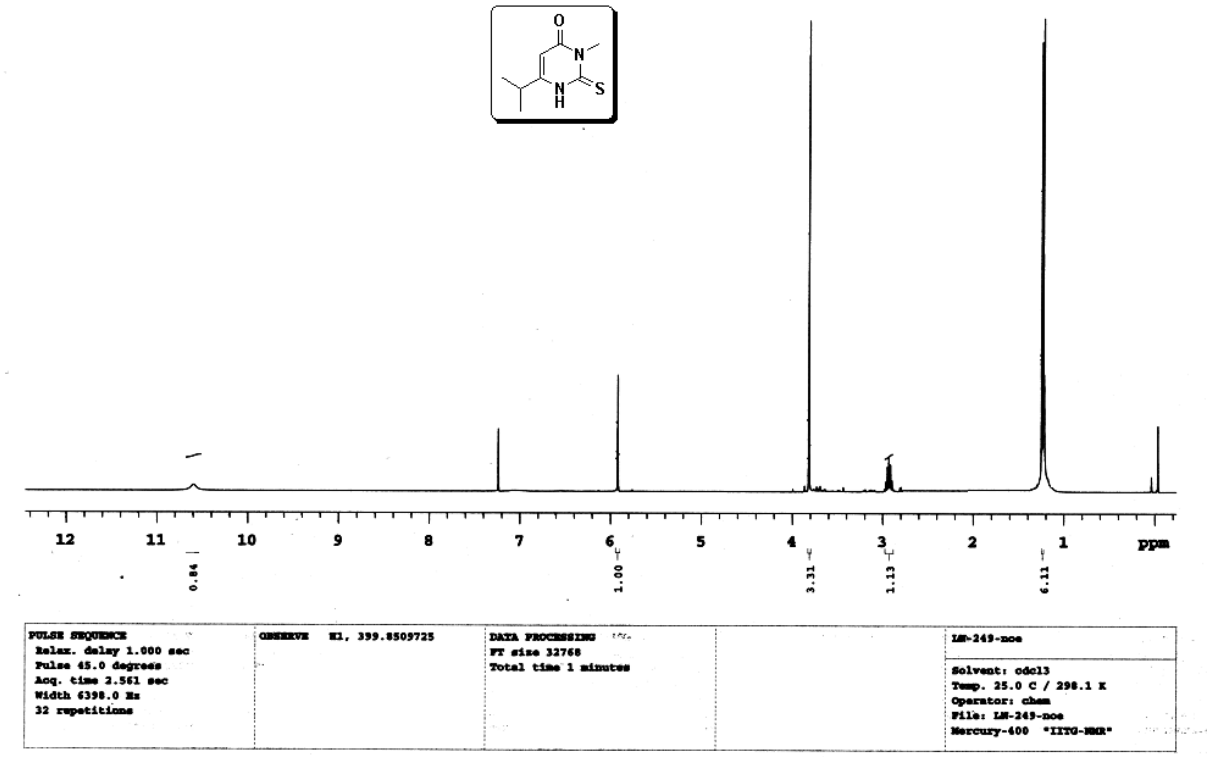
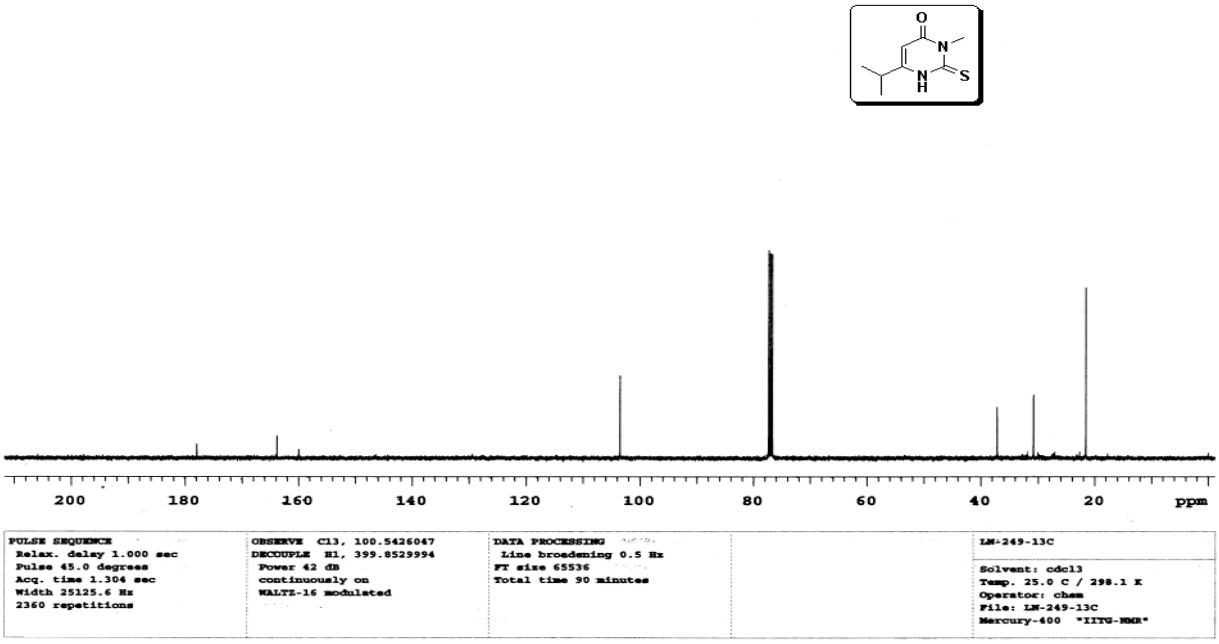
III.8. Selected spectra









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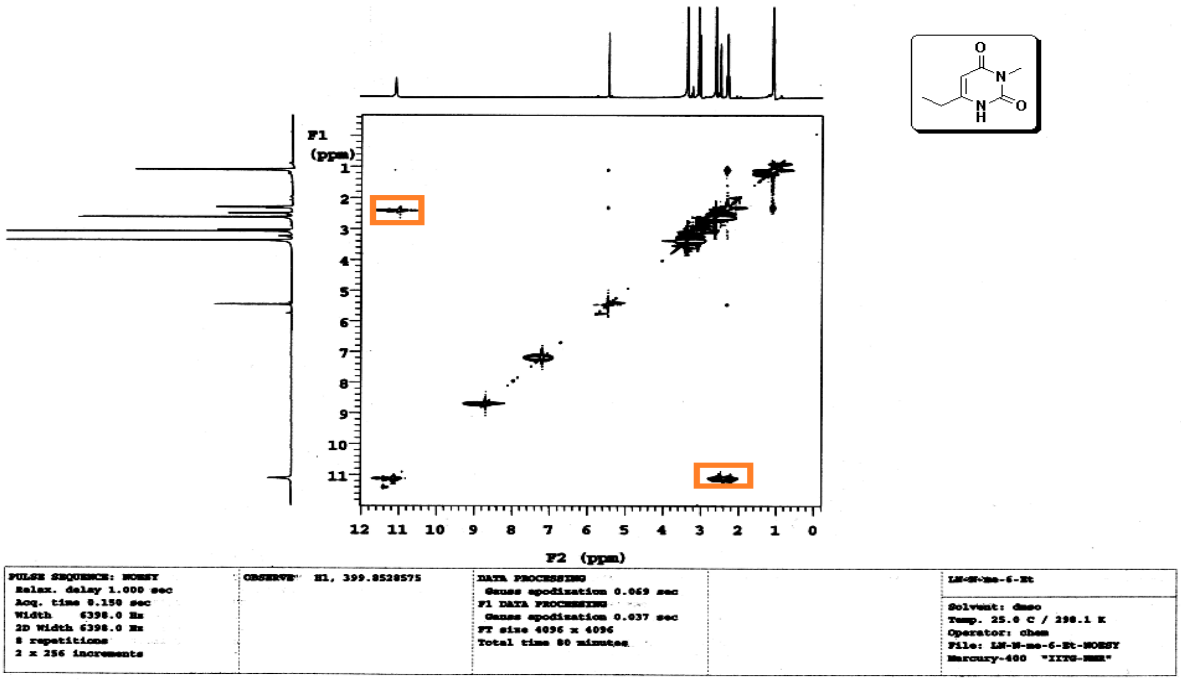
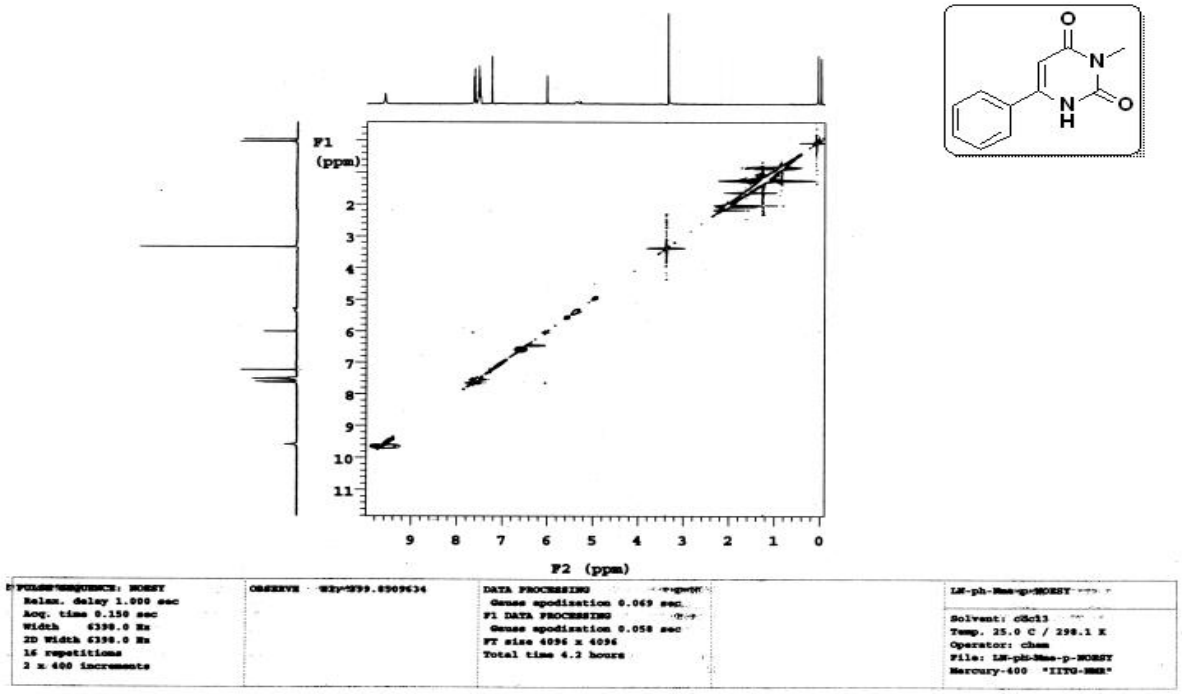
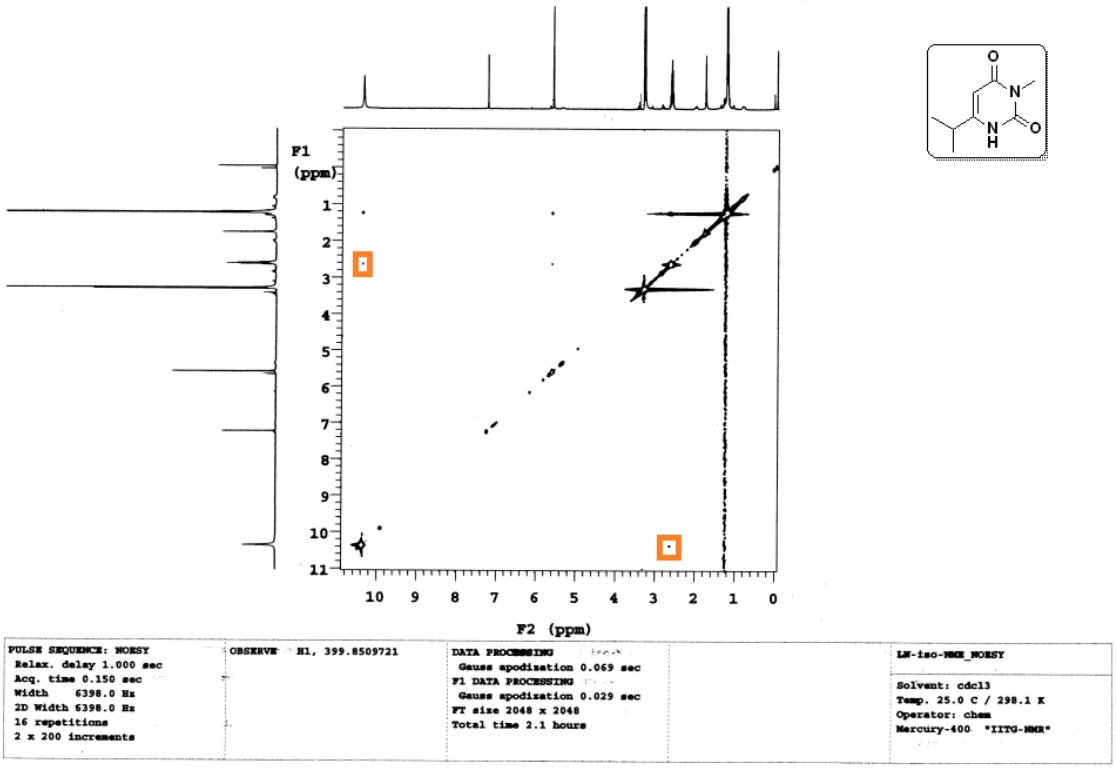


Compound (4):



Compound (5):



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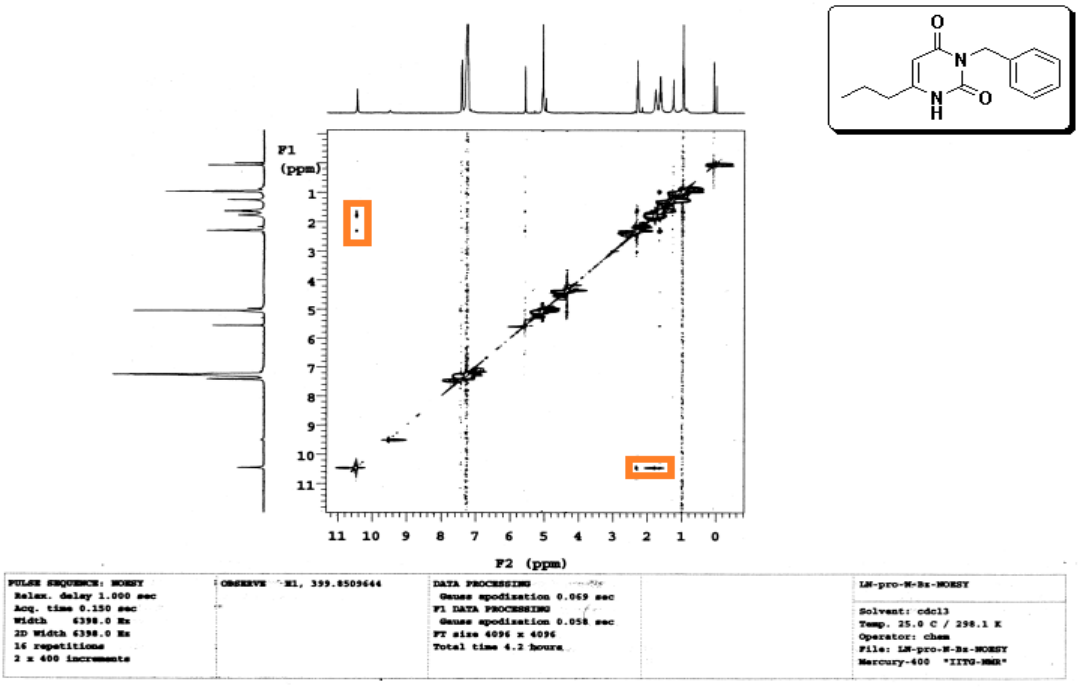
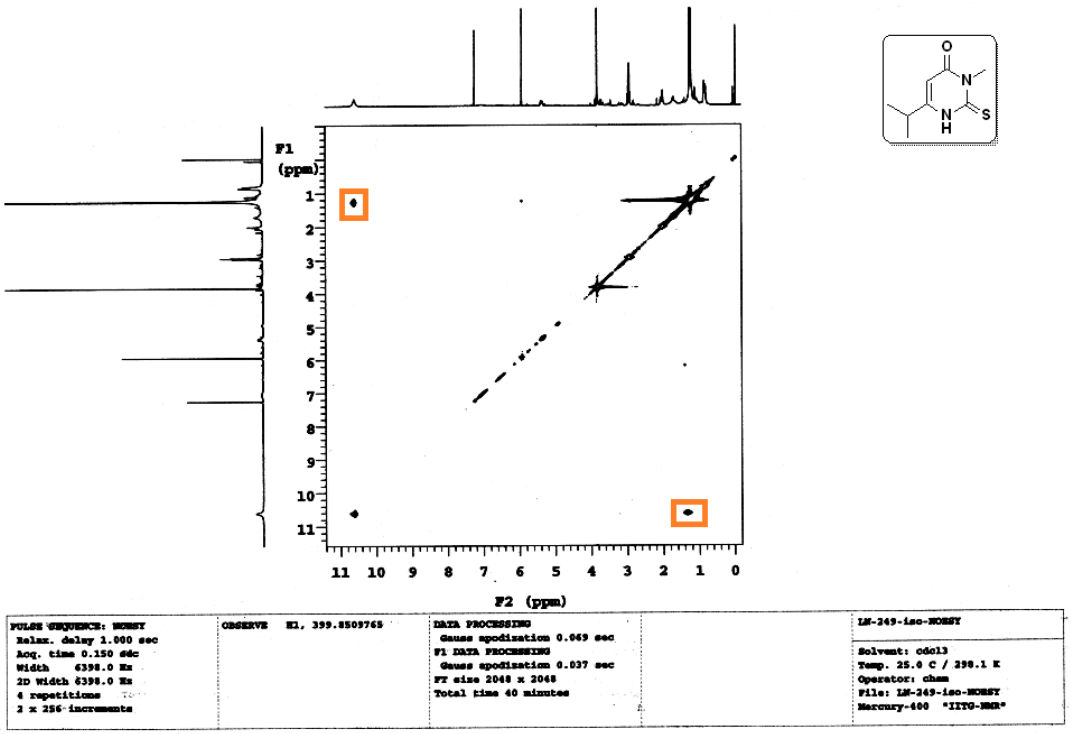


Compound (15):



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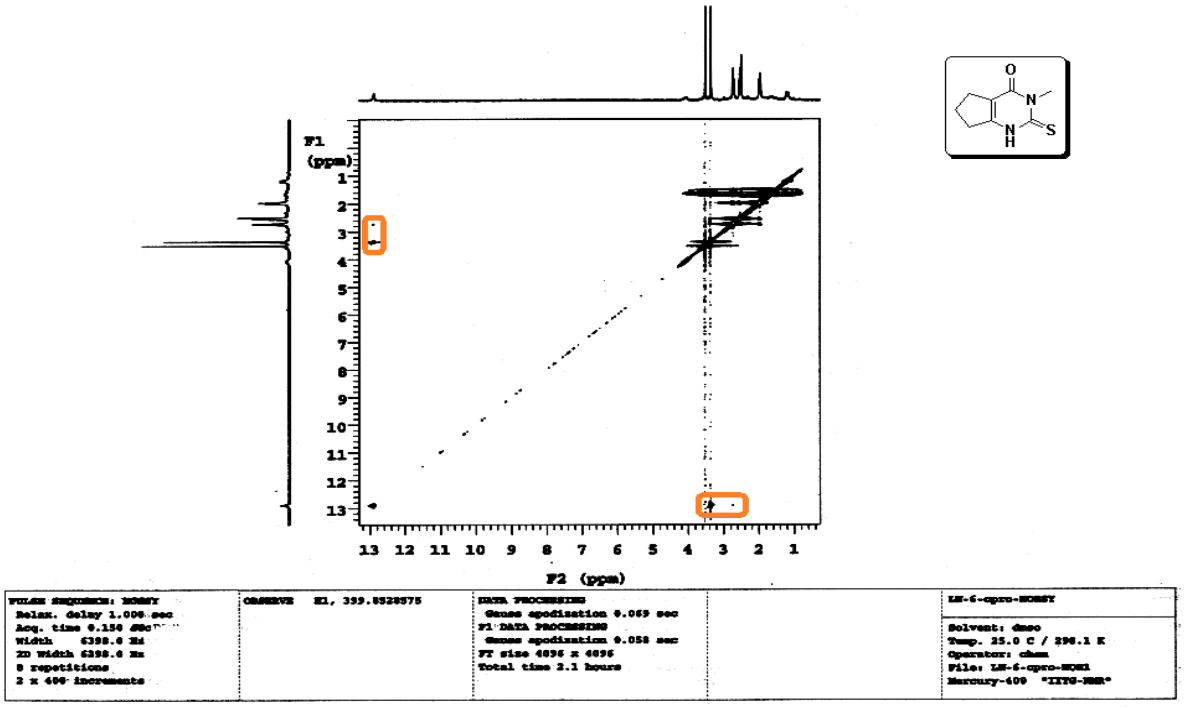


3-methyl-6-propylpyrimidine-2,4(1H,3H)-dione (3):

The crystal structure of **compond 3** was obtained from DMSO-d6 solution

Table 2: Crystallographic data of **compond 3**	CCDC# 991094
Chemical formula	C8 H12 N2 O2
Formula Mass	168.20
Temperature/K	296 K
Crystal system	Monoclinic
Space group	P21/c
a/Å	4.7152(8)
b/Å	21.823(3)
c/Å	8.8290(15)
α/°	90
β/°	94.553(11)
γ/°	90
Unit cell volume/Å	905.6(3)
Z	4
μ (mm-1)	0.090
*ρ*calcd (g cm-3)	1.234
No. of reflections measured	1627
No. of independent reflections	904

Final R1 values (I > 2σ(I))	0.0553
Final wR(F2) values (I > 2σ(I))	0.1193
Final R1 values (all data)	0.1520
Final wR(F2) values (all data)	0.1299
Goodness of fit (F^2)	0.922

3-methyl-6-phenylpyrimidine-2,4(1H,3H)-dione (4):

The crystal structure of **compound 4** was obtained from methanol/ethanol solution

Table 2: Crystallographic data of **compound 4**	CCDC# 991093
Chemical formula	C11 H10 N2 O2
Formula Mass	202.21
Temperature/K	296 K
Crystal system	Monoclinic
Space group	P21/n
a/Å	5.8924(19)
b/Å	21.161(6)
c/Å	8.054(3)
α/°	90
β/°	103.67(2)
γ/°	90

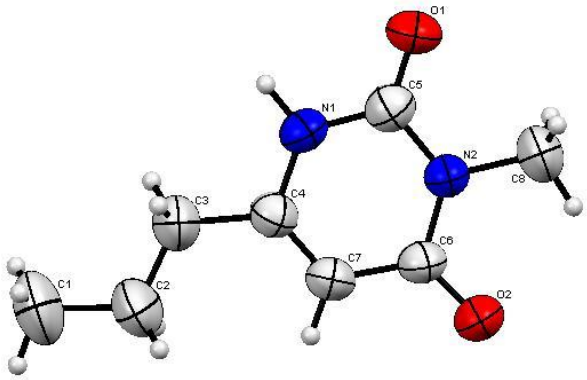
Unit cell volume/Å	975.8(5)
Z	4
μ (mm-1)	0.097
*ρ*calcd (g cm-3)	1.376
No. of reflections measured	1732
No. of independent reflections	1271
Final R1 values (I > 2σ(I))	0.0462
Final wR(F2) values (I > 2σ(I))	0.0952
Final R1 values (all data)	0.0621
Final wR(F2) values (all data)	0.1032
Goodness of fit (F^2)	0.968

6-isopropyl-3-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one(15):

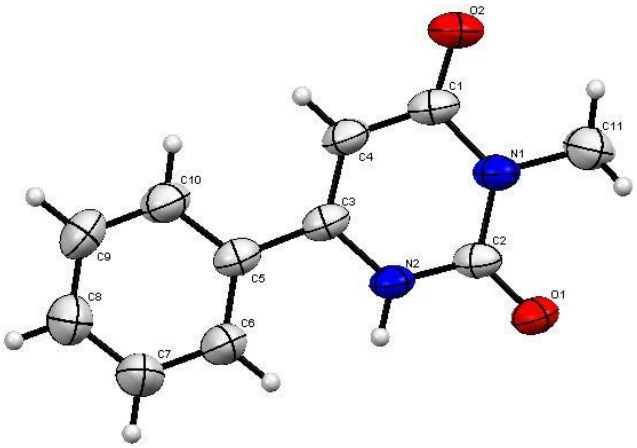
The crystal structure of **compound 15** was obtained from methanol/ethyl acetate solution

Table 2: Crystallographic data of **compound 15**	CCDC# 991092
Chemical formula	C8 H12 N2 O S
Formula Mass	184.26
Temperature/K	296 K
Crystal system	Monoclinic
Space group	C2/c

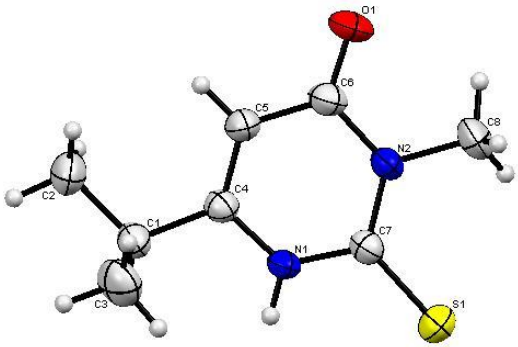
a/Å	21.5926(10)
b/Å	6.8375(3)
c/Å	14.9348(8)
α/°	90
β/°	122.333(4)
γ/°	90
Unit cell volume/Å	1863.09(16)
Z	8
μ (mm-1)	0.302
*ρ*calcd (g cm-3)	1.314
No. of reflections measured	1680
No. of independent reflections	1293
Final R1 values (I > 2σ(I))	0.0343
Final wR(F2) values (I > 2σ(I))	0.0739
Final R1 values (all data)	0.0424
Final wR(F2) values (all data)	0.0766
Goodness of fit (F^2)	1.083



ORTEP diagram of **compond 3:** The ellipsoid countour probablity level is 50%

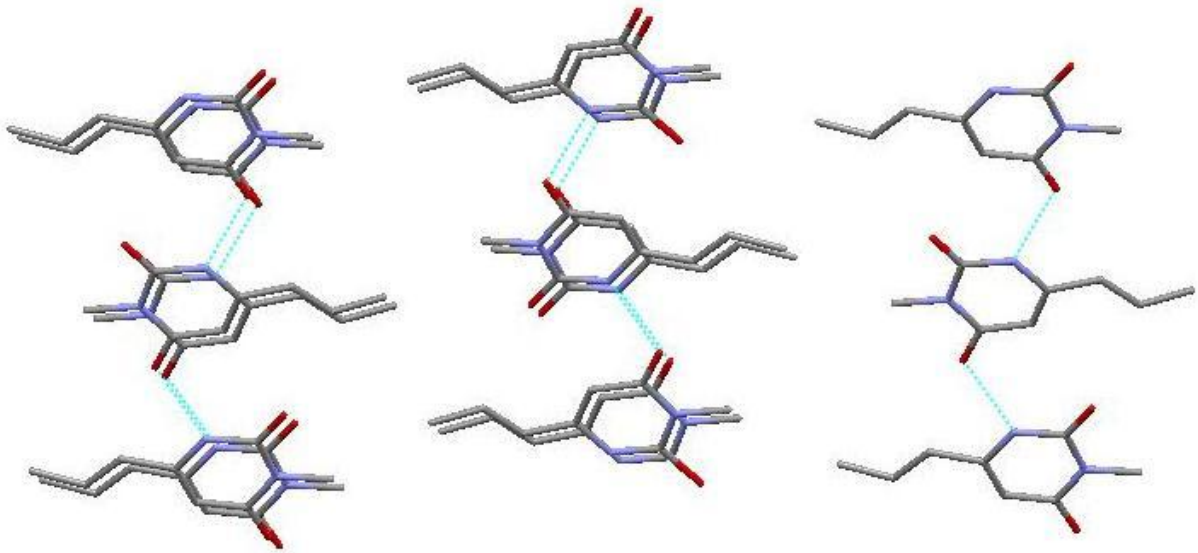


ORTEP diagram of **compound 4:** The ellipsoid countour probablity level is 50%

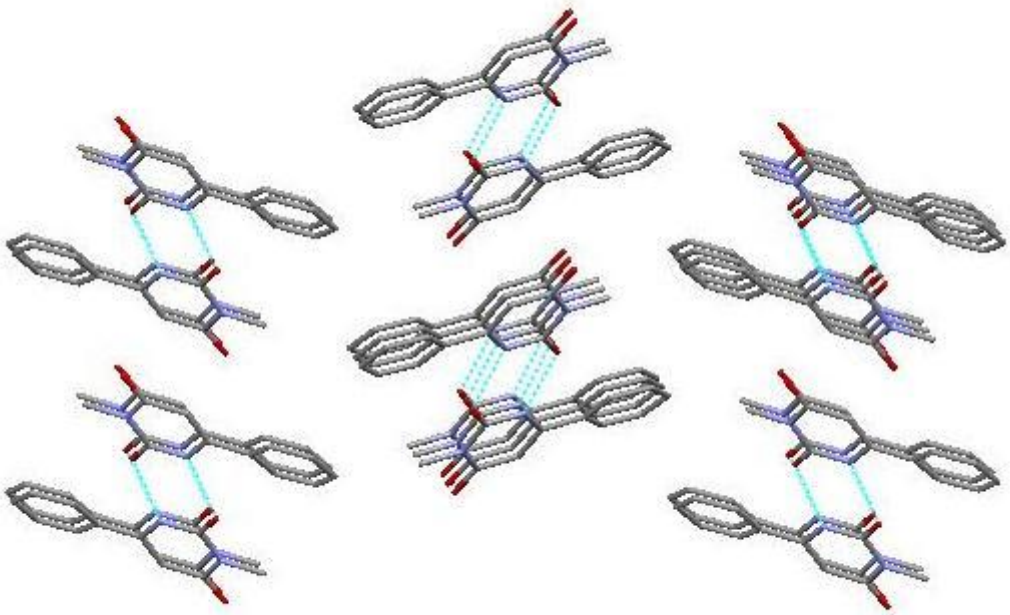


ORTEP diagram of **compound 15:** The ellipsoid countour probablity level is 50%

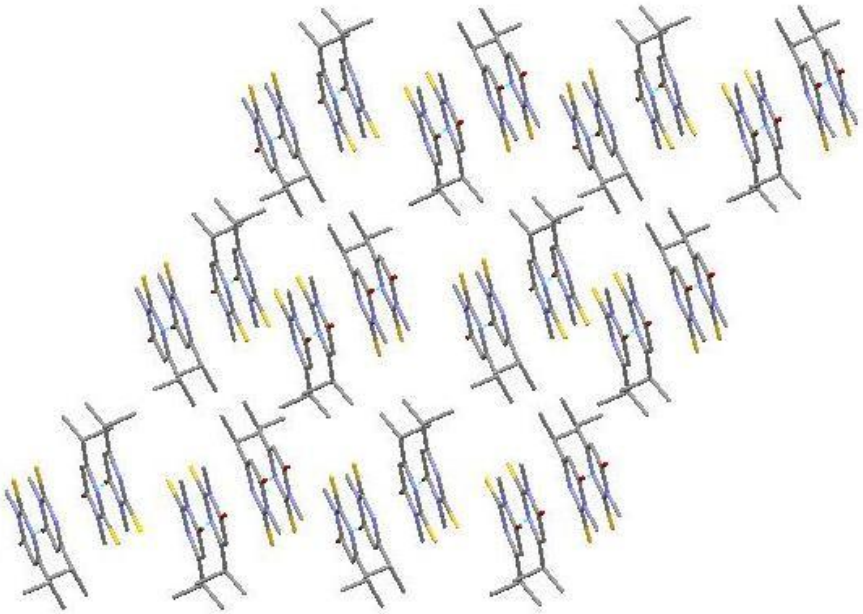
Compound (3) supramolecular architecture diagram:



Compound (4) supramolecular architecture diagram:



Compound (15) supramolecular architecture diagram:



CHAPTER-IV

Design and synthesis of self-assembled novel modified nucleobases for supramolecular architectures

IV.1. Molecular self-aggregation

Self aggregation is a spontaneous, thermodynamic process through which a molecule is organized into an ordered structure from a disorder state. These are of two types 1) *Intramolecular self-assembly* 2) *Intermolecular self-assembly*. In generally, the term molecular self-assembly refers to intermolecular self-assembly (*eg*: Quarternary structures, supramolecular assemblies) and an intramolecular analog refers to folding (*eg*: secondary and tertiary structures, Protein folding). Self aggregation is a key concept in supramolecular chemistry, because aggregation of molecules in systems is directed by non-covalent interactions like hydrogen bonding, hydrophobic forces, metal coordination, π-π interactions, *van der* Waals forces, and/or electrostatic as well as electromagnetic interactions. Example of self aggregation includes formation of crystals, micelles, liquid crystal phases, vesicles and Langmuir monolayer by surfactant molecules. Some examples of self-organization is depicted in ***Figure IV.1.1.***[1-4]

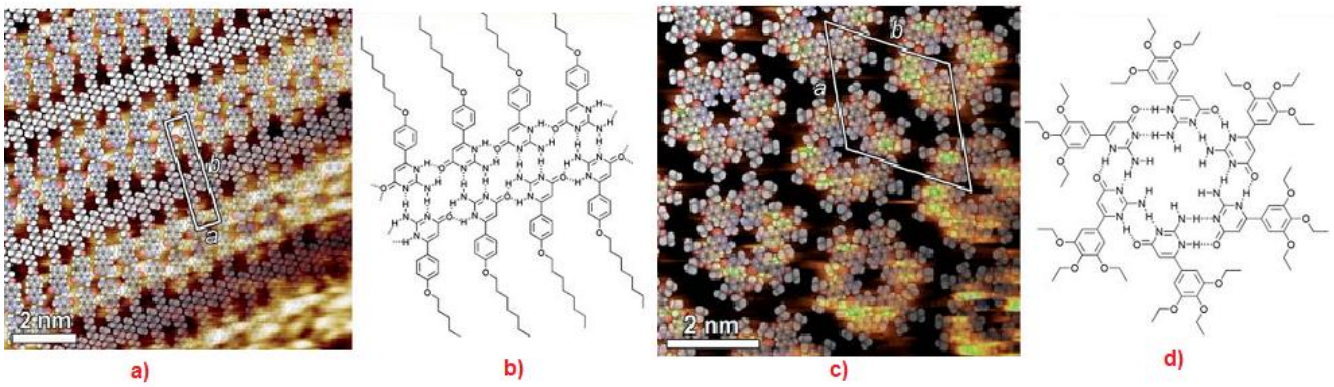


Figure IV.1.1: *Representation of molecular self aggregation of functionalized pyrimidine analogs*

IV.2. Applications

Watson–Crick base pairing interactions and π-stacking forces are major factors responsible for the formation of the helical structure of natural occurrence of DNA and it is a carrier of genetic information as well as it acts as a supramolecular scaffold unit for various applications.[5] Such natural DNA largely attributed with non covalent interactions involving nitrogenous heterocyclic nucleobases. The hydrogen bonding and π-stacking interactions of the nucleobases have been nicely utilized to develop supramolecular architectures.[6] Many modified nucleic acids have been synthesized for directionally controlled multiple hydrogen bonding and alteration of π-

interactions and have found applications in material science, bioorganic chemistry and drug delivery.[7]

Recently our group has demonstrated formation of co–crystals where, **a)** 6-isopropyluracil forms a 2:1 complex with adenine through Watson-Crick as well as Hoogsteen base pairing: **b)** 6-phenylisocytosine forms planar architecture with cytosine ***(Figure IV.2.1).*** [8, 9]

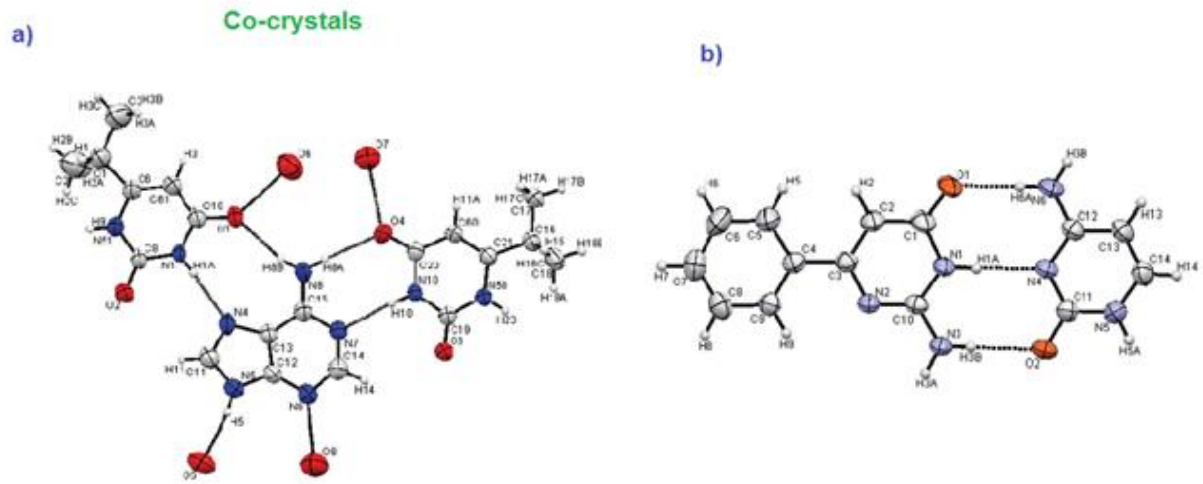


Figure IV.2.1: *a). Co-crystal of* ^{ipr}*U:Ade:*^{ipr}*U), b). Co-crystal of* ^{6ph}*Isocy:Cy.*

A classic example of nucleobase derived self aggregation is formation of G-quadruplex which showed plenty of biological applications.[10a-c] Four guanine molecules form a square-planar geometry *via* Hoogsteen hydrogen bonding, followed by stacking of the planes to form quadruplex structures.[5, 11] These structures could also be obtained through interaction of guanine residues with metal ions (***Figure IV.2.2a)***.[12a] Moreover, an array of modified nucleobases and nucleic acids has been recently used to show molecular architectures upon interaction with metal ions. Such molecules are used due to their good ligand nature to capturing the metal ions for forming various stable supramolecular architectures [12b-c]. For example, Okamoto, I. *et al.* showed that metal ions are used to stabilize the thiopyrimidine pairs in DNA duplexes ***(Figure IV.2.2b).*** [13] many of such complexes have been used for the development of new materials like DNA-based sensors [14, 15] and have significant conformational features in bio-molecules such as proteins, lipids, and nucleic acids. [16-19]

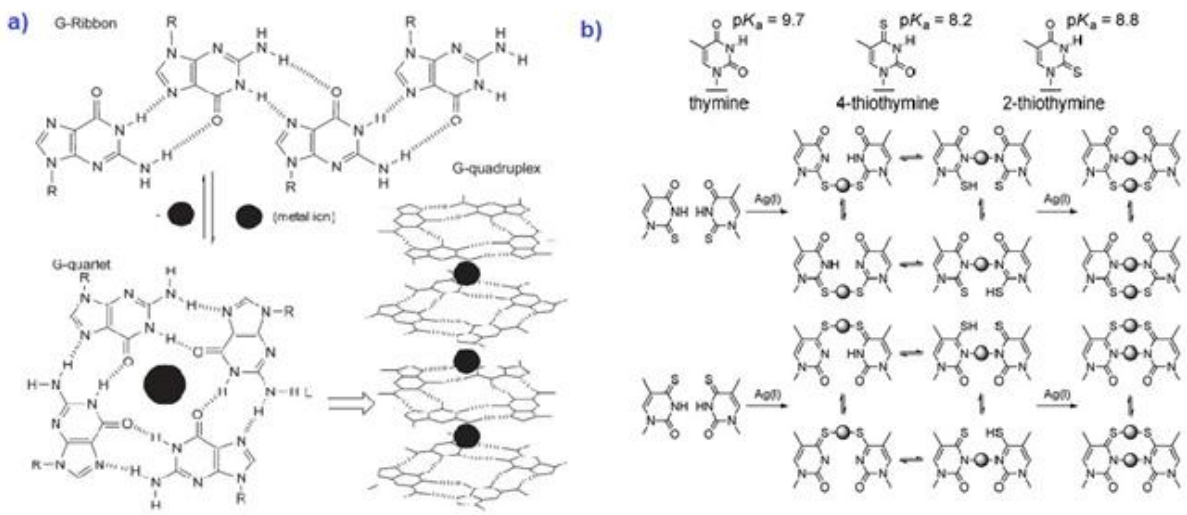
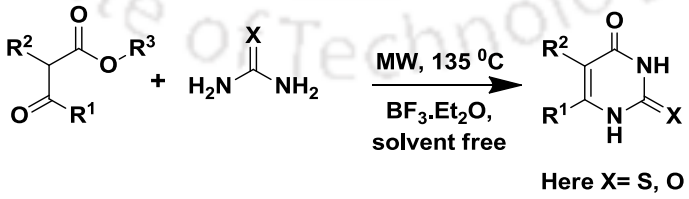


Figure IV.2.2: *a). G-quadruplex, b). Stable DNA duplexes.*

IV.3. Present work

In this chapter, we have developed supramolecular architectures (SMAs) with modified uracil and thiouracil moieties. A very few reports are available pertaining to crystal structure of modified nucleobases with controlled shapes and structures.[20] In this regard a number of uracil molecules, substituted with branched alkyl and/or aryl groups were synthesized. Crystals were mostly obtained from methanol solution. We demonstrate here that different molecular architectures could be produced by controlling the position and nature of the substitutions. A variety of nucleobase derivatives were synthesized under solvent-free condition in one pot using microwave-assisted method ***(Scheme IV.3.1).***



Scheme IV.3.1

Compounds **1-6** were synthesized in a closed vessel *CEM Discover Lab Mate* microwave reactor in absence of solvent, as shown in ***Table IV.3.1***. In general, 2 mmol of the substrate ester was

taken with 2.6 mmol of urea along with 2.4 mmol of $BF_3.Et_2O$ in a closed reaction vessel. The reaction attained completion upon irradiation for about 6-9 minutes and the temperature of the reaction vessel was kept at around 140^0C. Whereas the substrate β -ketoesters (**1-6**) were either purchased or synthesized following published procedures. [21] ***Table IV.3.1*** described the synthesis of uracil and thiouracil under microwave conditions.

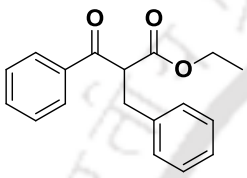
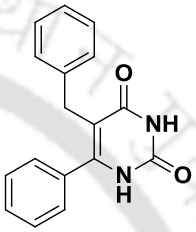
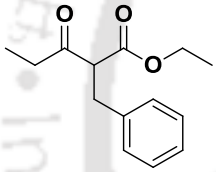
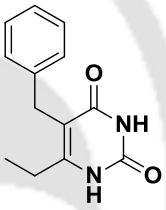
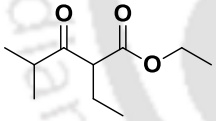
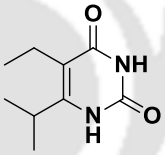
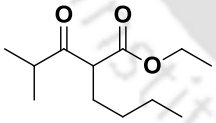
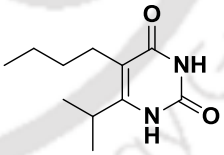
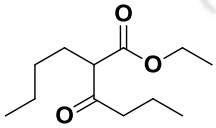
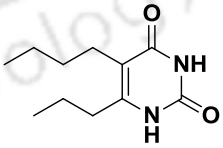
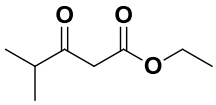
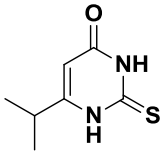
S. No.	Substrate 1a-6a	Microwave irradiation (Min)	%Yield 1-6	Product	%Yield with BF_2·Et_2O(Min)
1		15	25		55 (9)
2		12	60		80 (6)
3		10	63		85 (6)
4		12	65		78(8)
5		12	70		80 (6)
6		10	68		85 (6)

Table IV.3.1: *Synthesis of nucleobases to form molecular architectures*

It is very difficult to achieve the crystallization of free nucleobases for their poor solubility in organic solvents.[20] The above compounds were crystallized to study their supramolecular architecture and self-aggregation abilities. Here we have tried all the crystallization process in the different organic solvents, listed in ***Table IV.3.2.***

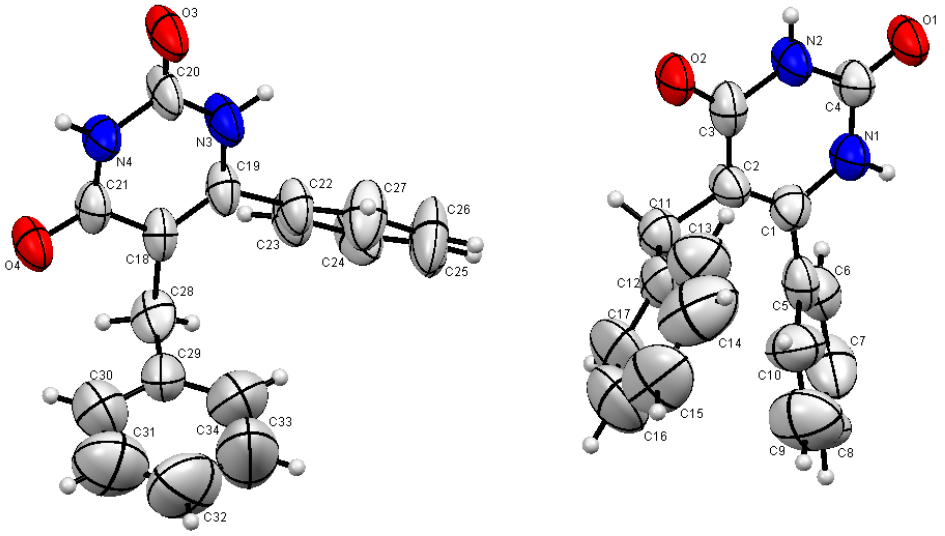
S. No.	Solvents (1:1% of all)	Crystals
01	Hexane/Methanol	Not obtained
02	Chloroform/ Methanol	Not obtained
03	Acetonitrile/ Methanol	Not obtained
04	Ethanol/ Methanol	Obtained
05	Ethyl acetate/ Methanol	Obtained
06	Dichloromethane/ Methanol	Not obtained
07	Methanol	Best crystals

Table IV.3.2: *Solvents used for crystallization*

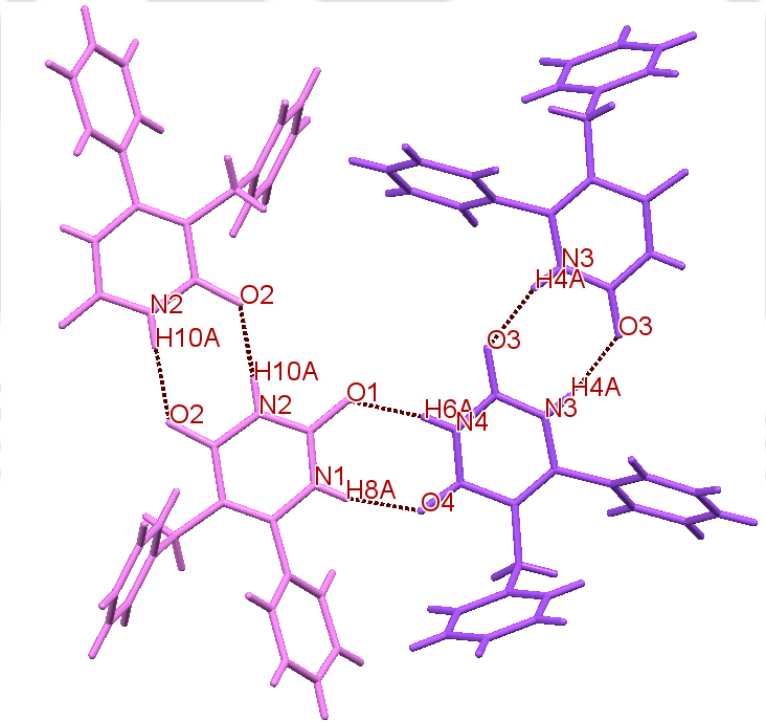
IV.3.1. ORTEP and supramolecular architecture images of nucleobases:

5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione(1):

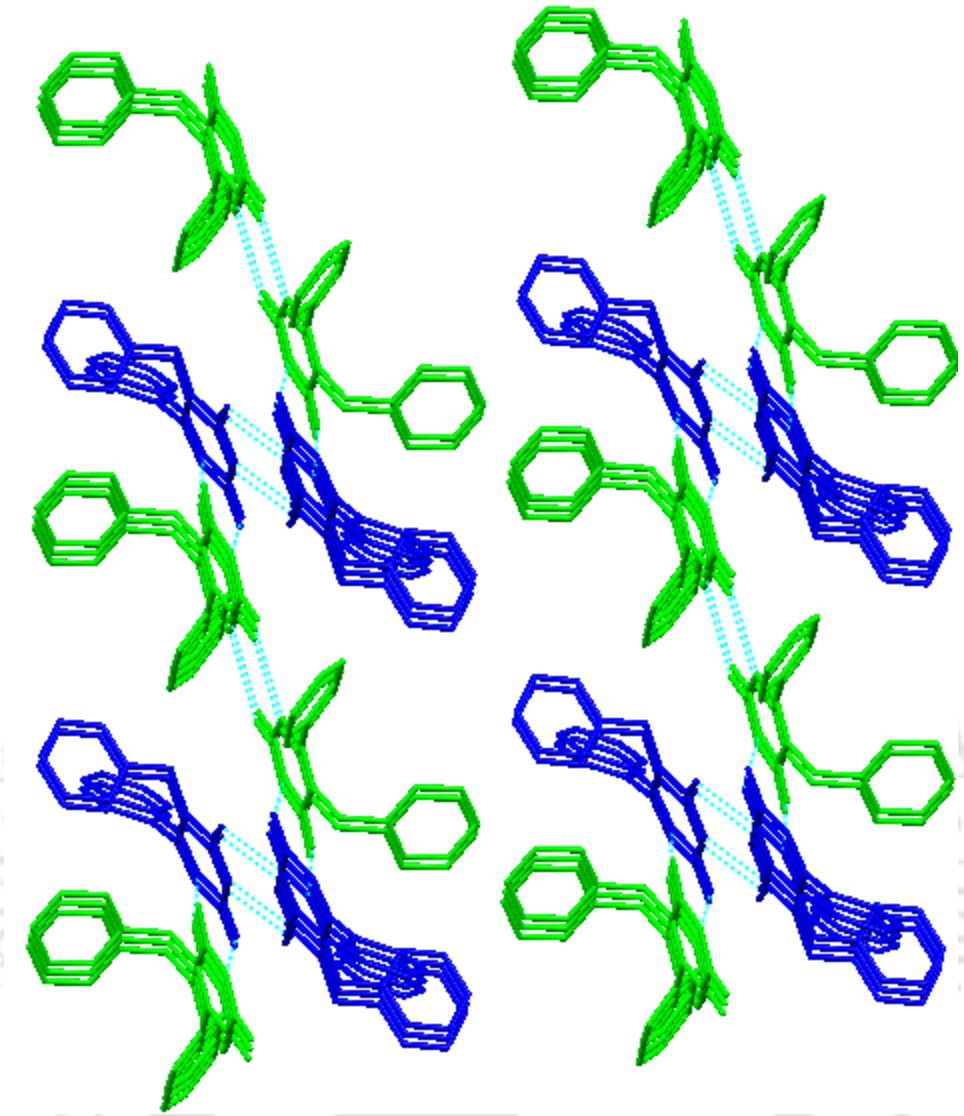
Compound (**1**) shows four types of H-bonds in between the heteroatoms of the nucleobase. These are N3–H4A···O3=1.970 Å N1–H8A···O4=1.960 Å, O1···H6A–N4=2.000 Å and O2···H10A–N2=1.990 Å. All the bond distances and other interactions of all the compounds **1-6** are tabulated in the characterization section. The molecular aggregation was found to generate two distinct layers. As can be evident from *figure* ***1c***, that π-stacking interactions between the benzene rings are very weak and do not contribute towards a properly organized pattern. It is also clear that no considerable π-stacking exist between the phenyl and the heterocyclic rings. The architecture shows two layers 1) hydrophobic layer, constructed with substituted aromatic rings on the nucleobases. 2) hydrophilic layer, formed by hetero atoms of the nucleobase. These molecules are symmetrically unequal and packing pattern grows along the crystallographic a-axis.



*1a). ORTEP diagram of 5-benzyl-6-phenyl uracil (**1**)*



*1b). Various H-bond interactions in compound **1***



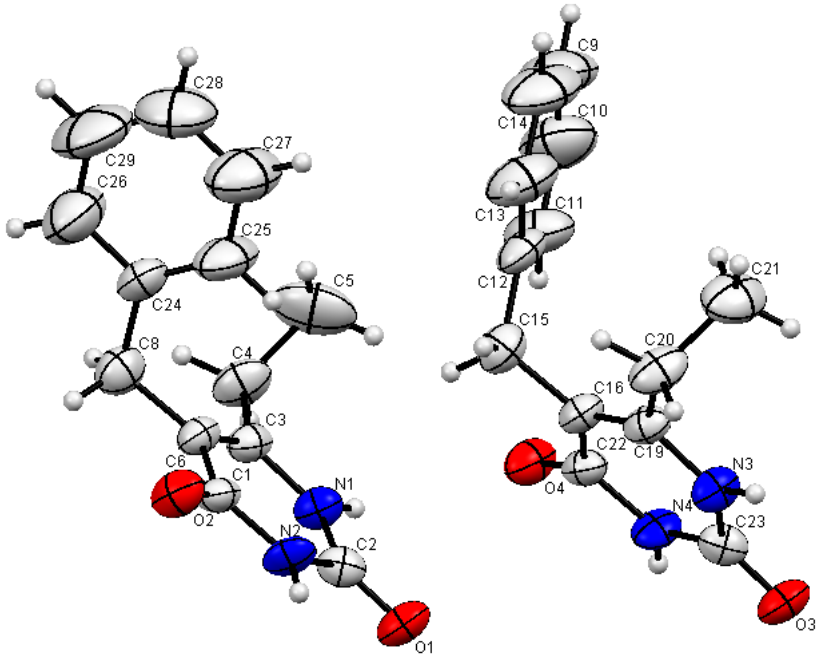
1c). 5-benzyl-6-phenyl uracil packing diagram along the crystallographic a-axis

Figure IV.3.1.1: *a) ORTEP, b) H-bond interactions, C) Supramolecular arrangement of compound* ***1***

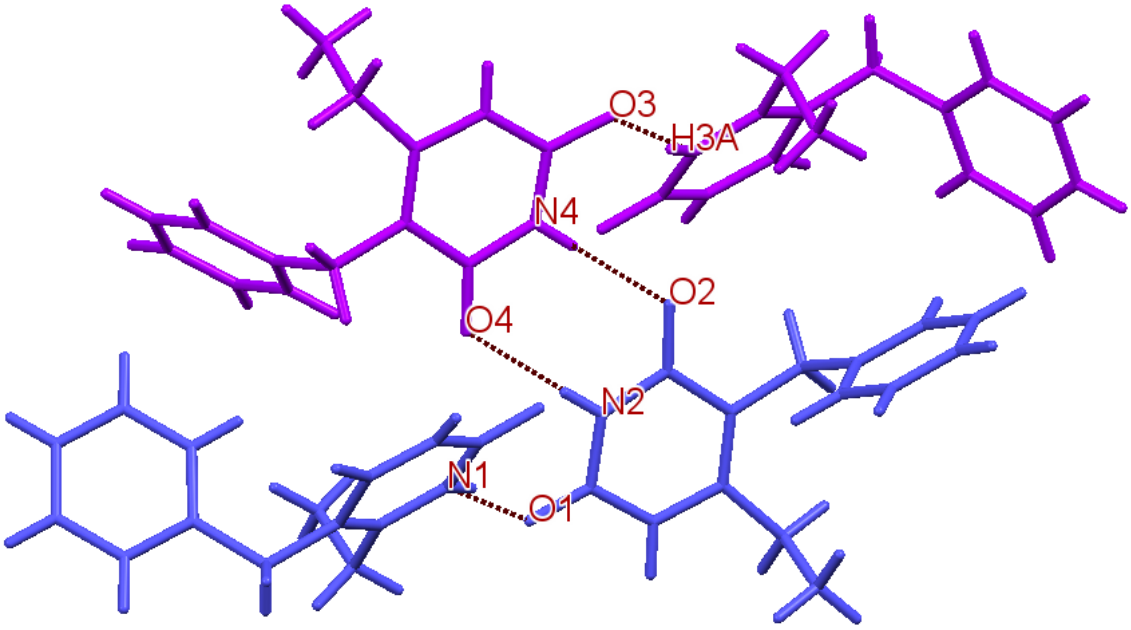
5-benzyl-6-ethylpyrimidine-2,4(1H,3H)-dione(2):

Compound (**2**) contains a benzyl group at C-5 and an ethyl group at C-6 position. It also shows four types of H-bonds. These are O1····H1–N1=2.000(4) Å, N2–H2····O4=2.08(3) Å, O3····H3–N3=2.06(4) Å and O2····H4–N4=2.02(3) Å *Figure 2c* represents a well-organized molecular architecture. The layers are inter-connected via strong hydrogen bondings between the heterocycles. It clearly forms a *chair structure*. These molecules are symmetrically unequal and

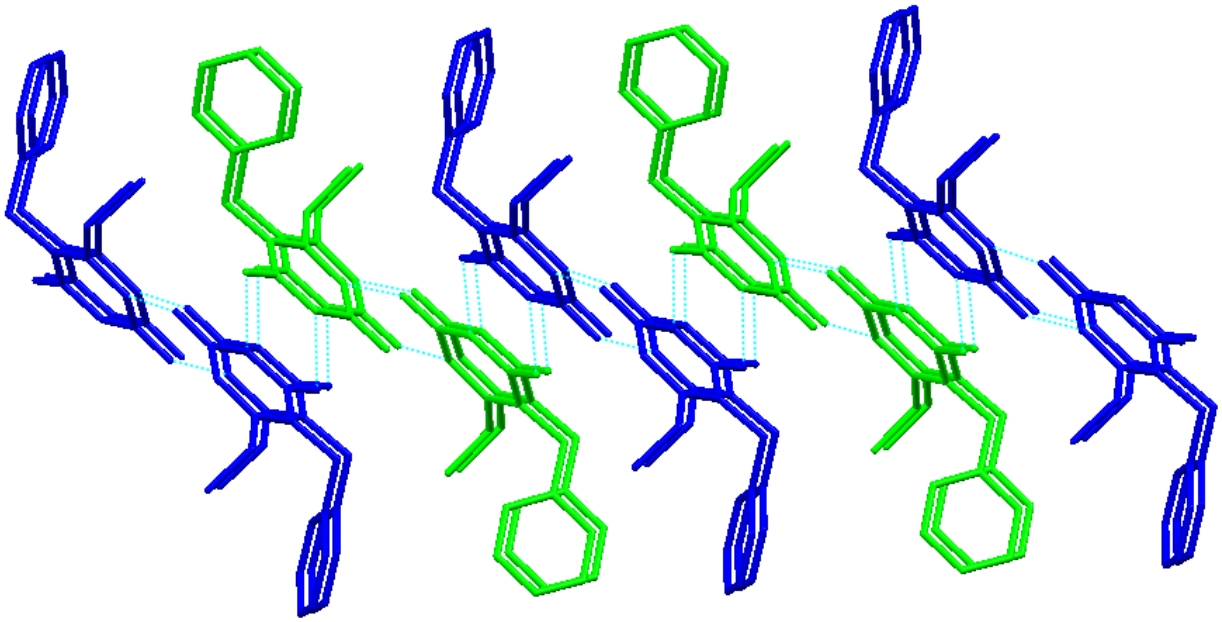
packing pattern packing pattern grows along the crystallographic b-axis.



2a). ORTEP diagram of 5-benzyl-6-ethyl uracil



2b). Various H-bond interactions in between the unequal symmetrical molecules of compound ***2***

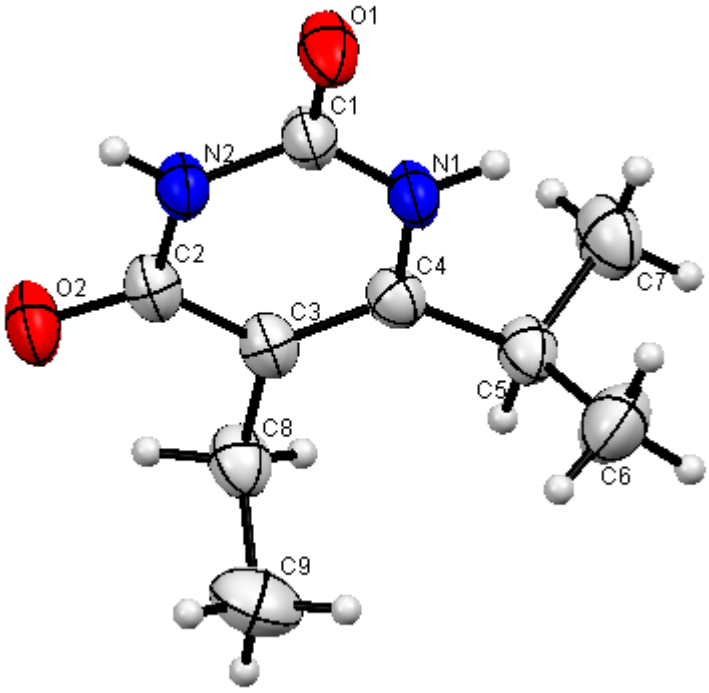


2c). 5-benzyl-6-ethyl uracil packing diagram along the crystallographic b-axis

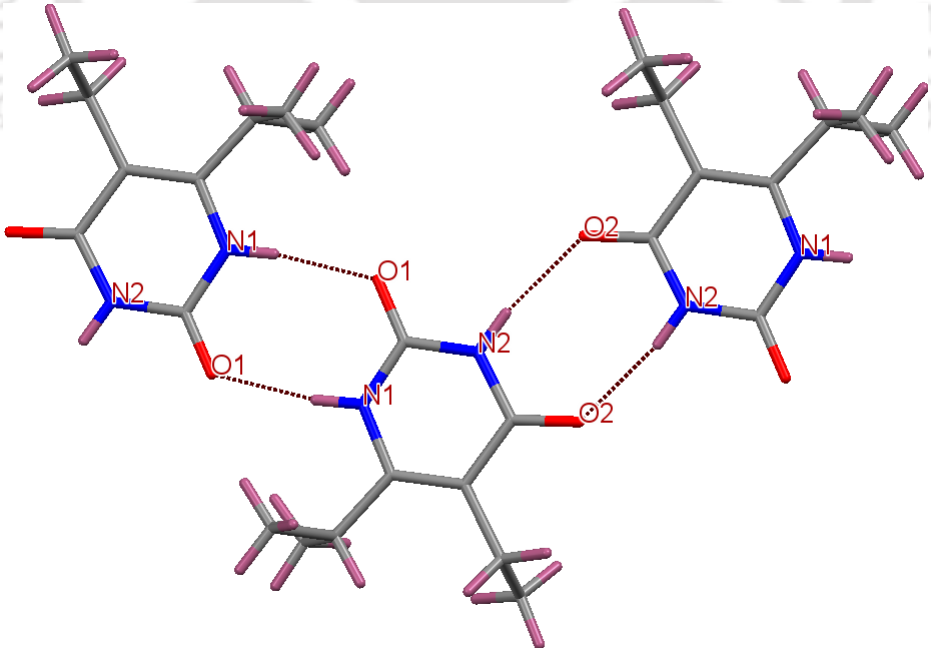
Figure IV.3.1.2: *a) ORTEP, b) H-bond interactions, c) Supramolecular arrangement of compound* ***2***

5-ethyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(3):

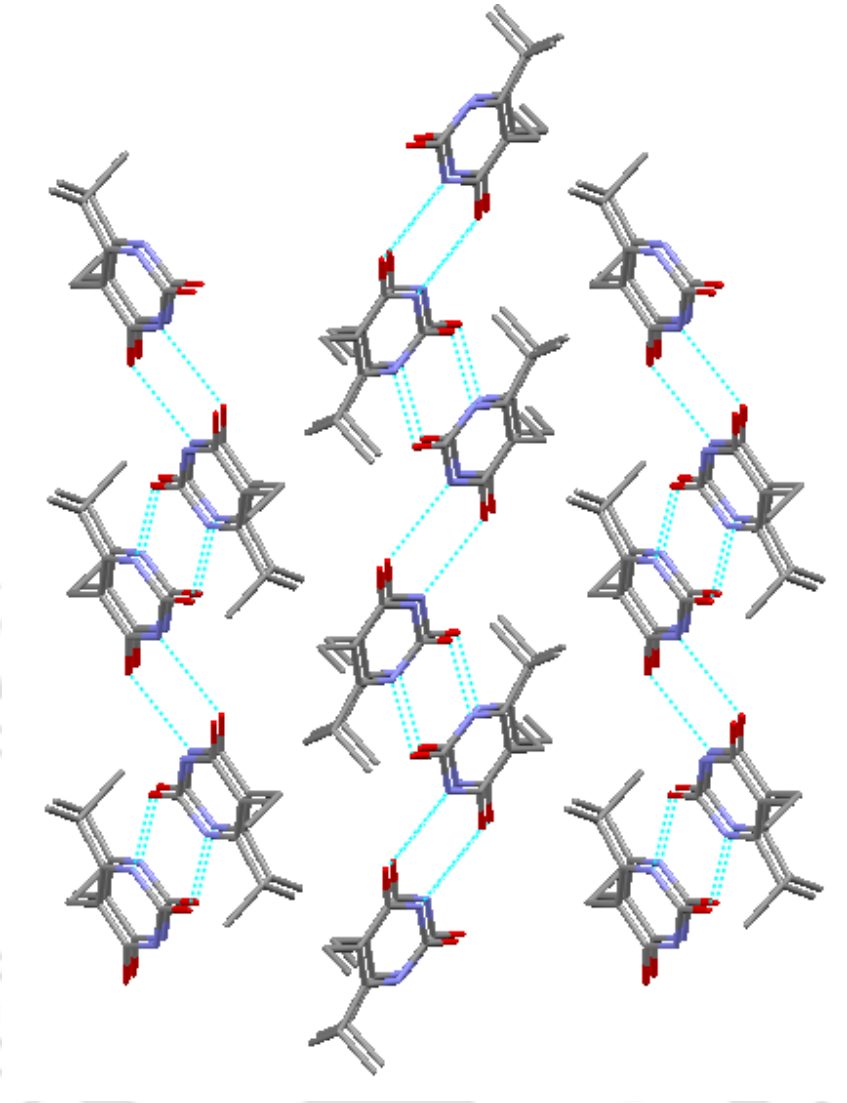
Compound **3** which contain alkyl substitutions at both C-5 and C-6 positions, show a helical like structure. No hydrogen bonding is observed between the layers which are presumably stabilized by hydrophobic interactions. The ordered structure is very symmetric and shows two types of H-bonds in between the heteroatoms of the nucleobase, which are almost same in distance. These are O1····H1–N1=2.010 Å, and O2····H2–N2=2.020 Å. These molecules are symmetrically unequal and packing pattern is shown along crystallographic a-axis.



3a). ORTEP diagram of 5-ethyl-6-isopropyl uracil



3b). Various H-bond interactions in between the unequal symmetrical molecules of compound ***3***



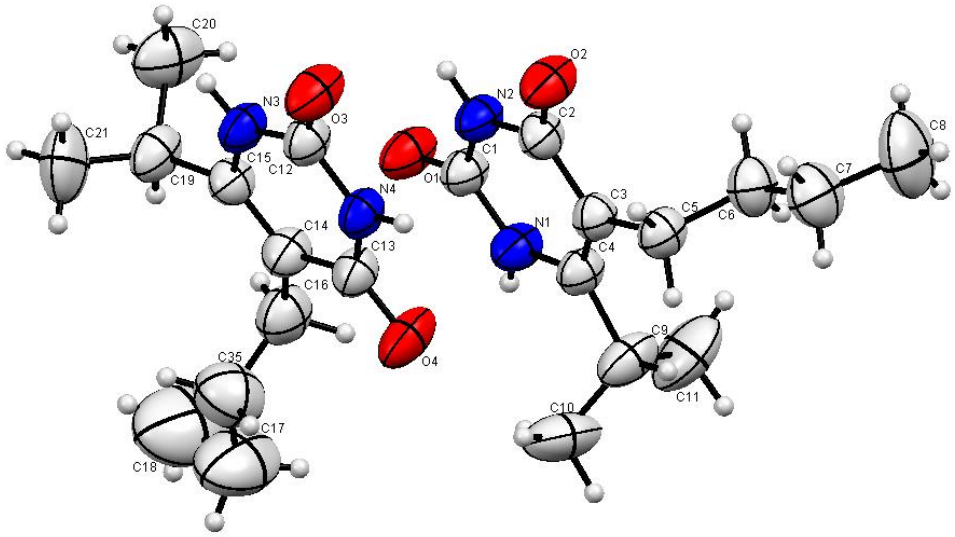
3b). 5-ethyl-6-isopropyl uracil packing diagram along the crystallographic a-axis

Figure IV.3.1.3: *a) ORTEP, b) H-bonding interactions, c) Supramolecular arrangement of compound* ***3***

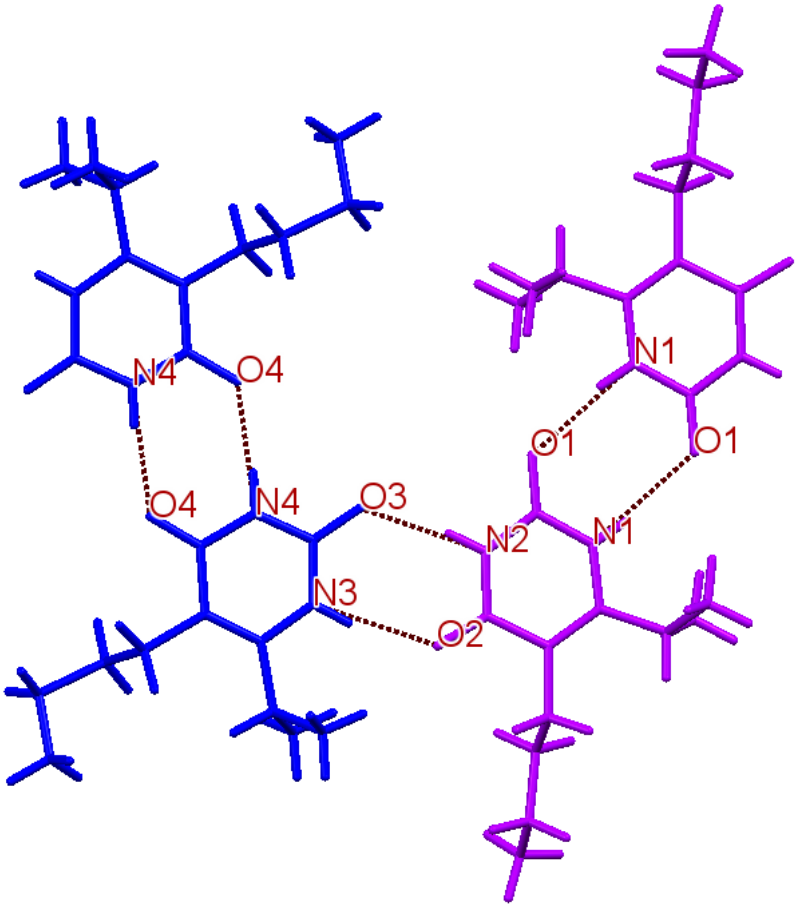
5-butyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(4):

Like compound (**3**), compound (**4**) also contains a C-6 isopropyl moiety. However, unlike **3** it contains a long butyl chain at the C-5 position. The crystal structure and self-assembled pattern of compound **4** shows four different types of H-bonds. These are O1···H1–N1=2.080 Å, N2–H2···O3=2.010 Å, O2···H3–N3 or O4···H4–N4=1.98 Å. It also shows two distinct hydrophobic channels generated due to interactions of the long alkyl chains. On the other hand hydrophilic channels are due to H-bondings between the heteroatoms. If we compare compound **3** and **4**, it

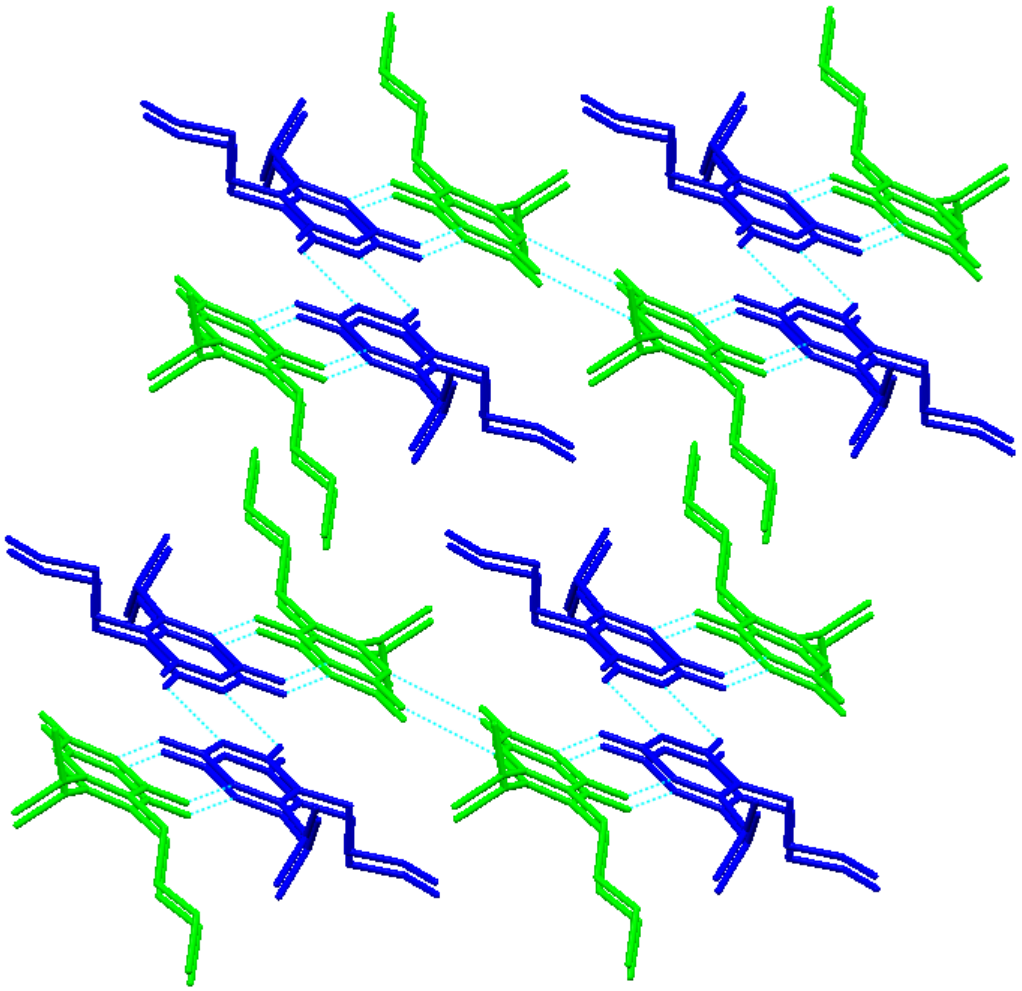
can be inferred that compound **3** has more symmetrical and ordered molecular assembly.



4a). ORTEP diagram of 5-propyl-6-isopropyl uracil



4b). Various H-bond interactions in between the unequal symmetrical molecules of compound ***4***



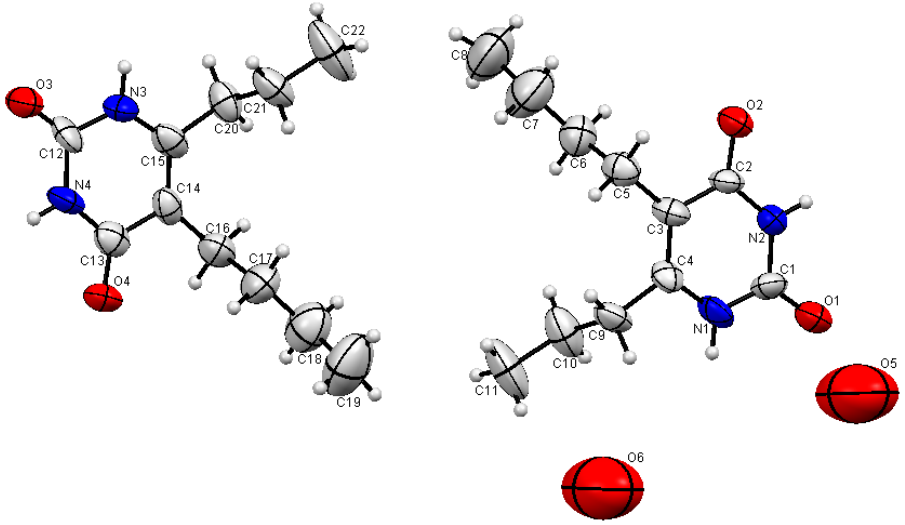
4b). 5-propyl-6-isopropyl uracil packing diagram along the crystallographic a-axis

Figure IV.3.1.4: *a) ORTEP b) H-bond interactions, c) Supramolecular arrangement of compound* ***4***

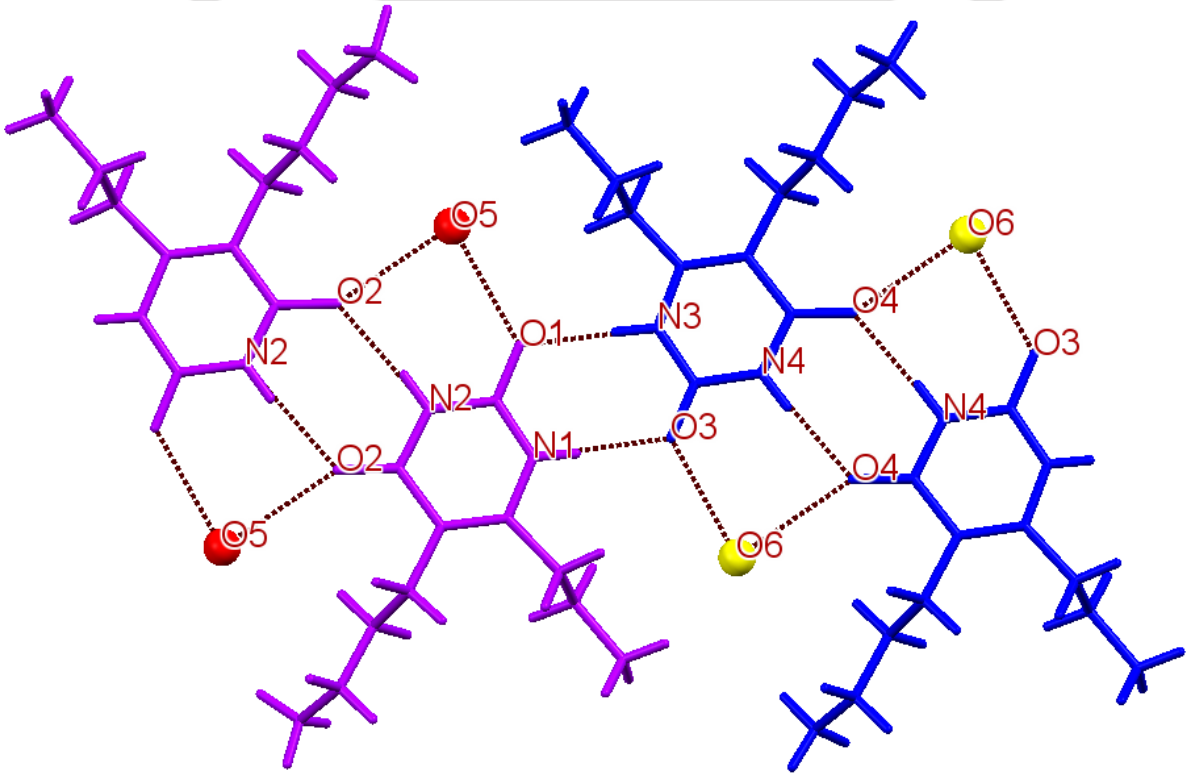
5-butyl-6-propylpyrimidine-2,4(1H,3H)-dione(5):

In compound **5**, the butyl chain at C-5 is retained as that of compound **4**, but at the C-6 position a propyl chain was inserted instead of isopropyl group. Compound (**5**) shows following H-bonds in between the heteroatom of the nucleobase. These are N3–H3···O1 or N4–H4···O4=1.970 Å, O3···H1–N1=1.960 Å, N2–H2···O2=2.000 Å, O1···· O5 (H_2O)=2.847 Å and O2···· O5(H_2O)=2.895 Å. The crystal structure shows highly symmetric and ordered molecular architectures. It could be clearly noted that water molecules act as bridges to stabilize the structure. A well recognized hydrophobic channel is observed between the layers, due to

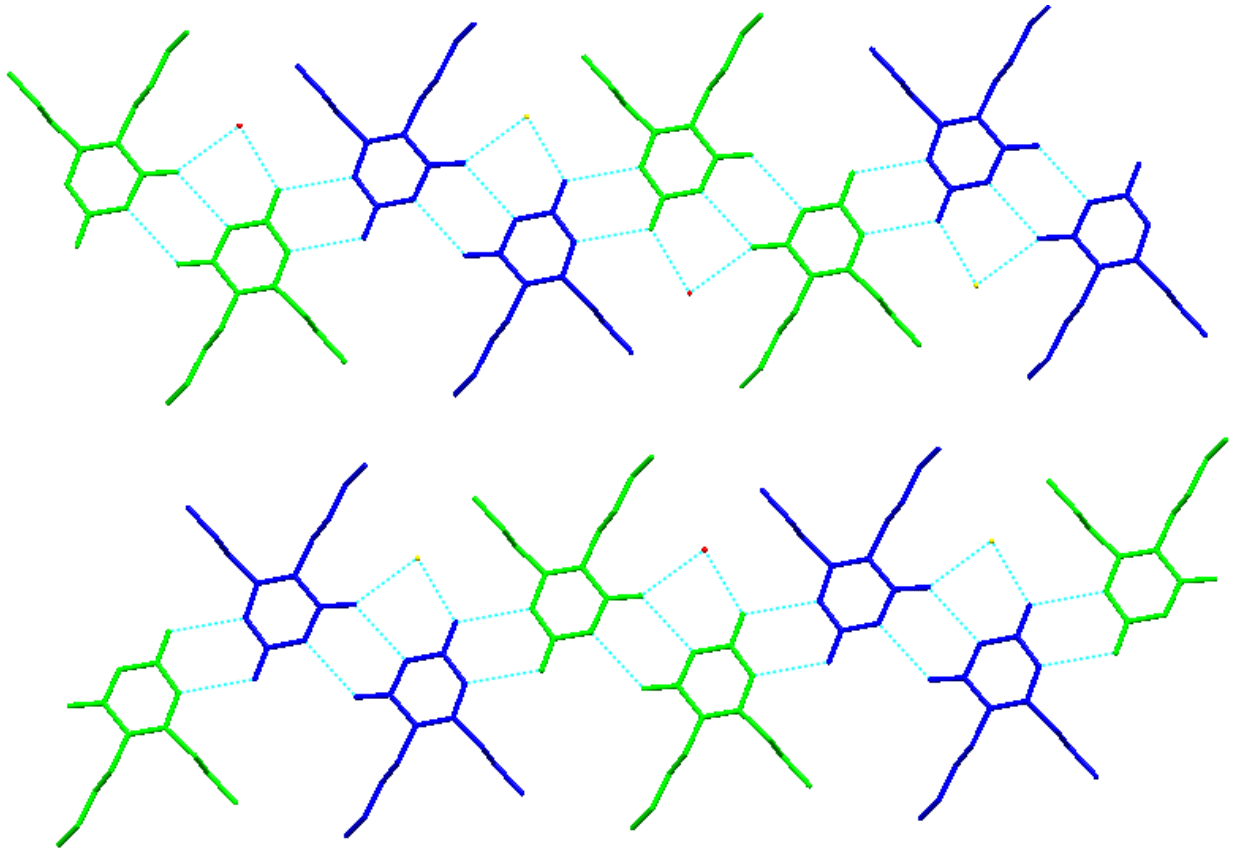
presence of the alkane chains at C-5 position. The molecule also shows a helical-like pattern. Vertical layers are stabilized by strong pi-stacking between the heterocyclic rings. The molecules are symmetrically unequal and packing pattern is shown along crystallographic a-axis.



5a) ORTEP diagram of 5-buthyl-6-propyl uracil



5b) Various H-bond interactions in between the unequal symmetrical molecules of compound ***5***

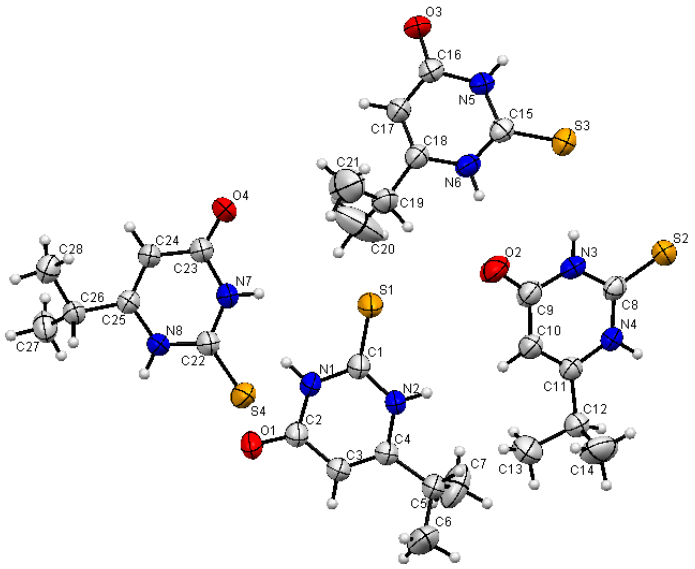


5b). 5-buthyl-6-propyl uracil packing diagram along the crystallographic a-axis. The unit cell contains two water molecules.

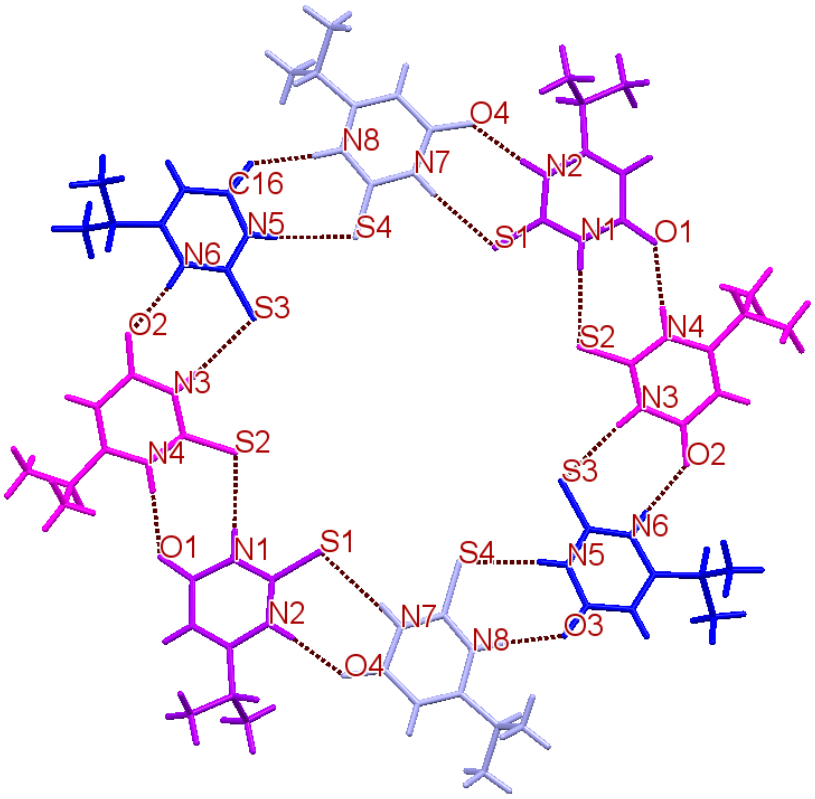
Figure IV.3.1.5: *a) ORTEP b) H-bonding interactions, c). Supramolecular arrangement of compound* ***5***

2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one(6):

Compound (**6**) is an analogue of thiouracil. Crystal was isolated from a methanol/ethylacetate mixture and shows the following H-bonds in between the heteroatoms of the nucleobase. These are N2–H2····O4=1.96 Å, N4–H4····O1=2.050 Å, N6–H6····O2=1.98 Å, N1–H1····S2=2.500 Å, N3–H3····S3=2.47 Å, N5–H5····S5=2.53 Å, N7–H7····S1=2.500 Å The compound forms a cyclic arrangement of the units.



6a) ORTEP diagram of 6-isopropyl thiouracil



6b) Packing diagram and H-bond interactions in between the unequal symmetrical molecules of compound ***6***

Figure IV.3.1.6: *a) ORTEP b) Supramolecular arrangement of compound* ***6***

In conclusion, a series of substituted pyrimidine nucleobases were designed and synthesized. A set of crystal structures of the free nucleobases were achieved. Systematic variation of the aromatic and aliphatic side chains resulted distinct molecular self-aggregations. The supramolecular architectures of the free nucleobases, therefore could be controlled by the nature of substituent.

IV.4. Experimental section

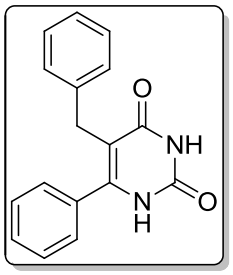
IV.4.1. General Information: All chemicals were purchased from SRL, Merck, and Sigma Aldrich and were used without further purification. All microwave-directed reactions were carried out in a *CEM Discover Lab* closed vessel microwave reactor at about 135°C for variable durations. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were all recorded from a *DRX-400 Varian spectrometer* using $CDCl_3$, D_2O or DMSO-D_6 as solvents. Chemical shifts are reported in parts per million (ppm). Melting points were determined using *Büchi B-545* apparatus and are uncorrected. High resolution mass spectrometry was analyzed from *Agilent Q-TOF 6500 LC/MS* system and *Micromass Q-TOF ESI-MS* instrument (model HAB 273). X-Ray data were collected from a *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structures were solved by direct method using *SHELLX-97* (Göttingen, Germany).

IV.4.2. General Procedure (compound 1-6): A β-ketoester (**1a-6a**, 2 mmol), taken in a reactor vessel was mixed thoroughly for 1 min with urea/thiourea (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at about 140°C. The compound (**1-6**) was further purified by column chromatography. **Yield:** as shown in ***Table IV.3.1***.

Synthesis using Lewis Acid (1-6): A β-ketoester (**1a-6a**, 2mmol), taken in a reactor vessel with BF_3 Et_2O (2.4 mmol) was mixed thoroughly for 1 min with urea (2.6 mmol). The vessel was closed immediately and was subjected to microwave irradiation at 140°C. The compound (**1-6**) was further purified by column chromatography. **Yield:** as shown in ***Table IV.3.1***.

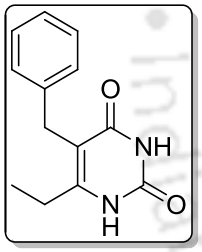
IV.5. Characterization Data

5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione(1)



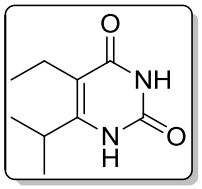
Yield 55%, White solid, m.p 252°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 11.17 (s, 1H), 7.98 (d, 2H, *J*= 8.4 Hz), 7.71 (d, 2H, *J*= 7.2 Hz), 7.53-7.49 (m, 3H), 7.36-724 (m, 3H), 3.80 (s, 2H). FT-IR (KBr ν/cm^{-1}). 3436, 3219, 2914, 2853, 1741, 1724, 1469, 1053. UV 285 nm**.** HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_2$: 279.1128; observed: 279.1135.

5-benzyl-6-ethylpyrimidine-2,4(1H,3H)-dione(2)



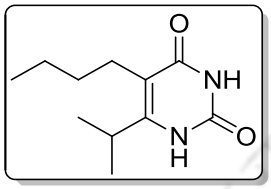
Yield 80%, White solid, m.p 230°C, ^{1}H NMR (400 MHz, DMSO-d_6 +$CDCl_3$) δ 10.52 (s, 1H), 9.99 (s, 1H), 7.40-7.14 (m, 5H), 3.72 (s, 2H), 2.45 (q, 2H, *J*= 7.4 Hz), 1.07 (t, 3H, *J*= 7.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 165.1, 154.0, 151.5, 139.9, 128.0, 127.6, 125.6, 108.0, 29.1, 23.6, 12.0. FT-IR (KBr ν/cm^{-1}). 3446, 3172, 2926, 2849, 1734, 1646, 1455, 1336, 1065, 696, 529. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{13}H_{15}N_2O_2$: 231.1128; observed:231.1135.

5-ethyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(3)



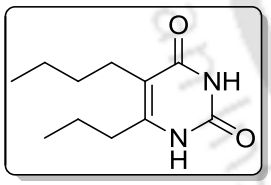
Yield 85%, White solid, m.p 205°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 9.14 (s, 1H), 3.13-3.07 (m, 1H), 2.42 (q, 2H, *J*= 7.2 Hz), 1.24 (d, 6H, *J*= 7.2 Hz), 1.04 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 164.8, 155.0, 151.8, 110.0, 28.2, 19.5, 17.2, 14.1. FT-IR (KBr ν/cm^{-1}). 3567, 3238, 2970, 2817, 1707, 1454, 1213, 1092, 883, 539. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_9H_{15}N_2O_2$: 183.1128; observed: 183.1128.

5-butyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(4)



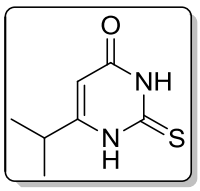
Yield 78%, White solid, m.p 220°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 1H), 8.86 (s, 1H), 3.27-3.07 (m, 1H), 2.51 (t, 2H *J*=.4 Hz), 1.35 (m, 2H), 1.23 (d, 6H, *J*= 6.8 Hz), 0.91 (t, 3H, *J*= 6.4 Hz). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 164.9, 155.1, 151.8, 108.6, 31.5, 28.1, 23.5, 22.1, 19.3, 13.5. FT-IR (KBr ν/cm^{-1}). 3455, 3165, 2925, 1697, 1641, 1446, 1095, 873, 552. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{19}N_2O_2$: 211.1441; observed: 211.1444.

5-butyl-6-propylpyrimidine-2,4(1H,3H)-dione(5):



Yield 83%, White solid, m.p 260°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (s, 1H), 9.26 (s, 1H), 2.45 (t, 2H, *J*= 7.8 Hz), 2.36 (t, 2H, *J*= 7.2 Hz), 1.69-1.64 (m, 2H), 1.42-1.34 (m, 4H), 1.04 (t, 2H, *J*= 7.2 Hz), 0.95 (t, 2H, *J*=.4 Hz). FT-IR (KBr ν/cm^{-1}). 3586, 3245, 1720, 1633, 1465, 1194, 103, 774, 543. HRMS (ES) m/z $[M+H]^+$ calcd for $C_{11}H_{19}N_2O_2$: 211.1441; observed: 211.1443.

2,3-dihydro-6-isopropyl-2-thioxopyrimidin-4(1H)-one(6):



Yield: 80%, white solid, m.p: 176-180°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.18 (s, 1H), 5.66 (s, 1H), 2.69-2.62 (m, 1H), 1.13 (d, 6H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.5, 163.4, 162.6, 100.8, 31.0, 20.9. FT-IR (KBr ν/cm^{-1}. 3415, 3281, 2916, 2844, 1744, 1680, 1430, 1169, 1080, 828, 557. HRMS (ESI) m/z $[M+Na]^+$ calculated ($C_7H_{10}N_2OSNa$): 193.0406; observed: 193.0408.

IV.6. References

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IV.7. Crystallographic data

5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione(1):

The crystal structure of 5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione was obtained from methanol solution.

Table 1: Crystallographic data of 5-benzyl-6-phenyl pyrimidine-2,4(1H,3H)-dione (**1**)	
Chemical formula	$C_{34}H_{28}N_4O_5$
Formula Mass	576.64
Temperature/K	296(2)
Crystal system	'Triclinic'
Space group	'P-1 '
a/Å	10.0681(15)
b/Å	12.7028(17)
c/Å	14.2914(19)
α/°	83.126(8)
β/°	84.679(11)
γ/°	78.853(9)

Unit cell volume/Å	1775.8(4)
Z	2
μ (mm-1)	0.087
*ρ*calcd (g cm-3)	1.301
No. of reflections measured	6684
No. of independent reflections	1639
Final R1 values (I > 2σ(I))	0.1848
Final wR(F2) values (I > 2σ(I))	0.4129
Final R1 values (all data)	0.4159
Final wR(F2) values (all data)	0.5032
Goodness of fit (F^2)	1.650

5-benzyl-6-ethylpyrimidine-2,4(1H,3H)-dione(2):

The crystal structure of 5-benzyl-6-ethyl pyrimidine-2,4(1H,3H)-dione was obtained from methanol solution.

Table 2: Crystallographic data of 5-benzyl-6-ethyl pyrimidine-2,4(1H,3H)-dione.	
Chemical formula	C_{13} H_{14} N_2 O_2
Formula Mass	230.26
Temperature/K	296 K
Crystal system	Monoclinic
Space group	P2(1)/c
a/Å	10.9279(6)
b/Å	7.2337(4)
c/Å	30.5821(17)
α/°	90
β/°	96.801(2)
γ/°	90
Unit cell volume/Å	2400.5(2)
Z	8

μ (mm-1)	0.087
*ρ*calcd (g cm-3)	1.274
No. of reflections measured	4350
No. of independent reflections	2857
Final R1 values (I > 2σ(I))	0.0472
Final wR(F2) values (I > 2σ(I))	0.1060
Final R1 values (all data)	0.0610
Final wR(F2) values (all data)	0.1106
Goodness of fit (F^2)	1.174

5-ethyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(3):

The crystal structure of 5-ethyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(4) was obtained from methanol solution.

Table 3: Crystallographic data of 5-ethyl-6-isopropyl pyrimidine-2,4(1H,3H)-dione	
Chemical formula	$C_9 H_{14} N_2 O_2$
Formula Mass	182.22
Temperature/K	296(2)
Crystal system	Monoclinic
Space group	P2(1)/c
a/Å	8.0591(2)
b/Å	14.6588(5)
c/Å	8.9323(3)
α/°	90.00
β/°	104.2890(10)
γ/°	90.00
Unit cell volume/Å	1022.59(6)
Z	4
μ (mm-1)	0.085
*ρ*calcd (g cm-3)	1.184

No. of reflections measured	1745
No. of independent reflections	1475
Final R1 values (I > 2σ(I))	0.0424
Final wR(F2) values (I > 2σ(I))	0.1175
Final R1 values (all data)	0.0762
Final wR(F2) values (all data)	0.0489
Goodness of fit (F^2)	1.086

5-butyl-6-isopropylpyrimidine-2,4(1H,3H)-dione(4):

The crystal structure of 5-butyl-6-isopropylpyrimidine-2,4(1H,3H)-dione was obtained from methanol solution

Table 4: Crystallographic data of 5-butyl-6-isopropyl pyrimidine-2,4(1H,3H)-dione	
Chemical formula	C_{11} H_{18} N_2 O_2
Formula Mass	210.27
Temperature/K	296(2)
Crystal system	'Triclinic”
Space group	'P-1'
a/Å	8.8857(4)
b/Å	12.3826(6)
c/Å	12.9351(6)
α/°	99.082(3)
β/°	108.473(3)
γ/°	109.130(3)
Unit cell volume/Å	1219.75(10)
Z	4
μ (mm-1)	0.079
*ρ*calcd (g cm-3)	1.145
No. of reflections measured	4390

No. of independent reflections	3269
Final R1 values (I > 2σ(I))	0.0645
Final wR(F2) values (I > 2σ(I))	0.1839
Final R1 values (all data)	0.0762
Final wR(F2) values (all data)	0.1959
Goodness of fit (F^2)	1.092

5-butyl-6-propylpyrimidine-2,4(1H,3H)-dione(5):

The crystal structure of 5-butyl-6-propylpyrimidine-2,4(1H,3H)-dione was obtained from methanol solution.

Table 5: Crystallographic data of 5-butyl-6-propylpyrimidine-2,4(1H,3H)-dione	
Chemical formula	C_{22} H_{36} N_4 O_5
Formula Mass	436.55
Temperature/K	296(2)
Crystal system	Triclinic
Space group	P-1
a/Å	5.1911(9)
b/Å	16.192(3)
c/Å	17.301(3)
α/°	62.671(11)
β/°	87.668(12)
γ/°	83.861(13)
Unit cell volume/Å	1284.4(4)
Z	4
μ (mm-1)	0.085
*ρ*calcd (g cm-3)	1.170
No. of reflections measured	4521
No. of independent reflections	3293
Final R1 values (I > 2σ(I))	0.0963

Final wR(F2) values (I > 2σ(I))	0.2099
Final R1 values (all data)	0.2431
Final wR(F2) values (all data)	0.2526
Goodness of fit (F^2)	1.223

6-isopropyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one(6):

The crystal structure of 6-isopropyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one was obtained from methanol solution.

Table 6: Crystallographic data of 6-isopropyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one.	
Chemical formula	C_{32} H_{48} N_8 O_6 S_4
Formula Mass	769.02
Temperature/K	296(2)
Crystal system	Triclinic
Space group	P-1
a/Å	12.6361(4)
b/Å	13.0190(4)
c/Å	13.9519(8)
α/°	98.480(2)
β/°	97.299(2)
γ/°	115.2350(10)
Unit cell volume/Å	2006.68(15)
Z	2
μ (mm-1)	0.287
*ρ*calcd (g cm-3)	1.273
No. of reflections measured	7228
No. of independent reflections	4971
Final R1 values (I > 2σ(I))	0.0438
Final wR(F2) values (I > 2σ(I))	0.0807

Final R1 values (all data)	0.0609
Final wR(F2) values (all data)	0.0873
Goodness of fit (F^2)	1.019

Hydrogen bond and π-stacking parameters

Compound 1

D-H··A	$d_{D...H(Å)}$	$d_{H\cdots A(Å)}$	$d_{D\cdots A(Å)}$	∠ D-H···A(°)	symmetry
N3–H4····O3	0.8600	1.9700	2.813(9)	166.00	-x,1-y,-z
N4 –H6A····O1	0.8600	2.0000	2.851(9)	171.00	-x,-y,1-z
N1–H8A ····O4	0.8600	1.9600	2.803(9)	166.00	-x,-y,1-z
N2–H10A ····O2	0.8600	1.9900	2.848(9)	173.00	1-x,-y,2-z
C6–H6····O1	0.9300	2.3600	3.264(13)	164.00	-x,-y,2-z
C23–H2····O2	0.9300	2.4900	3.414(11)	170.00	x,y,-1+z
C24–H24 ····O3	0.9300	2.6000	3.381(13)	142.00	1+x,y,z

Compound 2

D-H··· A	$d_{D...H(Å)}$	$d_{H\cdots A(Å)}$	$d_{D\cdots A(Å)}$	∠ D-H···A(°)	symmetry
N1 –H1A ····O1	0.95(4)	2.00(4)	2.920(4)	164(3)	1-x,-1/2+y,1/2-z
N2 –H2A····O4	0.79(3)	2.08(3)	2.866(5)	173(3)	1-x,1/2+y,1/2-z
N3 –H3A ····O3	0.85(4)	2.06(4)	2.895(5)	166(3)	2-x,1/2+y,1/2-z
N4 –H4C····O2	0.83(3)	2.02(3)	2.835(5)	17)	1-x,-1/2+y,1/2-z
C15 –H15B····O4	0.9700	2.5000	2.859(5)	102.00	

Compound 3

D-H···A	$d_{D...H(Å)}$	$d_{H\cdots A(Å)}$	$d_{D\cdots A(Å)}$	∠ D-H···A(°)	symmetry
N1 –H1····O1	0.8600	2.0100	2.8574(17)	168.00	1-x,-y,1-z
N2 –H2····O2	0.8600	2.0200	2.8595(17)	165.00	2-x,-y,2-z
C7 –H7C····O1	0.9600	2.5800	3.454(2)	151.00	1-x,-y,1-z
C8 –H8B····O2	0.9700	2.4900	2.851(2)	102.00	

Compound 4

D-H···A	$d_{D...H(Å)}$	$d_{H\cdots A(Å)}$	$d_{D\cdots A(Å)}$	∠ D-H···A(°)	symmetry
N1 –H1N····O1	0.8600	2.0800	2.927(3)	168.00	2-x,2-y,1-z
N2–H2J····O3	0.8600	2.0100	2.855(4)	167.00	
N3–H3····O2	0.8600	1.9800	2.822(4)	167.00	

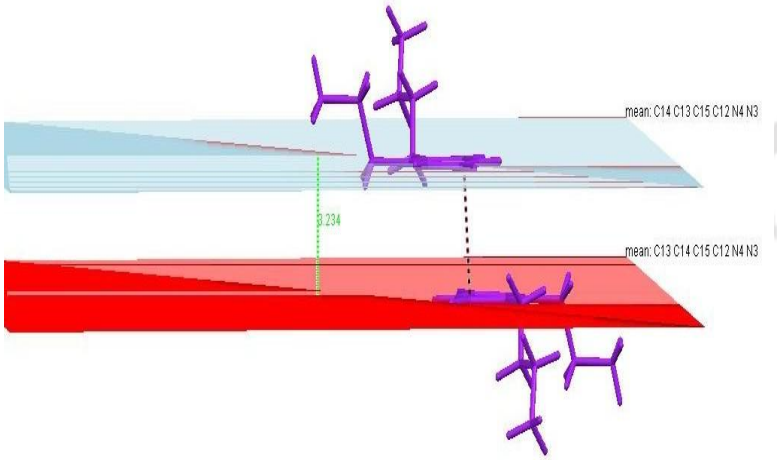
N4–H4····O4	0.8600	1.9800	2.828(4)	171.00	-x,1-y,1-z
C10–H10C····O1	0.9600	2.6000	3.497(4)	157.00	-x,2-y,1-z

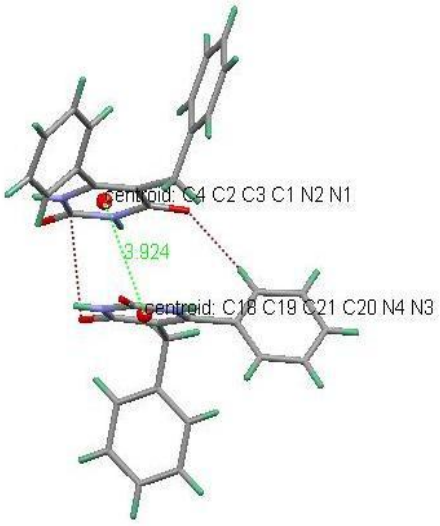
Compound 5

D-H···A	$d_{D...H(Å)}$	$d_{H...A(Å)}$	$d_{D...A(Å)}$	∠ D-H···A(°)	symmetry
N1 –H1····O3	0.8600	1.9600	2.819(7)	174.00	-1+x,1+y,z
N2 –H2····O2	0.8600	2.0000	2.849(7)	172.00	-1-x,1-y,1-z
N3–H3····O1	0.8600	1.9700	2.830(8)	177.00	1+x,-1+y,z
N4 –H4····O4	0.8600	1.9700	2.832(7)	175.00	3-x,-y,-z
C11–H11A····O6	0.9600	2.4300	3.31(2)	153.00	
C22 –H22C····O5	0.9600	2.4400	3.32(2)	153.00	1-x,-y,1-z

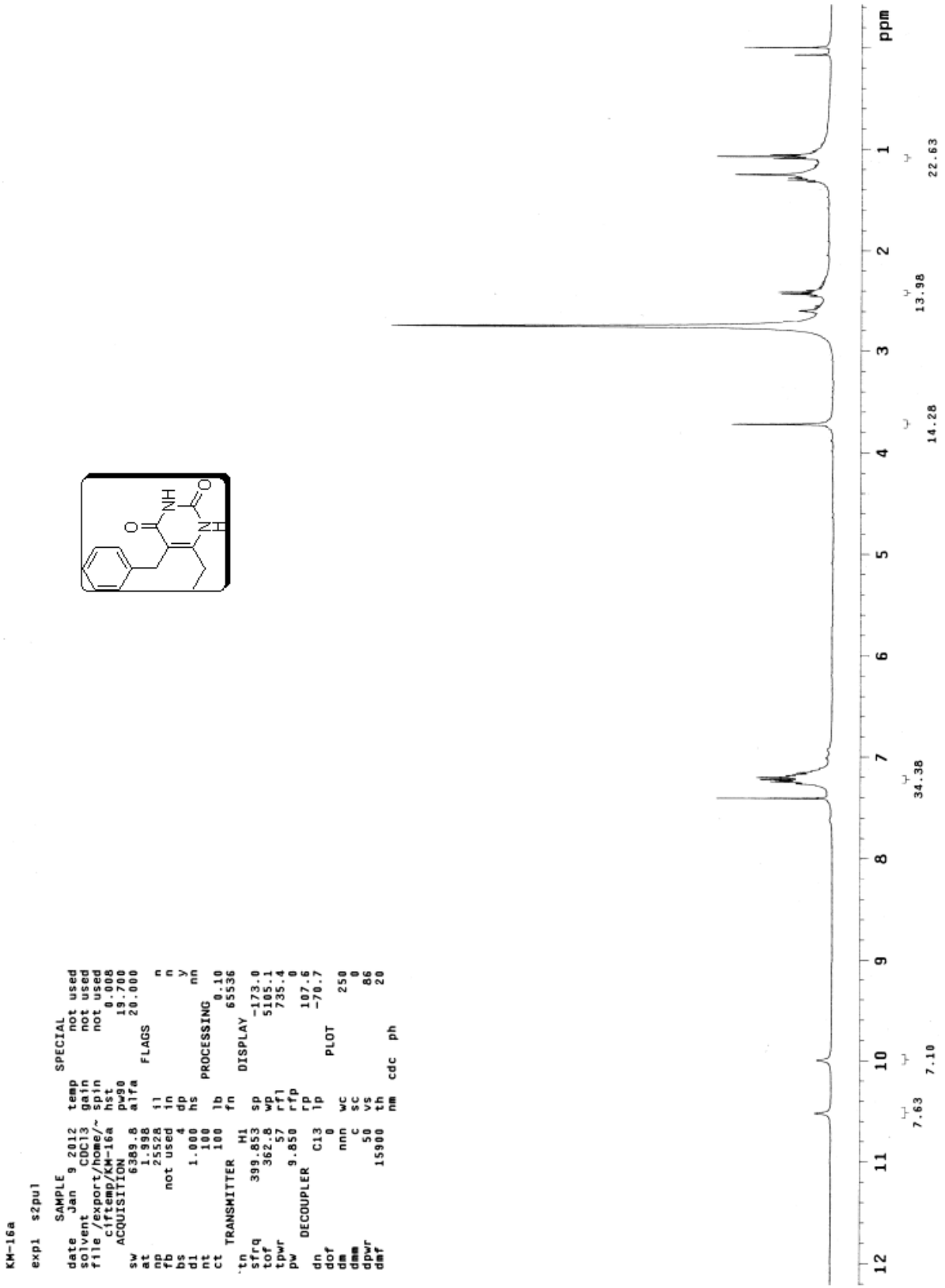
Compound 6

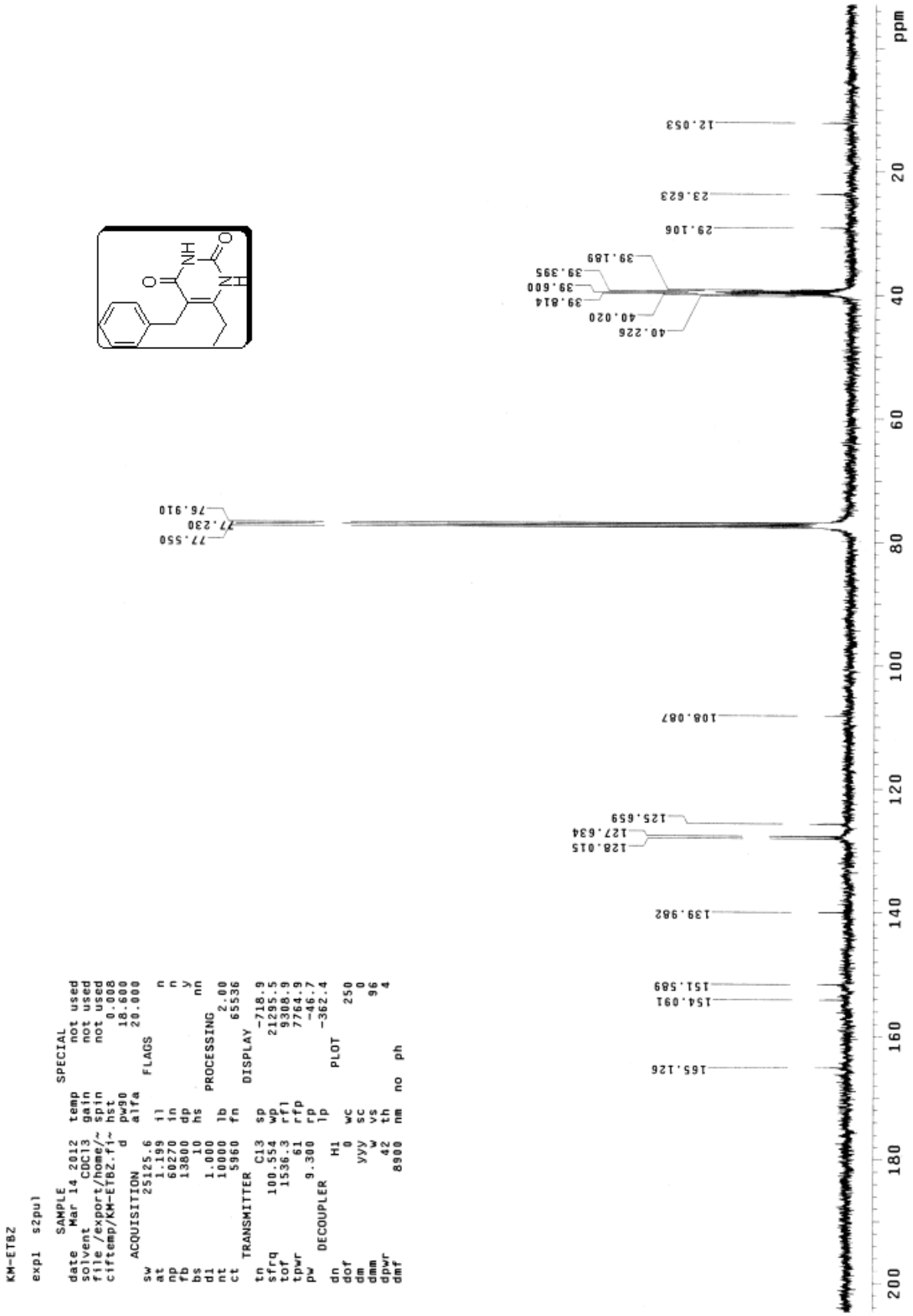
D-H···A	$d_{D...H(Å)}$	$d_{H...A(Å)}$	$d_{D...A(Å)}$	∠ D-H···A(°)	symmetry
N1 –H1M····S2	0.8600	2.5000	3.351(2)	171.00	x,-1+y,z
N2 –H2M ····O4	0.8600	1.9600	2.802(3)	166.00	
N3 –H3M ····S3	0.8600	2.4700	3.308(3)	166.00	1-x,1-y,1-z
N4 –H4M .. O1	0.8600	2.0500	2.872(3)	160.00	x,1+y,z
N5–H5M ····S4	0.8600	2.5300	3.387(2)	174.00	-1+x,y,z
N6–H6M ····O2	0.8600	1.9800	2.828(3)	169.00	1-x,1-y,1-z
N7–H7M ····S1	0.8600	2.5000	3.339(2)	166.00	
N8 –H8M ····O3	0.8600	1.9600	2.813(3)	173.00	1+x,1+y,z
C27 –H27B····O5	0.9600	2.5600	3.503(5)	167.00	

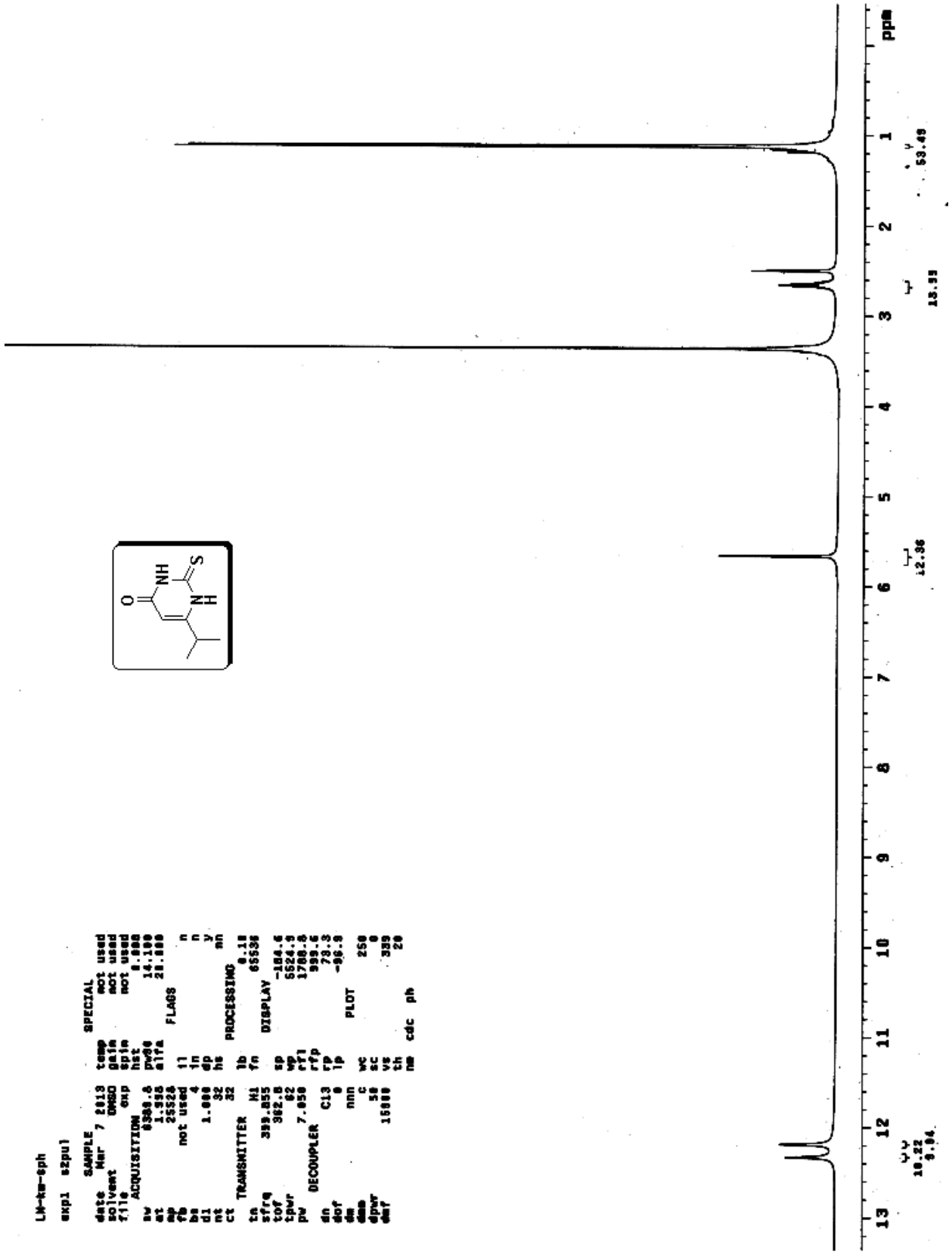


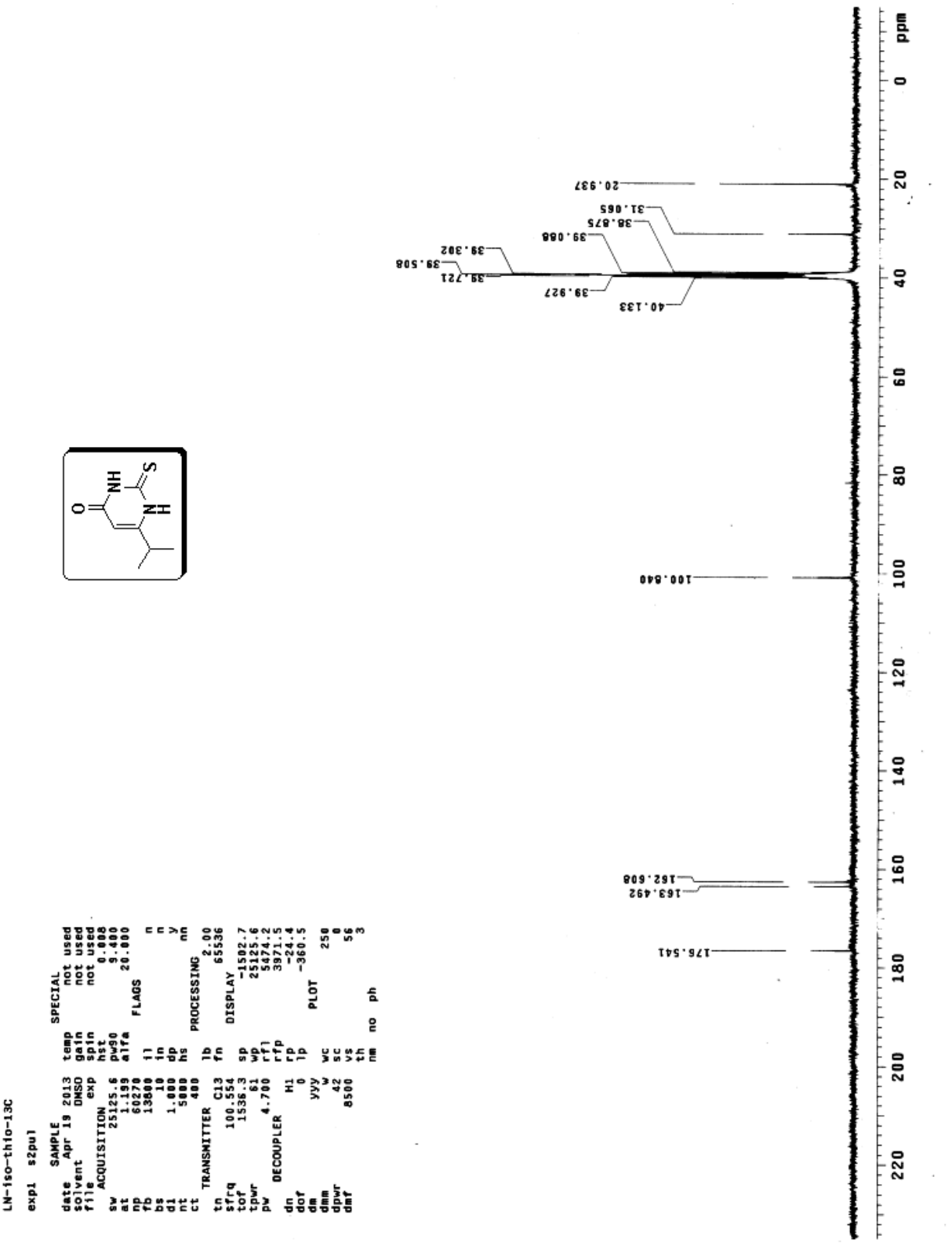


IV.8. selected spectra









CHAPTER-V

Synthesis of C-5 and C-6 substituted *bis*-pyrimidine dimers and their photochemical evaluations show remarkable stabilities under UVC radiation

V.1. Introduction

Gradual depletion of ozone layer and consequently increased ultraviolet (UV) radiation on the Earth's surface induces DNA-lesions inside the genome.[1] The effect of UV (200-290 nm) radiation in causing damages to genomic DNA is well-known and the mechanisms of formation of the photoproducts are well-established.[2-7] This is largely attributed to formation of dimeric photoproducts and oxidative lesions in DNA when exposed to UVC light.[8-11] Since DNA or RNA nucleobases mostly absorb light in the range of 250-270 nm, they become highly susceptible towards UVC light. The damages, if not repaired, leads to genomic mutations which are a major cause of various skin cancer, apoptosis and aging. Pyrimidine nucleobases are the prime targets that promptly undergo intra-strand dTpdT, dCpdC and dTpdC lesion formation,[12] although inter-strand dimeric lesions have also been reported.[13, 14] It is now widely accepted, as is illustrated is ***Fig V.1.1***, that *cis-syn* cyclobutane pyrimidine dimer (CPD) is formed as the major lesion via [2Π+2Π] cycloaddition along with highly mutagenic (6-4) photoproduct and Dewar lesion via Paternó-Büchi reaction, when thymine is exposed to UVC radiation.[15]

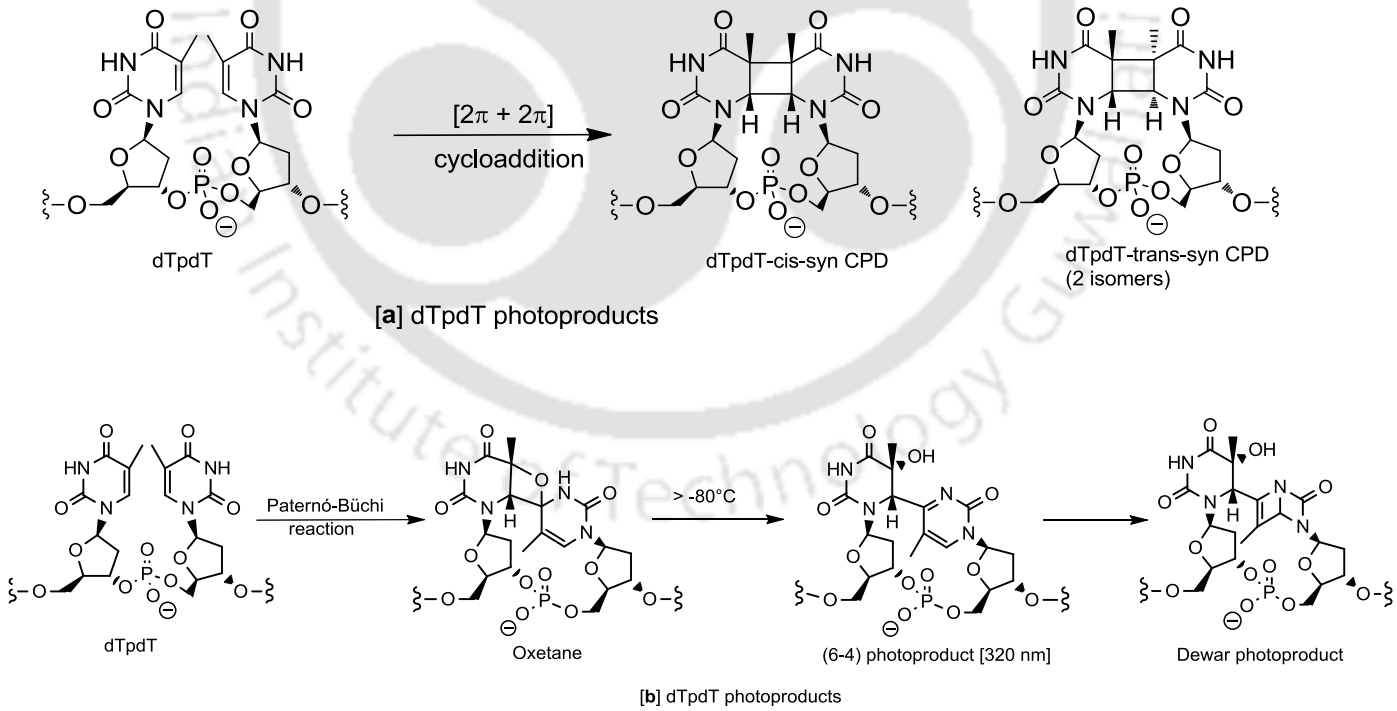


Fig V.1.1*: UV-Photodimers*

Since the past two decades, an extensive amount of research have been performed to understand

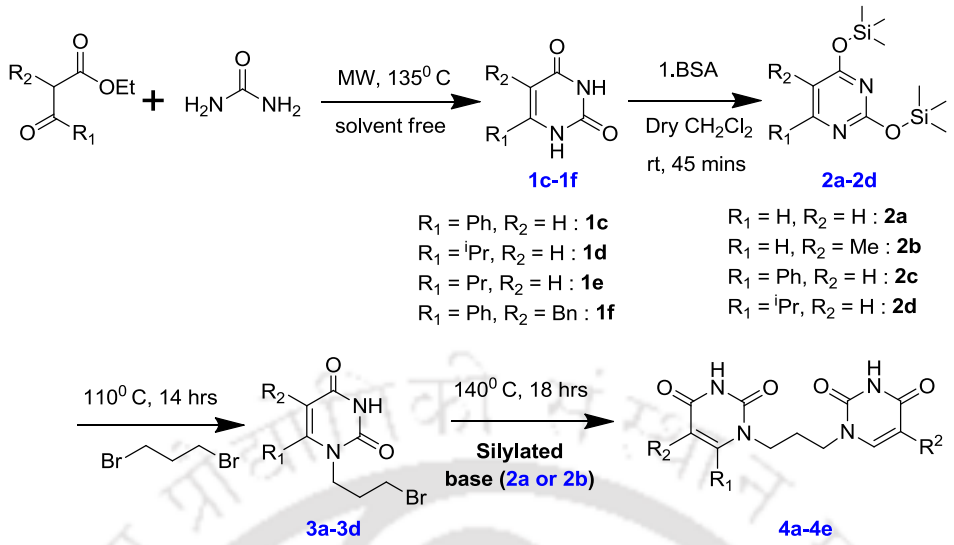
the chemistry, mechanistic pathway and repair mechanisms using free nucleobases and model dinucleotides,[16] excited state dynamics of the free pyrimidine nucleobases as well as that of DNA,[17-20] sequence and conformation dependence on photoproduct formation,[12, 21-23] multiplex detection method,[24] to mention just a few. Development of novel characterization techniques, such as LC-MS/MS for identification of new lesions by *Cadet* and *Douki* [25-28] and synthesis of model dimeric lesions for repair studies by *Carell* [29-31] have made significant contribution into this research area. Recently *Loppnow et al.* have used fluorescence based probes for quantitative estimation of various photolesions in DNA.[32-35]

However, systematic studies and propensity of photochemical reactions of synthesized, non-natural pyrimidine nucleobases have not been evaluated properly.[36-38] Synthesis of model compounds with modification of the nucleobases, in this regard, could be a useful approach to study the effect of substitution on photolesion formation.[39, 40]

V.2. Present work

In this chapter we demonstrate synthesis and comparison of photochemical behaviour of *bis*-pyrimidines, modified at C-5 and/or C-6 positions. A set of model homo- and hetero- *bis*-pyrimidines were synthesized with a propane backbone. Quantitative estimation of total amount of damage upon time-dependent UVC exposure unveils new and interesting results. A number of C-6 substituted uracil derivatives, both in free nucleobase form as well as in *bis*-pyrimidine form, exhibited remarkable stabilities under UVC radiation. To the best of our knowledge such nature of these pyrimidine analogues was not reported before and could have the potential applications as skin saving agents.

The homo- and hetero- *bis*-pyrimidine dimers **(4a-4e)** were synthesized according to ***Scheme V.2.1***



Scheme V.2.1: *Schematic route for the synthesis of bis-pyrimidine dimers (**4a-4e**). Nature nucleobases uracil (**1a**) and thymine (**1b**)*

Although a few protocols are reported for the synthesis of *bis*-pyrimidine dimers, most of them gave very poor yield, especially for C-6 substituted analogues.[39, 42] Earlier, uracil dimers with propane backbone were synthesized by direct alkylation using 1,3-dibromopropane in presence of base.[43] The method was improved by Falvey *et al.* where silylated pyrimidine was used to increase the nucleophilicity of the pyrimidine base.[36] However, synthesis of bis-pyrimidine dimers with modification at C-6 position of uracil was never reported. Regioselective nucleophilic reaction to N-1 of C-6 substituted uracil is indeed difficult to achieve, presumably due to steric hindrance. Reported silylating agents such as *trimethylsilyl chloride* and *hexamethyldisilazane* failed to produce the target *bis*-pyrimidine dimers in measurable quantity. In the present method, therefore, strong silylating agent, such as Bis(trimethylsilyl)acetamide (BSA) was employed. The reactions were found to proceed with moderate to high yield ***(Table V.2.1)*** and were fairly regioselective.

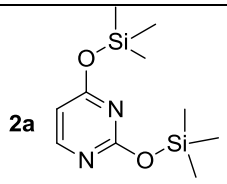
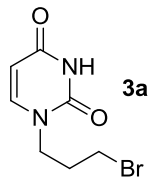
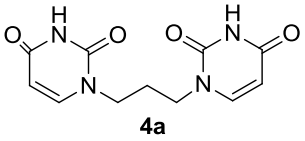
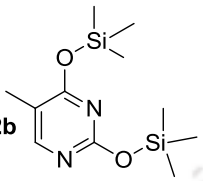
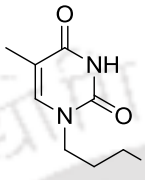
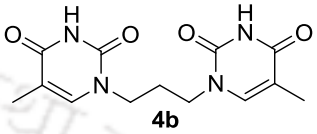
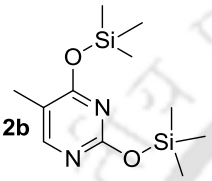
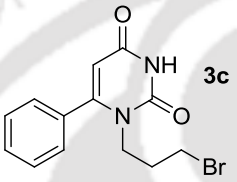
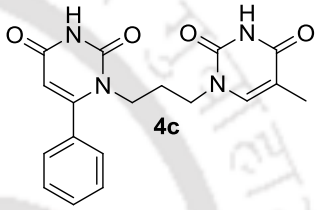
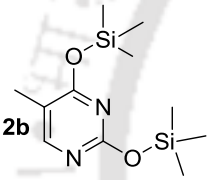
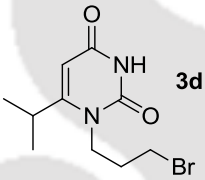
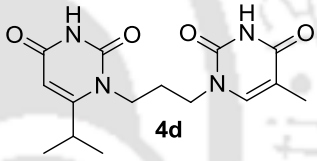
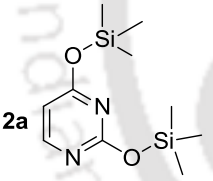
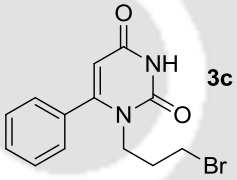
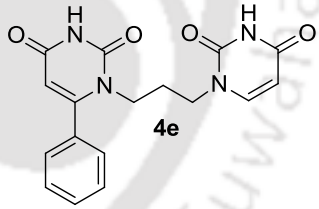
S.No.	Substrate (2a, 2b)	Substrate (3a-3d)	Yield (%)	Product (4a-4e)	Yield (%)
01	 **2a**	 **3a**	63	 **4a**	73
02	 **2b**	 **3b**	65	 **4b**	75
03	 **2b**	 **3c**	57	 **4c**	68
04	 **2b**	 **3d**	61	 **4d**	71
05	 **2a**	 **3c**	57	 **4e**	63

Table V.2.1: *homo-or hetero-model dinucleotodes*

From ***Table V.2.1*** it can be evident that a number of homo- and hetero- bis-pyrimidines have been synthesized with a variety of C-5 and C-6 substitution. 6-isopropyluracil and 6-phenyluracil were specifically chosen to impose both steric as well as electronic properties to the heterocyclic rings.

V.2.1. Study of UV-induced lesion formation

Photochemical damages of natural nucleobases and oligonucleotides are well studied and formation of photoproducts was attributed to loss of UV absorption due to loss of aromaticity of the heterocycles. Herein, time dependent UV irradiations were performed under 254 nm lamp (8W*5) in a closed chamber. Total amount of degradation was quantified by measuring the loss of UV absorption as well as by HPLC (peak area integration). First of all assessment of photo-degradation of the free nucleobases were performed. Time dependent degradation ***(Figure V.2.1.2)*** of a set of C-5 and C-6 substituted uracils (200 μM, 2% DMSO in H_2O) were carried out under UVC dose. As can be evident, while the free uracil nucleobase rapidly degraded into photoproducts, most of the synthesized C-6 substituted analogues were found to be highly photo-stable. ***Figure V.2.1.2*** demonstrates time of irradiation versus absorbance plot at the respective λ_{max} values of the free nucleobases. As can be noted that both the aromatic and alkyl substituted pyrimidines at C-6 position were inert to lesion formation, independent on their stereo-electronic characters. Similar results were obtained from two other analogues, 5-dodecyl-6-isopropyluracil and 5-naphthyl-6-phenyluracil which also show high stabilities upon exposure to 254 nm.

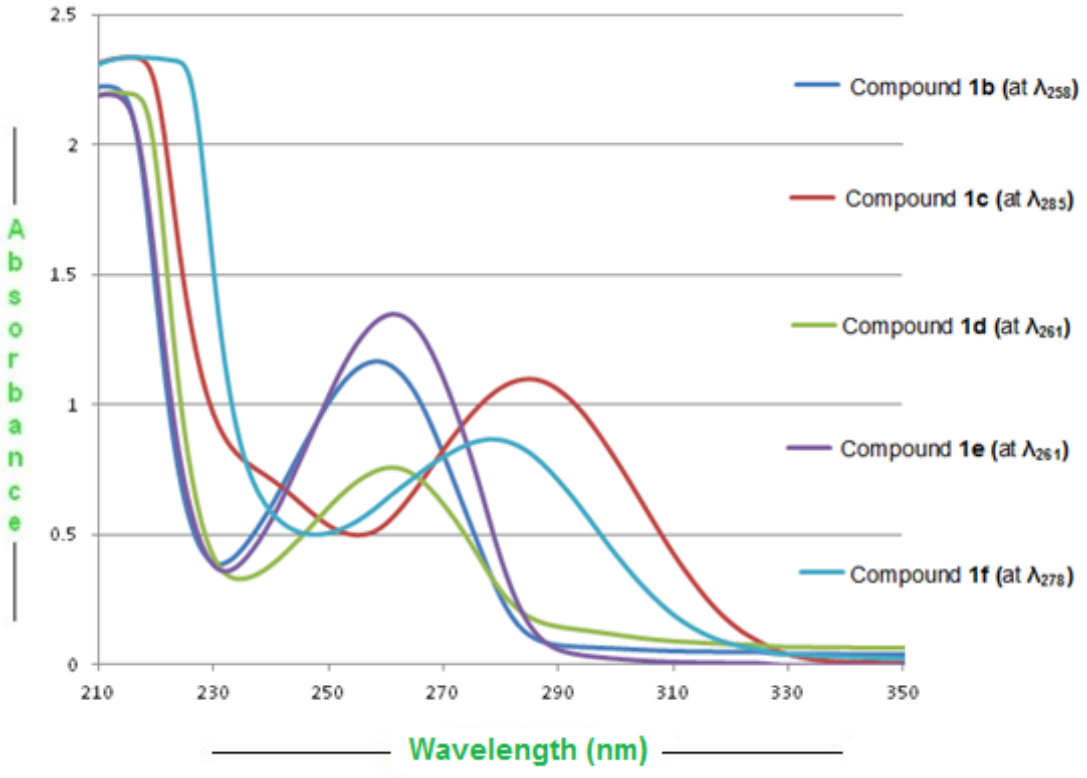


Figure V.2.1.1: *UV data of the free nucleobases.*

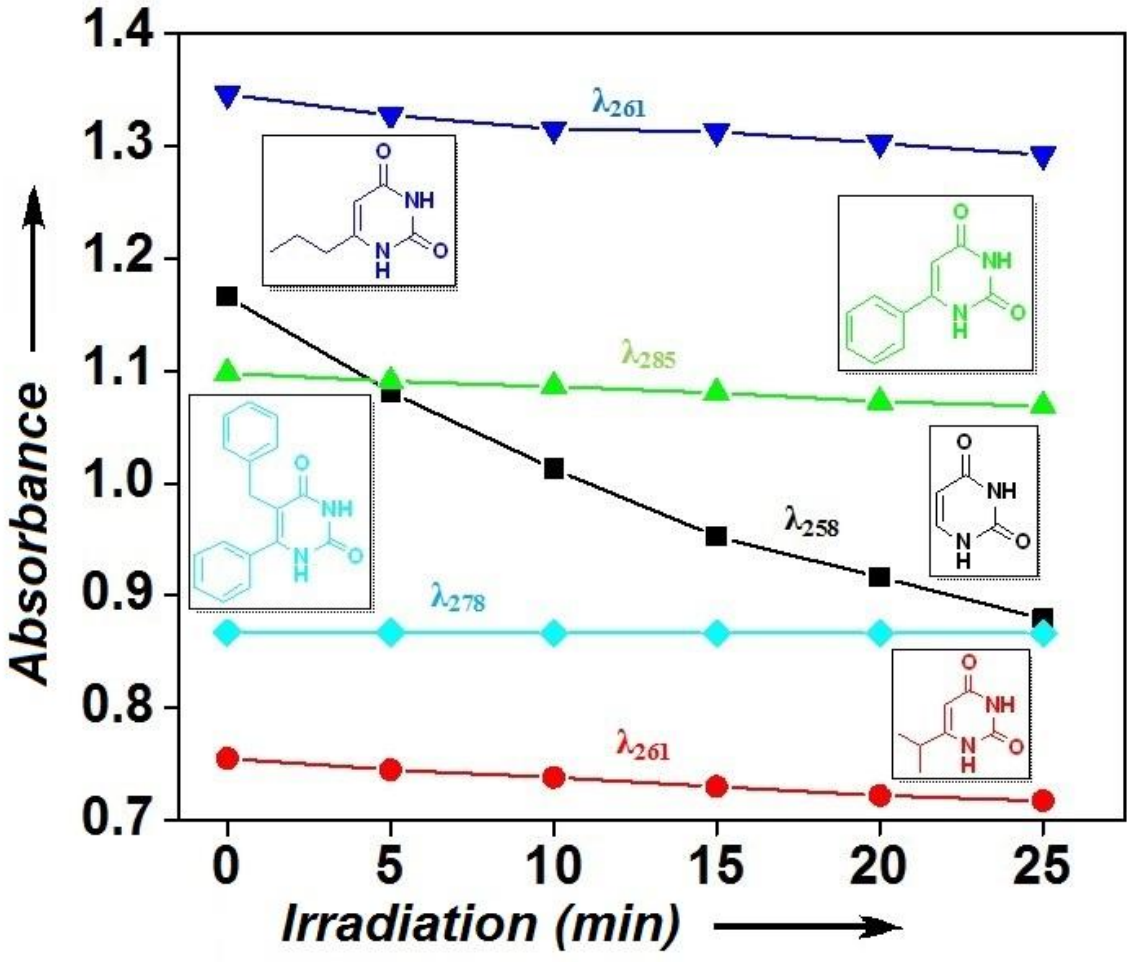


Figure V.2.1.2: *Gradual loss of UV absorbance of the free nucleobases (200 µM, 2% DMSO in H_2O) measured at their λ_{max} values*

This could further be clarified when the homo- and hetero- bis-pyrimidine dimers were subjected to UVC irradiation (***Figure V.2.1.4***). A significant degradation was observed when solutions (100 µM, 2% DMSO in H_2O) of uracil and thymine dimers (**4a** and **4b**) were exposed to UVC light. The irradiation *vs* absorbance plot clearly indicates that bis-uracil compound undergoes rapid degradation (**4a** and **4b**) whereas compounds **(4c, 4d and 4e**), containing either phenyl or alkyl substitutions at C-6 positions are very much stable and undergoes only very slow degradation under the UV-radiation. ***Table V.2.2*** shows the overall degradation of the compounds estimated by HPLC analysis which match well with chromophore loss study in ***Figure V.2.1.4***

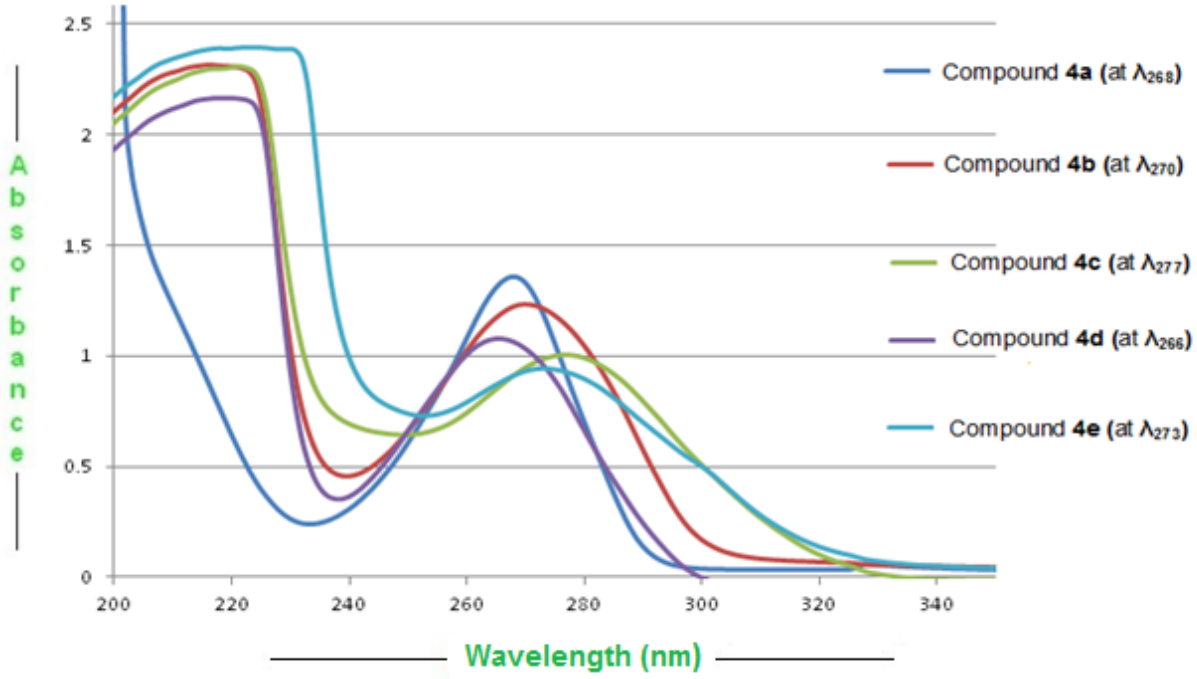


Figure V.2.1.3: *The UV-profile for the bis-pyimidines*

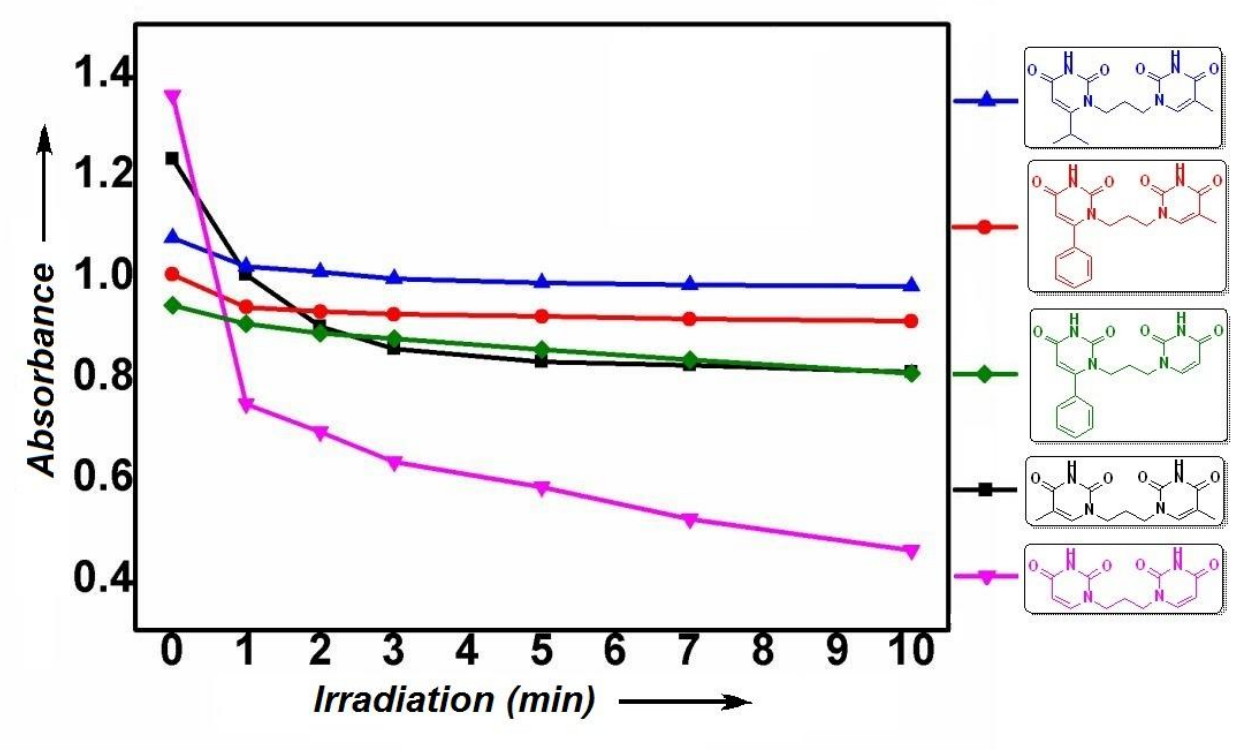


Figure V.2.1.4: *photo-degradation of the homo and hetero bis-pyrimidine dimers (**4a-4e**)*

That the degradations were indeed due to formation photo-dimeric lesions could be established

from HPLC and subsequent LC-MS analyses of the *bis*-pyrimidine dimers. HPLC profiles of **4b** and **4c** before and after 10 min of irradiation are shown in ***Figure V.2.1.5***. When the two dimers were irradiated, a new peak appeared in HPLC, at around 3 min for **4b** and around 1.4 min for **4c**, respectively. These peaks were isolated and subjected to LC-MS analyses which confirmed that the new peaks have identical *m/z* values as that of their un-irradiated counterparts. It should be mentioned here that the photodimers are formed mainly due to 2π+2π cycloaddition reactions and have identical mass as that of the parent dinucleotides. It was also established that the photodimers, due to change of polarity, should have different retention time compared to un-damaged dinucleotide.[11, 12] Therefore, the new peacks in the HPLC (at 3 min for **4b** and 1.4 min for **4e**), which show same m/z values as that of the un-irradiated **4b** and **4c**, should be due to formation of the dimeric lesions. Moreover, integration of peak area revealed that while about 33% damage occurred to **4b** upon irradiation, merely 7% lesions were formed for **4c (**data given in **V.6**.) This is also in good agreement with the calculations from ***Figure V.2.1.4***. Hence it could be inferred that presence of C-6 substitution drastically reduces the rate of photochemical degradation of the pyrimidine nucleobases.

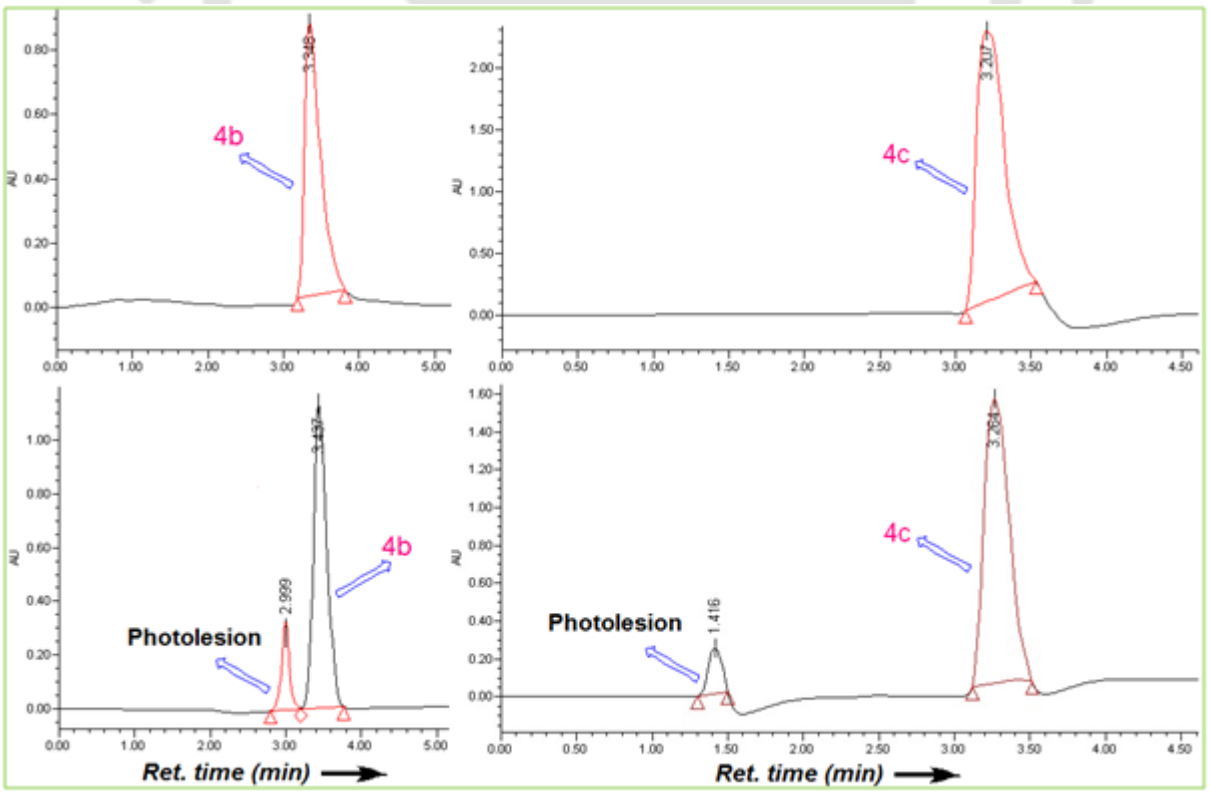


Figure V.2.1.5: *Representative HPLC profile of* ***4b*** *(left) and* ***4c*** *(right) before and after 10 min of irradiation under 254 nm lamp. Identity of the lesion was confirmed by LC-MS. Ascentis® C-18 (5 µm, 250×4.6 mm) column was used. Gradient: 5-30% of CH_3CN in H_2O in 0.1% formic acid in 8 min.*

Compound	λ_{max}	Irradiation (min) at 254 nm	% damage from UV absorption	% damage from HPLC profile
4a	268	10	66	64
4b	270	10	34	33
4c	277	10	8	7
4d	266	10	9	8
4e	273	10	13	12

Table V.2.2: *Quantitative estimation of 'total damage' of compounds* ***4a-4e****, obtained from HPLC analyses*

In conclusion, UV irradiation to pyrimidine nucleobases and DNA leads to photochemical lesions, mainly via [2Π+2Π] cycloadditions. With an aim to study the effect of various substitutions on photo-stabilities of the pyrimidine nucleobases, a set of homo and hetero *bis*-pyrimidine dimers, covalently linked through a propane backbone, were synthesized. The synthesized dimers contain C-5 and C-6 modified uracil analogues. A methodical estimation of total amount of damages, under UVC radiation, has been carried out. Very interestingly C-6 substituted uracil analogues, both in their free nucleobase form and as *bis*-pyrimidine dimers, show remarkable photo-stabilities under UVC light. Such pyrimidine compounds could have potential applications, *e.g*: as sunscreen agents, in preventing skin damage and aging.

V.3. Experimental Details

V.3.1. General Information: All chemicals were purchased from reputed pharmaceuticals and were used without further purification. All microwave-directed reactions were carried out in a *CEM Discover Labmate* closed vessel microwave reactor at 135-140°C. 1H NMR (400 MHz) and 13C NMR (100 MHz) were all recorded from a *DRX-400 Varian spectrometer* using $CDCl_3$ or DMSO-D_6 as solvents. Chemical shifts are reported in parts per million (ppm). Melting points

were determined using *Büchi B-545* apparatus and are uncorrected. Infrared (IR) spectra were recorded on a *Perkin Elmer Spectrum one* FT-IR spectrometer. High resolution mass spectrometry (HRMS) was analyzed from *Agilent Q-TOF 6500 LC/MS* system and *Micromass Q-TOF ESI-MS* instrument (model HAB 273). HPLC analysis was carried out with an Ascentis® C-18 analytical column (5 μm, 250×4.6 mm) coupled to a UV-visible detector whereas LC-MS was measured using shield RP18 (1.7 μm, 1 × 50 mm) column. HPLC grade solvents were used for HPLC analysis. The reactions were monitored by analytical TLC on Merck silica gel G/GF 254 plates. The column chromatography was performed with Merck silica gel (60-120 mesh). UV data were collected from a *PerkinElmer Lambda 25* double beam spectrophotometer.

V.3.2. Procedure for synthesis of compounds 1(c-f) to 4(a-e):

***A) General Procedure (compound 1c-1f)*:** As reported earlier, a β-ketoester (2 mmol), taken in a reactor vessel was mixed thoroughly for 1 min with urea (2.6 mmol) and $BF_3.Et_2O$ (2.4 mmol). The vessel was closed immediately and was subjected to microwave irradiation at about 135-140°C. The compound (**1c-1f**) was further purified by column chromatography.[41]

B) Representative Procedure for synthesis of compound 2c: Bis(trimethylsilyl)acetamide (BSA, 1.22 g, 6 mmol) was added to a solution of 6-phenyluracil (**1c**, 376 mg, 2 mmol) in dichloromethane (4 ml) under inert atmosphere at room temperature. Then the reaction mixture was stirred for 45 min until the solid mixture turns to clear liquid. This indicates that silylation was completed. The reaction mixture was directly used for the next step without purification. Silylated pyrimidines were found to be highly sensitive to moisture and exposure of the reaction mixture in open air readily reverted it back to the reactant. This methodology was also applied to synthesize compounds **2a-2d**.

C) Representative Procedure for compound 3c: In a 25 ml two-neck round bottom flask, 332 mg (1 mmol) of **2c** in 1.7 ml of 1,3-dibromopropane (16 mmol) was taken and stirred at 110°C under nitrogen atmosphere for 14 hours. The completion of the reaction was monitor by TLC. Then it was poured into 60 ml of water and extracted in CH_2Cl_2. The organic layer was dried over $MgSO_4$. The solvent was removed by rotary evaporation and excess of 1,3-dibromopropane

was removed by high vacuum pump. The compound was purified from crude reaction mixture through column chromatography by using 50-60% ethyl acetate in hexane. Compounds **3a-3d** was all synthesized following the same procedure.

D) Procedure for compound 4c: 154 mg (0.5 mmol) of **3c** was taken in a dry, two-neck round bottom flask in CH_2Cl_2 (2 ml). Then the reaction mixture was stirred for 5 min followed by the addition of freshly prepared silylated compound (**2b**, 540 mg, 2 mmol) and stirred for more than 16h at 140°C under nitrogen atmosphere. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was concentrated under reduced pressure and the excess BSA was removed under high vacuum. The residue was purified by column chromatography (20% $CHCl_3$/MeOH). Other bis-pyrimidine dimers (**4a-4e**) were synthesized accordingly.

UVC irradiation and quantification of total damage: all UV irradiations were performed in a closed chamber equipped with a 254 nm lamps (5*8W). For the free nucleobases, about 1 ml solution (200 μM, 2% DMSO in H_2O) was taken in a UV quartz cuvette and was subjected to irradiation while slow stirring. For each nucleobases (1a-1d) UV absorbance (at the λ_{max}) was measured at a fixed time interval. Similarly, about 100 μM solution of 4a-4e was exposed to UV light and time-dependent loss of UV absorbance (at the λ_{max}) was studied.

V.4. Characterization data:

6-phenylpyrimidine-2,4(1H,3H)-dione (1c):

Yield: 92%, White powder, m.p: 260-264°C, ^{1}H NMR (400 MHz,$CDCl_3$+DMSO-d_6) δ 10.64 (s, 2H), 7.40-7.20 (m, 5H), 5.52 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6+$CDCl_3$) δ 164.1, 152.3, 151.6, 131.1, 130.3, 128.1, 126.0, 97.7. FT-IR (KBr ν/cm^{-1}) 3407, 3166, 3001, 2924, 1717, 1651, 1487, 1451, 1236, 1037. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{10}H_9N_2O_2$: 189.0659; observed: 189.0662.

6-isopropylpyrimidine-2,4(1H,3H)-dione (1d):

Yield: 85%, White powder, m.p: 263-265 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.75 (s, 1H), 5.30 (s, 1H), 2.53 (m, 1H), 1.12(d, 6H, *J*= 6.8 Hz).

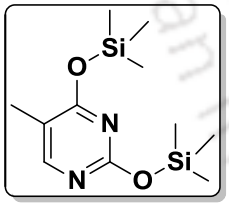
6-propylpyrimidine-2,4(1H,3H)-dione (1e):

Yield: 83%, White solid, m.p: 268-272°C, ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ 10.67 (s, 1H), 10.24 (s, 1H) 5.32 (s, 1H), 2.27 (t, 2H, *J*= 7.4 Hz), 1.62 (m, 2H), 0.97 (t, 3H, *J*= 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6) δ 164.7, 156.4, 152.1, 98.4, 34.1, 20.2, 13.0. FT-IR **(**KBr ν/cm^{-1}) 3426, 3342, 2959, 2926, 2854, 1727, 1698, 1508, 1609, 1426, 1354, 1084, 541. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_7H_{11}N_2O_2$: 155.0776; observed: 155.0780.

5-benzyl-6-phenylpyrimidine-2,4(1H,3H)-dione (1f):

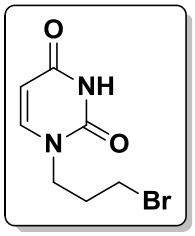
Yield: 55%, White solid, m.p: 210-215°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 11.17 (s, 1H), 7.98 (d, 2H, *J*= 8.4 Hz), 7.71 (d, 2H, *J*= 7.2 Hz), 7.53-7.49 (m, 3H), 7.36-724 (m, 3H), 3.80 (s, 2H). FT-IR (KBr ν/cm^{-1}). 3436, 3219, 2914, 2853, 1741, 1724, 1469, 1053. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{17}H_{15}N_2O_2$: 279.1128; observed: 279.1135.

5-methyl-2,4-bis((trimethylsilyl)oxy)pyrimidine (2b)



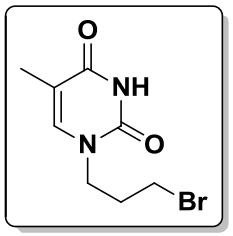
Yield: 100%, Clear liquid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 1.97 (s, 3H), 0.093 (s, 9H), 0.074 (s, 9H).

1-(3-bromopropyl)pyrimidine-2,4(1H,3H)-dione (3a):



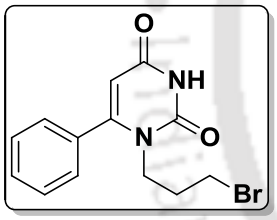
Yield: 63%, White solid, m.p: 100-104°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 1H), 7.30 (d, 1H, *J*= 7.6 Hz), 5.75 (d, 1H, *J*= 8.0 Hz), 3.94 (t, 2H, *J*= 6.4 Hz), 3.46 (t, 2H, *J*= 6.4 Hz), 2.31-2.24 (m, 2H).

1-(3-bromopropyl)-5-methylpyrimidine-2,4(1H,3H)-dione (3b):



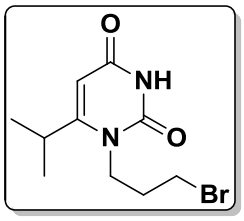
Yield: 65%, White solid, m.p: 134-136°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.04 (d, 1H, *J*= 1.6 Hz), 3.86 (t, 2H, *J*= 6.4 Hz), 3.41 (t, 2H, *J*= 6.4 Hz), 2.26-2.21 (m, 2H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 164.6, 151.2, 140.8, 111.1, 47.4, 31.3, 30.0, 12.4

1-(3-bromopropyl)-6-phenylpyrimidine-2,4(1H,3H)-dione (3c):



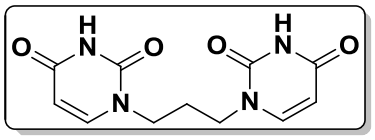
Yield: 57%, White solid, m.p: 142-146°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 7.61-7.50 (m, 5H), 5.99 (d, 1H, *J*= 1.6 Hz), 4.09 (t, 2H, *J*= 6.8 Hz), 3.44 (t, 2H, *J*= 6.8 Hz), 2.28-2.21 (m, 2H).

1-(3-bromopropyl)-6-isopropylpyrimidine-2,4(1H,3H)-dione (3d):



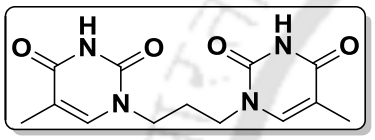
Yield: 61%, White solid, m.p: 160-165°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 5.57(d, 1H, 1.6 Hz), 4.04 (t, 2H, *J*= 7.2 Hz), 3.42 (t, 2H, *J*= 6.8 Hz), 2.65-2.58 (m, 1H), 2.23-2.16 (m, 2H), 1.25 (d, 6H, *J*= 7.2 Hz).

1-(3-(3,4-dihydro-2,4-dioxopyrimidin-1(2H) yl)propyl)pyrimidine-2,4(1H,3H)-dione (4a):



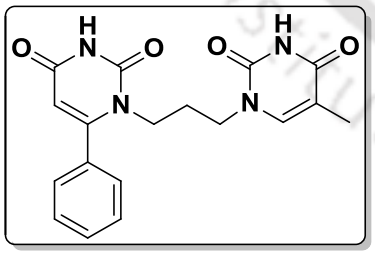
Yield: 73%, White solid, m.p: 285-288°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 2H), 7.66 (d, 2H, *J*= 8.0 Hz), 5.55 (d, 2H, *J*= 8.0 Hz), 3.63 (t, 4H, *J*= 6.8 Hz), 1.90 (m, 2H)**.** FT-IR (KBr ν/cm^{-1}). 3521, 3102, 2935, 2857, 2736, 1454, 1419, 1235, 998, 855, 544. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{11}H_{13}N_4O_4$: 265.0931; observed: 265.0817.

1-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)propyl)-5-methylpyrimidine-2,4(1H,3H)-dione (4b):



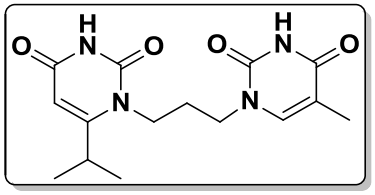
Yield: 75%, White solid, m.p: 260-265 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 2H), 7.50 (s, 2H), 3.62 (t, 4H, *J*= 6.8 Hz), 1.85 (m, 2H), 1.66 (s, 6H)**.** FT-IR (KBr ν/cm^{-1}). 3497, 3176, 2929, 2892, 2818, 1684, 1478, 1379, 1241, 1216, 1075, 876, 559. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{13}H_{17}N_4O_4$: 293.1244; observed: 293.1241.

1-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)propyl)-6-phenylpyrimidine-2,4(1H,3H)-dione (4c):



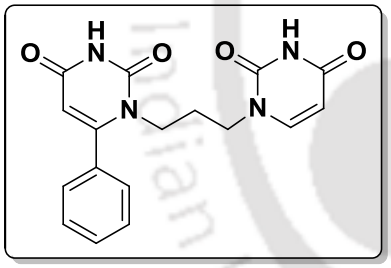
Yield: 68%, White solid, m.p: 251-256°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 11.21 (s, 1H), 7.72 (d, 2H, *J*= 6.8 Hz), 7.54-7.47 (m, 4H), 5.96 (s, 1H), 3.86 (t, 2H, *J*= 6.8 Hz), 3.72 (t, 2H, *J*= 6.8 Hz), 1.89-1.84 (m, 2H), 1.74 (s, 3H). FT-IR (KBr ν/cm^{-1}). 3619, 3526, 3214, 2943, 2810, 1749, 1451, 1421, 1026, 1247, 1210, 846, 557. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{18}H_{19}N_4O_4$: 355.1401; observed: 355.1401.

1-(3-(3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2H)-yl)propyl)-6-isopropylpyrimidine-2,4(1H,3H)-dione (4d):



Yield: 71%, White solid, m.p: 234-236°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 11.06 (s, 1H), 7.56 (s, 1H), 5.44 (s, 1H), 3.74 (t, 2H, *J*= 6.8 Hz), 3.64 (t, 2H, *J*= 6.8 Hz), 2.58 (m, 1H), 1.83-1.80 (m, 2H), 1.73 (s, 3H), 1.01 (d, 6H, *J*= 6.8 Hz), ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1, 165.6, 160.4, 151.7, 150.1, 141.6, 108.5, 95.3, 45.6, 37.4, 30.5, 27.0, 20.3, 12.0. FT-IR (KBr ν/cm^{-1}) 3512, 3209, 2929, 2813, 1762, 1489, 1450, 1247, 1209, 1026, 845, 559. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{15}H_{21}N_4O_4$: 321.1557; observed: 321.1551.

1-(3-(3,4-dihydro-2,4-dioxopyrimidin-1(2H)-yl)propyl)-6-phenylpyrimidine-2,4(1H,3H)-dione (4e):



Yield: 63%, White solid, m.p: 244-248°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (s, 1H), 11.22 (s, 1H), 7.74 (d, 2H, *J*= 6.8 Hz), 7.56-7.53 (m, 4H), 5.95 (s, 1H), 5.65 (s, 1H), 3.84 (t, 2H, *J*= 7.2 Hz), 3.68 (t, 2H, *J*= 6.8 Hz), 1.89-1.86 (m, 2H). FT-IR (KBr ν/cm^{-1}). 3617, 3523, 3211, 2942, 2814, 1745, 1454, 1422, 1022, 1249, 1208, 845, 553. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{17}H_{17}N_4O_4$: 341.1244; observed: 341.1253.

After irradiation (lesion) of compound 4b: LRMS (ESI) m/z $[M+H]^+$ calcd for $C_{13}H_{17}N_4O_4$: 293.1244; observed: 293.1663.

Compound 4c after irradiation (lesion): LRMS (ESI) m/z $[M+H]^+$ calcd for $C_{18}H_{19}N_4O_4$: 355.1401; observed: 355.1395.

V.5. References

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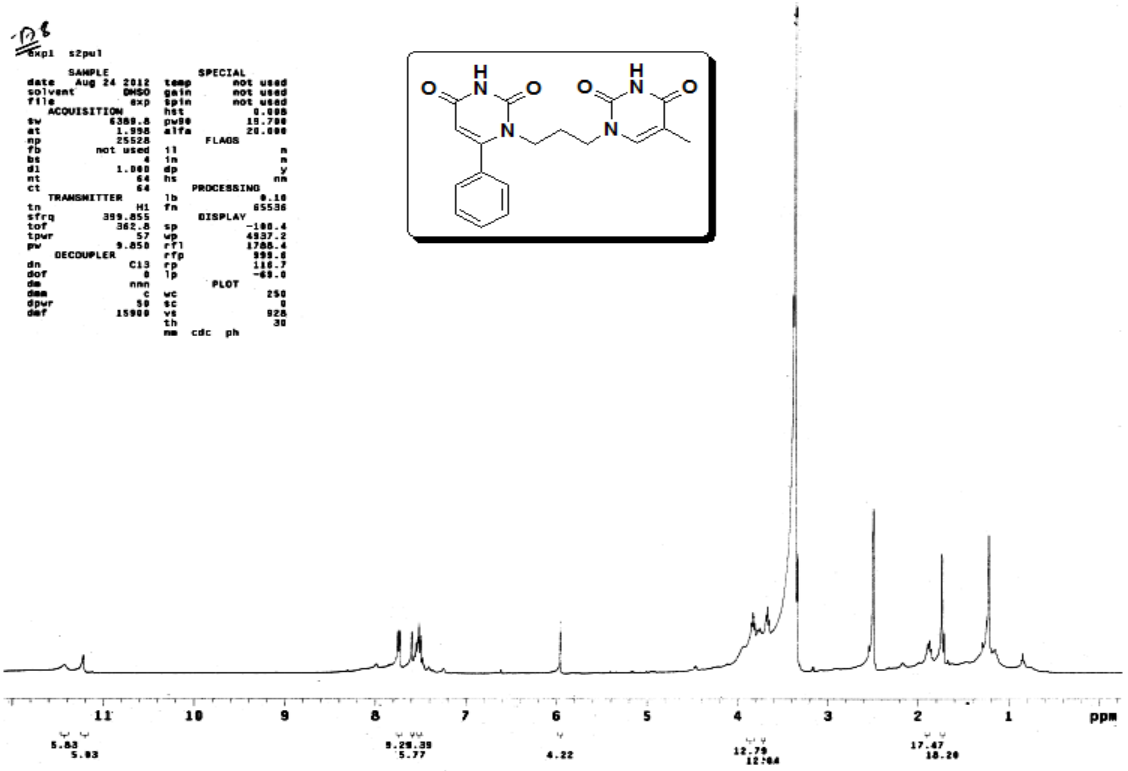
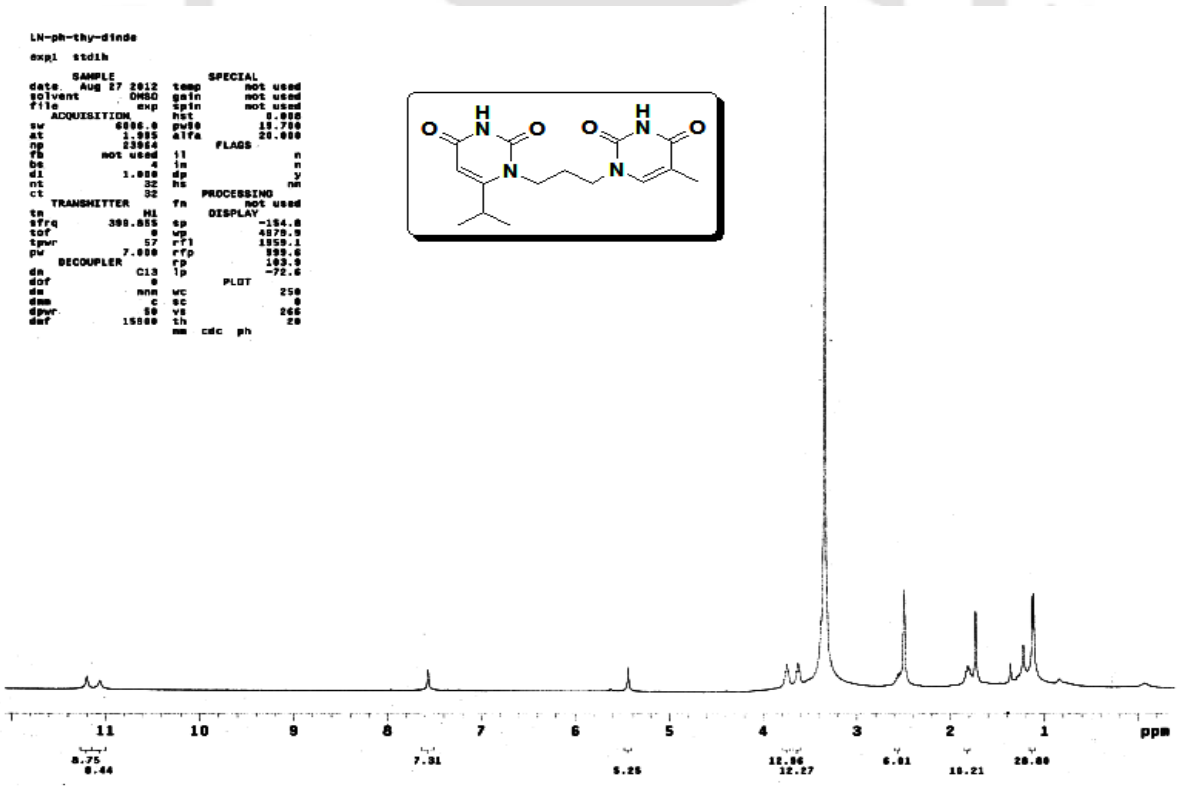
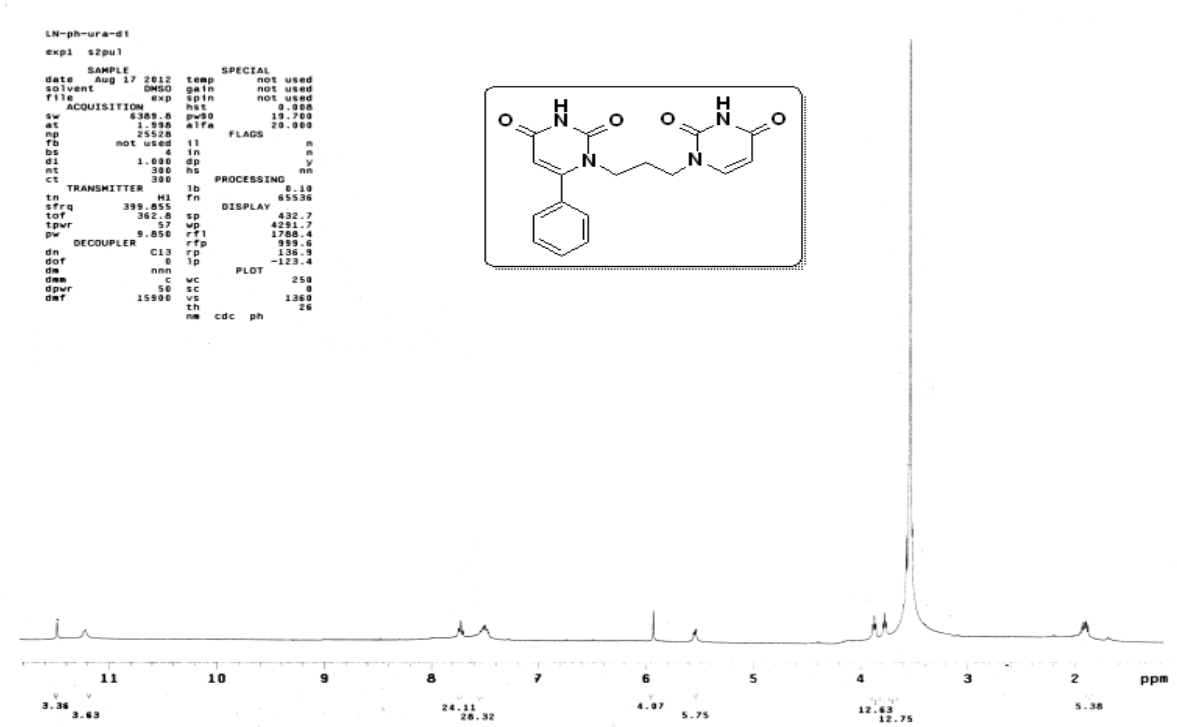
V.6. Selected spectra:

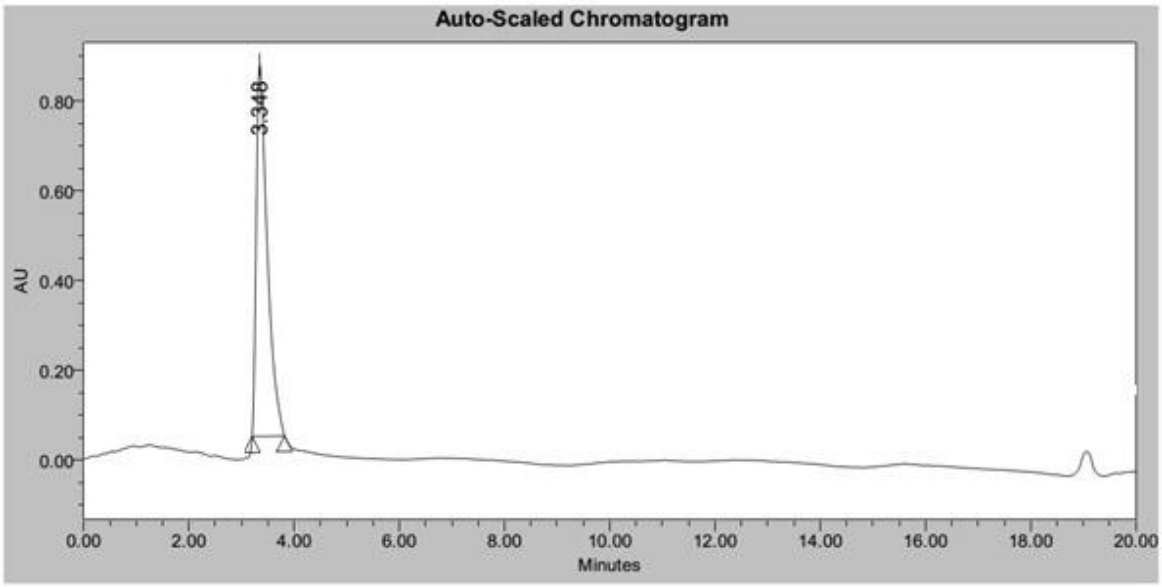






V.6.1. LCMS, HPLC and UV data

Compound (4b): a) Before UV irradiation:

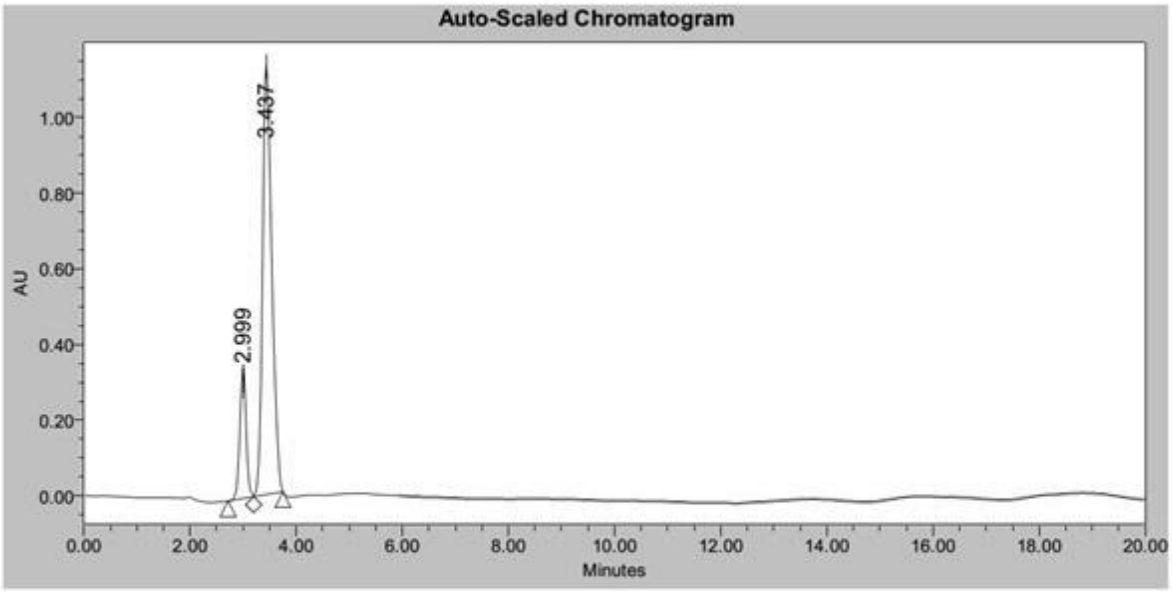


Peak Results

	Name	RT	Area	Height	% Area
1		3.348	12025073	828370	100.00

HPLC profiles of model dinucleotides, reverse phase, run time 20 min (linear gradient of 5 to 30% CH_3CN in H_2O with 0.1% formic acid up to 8 min, then 8 to 10 min 100% CH_3CN)

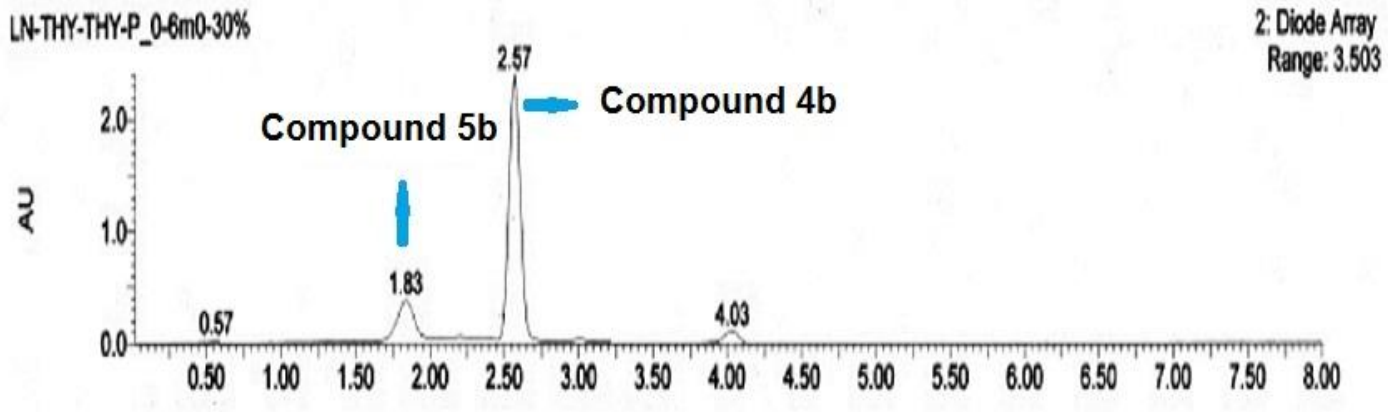
b) After UV irradiation:

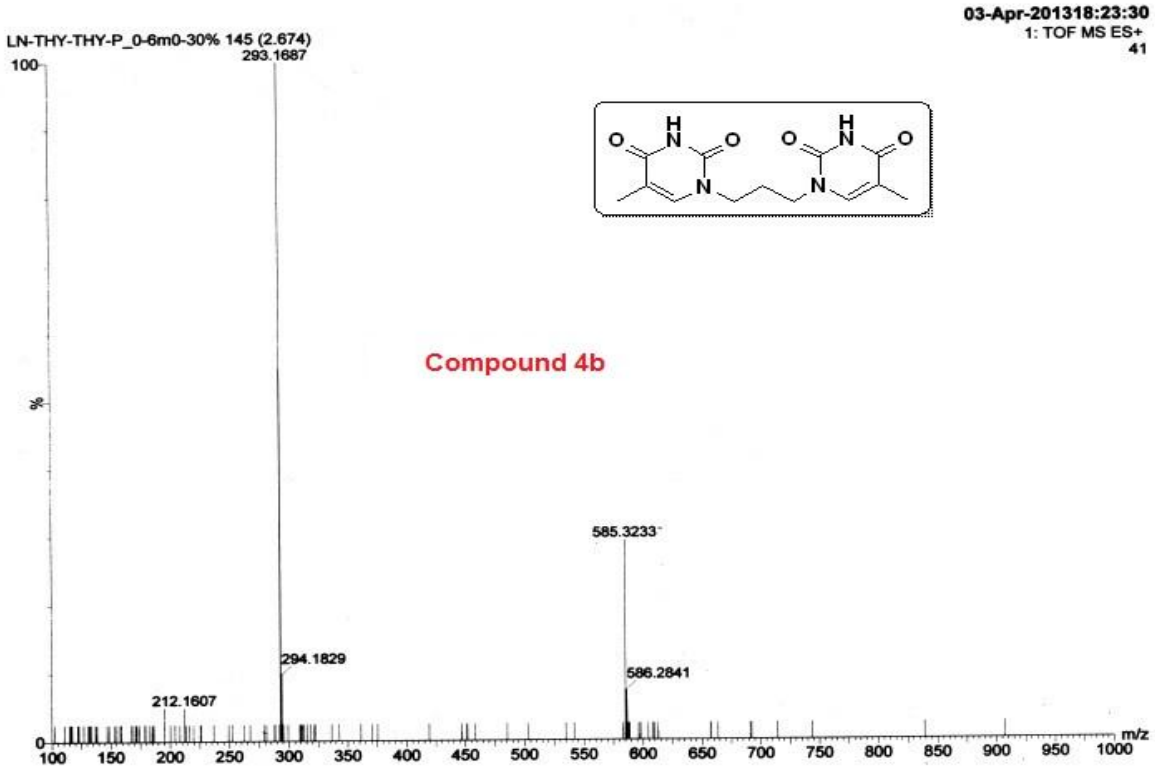


Peak Results

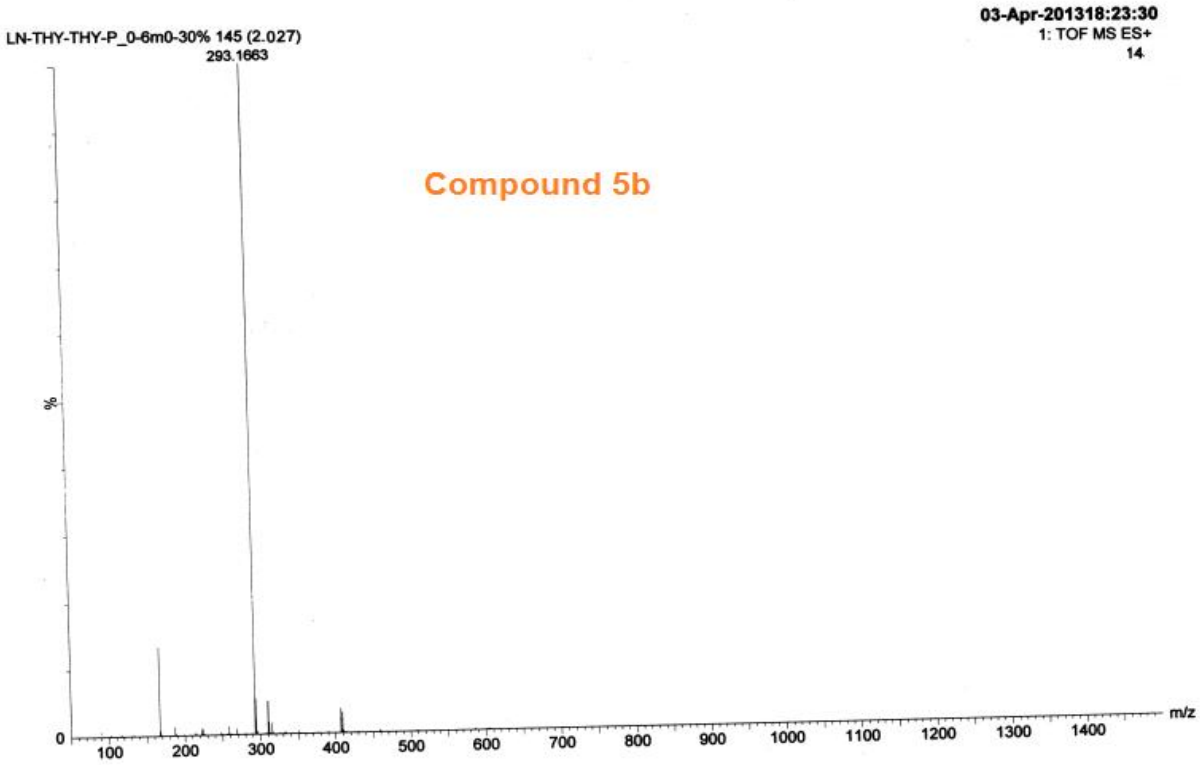
	Name	RT	Area	Height	% Area
1		2.999	1507786	163553	33.01
2		3.437	3059372	503205	66.99

b) After UV irradiation (LCMS):





Compound 4b

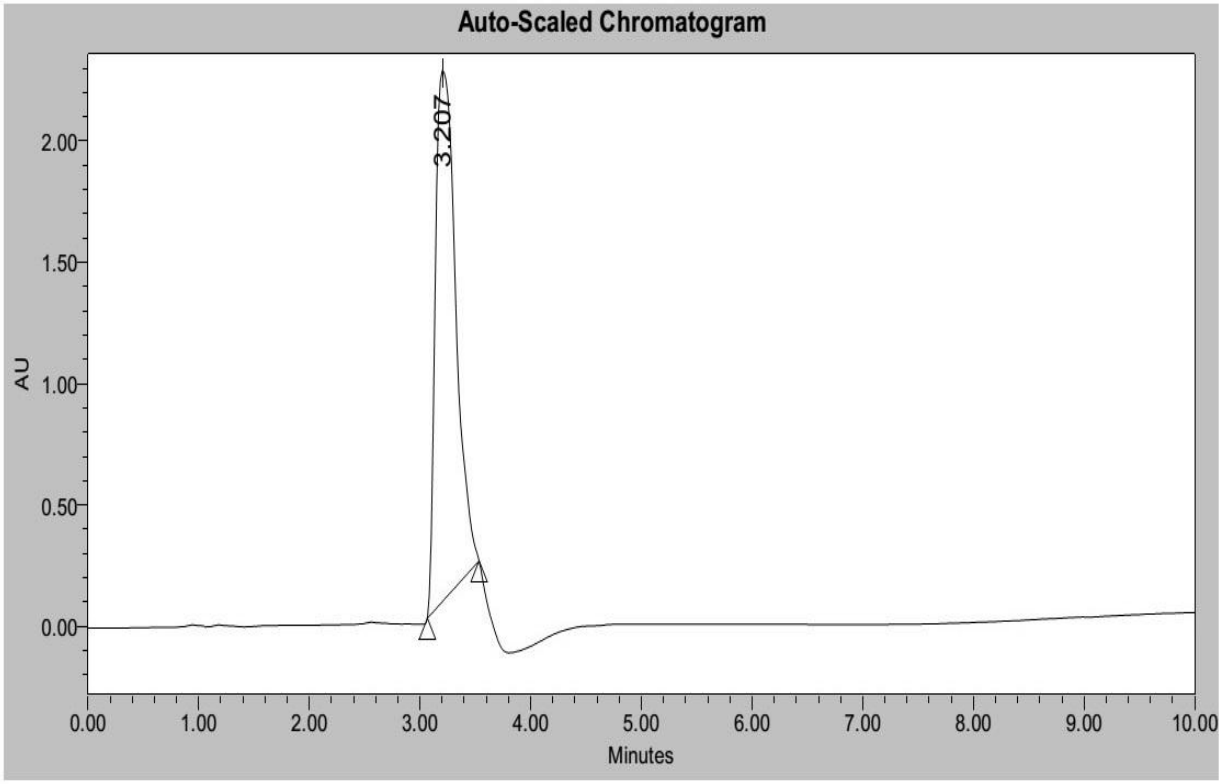


Compound 5b

Compound 4c:

HPLC profiles of **4c**, reverse phase, run time 10 min (linear gradient of 5 to 30% CH_3CN in H_2O with 0.1% formic acid up to 8 min, then 8 to 10 min 100% CH_3CN)

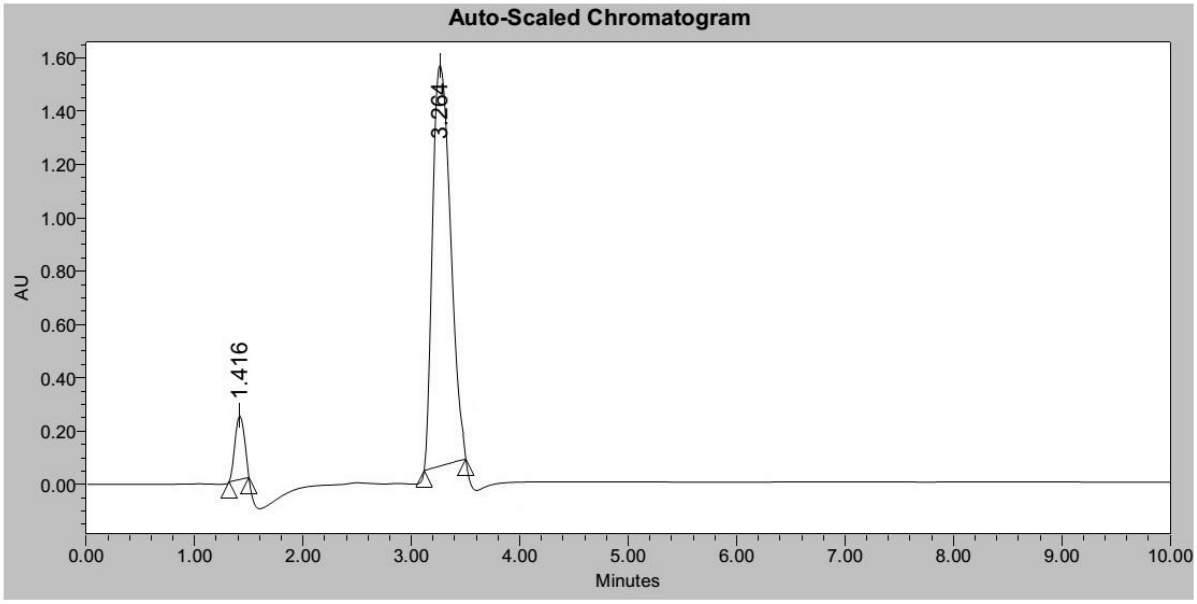
a) Before UV irradiation:



Peak Results

	Name	RT	Area	Height	% Area
1		3.207	28064816	2195411	100.00

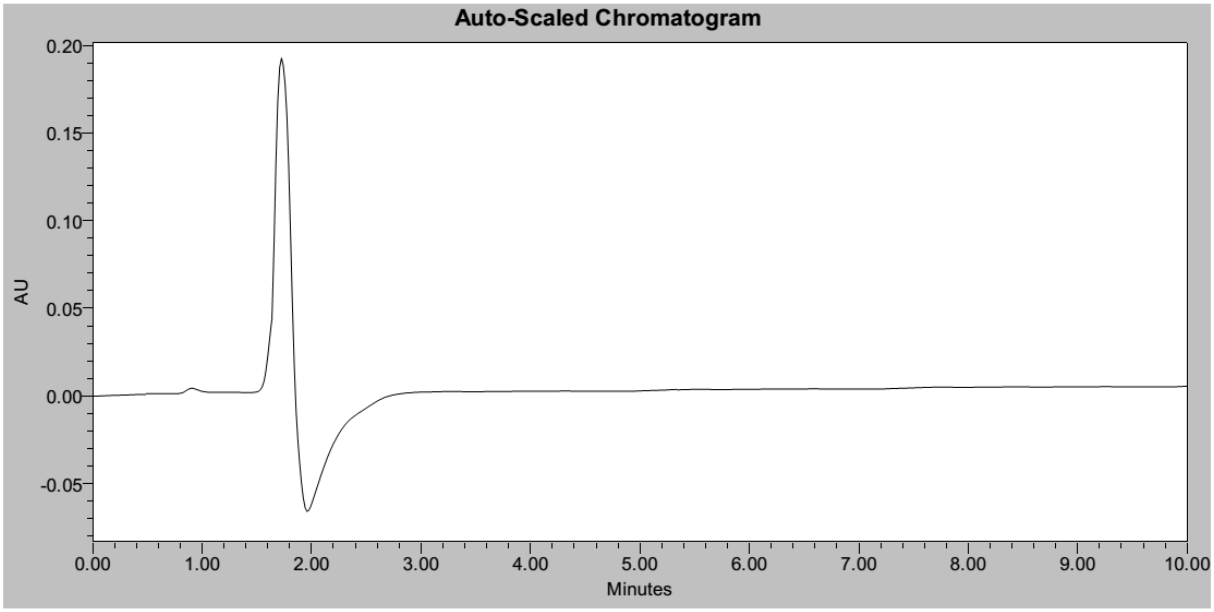
b) After UV irradiation:

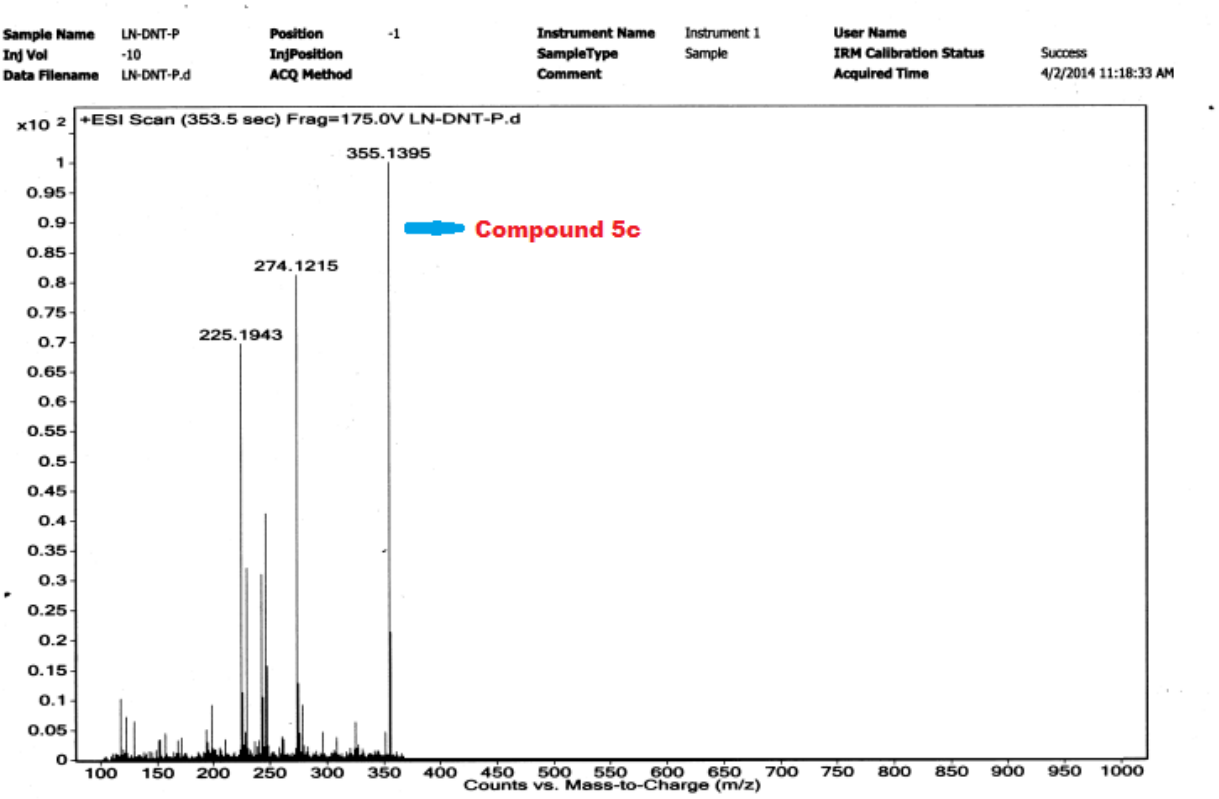


Peak Results

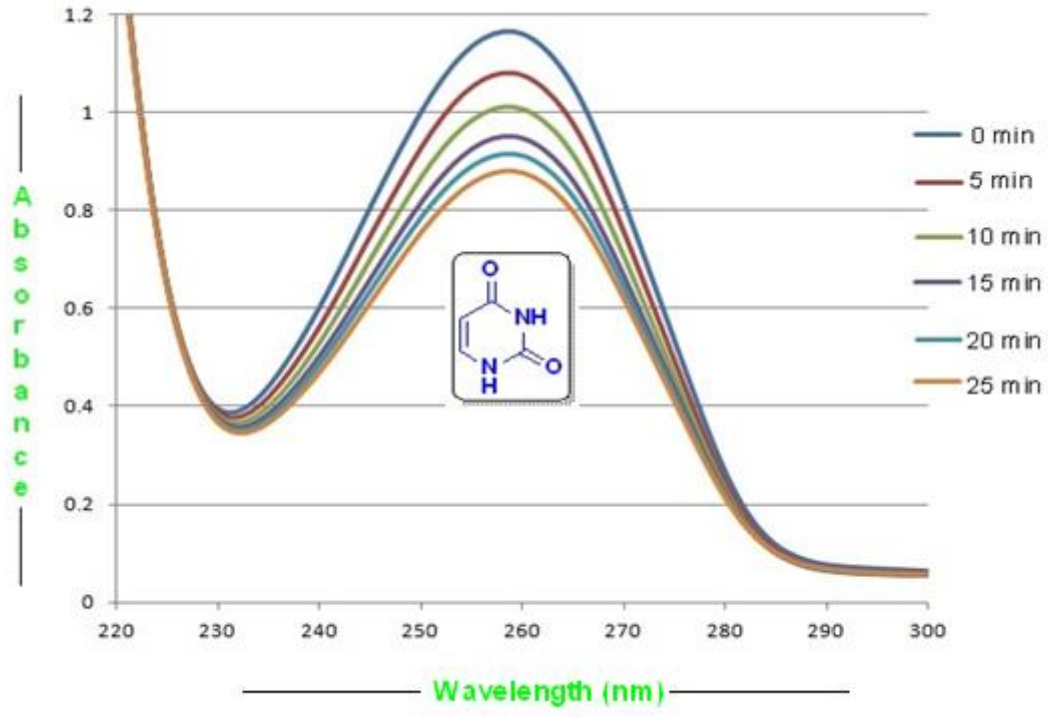
	Name	RT	Area	Height	% Area
1		1.416	1446293	239516	7.67
2		3.264	17421170	1506089	92.33

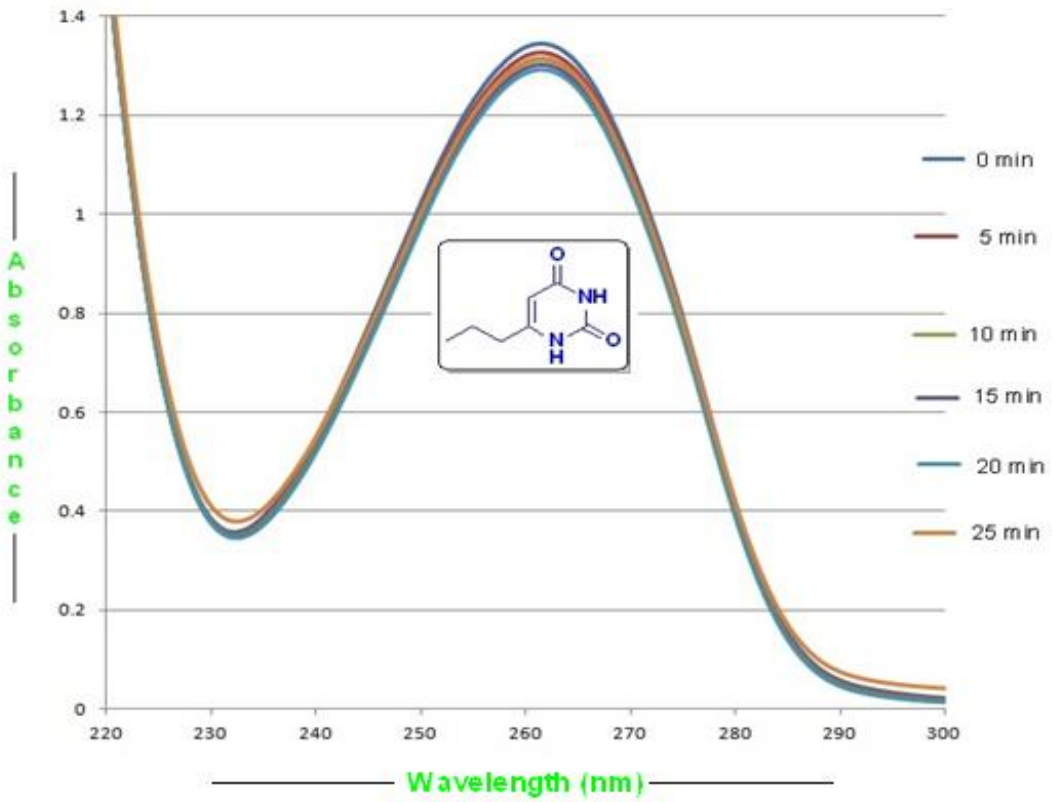
HPLC chromatogram for 5c:

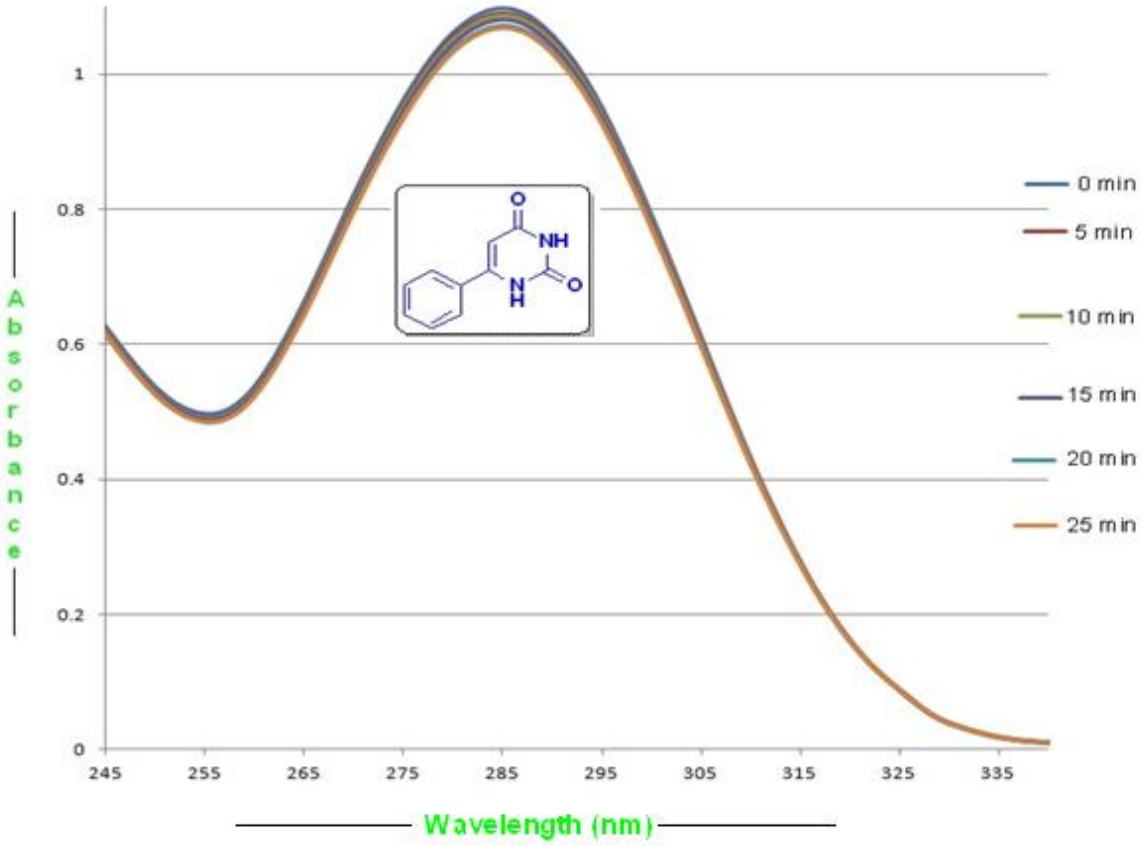


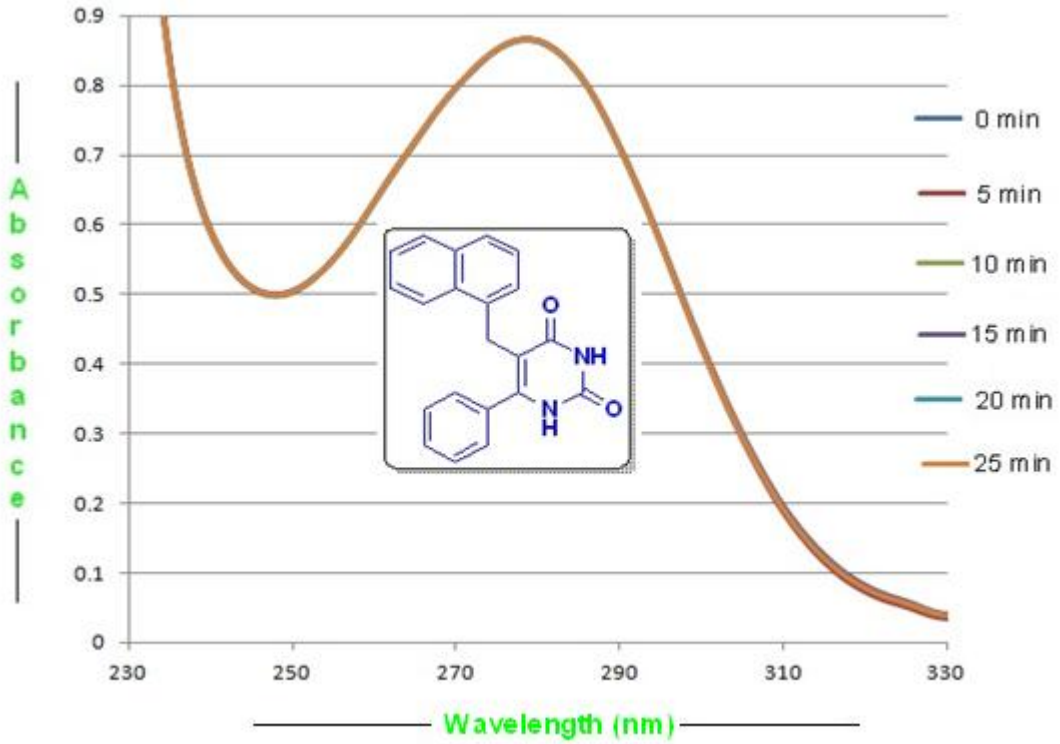


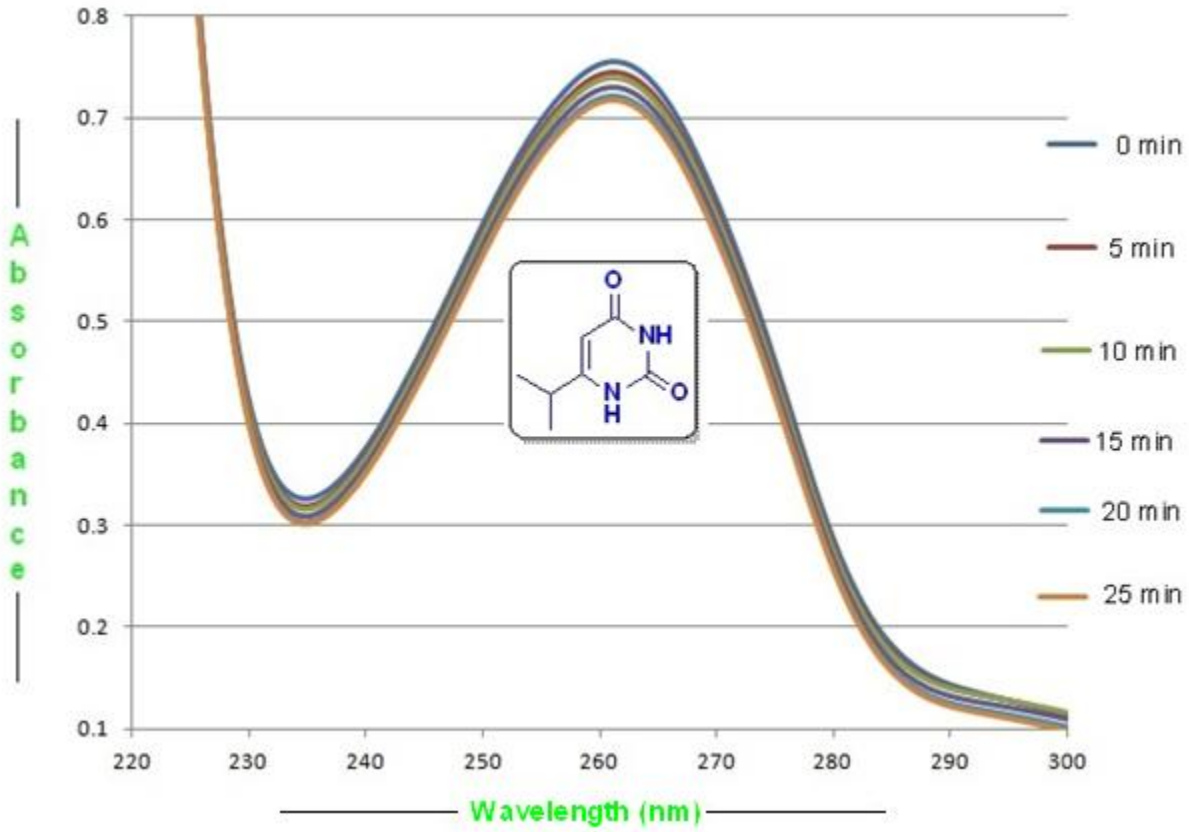
UV spectra of free nucleobases:



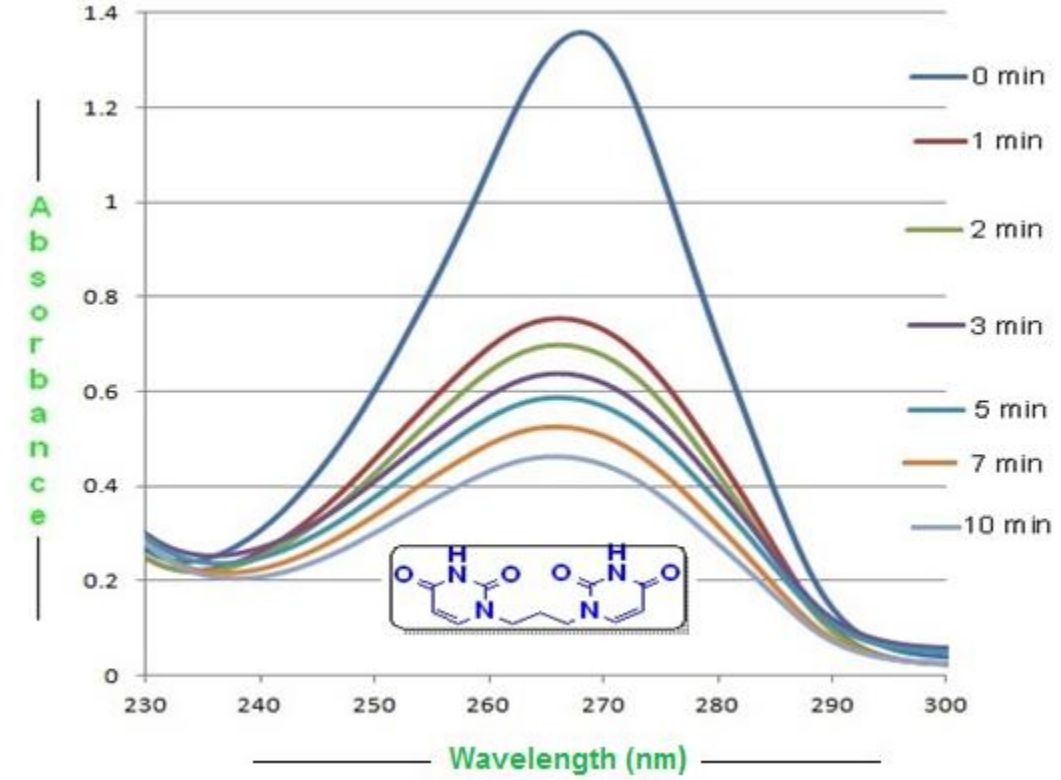


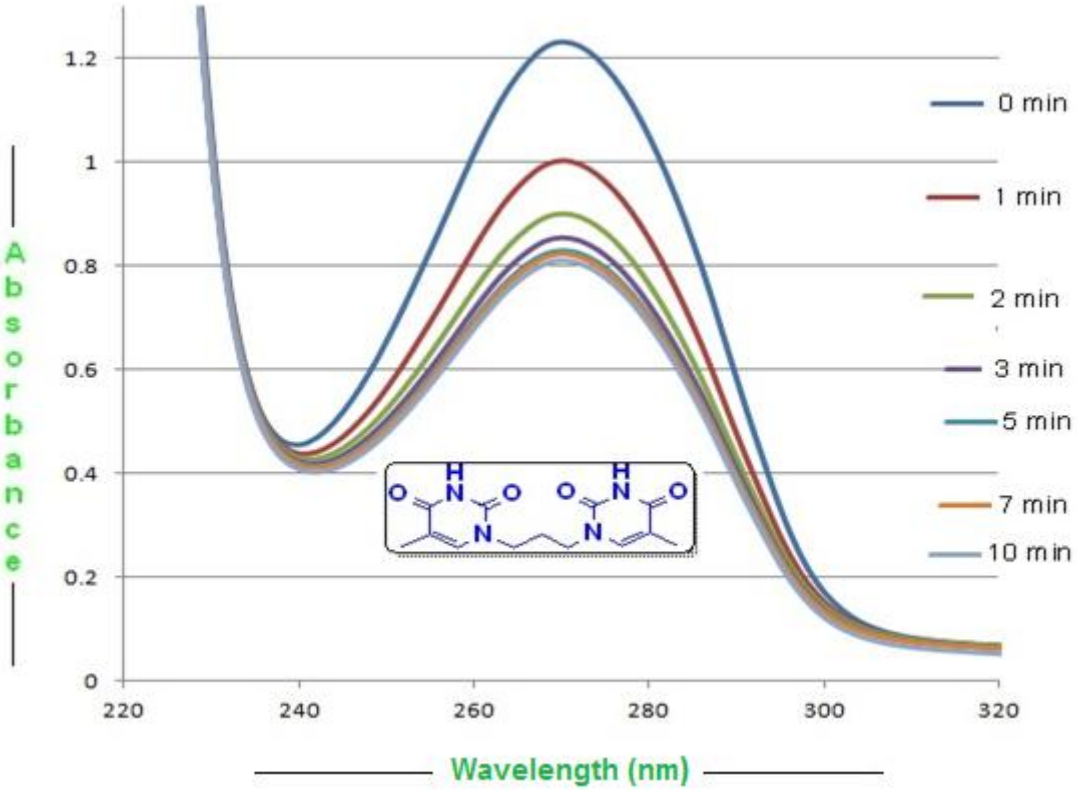


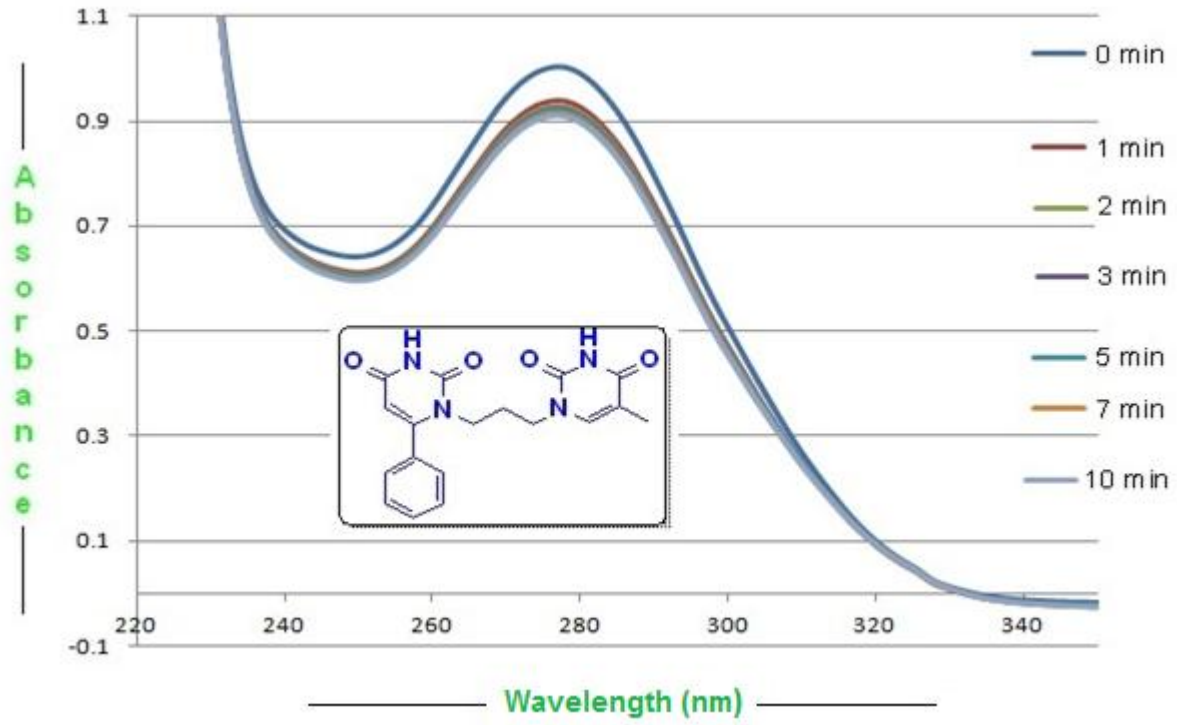


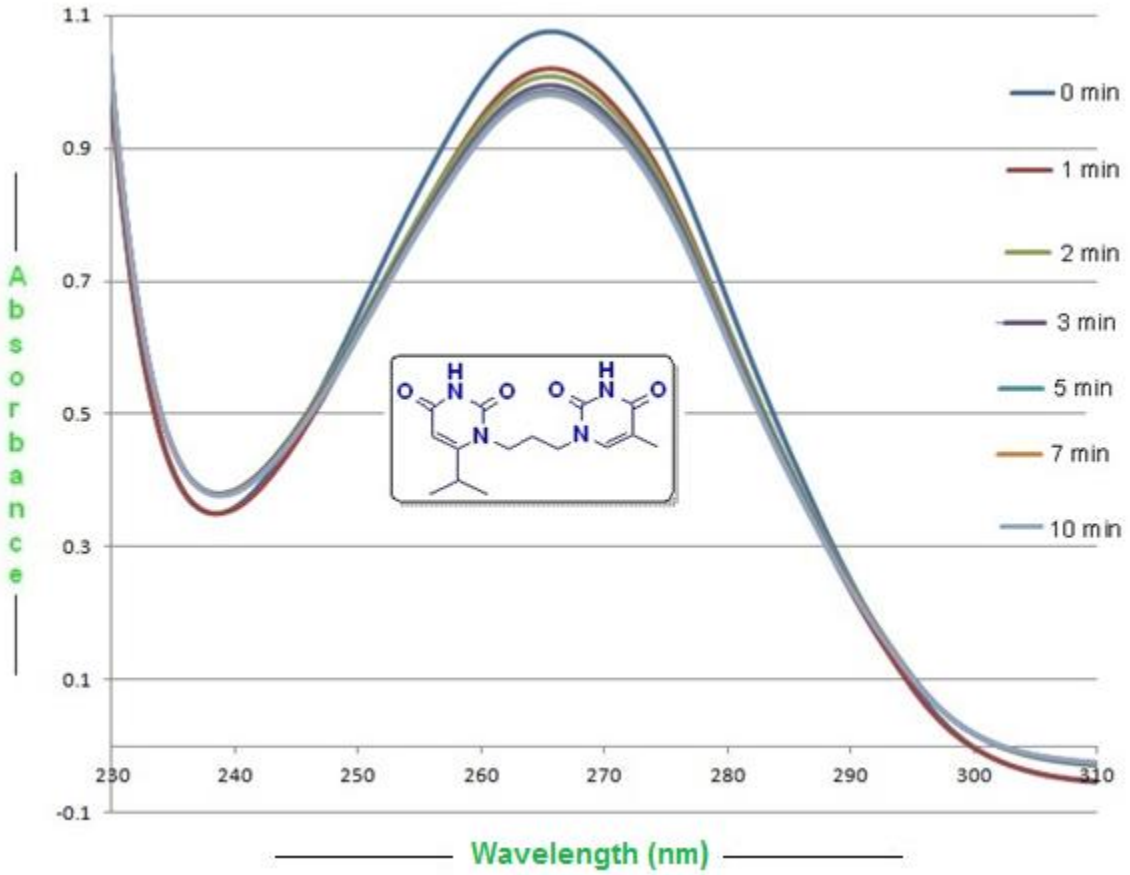


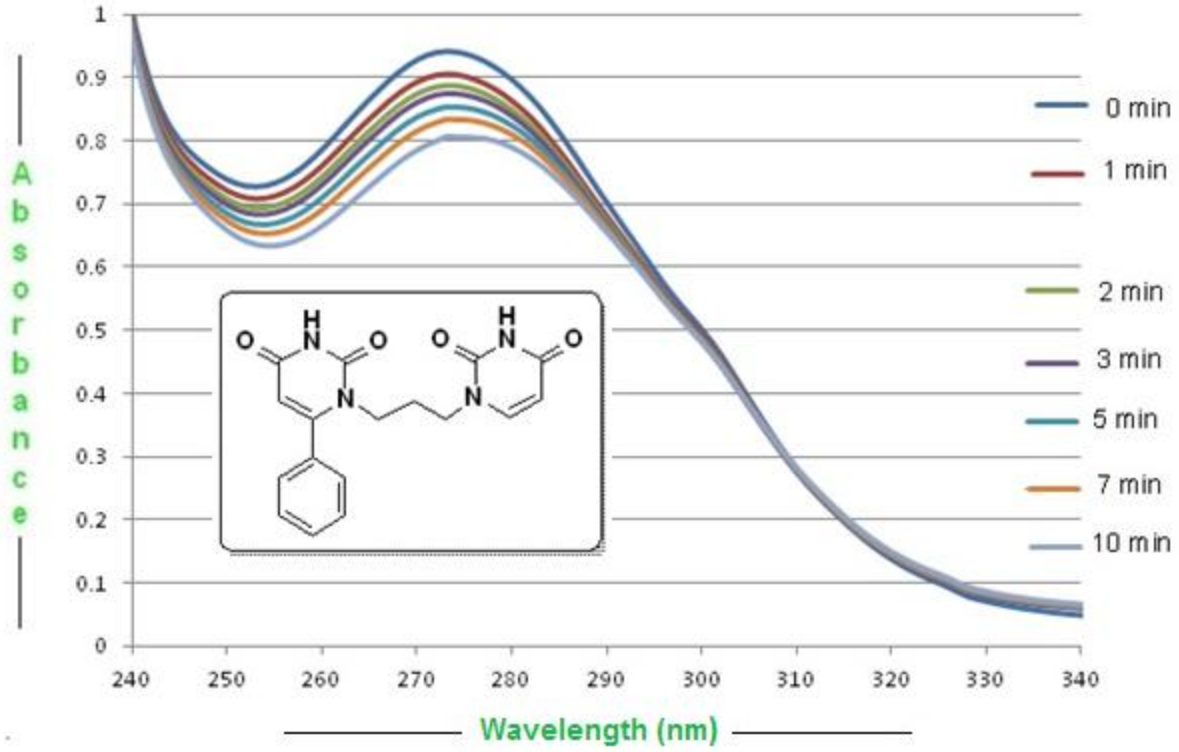
UV spectra of *Bis*-pyrimidine:











CHAPTER-VI

Synthesis of modified peptide nucleic acid which forms stable base-pair with DNA

VI.1. Introduction

Peptide nucleic acid (PNA) is artificially synthesized oligonucleotides with peptide backbone invented by Peter E. Nielsen in 1991. PNA is an unnatural mimic of DNA, where the sugar-phosphate backbone is replaced by a polyamide chain (***Figure VI.1.1)*** in order to bring in new characteristics and properties into the oligonucleotides. [1] The main advantages of PNA are:

1) Neutral in nature: The phosphate charges are minimized with peptide replacement.
2) Nuclease stable: The enzyme nucleases rapidly degrade foreign DNA in cell. Therefore, DNA based molecular probes and carriers have disadvantages of getting destroyed by cell nucleases. PNA, on the other hand, is very stable towards nucleases and could be used as effective moluclar probes or carriers in cells.
3) Better target specific: Since PNA is neutral in nature it forms strong hybridization with a target DNA. The melting temperature pattern of a probe PNA is as follows.[2]

PNA-PNA > PNA-DNA > DNA-DNA

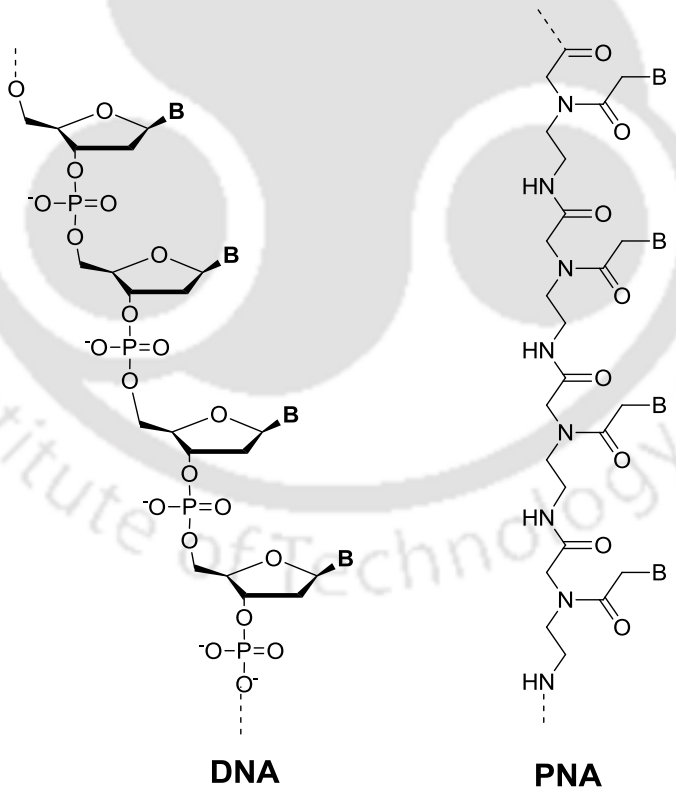


Figure VI.1.1: *Diagrammatically presentation of PNA and DNA.*

Since, the binding constant of a PNA probe is very high with a complementary DNA, presence of a single base mismatch offers a considerable change in melting temperature, which makes PNA a better target specific probe. Due their target specificities these modified oligonucleotides are widely used in detecting point mutations as well as in many drug delivery systems.[3a-c]

VI.2. Biomolecular Applications

Peptide Nucleic Acid (PNA) is a very potent DNA mimic, it's oligonucleotides analogues have most successful potential applications in antisense, antigene strategy and have high binding constants with complementary DNA or RNA analogues. [3a, 4] As a result a PNA-DNA duplex shows higher thermal stability than the corresponding DNA-DNA duplex. A covalent conjugation of PNA and DNA results in PNA-DNA chimeras, which found many applications for sequence detection and gene silencers.[5b] In the last decade PNA probes or PNA-DNA chimeras have been applied for detection of point mutations or single nucleotide polymorphisms (SNPs), [5, 6] as hybridization probes, where the identity of one or more bases in the target sequence is unknown, [7-10] as well as for sequence-specific recognition of duplex DNA, thereby blocking gene expression.[11, 12a, b] They were also used as powerful tools in molecular biology, biotechnology, potential drugs deliveries and many research applications.[13a] Several derivatives and analogs of PNA were designed for investigation of structure-function relationships using combinatorial chemical strategies and physico-chemical properties. [13b-c]

Nielsen. et al. have demonstrated synthesis of PNAs by using *achiral N-(2-aminoethyl) glycine units* (backbone) in 1991. Further, *K. N. Ganesh and his co workers* have developed many modifications on the backbone to generate conformational constrained PNA analogues and evaluated their hybridization specificities (***Figure I.3.5.2)***. However, in the last decade the focus was shifted towards the modifications on nucleobases or replacing nucleobases with abasic molecules such as polar molecules or heterocyclic compounds. For example, *Hayakawa et al.* synthesized pyrimido[4,5-d]pyrimidine-2,4,5,7-(1H,3H,6H,8H)-tetraone (PPT) **(a)** in 2009. Later *Yao et al.* also demonstrated aromatic fused pyrimidine-2,4-[1H,3H]-diones **(b-e)** in 2012. *K. N. Ganesh, et al.* have synthesized cyanuryl-PNA **(h)** to study their stable complementary PNA-DNA interactions. *Nielsen et al.* have found cholesterol conjugated PNA could be used in

antisense activity **(g)**. Co-crystal studies have revealed that a few of the PNA monomers, in their free forms, were able to alter base pairing properties **(f)** (***Figure VI.2.1)***[13d-i]

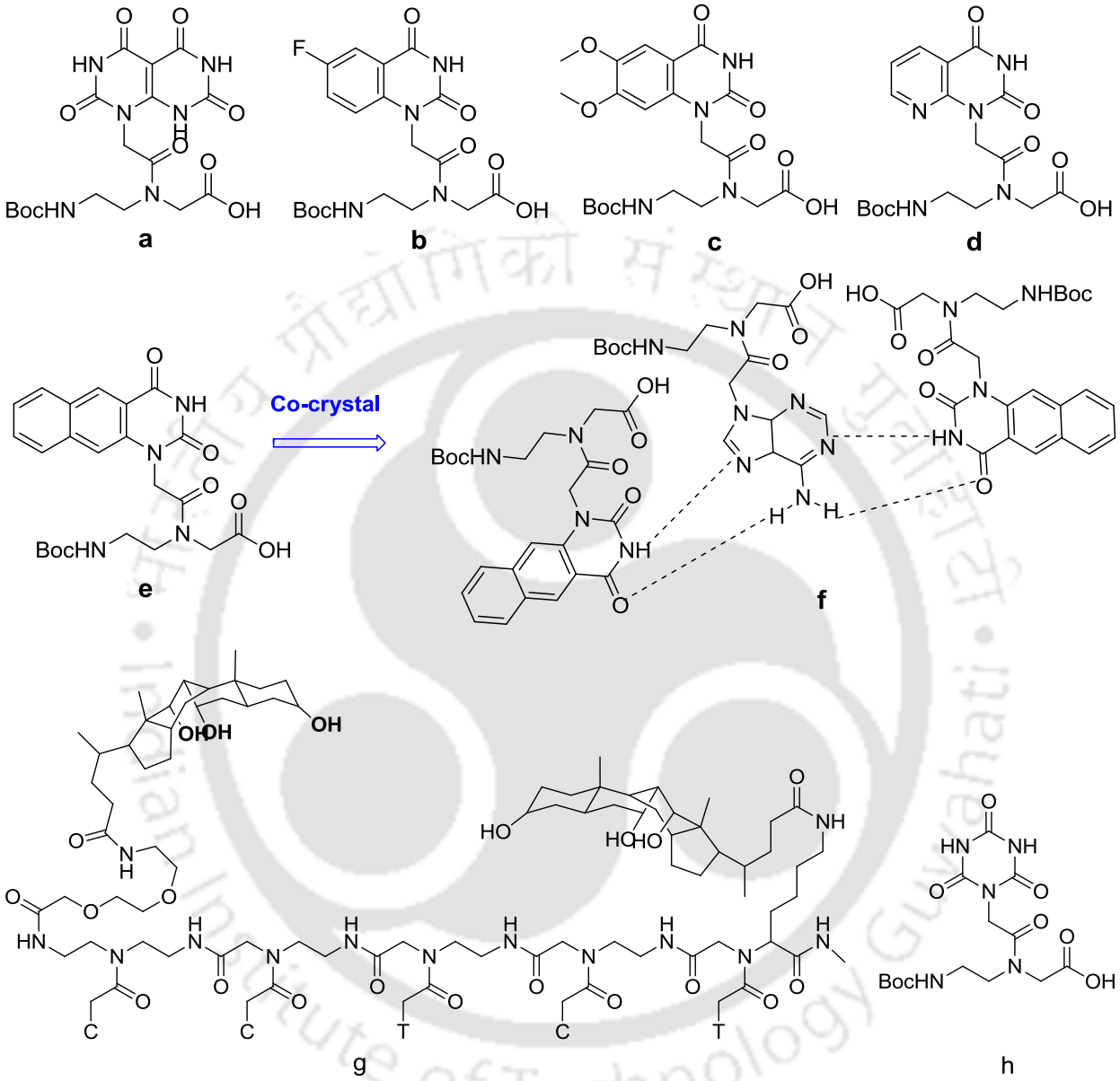
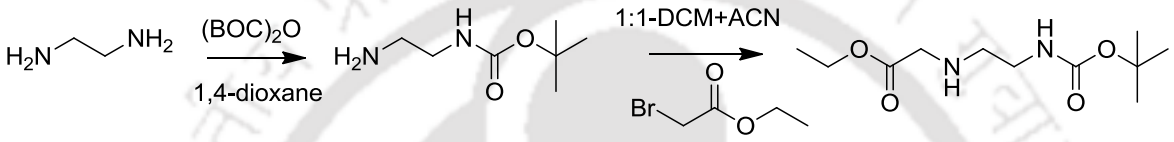


Figure VI.2.1: *Reported PNA monomers*

PNA oligonucleotides or PNA-peptide chimeras are most conveniently synthesized by solid phase peptide synthesis (**SPPS**). The protocol involves multi-step reactions on a resin based solid support. The monomers for PNA usually have a glycine based backbone which is conjugated to a nucleobase. ^{t}Boc or Fmoc strategy is usually used for **SPPS** [13e-f]

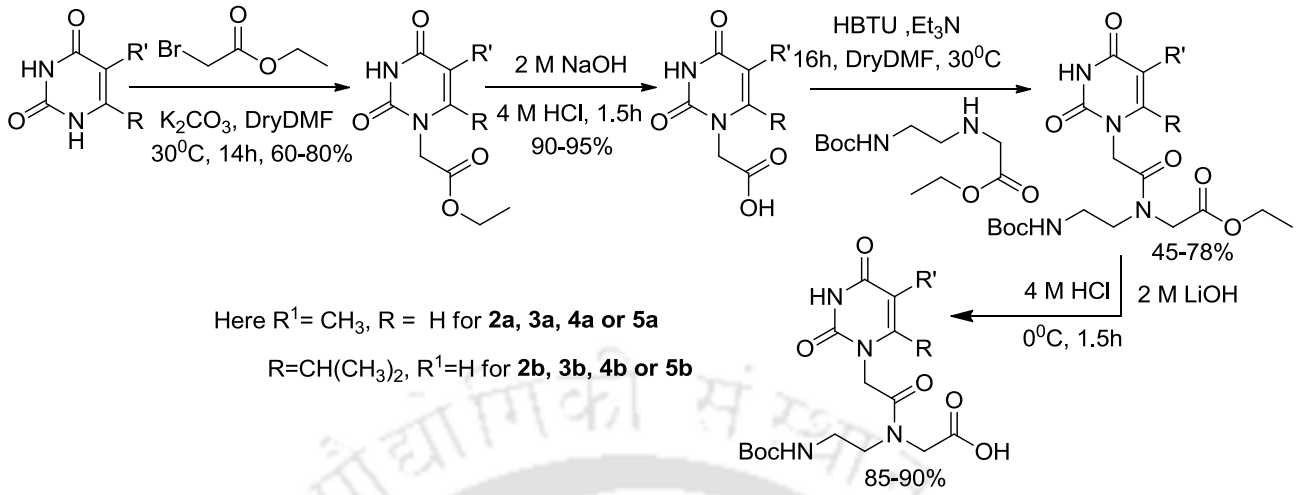
VI.3. Present work

The synthesized nucleobases with altered steric and electronic properties are expected to change the base-pairing properties and could be useful as probe oligonucleotides for detection of point mutation and disease diagnosis. The aim of this chapter is to develop oligonucleotides with above mentioned nucleobases in a peptide backbone (PNA). In this pathway we have synthesized PNA monomers with our nucleobase analogues. First peptide backbone was synthesized in two steps following procedure described by *Komiyama et.al.* and presented in ***Scheme VI.3.1.*** [14-18]



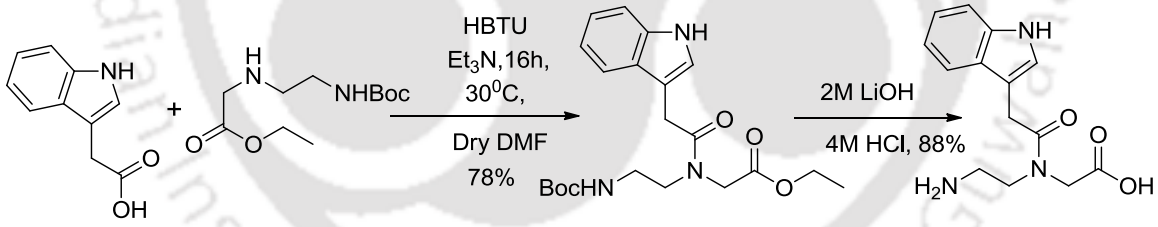
Scheme VI.3.1: *Schematic representation for synthesis of back bone*

In order to construct novel modified oligonucleotides, PNA monomers containing the modified nucleobases need to be synthesized. However, the synthesis of PNA monomers first involved alkylation of nucleobases (6-isopropyl uracil, **1b**) with ethyl bromoacetate followed by hydrolysis of the resultant ethyl ester to give respective acids (6 isopropyl-1-ylacetic acid, **3b**) as shown in ***Scheme VI.3.2***. The other way is the alkylation of nucleobases with 2-bromoacetic acid with water as the reaction medium and with KOH as the base. This method gave a typical yield of around 40%, and therefore was avoided. Then the next step is attachment of the acids **(3b)** to the backbone. Here ethyl N-(2-Boc-aminoethyl)glycinate was used as a backbone. For this compounds **3a**, **3b**, or **3c** (shown in ***Table VI.3.1***) were activated with HBTU (*N,N,N′,N′*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate) *in situ* followed by addition of the backbone and triethylamine to the reaction mixture. The resultant monomer ethyl esters (**4a**, **4b**, or **4c**) were hydrolyzed with LiOH in water/THF to afford the pure monomers (**5a**, **5b**, or **5c**, respectively, ***Scheme VI.3.2***).



Scheme VI.3.2: *Schematic representation for synthesis of modified PNA monomers*

The PNA monomers that we have synthesized (in ***Table VI.3.1)*** were designed to alter the base pairing properties, by changing the steric and electronic characters of the nucleobases. For example monomer **5b** was selected for its increased steric parameters where as monomer **5c** was chosen due to better π-stacking ability.



Scheme VI.3.3: *Schematic representation for synthesis of modified PNA monomers*

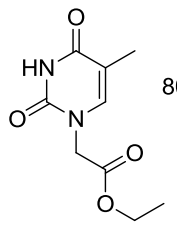
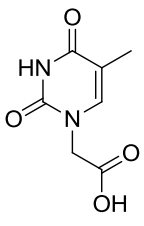
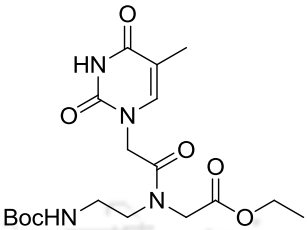
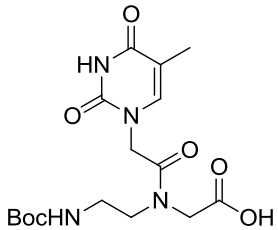
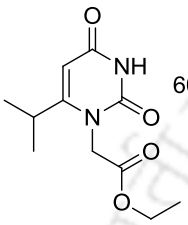
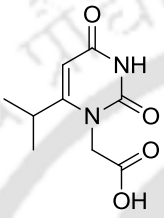
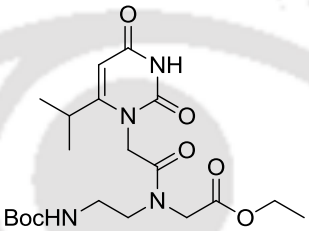
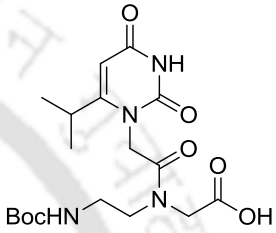
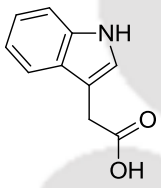
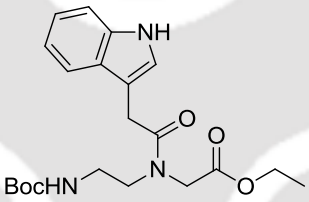
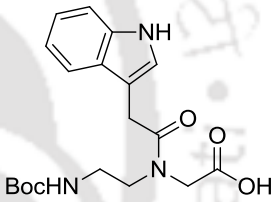
S.No.	Substrate (2a-2c)	Substrate (3a-3c)	Substrate (4a-4c)	Yield (%)	Product (5a-5c)	Yield (%)
01	 **2a**	 **3a**	80	95	 **4a**	78	 **5a**	90
02	 **2b**	 **3b**	60	90	 **4b**	45	 **5b**	85
03	—	 **3c**	—	78	 **4c**	88	 **5c**	

Table VI.3.1: Variety of PNA monomers; 3c was purchased.

VI.3.1. Synthesis of PNA Oligomers by Using Solid Phase:

Oligomer **1**, **2** and **3** were synthesized on MBHA (4-Methylbenzhydrylamine hydrochloride) resin (0.076 mmol, 50 mg) with a loading capacity of 0.7-1.4 mmol/gm. All the oligomers were synthesized according to standard Fmoc/^{t}Boc protection strategy on MBHA resin following a reported protocol. [19-22]

***Pretreatment of MBHA Resin (per 50 mg of dry resin)*:** The dry MBHA resin was suspended in DMF (2 mL) for 12h. The resin was washed with 30% DIPEA in DMF (2x2 mL, 10 min each time) and washed with 1:1 DCM/DMF solution (3 x 30 s, 2 mL).

***Coupling, Capping and Deprotection*.**

Coupling was carried out using two equivalents of Fmoc-PNA monomer, HBTU (**2.5** equivalents) and DIPEA (**5** equivalents) in the presence of 1:1 DCM/DMF solution. Coupling was monitored by using Kaiser's test and was found to be completed in 3h. In case of **5b** and **5c**, the coupling step was repeated twice to obtain better efficiency. In case of incomplete acylation, coupling cycle was repeated and washed with 1:1 DCM/DMF solution (3 x 30 s, 2 mL). Capping was done using 1:9 acetic anhydride/DMF for 30 minutes in two cycles, then washed with1:1 DCM/DMF solution (3 x 30 s, 2 mL). Next step is the deprotection of N-terminal Fmoc and was carried out using 30% piperidine in DMF (N-terminal Boc removal was carried out using 20% TFA in DMF) for 25 minutes (7 min x 3 times). After N-terminal Fmoc/Boc removal peptide was washed with 1:1 DCM/DMF solution (3 x 30 s, 2 mL). Here we have monitored every step by using Kaiser's test. It's shown systematically in ***Figure VI.3.1***.

Elongation

Above coupling and deprotection processes were repeated to produce the full sequence. Here also each step was followed by the Kaiser's test.

Cleavage of Oligomer for the resin

The resin was dried in vaccuo for 30 mins and washed with TFA (2x1 min, 0.5 mL). Later, the resin was subjected to freshly prepared precooled (0°C) solution of TFMSA/thioanisole/TFA (1:1:8) for 3 h. Then resin was removed by filtration and that filtrate was diluted with anhydrous Et_2O (15 mL). After the resulting mixture was centrifuged, a white solid (precipitate) was obtained. Further the oligomers were purified from the reaction mixture by using reversed-phase HPLC. Purified PNA was obtained in the form of white solid.

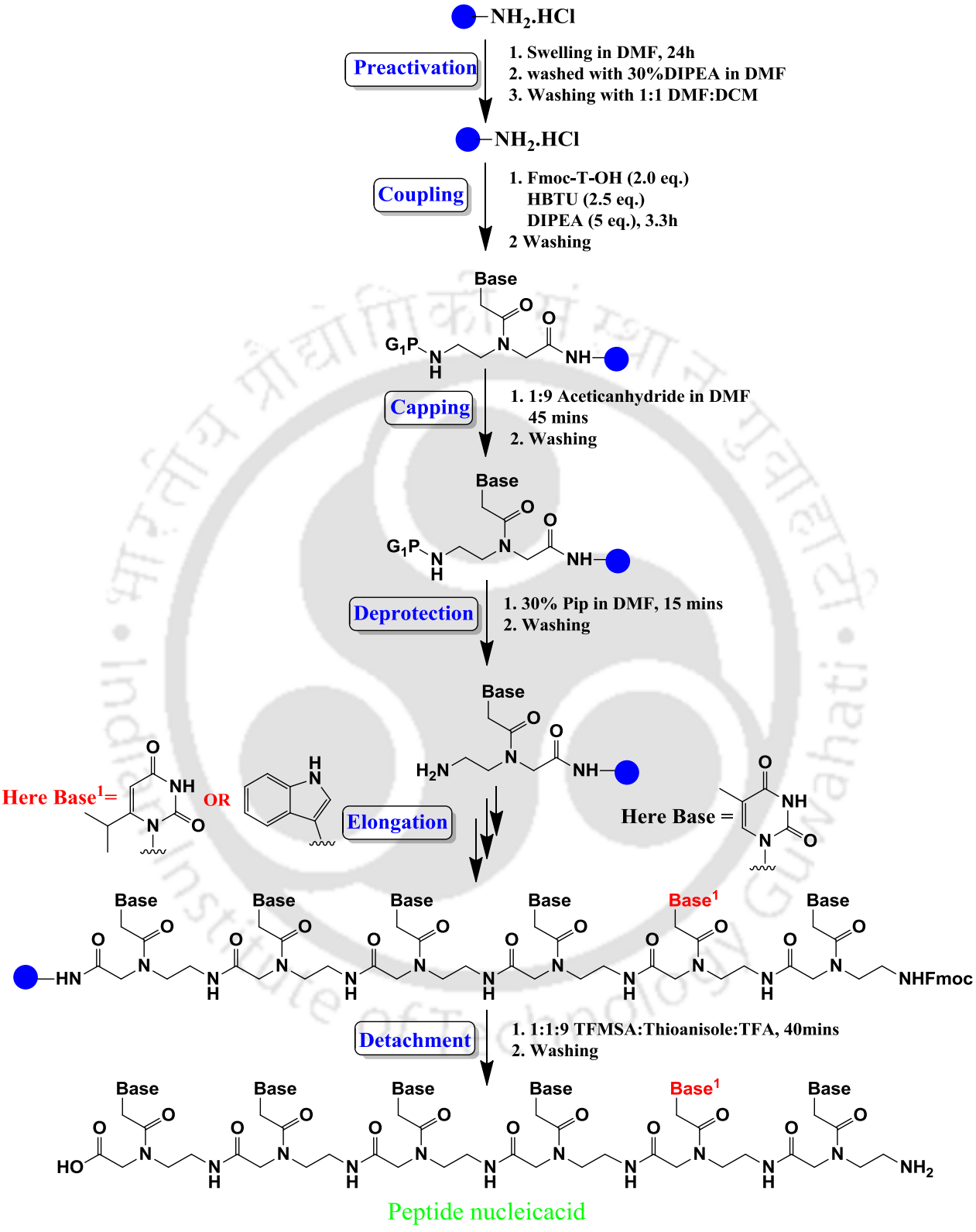
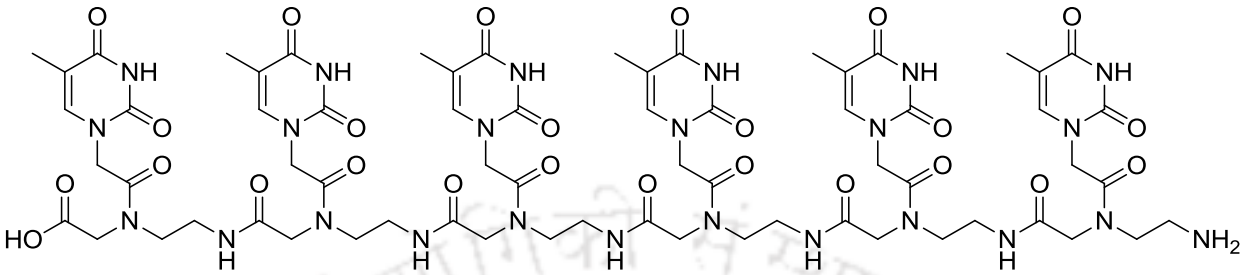
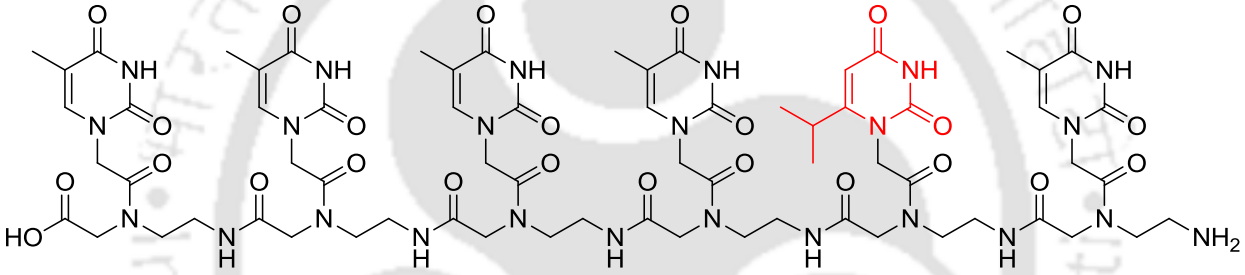


Figure VI.3.1: *Schematic representation for synthesis of peptide nucleic acid*

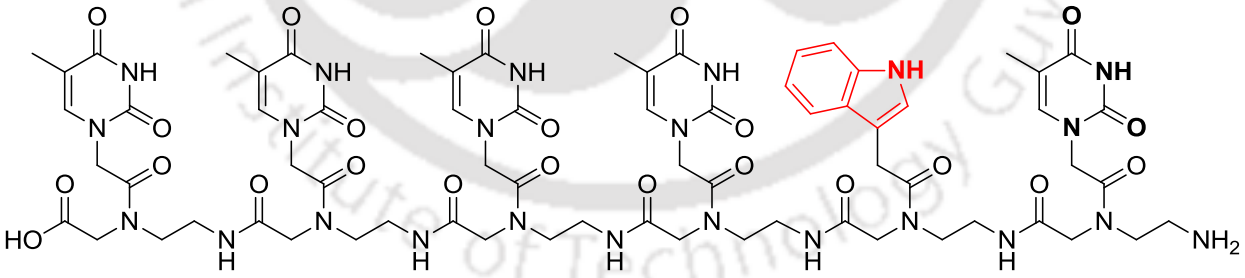
Following the described SPPS synthesis we have synthesized a few PNA oligomers (*Oligomer **1**, Oligomer **2** and Oligomer **3***). These are listed in ***Figure VI.3.2.***



a). Oligomer 1



b). Oligomer 2



c). Oligomer 3

Figure VI.3.2: a) *Represents the PNA Hexamer* ***(TTTTTT=Oligomer 1)****: (b) Represents modified PNA Hexamer* ***(TTTTT$^{(iso)}$T= Oligomer 2)*** *and (c) modified PNA Hexamer* ***(TTTTT$^{(indo)}$T= Oligomer 3).***

The purity of **Oligomer 2** was confirmed from LC-MS using C18 column using solvent A (water with 0.1% FA) and solvent B (acetonitrile with 0.1% FA). Mass was confirmed from MS (ESI +ve mode). A representative figure of the LC-MS profile of the pure **Oligomer 2** is shown below (***Figure VI.3.2***). The LC profile of the pure PNA is depicted as panel (**a**) in ***Figure VI.3.2***, whereas the peak corresponding to the mass of the PNA is shown in panel (**b**). Linear gradient of 5-50% CH_3CN in water for 6 min and 50-100% acetonitrile in water for 2 mins with total run time of 8 minutes was used. A representative picture of LC profile is shown in ***Figure VI.3.2***

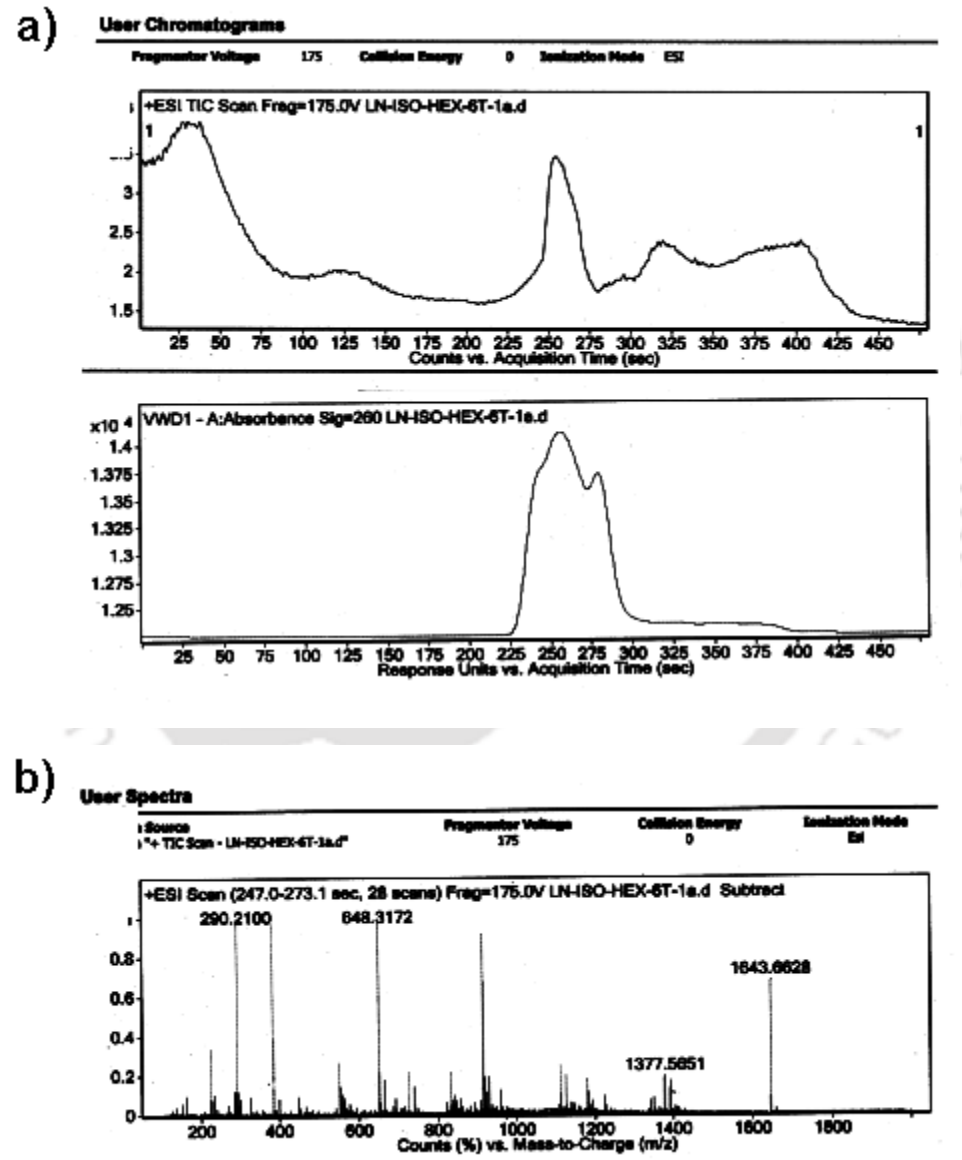
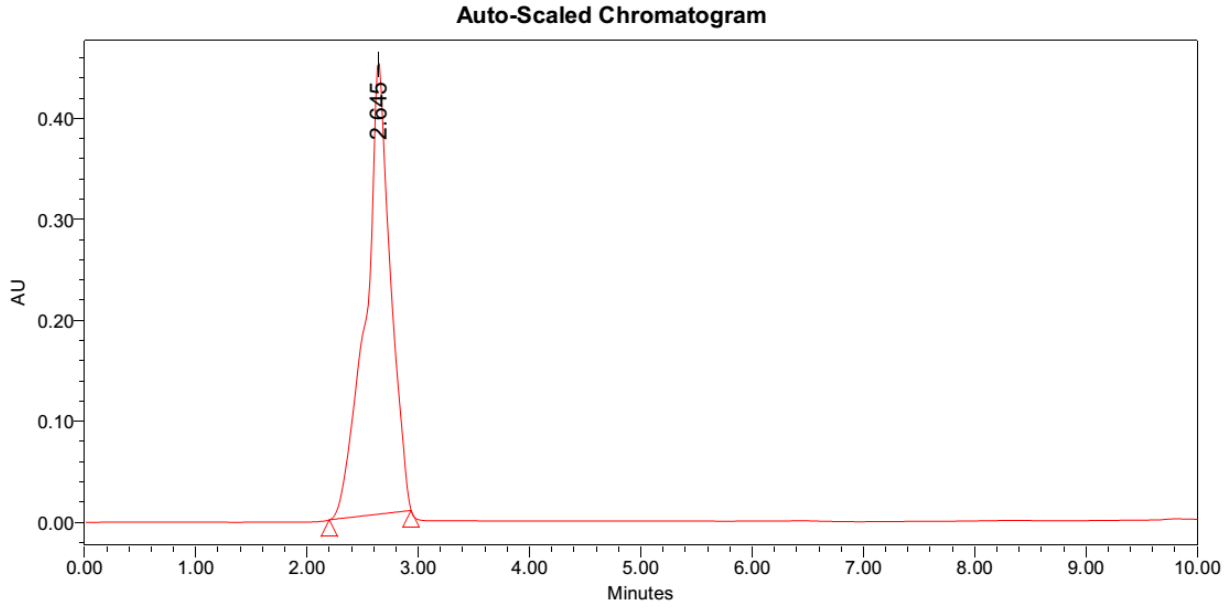


Figure VI.3.2: *LCMS profile for Oligomer 2*

Oligomer 2 was purified using C-18 Bondapak semi preparative HPLC column using solvent A (H_2O with 0.1% TFA) and solvent B (CH_3CN with 0.1% TFA). Linear gradient of 5-30 % acetonitrile in water for 8 min and 30-100 % acetonitrile in water for 2 minutes with total run time of 10 minutes was used. Dual wavelength was selected at 222 and 260 nm (***Figure VI.3.3)***

c)



d)

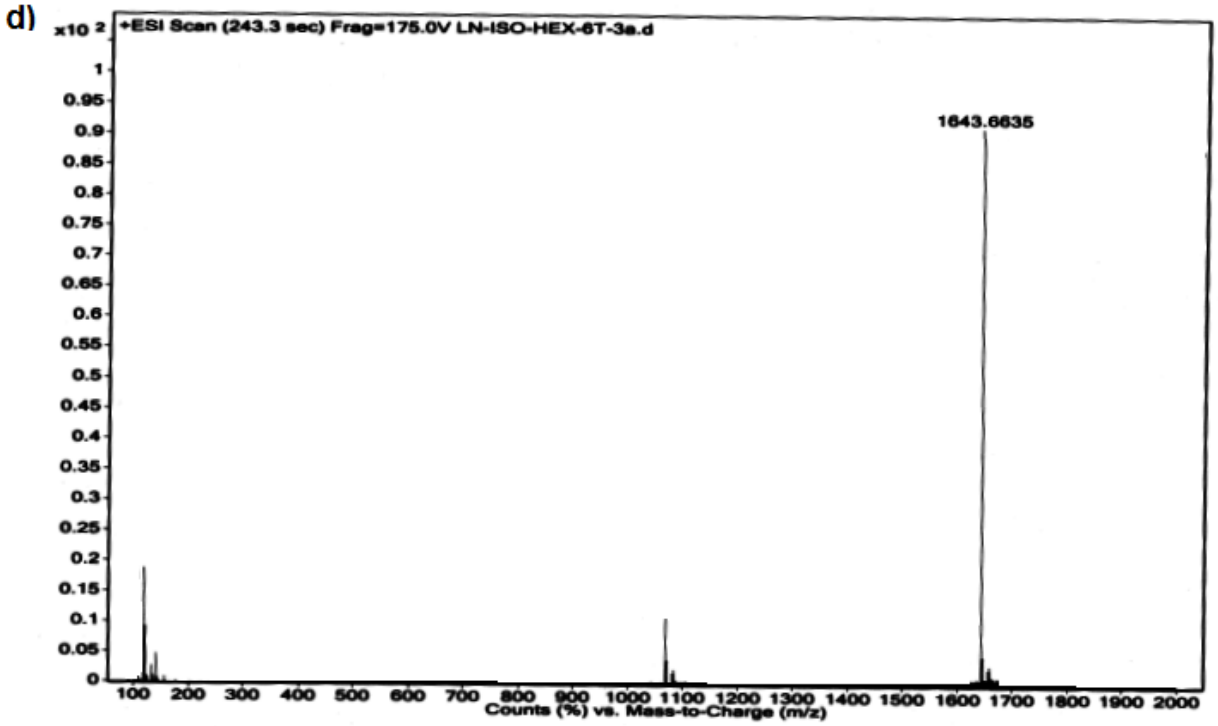


Figure VI.3.3: **(***c) HPLC picture of the purified oligomer 2: (d) ESI-MS data of oligomer 2 calculated mass for $C_{68}H_{91}N_{24}O_{25}$ is 1643.6582; observed: 164.6635*

In conclusion, we have synthesized PNA monomers containing C-6 substituted uracil nucleobase and also indole based monomer. Solid phase peptide synthesis protocol was used to synthesize the peptide nucleic acids. We have successfully synthesized the desired PNA oligomers containing modified uracil, in order to incorporate artificial base-pairing.

VI.4. Experimental section

VI.4.1. General Information: All chemicals were purchased from reputed pharmaceuticals and were used without further purification. All microwave-directed reactions were carried out in a *CEM Discover Labmate* closed vessel microwave reactor at 135^0C. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were all recorded from a *DRX-400 Varian spectrometer* using $CDCl_3$ or DMSO-D_6 as solvents. Chemical shifts are reported in parts per million (ppm). Melting points were determined using *Büchi B-545* apparatus and are uncorrected. High resolution mass spectrometry (HRMS) was analyzed from *Agilent Q-TOF 6500 LC/MS* system and *Micromass Q-TOF ESI-MS* instrument (model HAB 273). HPLC analysis was carried out with an Ascentis® C-18 analytical column (5 μm, 250×4.6 mm) coupled to a UV-visible detector whereas LC-MS was measured using shield RP18 (1.7 μm, 1 × 50 mm) column. HPLC grade solvents were used for HPLC analysis. The reactions were monitored by analytical TLC on Merck silica gel G/GF 254 plates. The column chromatography was performed with Merck silica gel (60-120 mesh). Oligomers were synthesized by using *STUART (SB_3)* rotator with *BAD PLASTIC* pak syringes (2 mL and 5 mL). Centrifuge was carried out in *SIGMA (3-30)* Instrument.

VI.4.2. Procedure for synthesis of compounds 2a-2b to 5a-5c

a) Representative Procedure for synthesis of compound 2b: In a 100 ml round bottom flask, 6-isopropyluracil (**1b**, 770 mg, 5 mmol), K_2CO_3 (690 mg, 5 mmol) were taken in dry DMF (6 ml) then ethyl bromo acetate (835 mg, 5 mmol) was added drop wise in 10 mins duration under inert atmosphere at 40^0C temperature. Then the reaction mixture was stirred for 14h. The completion

of the reaction was monitor by TLC. The reaction mixture was filtered with filter paper (excess of potassium carbonate was removed) and the filtrate was finally extracted in DCM (10 times). The organic layer was dried over Na_2SO_4. The solvent was removed by rotary evaporation and excess of DMF was removed by high vacuum pump. The compound was purified from crude reaction mixture through column chromatography by using 50-60% ethyl acetate in hexane. Compound **2a** were synthesized following the same procedure. The reaction yields are shown in ***table VI.3.1***

b) Representative Procedure for compound 3b*:* In a 100 ml round bottom flask, 960 mg (4 mmol) of **2b** in THF and around 2 ml of 2M NaOH was taken and stirred at room temperture for 1.5 hours. The completion of the reaction was monitored by TLC. Then the reaction mixture was cooled down to 0^oC temperature and was acidified with 4M HCl up to *pH=3*. The reaction mixture was filtered and the solid was washed with water and the precipitate collected (compound **3b**). Since **3b** is soluble in water, therefore the aqueous layer was also extracted with ethyl acetate repeatedly (10 times) to recover the compound **3b**. The yield is shown in ***table VI.3.1***. Compounds **3a** were synthesized following the same procedure.

c) Representative Procedure for compound 4b*:* In a 50 ml two-neck round bottom flask, 636 mg (3 mmol) of **3b,** HBTU (1.25 gm, 3.3 mmol), Backbone (812 mg, 3.3 mmol) and Triethylamine (455 mg, 4.5 mmol) in Dry DMF (3 ml) were taken and stirred at 30°C under nitrogen atmosphere for 16 hours. The completion of the reaction was monitor by TLC. Then it was poured into 25 ml of water and extracted in CH_2Cl_2. The organic layer was dried over $MgSO_4$. The solvent was removed by evaporation and excess DMF was removed by high vacuum. The compound was purified from crude reaction mixture through column chromatography by using 5% Chloroform in Methanol. Compounds **4a** and **4c** were synthesized following the same procedure. The reaction yields are mentioned in ***table VI.3.1***

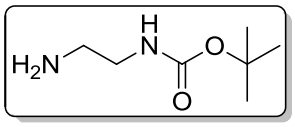
d) Procedure for compound 5b*:* 880 mg (2 mmol) of **4b** was taken in a dry, two-neck round bottom flask in THF (2 ml). Then the reaction mixture was stirred for 5 min, followed by the addition of freshly prepared 2M LiOH (2 ml) and stirred for more than 1.5h at room temperature. The progress of the reaction was monitored by TLC. After completion of the

reaction, the mixture was filtered, washed with excess of water and the precipitate collected. Compounds **5a** and **5c** were synthesized following the same procedure. The reaction yields are mentioned in ***table VI.3.1***

e) Representative Procedure for oligomer 2: PNA oligomer **2** was synthesized according to standard Fmoc/tBu protection strategy on MBHA resin following a reported protocol. [16-19.] Coupling was carried out using 2.5 equivalents of Fmoc PNA monomer, HBTU (2 equivalents) in the presence of DIPEA (5 equivalents). Coupling was monitored at each step using Kaiser's test. In case of incomplete acylation coupling cycle was repeated and capping was done using acetic anhydride for 30 minutes in two cycles. N-terminal Fmoc removal was carried out using 30% piperidine in DMF (N-terminal Boc removal was carried out using 20% TFA in DMF) for 25 minutes (7 min x 3 times). After N-terminal Fmoc/Boc removal peptide was washed with1:1 DCM/DMF solution (***Figure VI.3.1***). Peptide was cleaved from the resin using TFA:TFMSA:Thioanisole (8:1:1) for nearly 3 hours. Resin was washed three times with TFA. PNA was precipitated using cold diethyl ether to obtain white solid mass. Later on the resulting mixture was centrifuged we got a white solid (precipitate). Further the oligomers were purified from the reaction mixture by using reversed-phase HPLC. Purified PNA was obtained in the form of white solid.

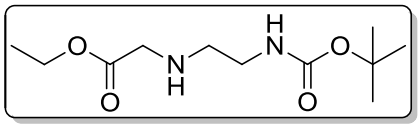
VI.5. Characterization data

Tert-butyl (2-aminoethyl)carbamate



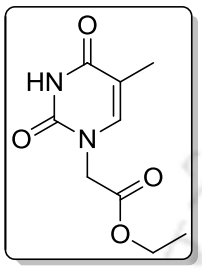
Yield: 65 %, thick oily. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (s, 1H), 3.01 (m, 2H), 2.63 (t, 2H, *J*= 6.0 Hz), 2.20 (s, 2H), 1.27 (s, 9H).

Ethyl 2-((2-((tert-butoxycarbonyl)amino)ethyl)amino)acetate



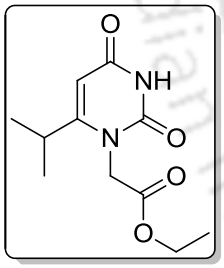
Yield: 70 %, thick oily. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 1H), 4.21 (q, 1H, *J*= 7.2 Hz), 3.40 (s, 2H), 3.23-3.17 (m, 2H), 2.76 (t, 2H, *J*= 6.0 Hz), 1.84 (s, 1H), 1.44 (s, 9H), 1.29 (t, 3H, *J*=7.2 Hz).

Ethyl 2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate(2a):



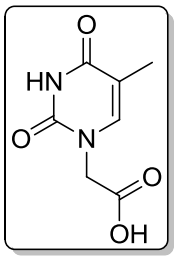
Yield: 80%, White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.40 (s, 1H), 7.67 (d, 1H, *J*= 1.6 Hz), 4.62 (s, 2H), 4.20 (q, 2H, *J*= 7.2 Hz), 1.85 (s, 3H), 1.08 (t, 3H, *J*= 6.8 Hz).

Ethyl 2-(6-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetate(2b):



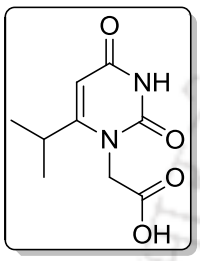
Yield: 60%, White solid, m.p 165°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (s, 1H), 5.64 (d, 1H, *J*= 1.6 Hz), 4.66 (s, 2H), 4.25 (q, 2H, *J*= 7.2 Hz), 2.64-2.61 (m, 1H), 1.03 (t, 3H, *J*= 6.8 Hz), 1.25 (d, 6H, *J*= 6.8 Hz).

2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (3a):



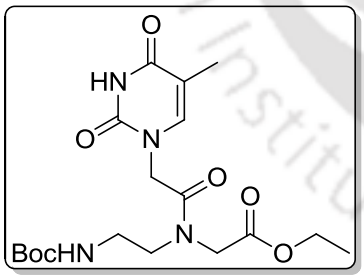
Yield: 95%, White solid, m.p 220°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 7.67 (s, 1H), 4.04 (s, 2H), 1.87 (s, 3H).

2-(6-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetic acid (3b):



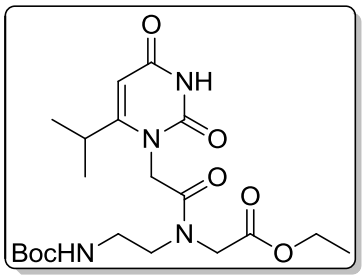
Yield: 90%, White solid, m.p 185°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11. 19 (s, 1H), 5.50 (s, 1H), 4.38 (s, 2H), 2.61-2.50 (m, 1H), 1.15 (d, 6H, *J*= 6.8 Hz).

Ethyl 2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(5-methyl-2,4-dioxo-3,4 dihydropyrimidin-1(2H)-yl)acetamido)acetate (4a):



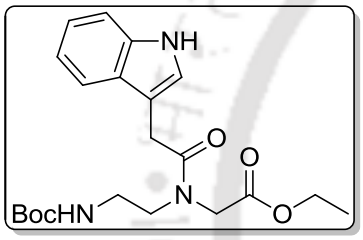
Yield: 78%, White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 6.94 (s, 1H), 4.54 (s, 2H), 4.24 (q, 2H, *J*= 6.8 Hz), 4.01 (s, 2H), 3.51 (m, 2H), 3.30 (m, 2H), 1.88 (s, 3H), 1.41 (s, 9H), 1.26 (t, 3H, *J*= 6.8 Hz).

Ethyl2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(6-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetate (4b):



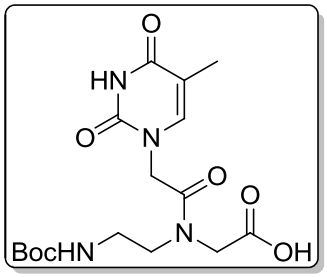
Yield: 45%, White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 5.58 (s, 1H), 4.79 (s, 2H), 4.19 (q, 2H, *J*= 7.2 Hz), 4.02 (s, 2H), 3.59 (m, 2H), 3.38 (m, 2H), 2.66-2.59 (m, 1H), 1.44 (s, 9H), 1.25 (t, 3H, *J*= 6.8 Hz), 1.15 (d, 6H, *J*= 6.8 Hz).

Ethyl 2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(1H-indol-3-yl)acetamido)acetate (4c):



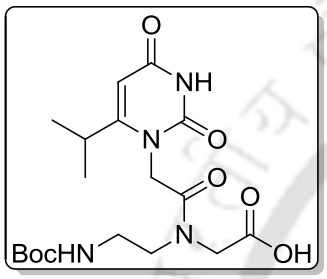
Yield: 78%, White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 7.62 (d, 1H *J*= 6.4 Hz), 7.24 (d, 2H, *J*= 6.4 Hz), 7.18 (s, 1H), 7.18 (t, 1H, *J*= 6.4 Hz), 6.87 (m, 1H), 4.16 (q, 2H, *J*= 7.2 Hz), 3.90 (s, 2H), 3.64 (s, 2H), 3.43 (m, 2H), 3.10 (m, 2H), 1.30 (s, 9H), 1.25 (t, 3H, *J*= 6.8 Hz).

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid (5a):



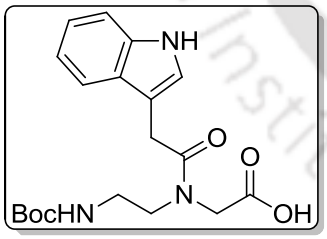
Yield: 90%, White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 7.29(s, 1H), 4.63 (s, 2H), 3.96 (s, 2H), 3.39 (m, 2H), 3.16 (m, 2H), 1.74 (s, 3H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 167.6, 164.5, 156.2, 151.4, 141.1, 110.9, 85.6, 80.1, 62.4, 61.8, 50.5, 49.2, 48.9, 48.0, 38.8, 29.8, 28.5, 14.2, 12.5

2-(N-(2-((tert-butoxycarbonyl)amino)ethyl)-2-(6-isopropyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic acid (5b):



Yield: 85%, White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 1H), 5.46 (s, 1H), 4.62 (s, 2H), 3.93 (s, 2H), 3.42 (m, 2H), 3.15 (m, 2H), 2.61-2.58 (m, 1H), 1.36 (s, 9H), 1.15 (d, 6H, *J*= 6.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ LRMS (ESI) m/z $[M+H]^+$ calcd for $C_{18}H_{28}N_4O_7$: 413.2031; observed: 413.2140.

2-(N-(2-aminoethyl)-2-(1H-indol-3-yl)acetamido)acetic acid (5c):



Yield: 88%, White solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 7.52 (d, 1H, *J*= 6.8 Hz), 7.34 (d, 2H, *J*= 6.0 Hz), 7.22 (s, 1H), 7.08 (t, 1H, *J*= 6.8 Hz), 6.97 (m, 1H), 3.94 (s, 2H), 3.74 (s, 2H), 3.43 (m, 2H), 3.13 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.19, 171.10, 155.7, 136.1, 136.0, 127.2, 123.3, 120.9, 118.6, 118.2, 111.2, 108.1, 77.9, 47.9, 47.2, 37.3, 29.8, 28.1. LRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{19}H_{25}N_3O_5Na$: 398.1686; observed: 398.1515.

VI.6. References

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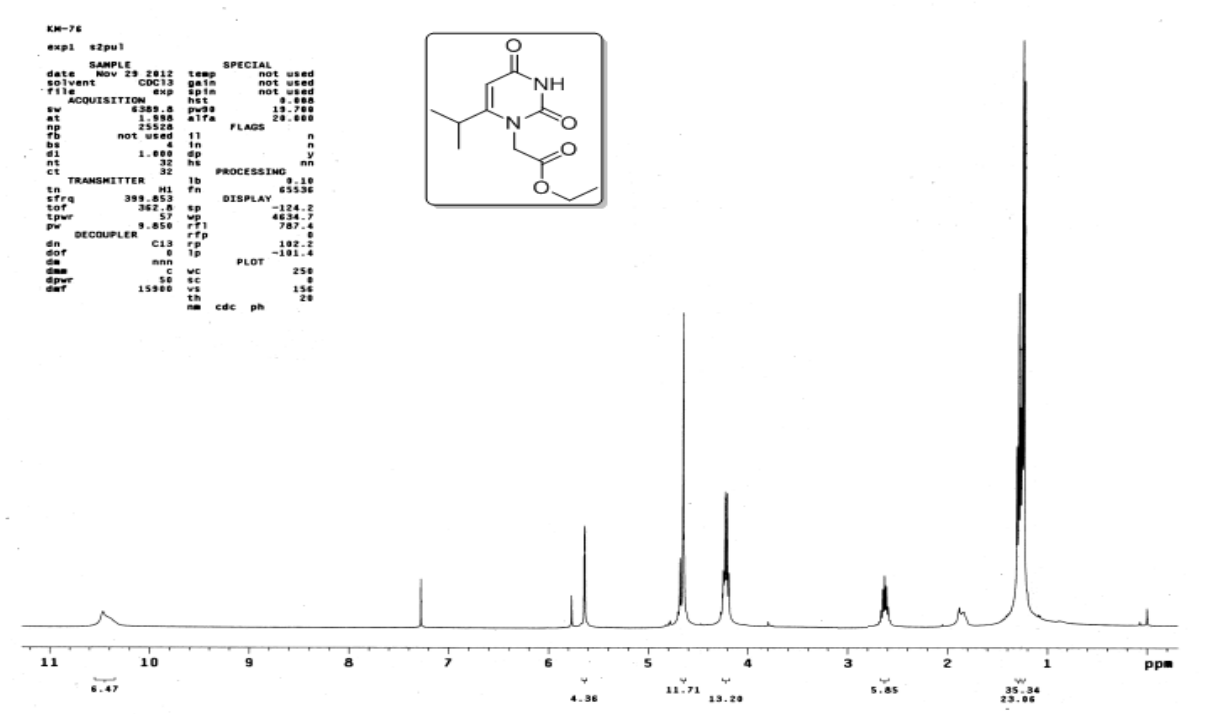
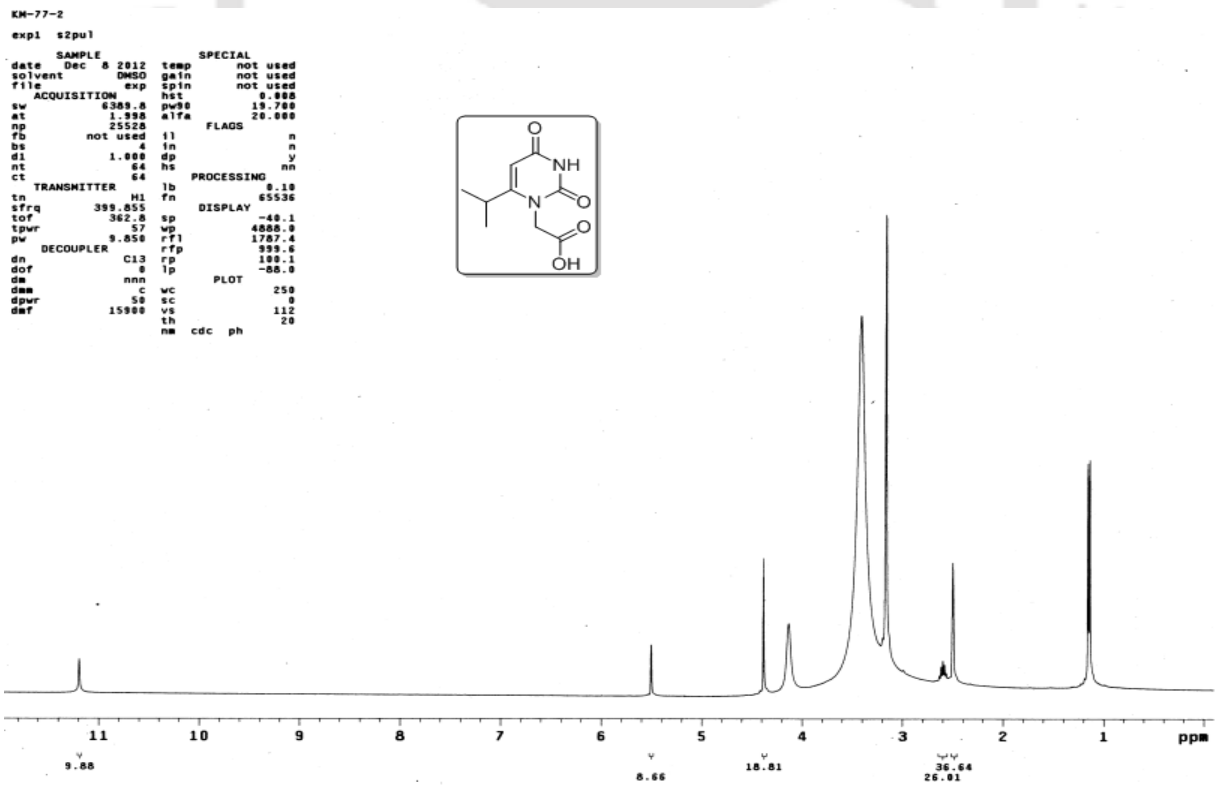
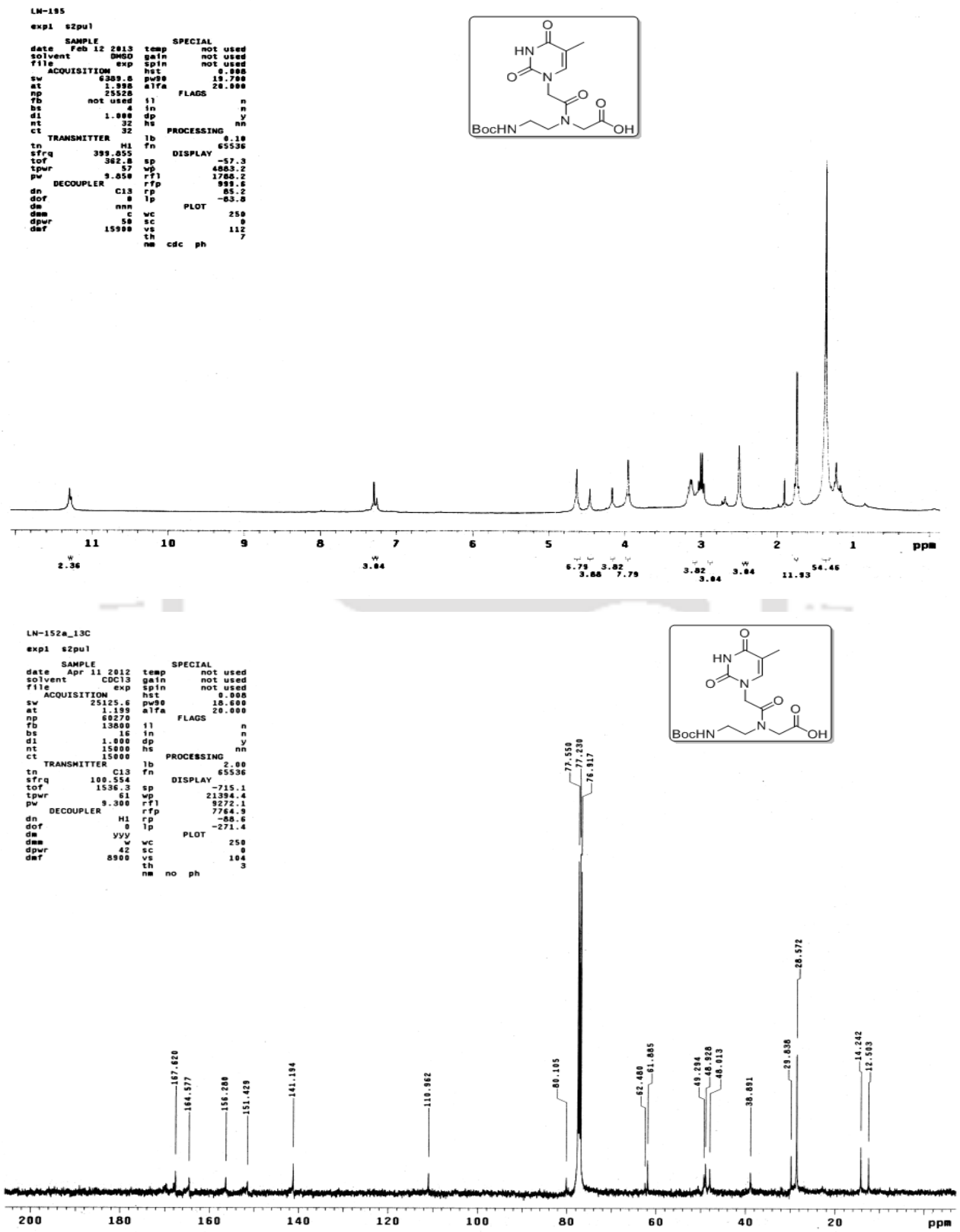
VI.7. Selected spectra

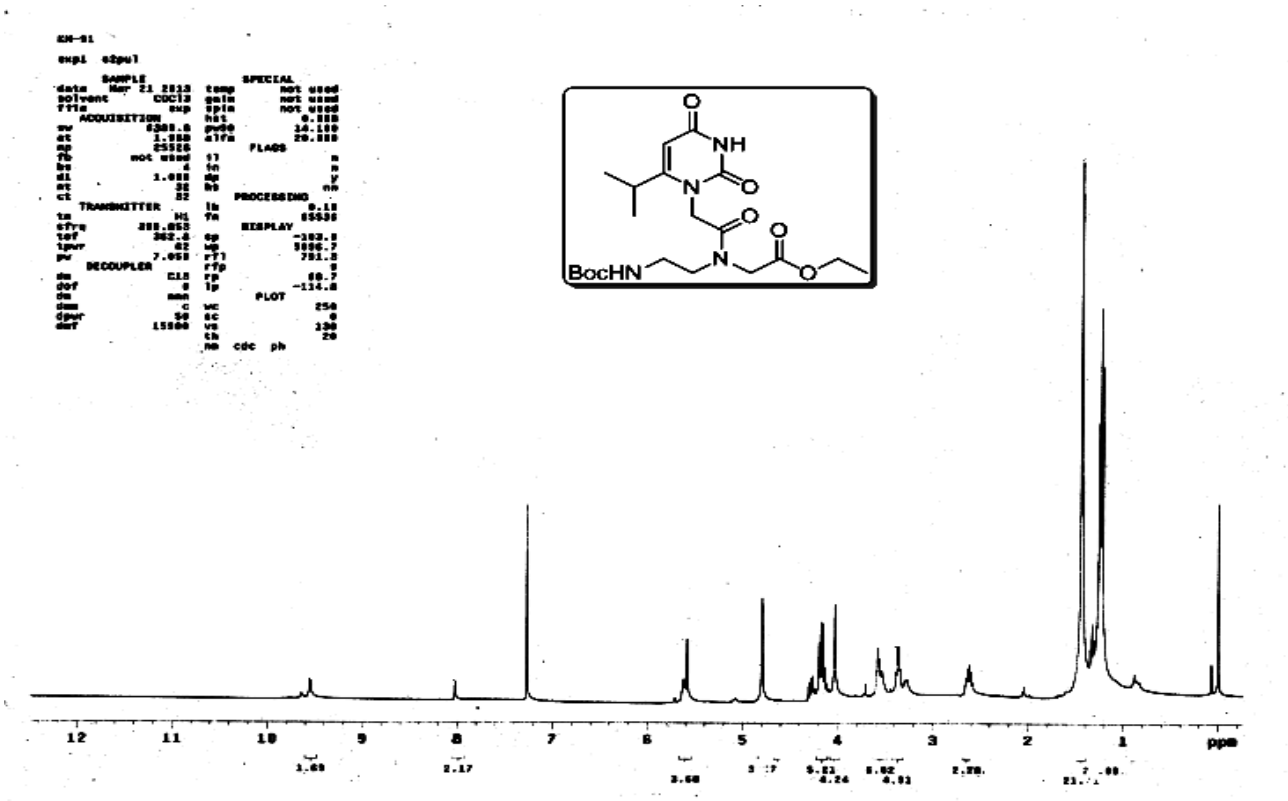


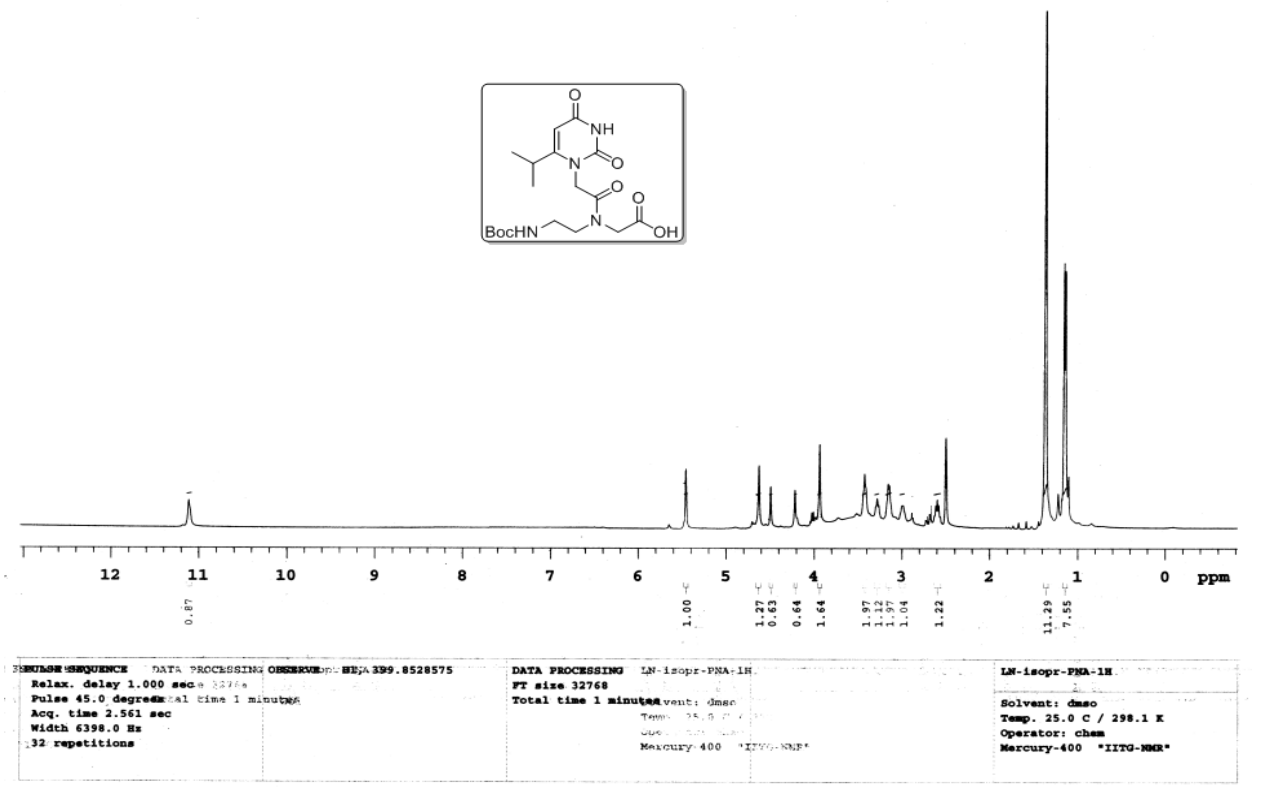


Compound 5a

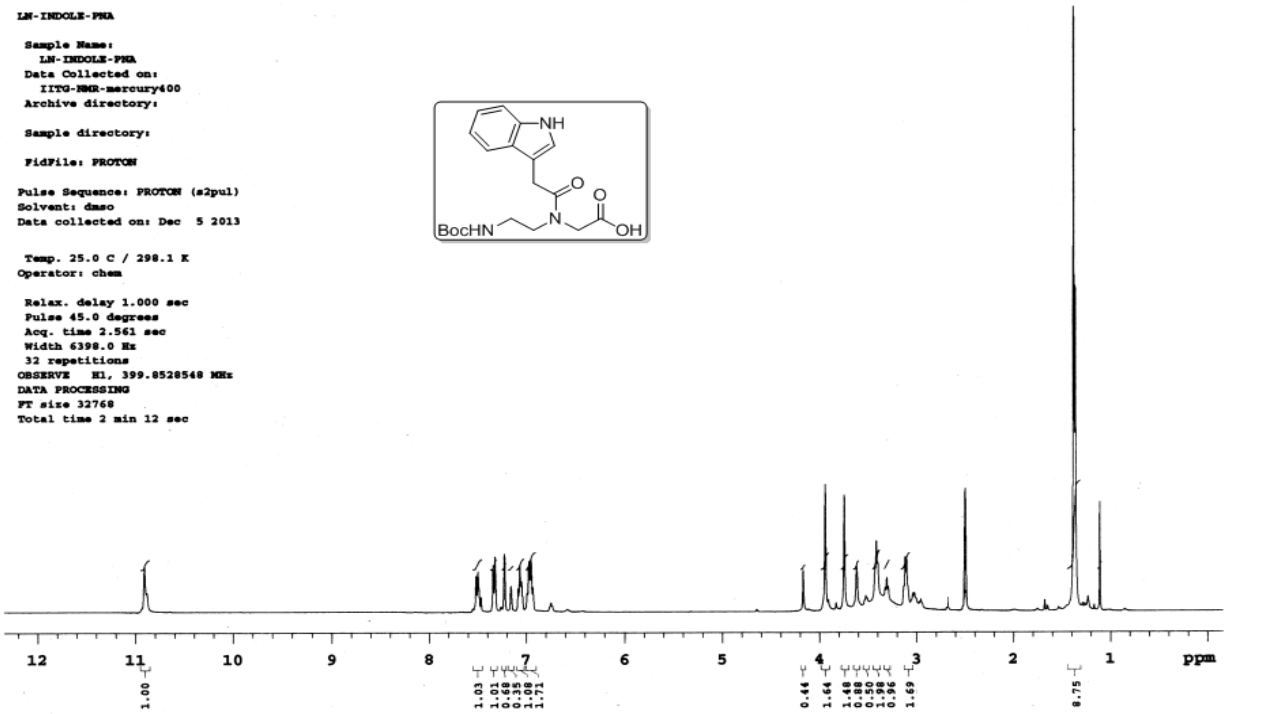


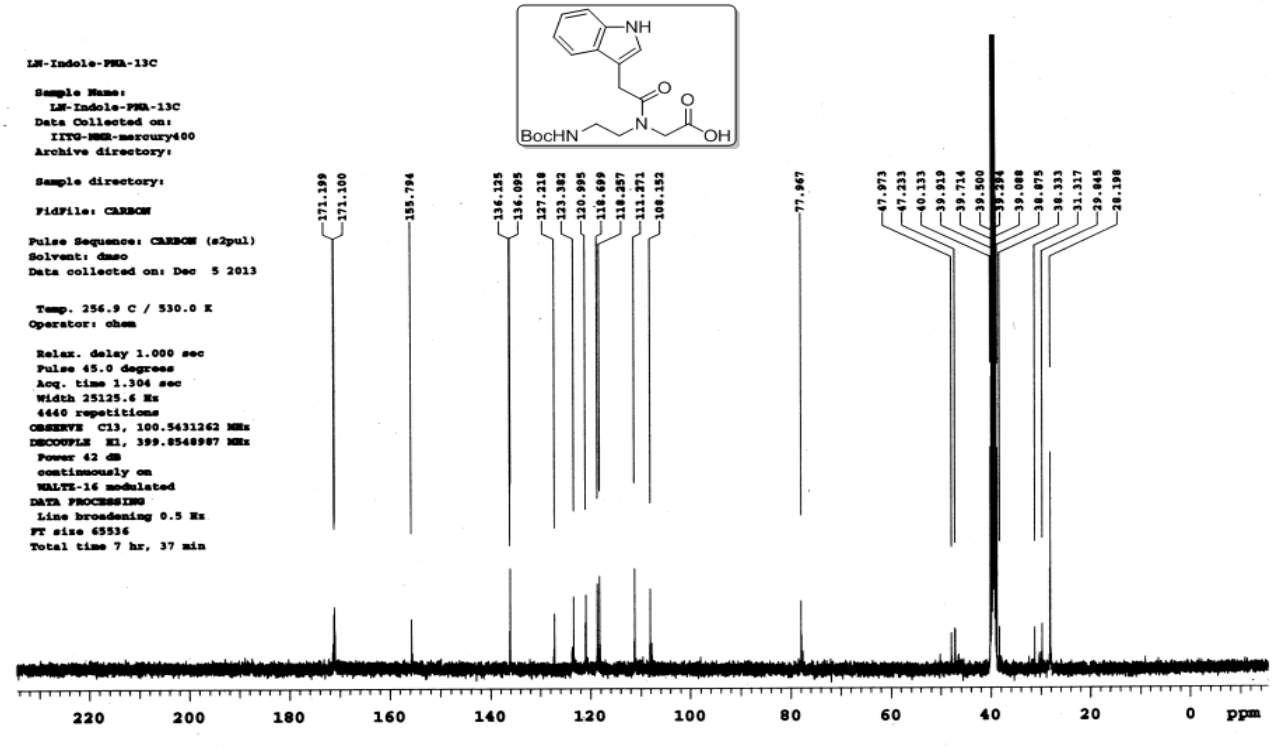
Compound 5b



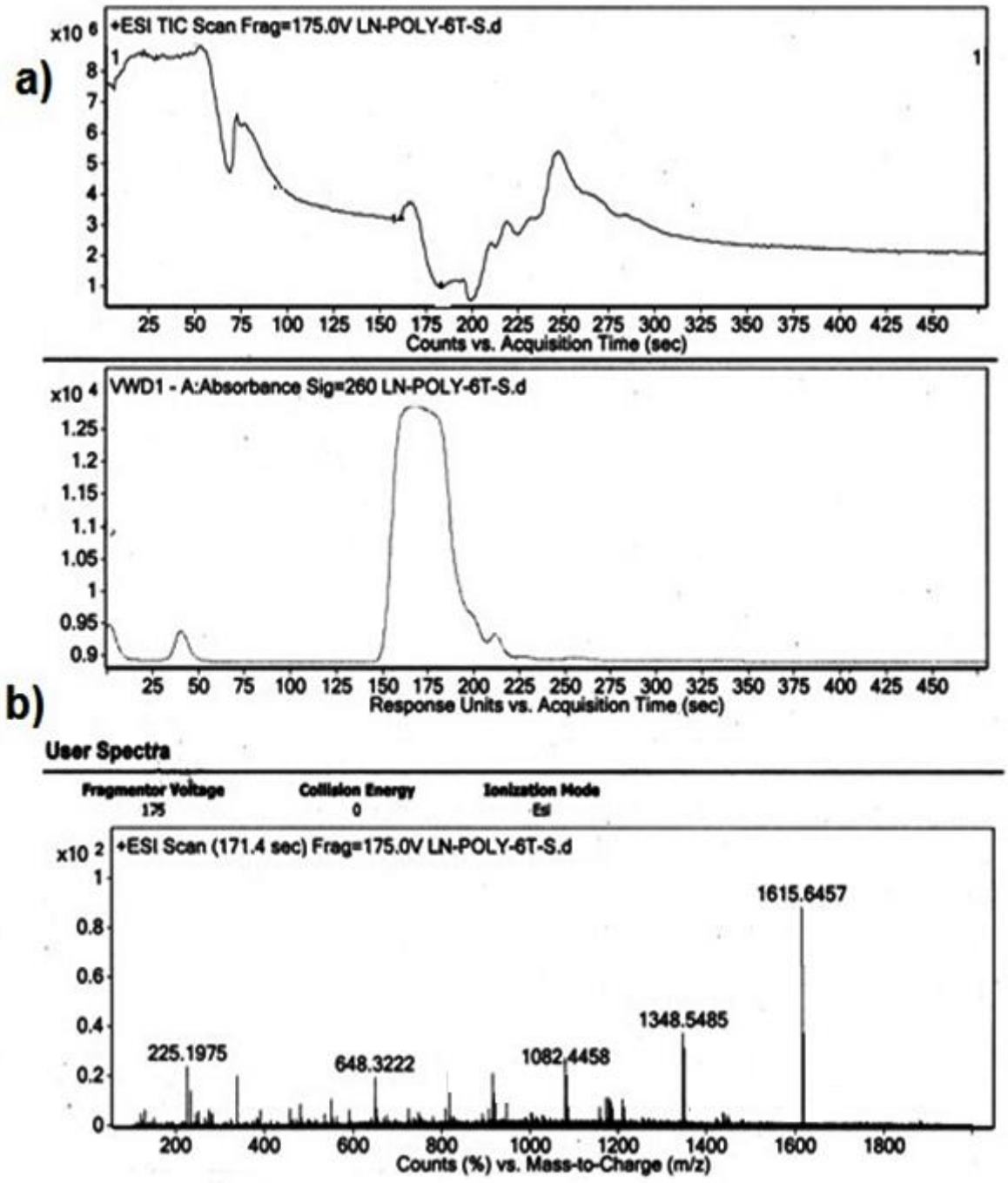


Compound 5c

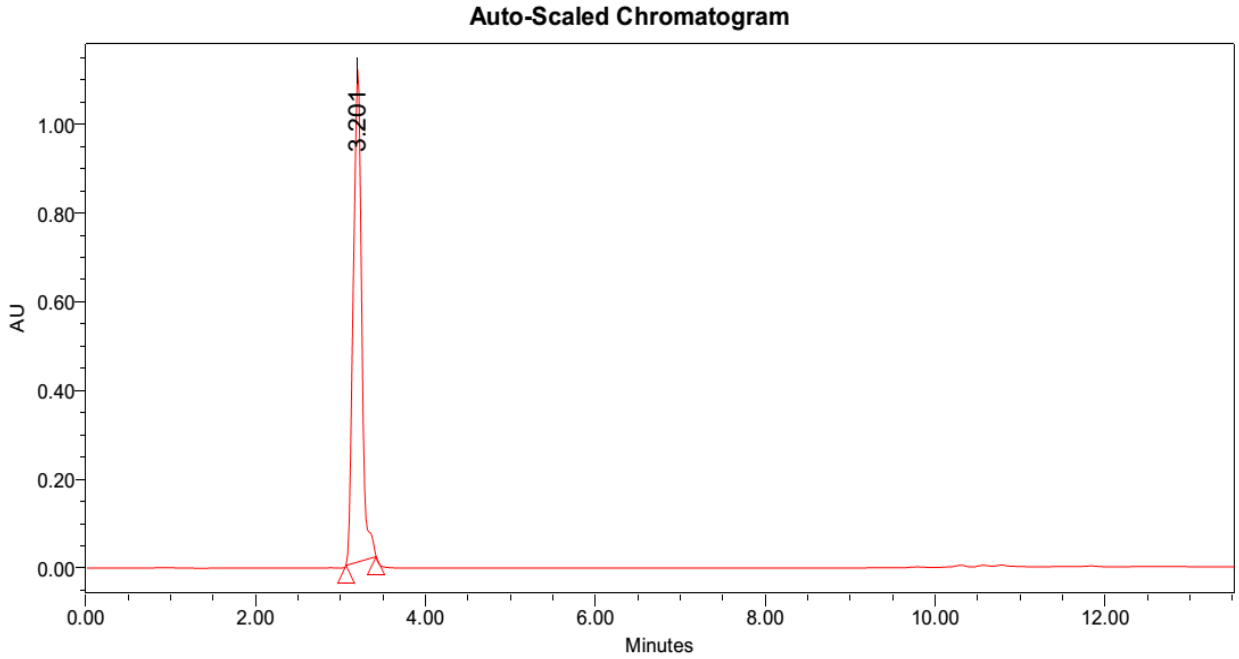




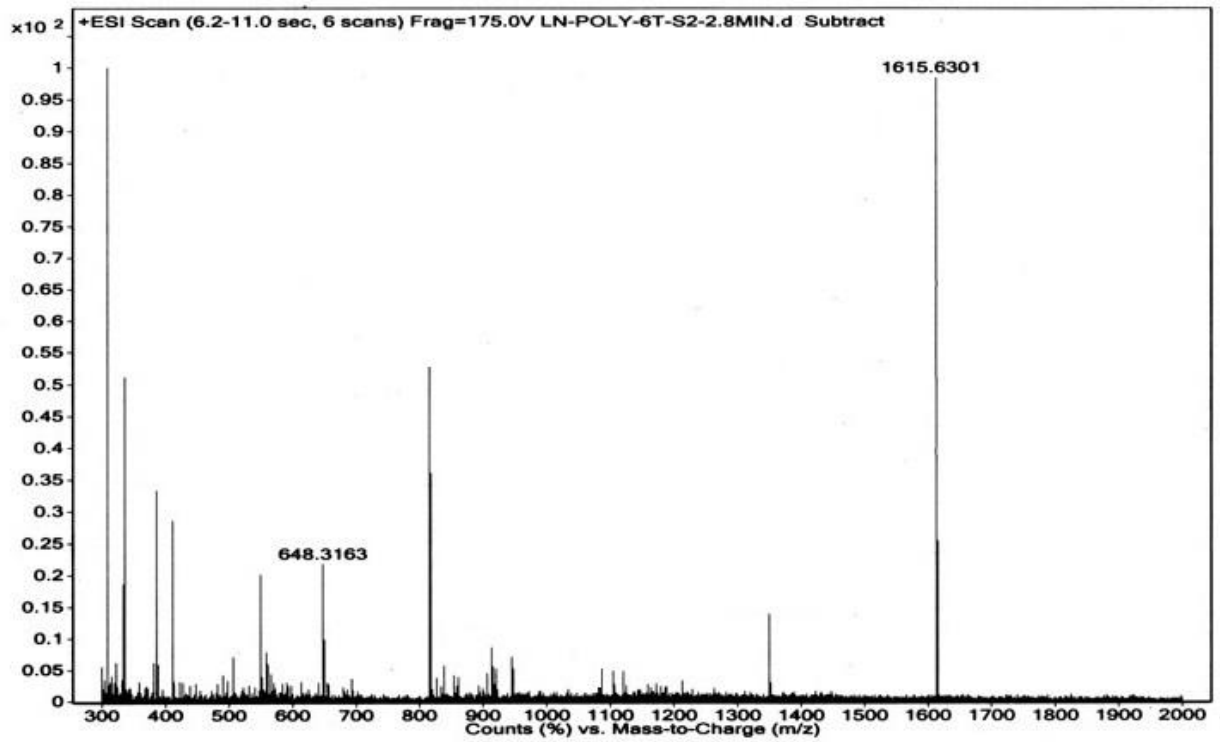
***Oligomer 1 (TTTTTT) LCMS profile**:*



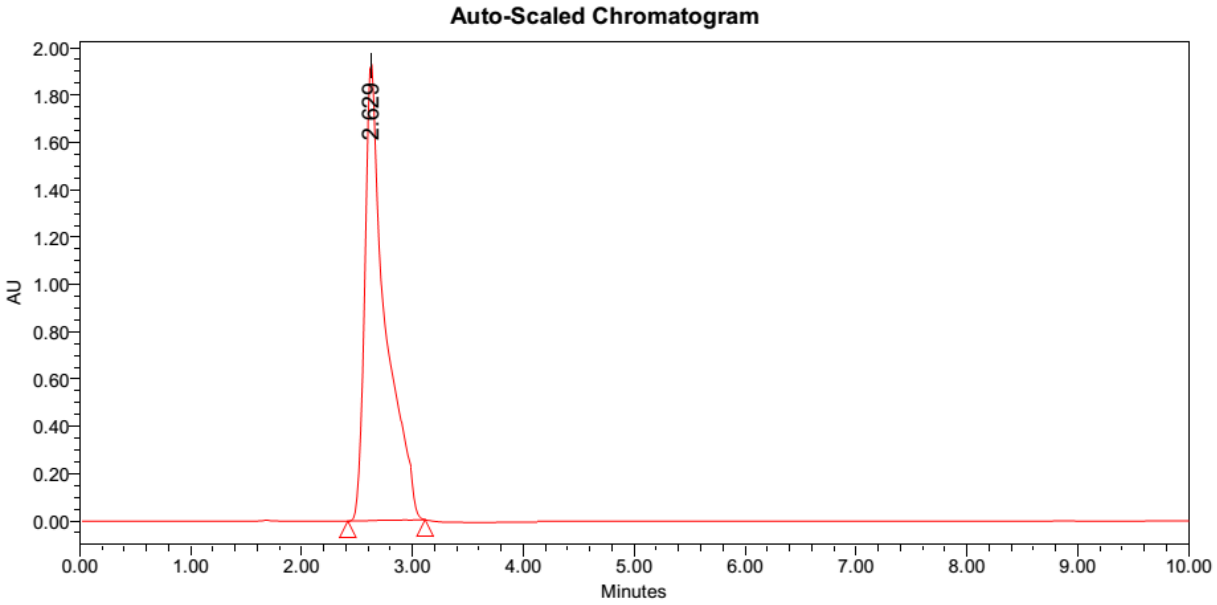
HPLC and MS profile of Oligomer 1 (TTTTTT):



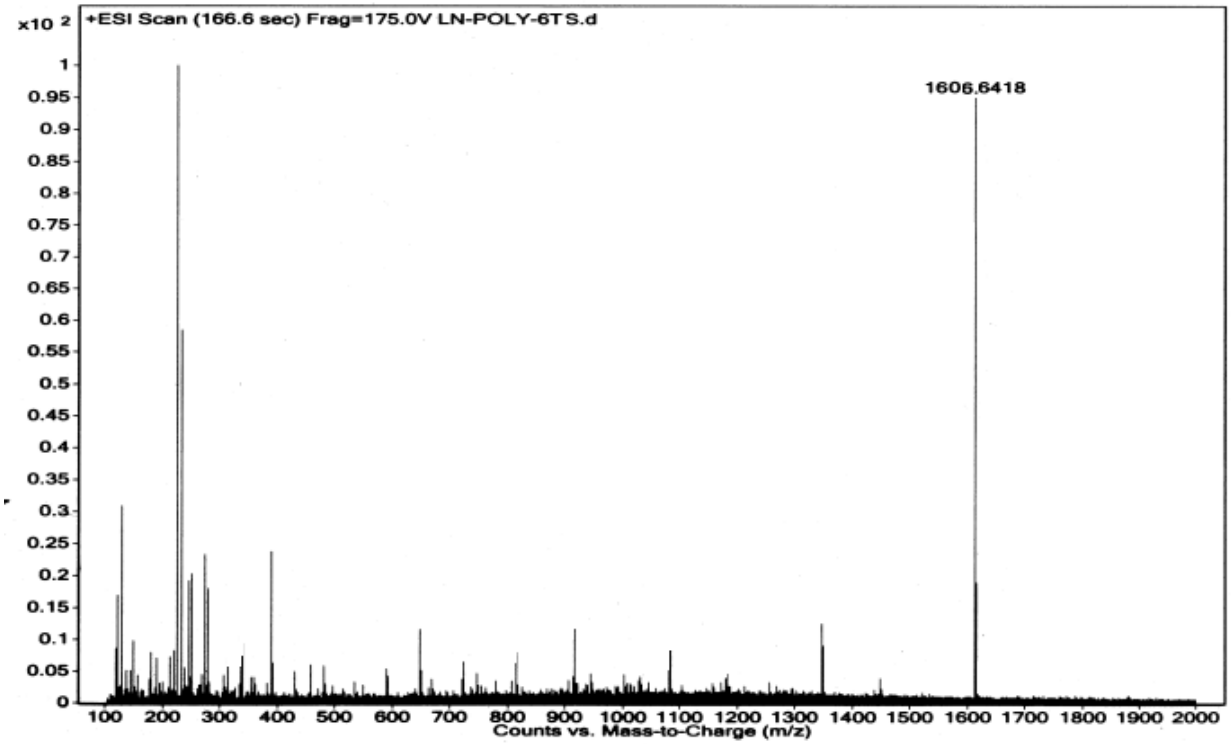
Oligomer 1: LRMS (ESI) m/z $[M+H]^+$ calcd for $C_{66}H_{87}N_{24}O_{25}$: 1615.6269; observed:1615.6301



HPLC and MS profile of Oligomer 3 (TTTTT$^{(indo)}$T):



Oligomer 3: HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{69}H_{88}N_{23}O_{23}$: 1606.6418; observed:1606.6418



List of publications:

6. **Burgula, L. N**.; Gogoi, K and Lal Mohan Kundu*

"Synthesis of modified peptide nucleic acid which forms stable base-pair with DNA" (*Manuscript under preparation*).

5. **Burgula, L. N** and Lal Mohan Kundu*

"An Efficient Chemoselective Microwave-Assisted Methodology for Direct Synthesis of N-3 Substituted Pyrimidinones and 2-Thiopyrimidines from Their β-Carbonylester Precursors" (Communicated).

4. **Burgula, L. N**.; Radhakrishnan, K and Lal Mohan Kundu*

"*Design and synthesis of self assembled novel modified nucleobases for supra-molecular architectures.*"(*Manuscript under preparation*)

3. **Burgula, L. N**.; Gogoi. K, and Lal Mohan Kundu*

"Synthesis of C-5 and C-6 modified bis-pyrimidine dimers and their photochemical evaluations show remarkable stabilities under UVC radiation."*(Communicated)*

2. Radhakrishnan, K.; **Burgula, L. N** and Lal Mohan Kundu*

"Watson-Crick and Hoogsteen tri base pairing of 6-isopropyl uracil with adenine "

RSC. Adv. 2013, 3, 7282.

1. **Burgula, L. N**.; Radhakrishnan, K and Lal Mohan Kundu*

"Synthesis of modified uracil and cytosine nucleobases using amicrowave-assisted method"

***Tetrahedron. Lett*. 2012, *53*, 2639-2642. (*Highlighted in cheminform*)**

Symposia and Conference(s)

International:

1) **Burgula, L. N**.; Radhakrishnan, K and Lal Mohan Kundu*

RSC-**2011** held at IITG, Guwahati, India.

2) **Burgula, L. N** and Lal Mohan Kundu*

***ISCB**-2014* held at university of delhi, Delhi, India.

National:

1 **Burgula, L. N**.; Radhakrishnan, K and Lal Mohan Kundu*

15th CRSI-**2013** held at BHU, Varanasi, India.

2 **Burgula, L. N**.; Gogoi, K and Lal Mohan Kundu*

JNOST-**2012** held at IITG, Guwahati, India.