Synthesis and Physical Studies of 2-Aminopyrimidine and Uracil Derivatives as Nucleobase Analogues and Oligonucleotides

A Dissertation Submitted in Partial Fulfillment for the Degree of Doctor of Philosophy

By

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STATEMENT

I do hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology Guwahati, India, under the guidance 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 the other investigators.

October, 2015.

IIT Guwahati**.** **K. Radhakrishnan**

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CERIFICATE

This is to certify that **K. Radhakrishnan** has been working under my supervision since July, 2010 as a regular registered Ph. D. student. His thesis entitled **"Synthesis and Physical Studies of 2-Aminopyrimidine and Uracil Derivatives as Nucleobase Analogues and Oligonucleotides"** is an authentic record of the results obtained from the research work in the Department of Chemistry, Indian Institute of Technology Guwahati, Assam, India. I am forwarding his thesis for the award of degree of Doctor of Philosophy, from 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.

October, 2015
Guwahati.

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Dedicated to My Family and Friends

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K. Radhakrishnan

Synopsis

The content of this thesis entitled **"Synthesis and Physical Studies of 2-Aminopyrimidine and Uracil Derivatives as Nucleobase Analogues and Oligonucleotides"** is divided into five chapters based on the results of experimental work performed during the research tenure.

Chapter 1:

This chapter discusses the literature review of various modifications in nucleic acids and their applications.

Chapter 2:

It describes the microwave-assisted synthesis of various C-5, C-6 substituted 2-aminopyrimidines as nucleobase analogues.

Chapter 3:

This chapter deals with metal-catalyzed synthesis of 2-aminoquinazolines using microwave-assisted method.

Chapter 4:

This chapter consists three parts which describes the fluorescence, gel properties and metal ion detection of modified pyrimidine nucleobases.

Chapter 5:

This chapter mainly focuses an artificial base-pair formation and peptide nucleic acid synthesis of modified pyrimidines.

Chapter 1: Introduction

This chapter elaborates the synthesis of modified nucleobases, nucleic acids and their various biomolecular applications, such as, gene targeting and gene silencing, through literature survey. This chapter also describes the role of metal ion and supramolecular interactions of nucleobases which are responsible for various biological processes.

Chapter 2: Direct synthesis of 5- and 6-substituted 2-aminopyrimidines as potential non-natural pyrimidine nucleobase analogues

The aim of this chapter is to demonstrate the synthesis of substituted pyrimidine derivatives by developing easy, one-pot synthetic methods. A class of pyrimidine analogues, *viz*. 2-amino pyrimidines, is important pharmaceutical molecules. They also show potential applications as molecular probes due to their altered base-pairing properties. Here, we are reporting one-pot synthesis of 2-amino pyrimidines and 2, 4-diaminopyrimidines using environment friendly microwave assisted method, under solvent free condition. Microwave reaction is one of the reliable methods to carry out various organic transformations in short time. A library of various 5- and 6- substituted 2-amino pyrimidines were synthesized with varying steric and electronic properties of the substituent. A number of such molecules were designed to obtain altered base-pairing properties as well fluorescence properties. Importance of this methodology is that large variations can be synthesized with high yield, in a very short time under mild condition.

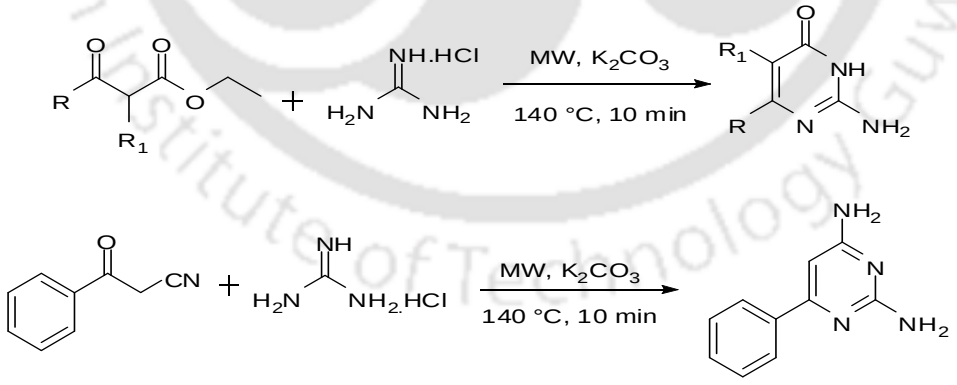


Figure 1. Schematic representation of 2-amino and 2, 4 diaminopyrimidine syntheses

Some representative examples:

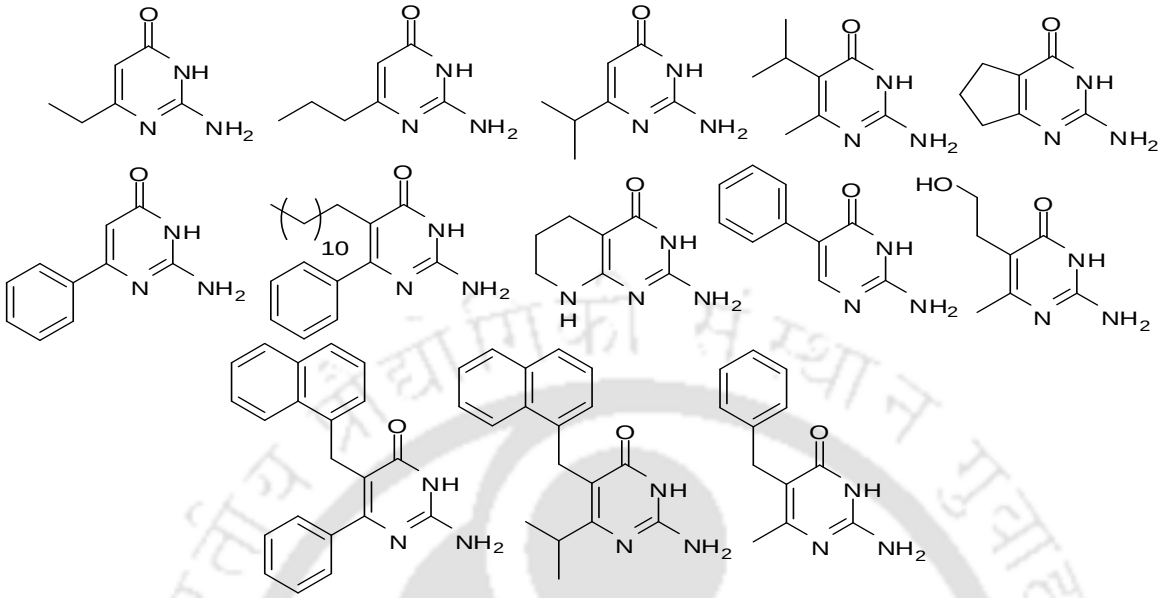


Figure 2. Table of some 2-aminopyrimidine (isocytosine) derivatives

The base pairing properties of selected 2-amino pyrimidines were achieved through co-crystal structures with cytosine as counterpart.

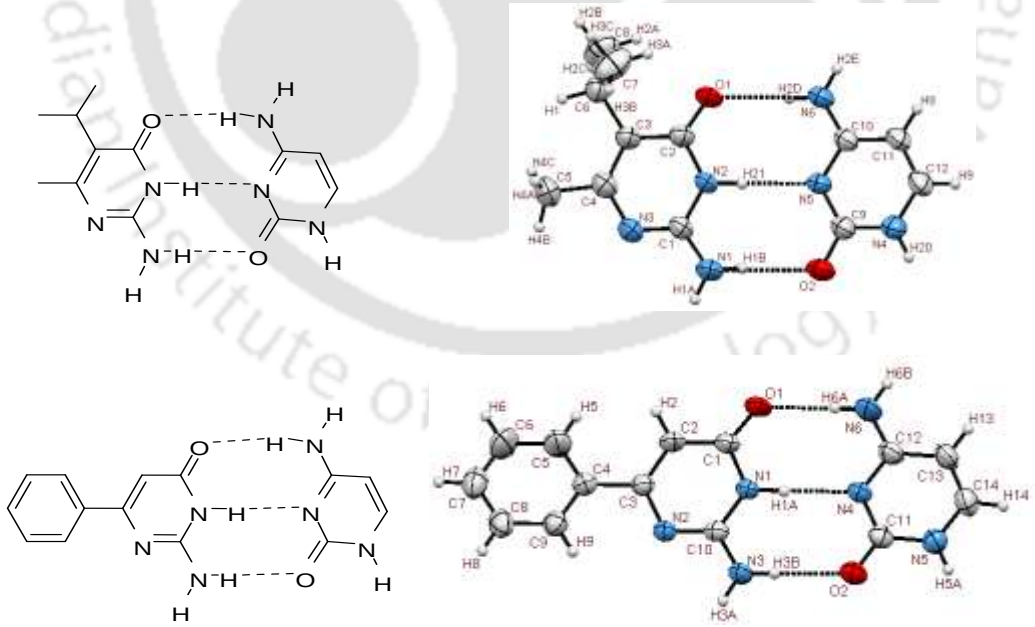


Figure 3. Base pairing interactions of 2-aminopyrimidines

Chapter 3: Copper catalyzed microwave-assisted synthesis of ring expanded pyrimidine nucleobase analogues

In this chapter a new methodology was developed for the synthesis of quinazoline derivatives as effective non-natural nucleobase analogues. Quinazolines are important molecules in heterocyclic chemistry which posses many medicinal and pharmaceutical applications. 2-amino quinazolinone is a biologically active derivative of quinazoline which has structural resemblance with nucleobase analogues, especially, pyrimidine analogues. So these can be called as expanded pyrimidine nucleobase analogues. Design of modified nucleobases can be made based on the following three important properties: 1) fluorescence, 2) base-pair selectivity and 3) pi-stacking. Even though variety of nucleobases were synthesized with many modification, ring expanded nucleobases play a crucial role to extent the genetic alphabet and DNA based sensing techniques. The duplex stability of ring-expanded nucleic acids is much higher than the unmodified duplex. These stability enhancements not only depend on hydrogen bonding but also depend on hydrophobic interactions.

Here we are presenting the synthesis of 2-aminoquinazolinone derivatives (ring expanded nucleobase analogues) through copper-catalyzed coupling reaction using microwave-assisted method. Such a methodology was never established before. Copper is one of the best catalysts for various coupling reactions, especially, cross-coupling reactions. 2-aminoquinazolinone derivatives were synthesized by treating substituted *ortho*-halobenzoic acid with guanidine hydrochloride, in presence of copper (I) oxide. Cesium carbonate was found to be a good basic source for these reactions. All the reactions were carried out using *Labmate CEM Discover* microwave reactor in a closed vessel. With a selected synthesized molecule, base-pairing properties were studied through NMR titration. A number of synthesized molecules were also found to be fluorescence active and showed selective sensing of Pd (II) metal ion **(Chapter 4)**.

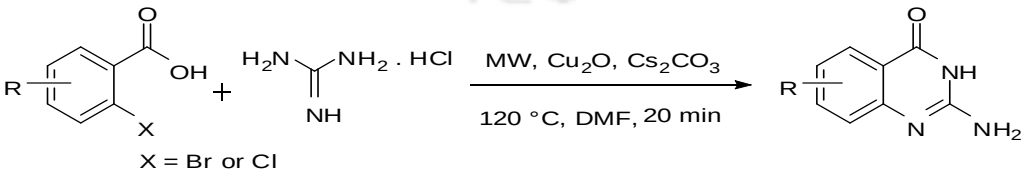


Figure 4. Schematic representation of 2-aminoquinazoline synthesis

Some representative examples:

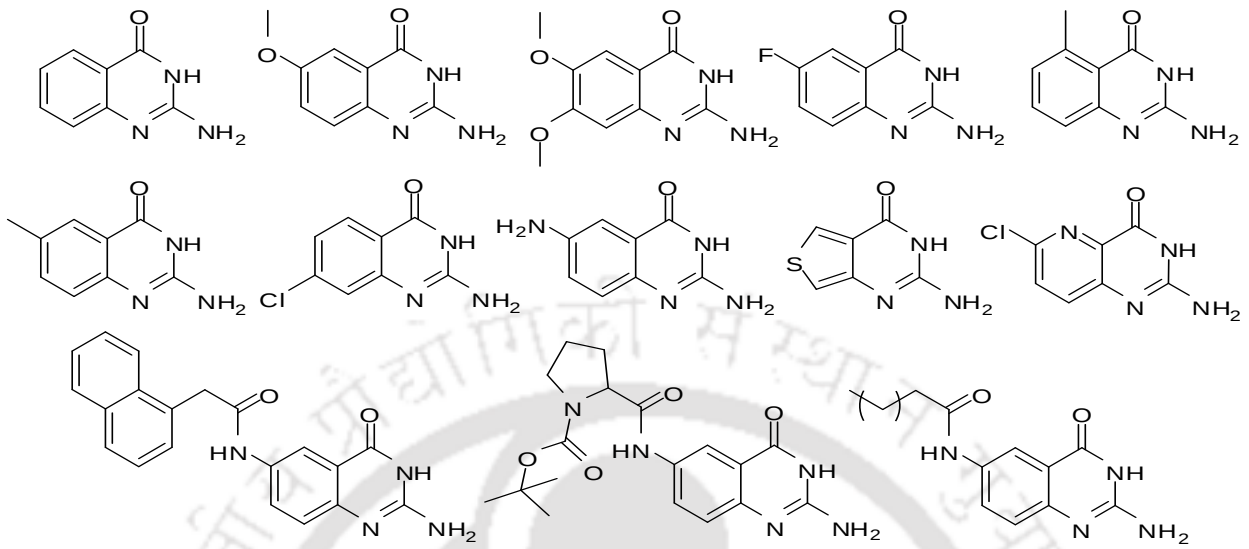
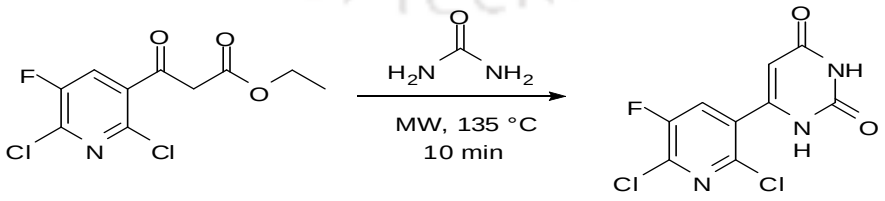


Figure 5. Table of some 2-aminoquinazoline derivatives

Chapter 4: Design and synthesis of pyrimidine and 2-aminopyrimidine nucleobases as molecular probes

(a) Fluorescence active nucleobases

In literature, almost all the DNA or protein based molecular probes that show fluorescence properties are due to labeling of the probes with a fluorescent molecule. In the present work we have developed nucleobase that is, in itself, fluorescence. The substituted pyridine moiety at the C-6 position of the nucleobase does not show any fluorescence. The fluorescence signal is obtained only when it is conjugated to the isocytosine or uracil nucleobases. Preliminary experiments show that the modified nucleobases still retain their base-pairing properties. Such a bioactive, fluorescent nucleobase could have enormous potential in biological chemistry, such as imaging, selective detection of mismatches, specific protein-DNA interactions etc.



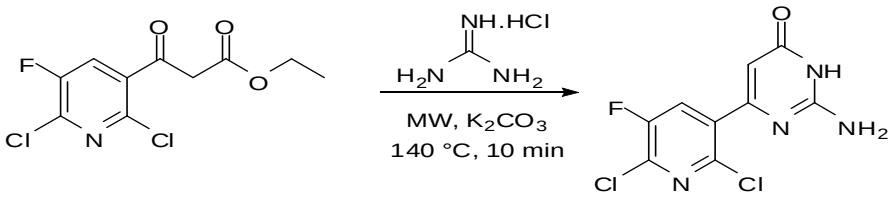


Figure 6. Schematic representation of fluorescence active pyrimidines synthesis

(b) Gel-forming nucleobases as selective probe

Small molecule gelator nucleobases were never reported in literature. Here we report 6-propyl isocytosine, a 2-amino pyrimidine derivative, which exhibit special feature to form gel in organic solvents such as DMSO, ethanol, and DMF. The critical gel concentration was achieved through a number of experiments. The goal of this chapter is to establish structural factors that could be responsible for such gel formation and how it is assisting or affecting polymeric network in organic solvents. For this purpose we have synthesized various 5- and 6- substituted 2-amino pyrimidines as described in **chapter 2**, using microwave-directed method. An extensive analysis and studies of crystal packing revealed that substituent at C-6 position is responsible for such gel formation.

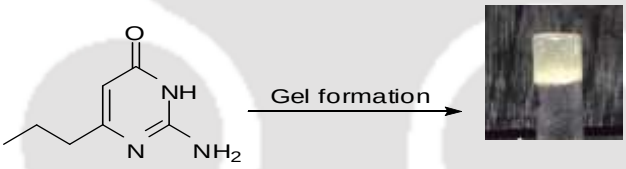


Figure-7: Representation of gel forming 2-aminopyrimidine

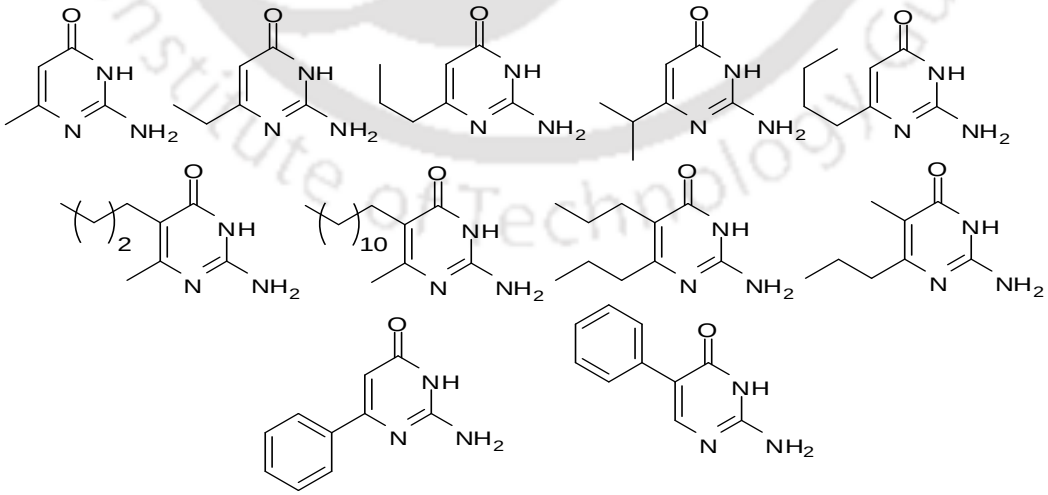


Figure 8. Some examples of 2-aminopyrimidines used for gel study

Base -paring study through gelation process (probe for cytosine detection)

We have explored the base-paring property of the synthesized gelator through gel formation and deformation processes. Upon addition of equimolar cytosine nucleobase, which is known to form strong base-pairs with isocytosine, the gel undergoes deformation. The strong H-bonding interaction between the synthesized isocytosine and natural cytosine nucleobase was confirmed by ^{1}H-NMR technique. Addition of other natural nucleobases, such as, thymine and adenine were found to have no effect on the gel. The result suggests that gelator isocytosine could be utilized as a molecular probe for selective detection of cytosine nucleobase.

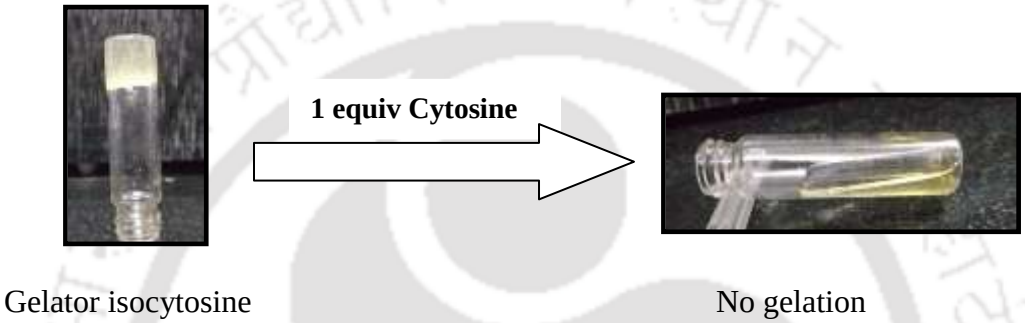


Figure 9. Representation of gel formation and deformation process

(c) Selective metal ion sensing using modified nucleobase analogue

In nucleic acid chemistry, metal ions are playing important role, especially, metal-mediated base-pair process. Here, the metal ions are binding with phosphate moiety or nucleobase to assist the base-pair formation. Based on these studies, chemists have developed nucleobase derived sensors which are useful to detect various metals such as Hg, Ag etc in biological system.

Here, we have developed a nucleobase derived fluorogenic sensor which selectively binds with Pd (II). The fluorescence of the synthesized 2-aminopyrimidine gradually decreases upon addition of the metal ion. The Job's plot also suggests that the binding ratio of the molecule to Pd (II) is 1:1.

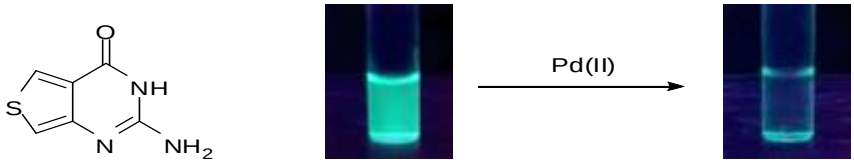


Figure 10. Representation of metal sensing

Chapter 5: Synthesis of pyrimidines for artificial base-pairing and peptide nucleic acid

The double helical structure of naturally occurring DNA is largely attributed to *Watson–Crick* base pairing interactions, along with pi-stacking forces. Usually, adenine forms a H-bonded complex with thymine, whereas guanine forms a base-pair with cytosine. *Hoogsteen* base-pairs were also found in nature, where the formation of a triple helix was more probable. The formation of such triple helices could be important to selectively modulate gene expression, by controlling gene transcription.

We present a *Watson–Crick* and *Hoogsteen* tri-base pairing co-crystal structure formed by adenine and 6-isopropyluracil in 1:2 ratio. 6-Isopropyluracil was used to replace thymine, since the 6-isopropyl substituent can render a higher hydrophobic property compared to the methyl group on thymine. The co-crystal was obtained when equimolar quantities of 6-isopropyluracil were mixed with adenine in water–methanol (2:1 v/v), followed by slow evaporation of the solvents at ambient temperature. One molecule of the adenine nucleobase was found to be flanked by two molecules of the uracil derivative, forming a tri-base pair. Careful crystal structure analysis showed that part of the adenine is hybridized to a 6-isopropyluracil, through *Watson–Crick* base-pairing. The other part of the adenine is bound to a second pyrimidine base via *Hoogsteen* interactions.

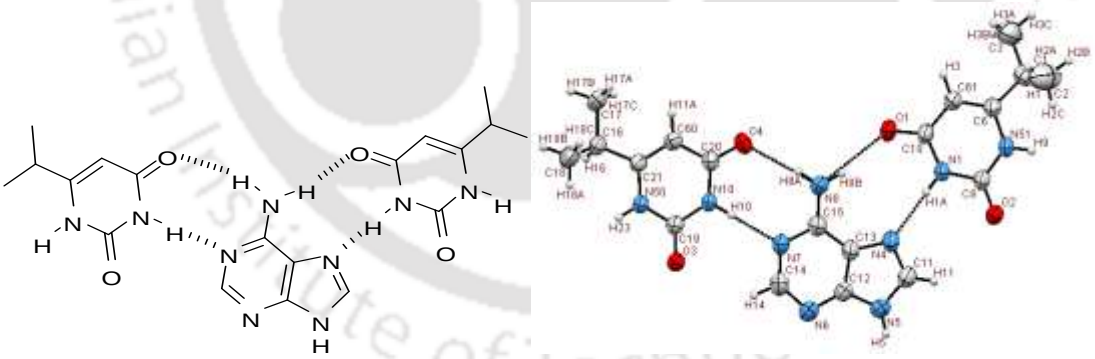


Figure 11. Co-crystal of 6-isopropyluracil-adenine

In this Chapter we also report the synthesis of peptide nucleic acid using solid phase peptide synthesis. Peptide nucleic acids have numerous applications in biology due to their thermal stability, and better hybridization properties. Here we demonstrate the synthesis of peptide nucleic acid contains modified nucleobase.

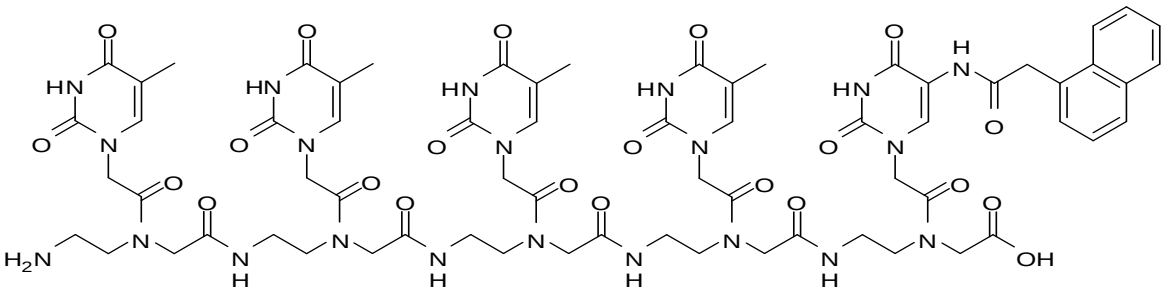
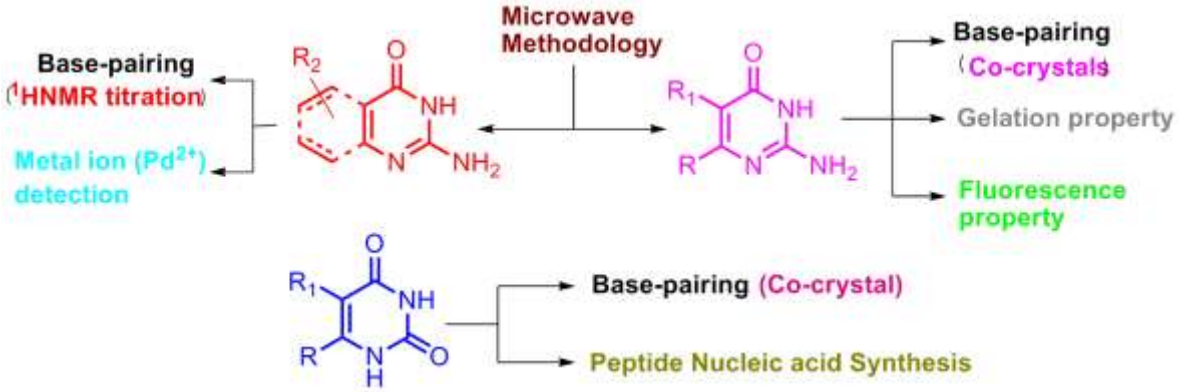


Figure 12. Representation of modified peptide nucleic acid

Summary

In this thesis, we have shown the synthesis of pyrimidine nucleobase analogues using microwave assisted method in short time. We have explored the base pairing interactions through co-crystal and NMR studies. Metal sensing ability of pyrimidine analogue was shown by fluorescence spectroscopy and gel forming property of pyrimidine was illustrated by various solvents and crystal structure studies.

Thesis Overview



Contents

Chapter 1

Introduction

I. Structure and Functions of DNA

DNA is the genetic material found in most of the living organisms. It is the storage unit and carries the genetic information in the living cells. DNA is polymeric structure of monomeric unit called nucleotide which consists of nucleobase, sugar, and phosphate as components. The double helical structure of DNA enables it to amplify and re-generating itself. The negatively charged phosphates present in outer surface of the double helix, making it highly water soluble. In the interior of the duplex aromatic nucleobases, attached to the 2'-deoxyribose sugar moiety, are stacked and paired with each other through hydrogen bonding. Strong hydrogen bonding and π-stacking interaction between the nucleobases enables stability to the double helix.

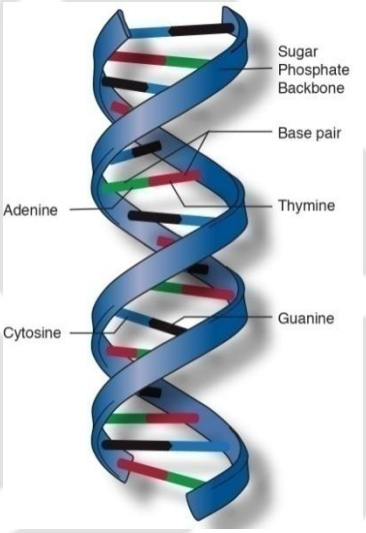


Figure I.a *DNA Double helical Structure*

The naturally available nucleobases in DNA are adenine, guanine (known as purine nucleobases), thymine and cytosine (known as pyrimidine nucleobases).

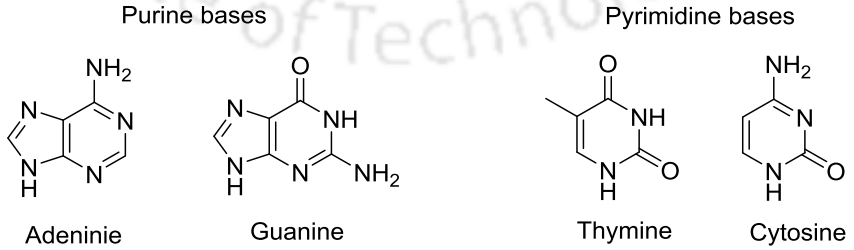


Figure I.b *Structure of natural nucleobases*

The base pairing was first introduced by *Watson* and *Crick* in 1953, showing that naturally abundant DNA preferably shows a B-type double helical structure, generated mainly due to base pairing between the purine and pyrimidine nucleobases.[1]

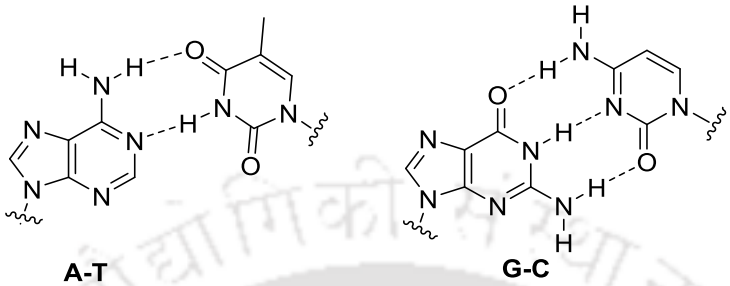


Figure I.c *Base pair of nucleobases (Watson-Crick interactions)*

Factors that influence the formation of a double helix (B DNA) are:

Hydrogen bonding: Hydrogen bonding can be considered as electrostatic interaction between an acidic proton and a good electron donor. In DNA, nucleobases act as good donor as well as acceptor to form stable double helix.[2] Normally, adenine-thymine base pair is formed by two hydrogen bonds whereas guanine-cytosine base pair is formed via three hydrogen bonds.

π-stacking interaction: This effect arises due to π-electrons present in the heterocyclic nucleobases. So neighbouring nucleobases exhibit strong π-stacking interaction and is a major force in stabilizing the helix.[2]

Sugar pucker conformation: Although the 2'-deoxyribose sugar can adopt several conformation, C2'-endo twist is the most favorable conformation that allows the formation of the duplex DNA.

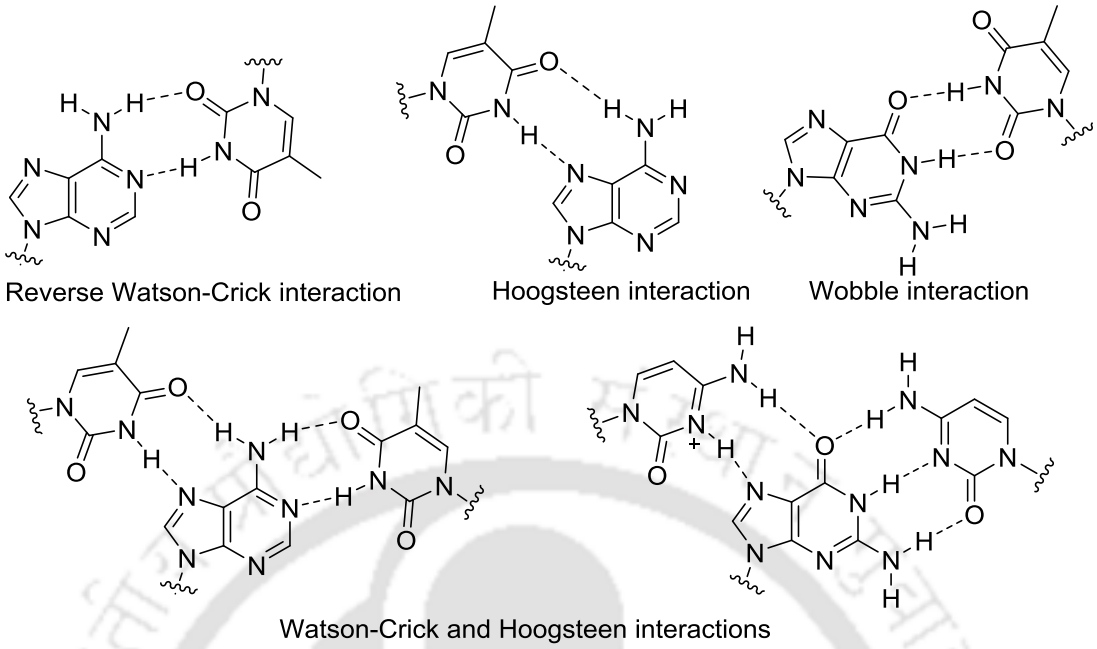


Figure I.d *Some other possible non-covalent interactions in DNA*

I.1 Modified Nucleic acids

Modified nucleic acids are important biomolecular probes for efficient detection of genetic mutations, gene targeting and to study other important biological processes.[3,4] Synthesis and development of novel nucleobases, therefore, have drawn significant research interest since past decade.

Modified nucleic acids are developed in three ways: 1) modification in nucleobases 2) modification in sugar moiety and 3) modification in the backbone. Modification in DNA structure can induce new properties which natural DNA and RNA do not have. These properties can be useful for the practical application of nucleic acids as biomolecular probes for detection of point-mutations in DNA, gene targeting, and DNA-protein interaction.

I.2 Modifications in nucleobases

Modified nucleobases are immensely important, apart from being used as molecular probes, as pharmaceutical agents ranging from anti-bacterial agents to anti-tumor drugs. Base modification in nucleic acid is the easiest way to find out structure, dynamics, and functions of nucleic acids. Various modified nucleobases have been synthesized with some unique properties.

I.2.1 Simple DNA bases

Substituted pyrimidines are more stabilizing to nucleic acid helices than the natural pyrimidine nucleobases. Halogen substitutions enhance the *van der Waals* interactions with neighbouring bases and aromatic substitutions enhance the stacking to give stability to the helices.[5-13] Besides, inclusion of functional groups also have much impact in nucleic acids because such nucleobases have the tendency to alter or create new base-pairs.[14-19] Some of the modified nucleobases, reported in literature, are given below.

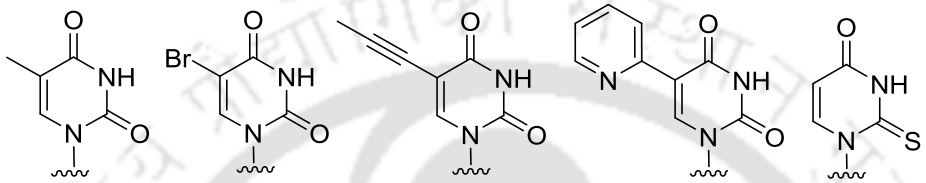


Figure I.2.1a *Modified Pyrimidine Nucleobases*

Modified nucleobases as Drugs

Modified nucleobases are used in drugs because of their potential application. They can act as antiviral agents and in the treatment of tumors.[20, 21]

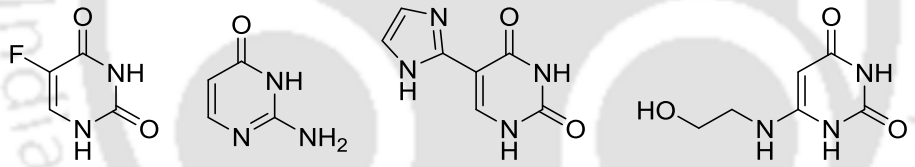


Figure I.2.1b *Nucleobase analogue drugs*

I.2.2 Fluorescence active nucleobases

Simple fluorescence active nucleobases: The synthesized nucleobases with minimum structural perturbations could be developed to introduce novel physical properties. Fluorescence is an important as well as a powerful tool to know the nucleic acid structure and functions.[22] The nucleobases commonly found in nucleic acids are naturally fluorescent inactive or non-emissive in neutral aqueous conditions. A number of fluorescence active pyrimidine nucleobases have been successfully synthesized and their applications were studied in nucleic acids. Tor *et al*. have reported various heterocyclic substituted fluorescent pyrimidine nucleobases in DNA.[23-29]

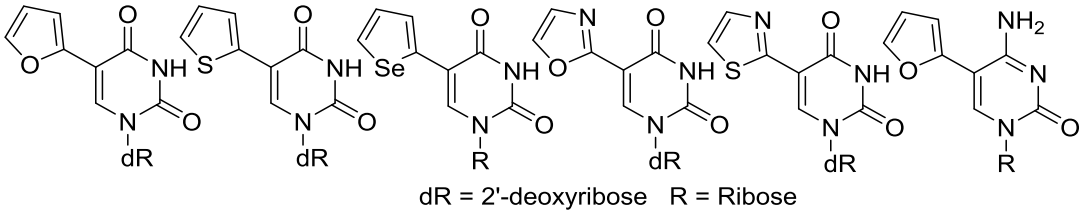


Figure I.2.2a *Fluorescence active pyrimidine nucleobases with heterocyclic substitution*

Size expanded nucleobases*:* The impressive work in changing the base portion of the nucleic acids has been undertaken by Kool and co-workers, who have synthesized a range of fluorescence active expanded nucleobases, primarily to see how these modifications affect the *Watson-Crick* pairing, but ultimately to expand the genetic code. These expanded nucleobases have very good π-stacking and increases the duplex stability.[30-34]

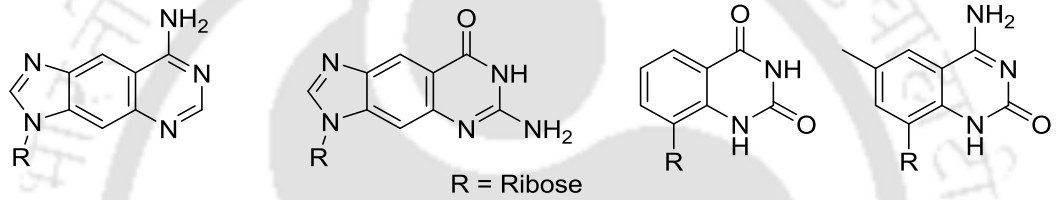


Figure I.2.2b *Kool's expanded DNA sets*

Various heterocyclic size-expanded nucleobases have also been synthesized and were efficiently studied in DNA sequences. The planarity nature of aromatic heterocyclic moieties also maintains the π-stacking properties in nucleic acids.[35,36]

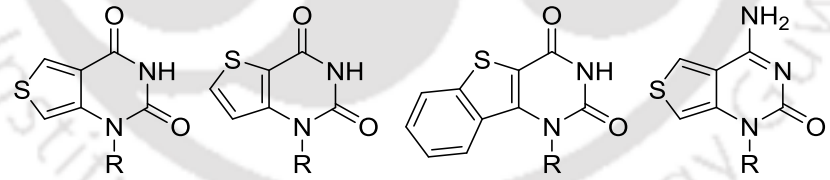
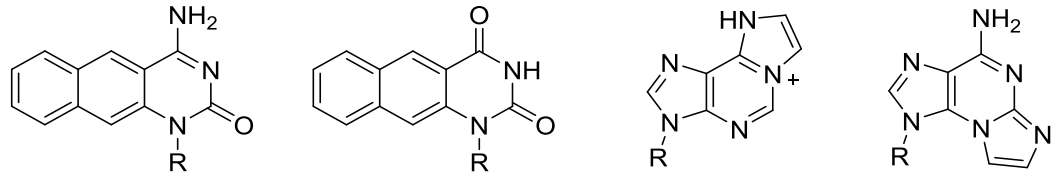


Figure I.2.2c *Heterocyclic expanded nucleobases*

The direction of the expansion has also been made in nucleobase that would have new properties in nucleic acids such as increase the stability and alter the base-pairing.[37-42]



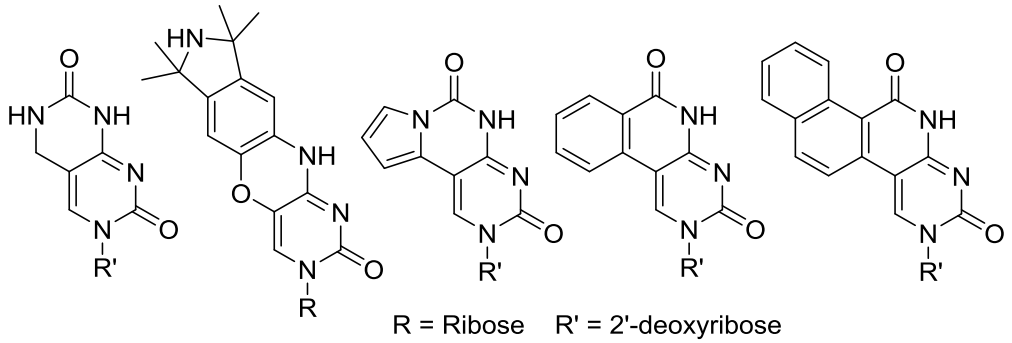


Figure I.2.2d *Nucleobases expansion in different direction*

Fluorophore labelled nucleobases: Fluorescent DNA bases play vital role as efficient biomolecular probes to elucidate structural studies of DNA and RNA and also DNA repair and replication.[43-50]

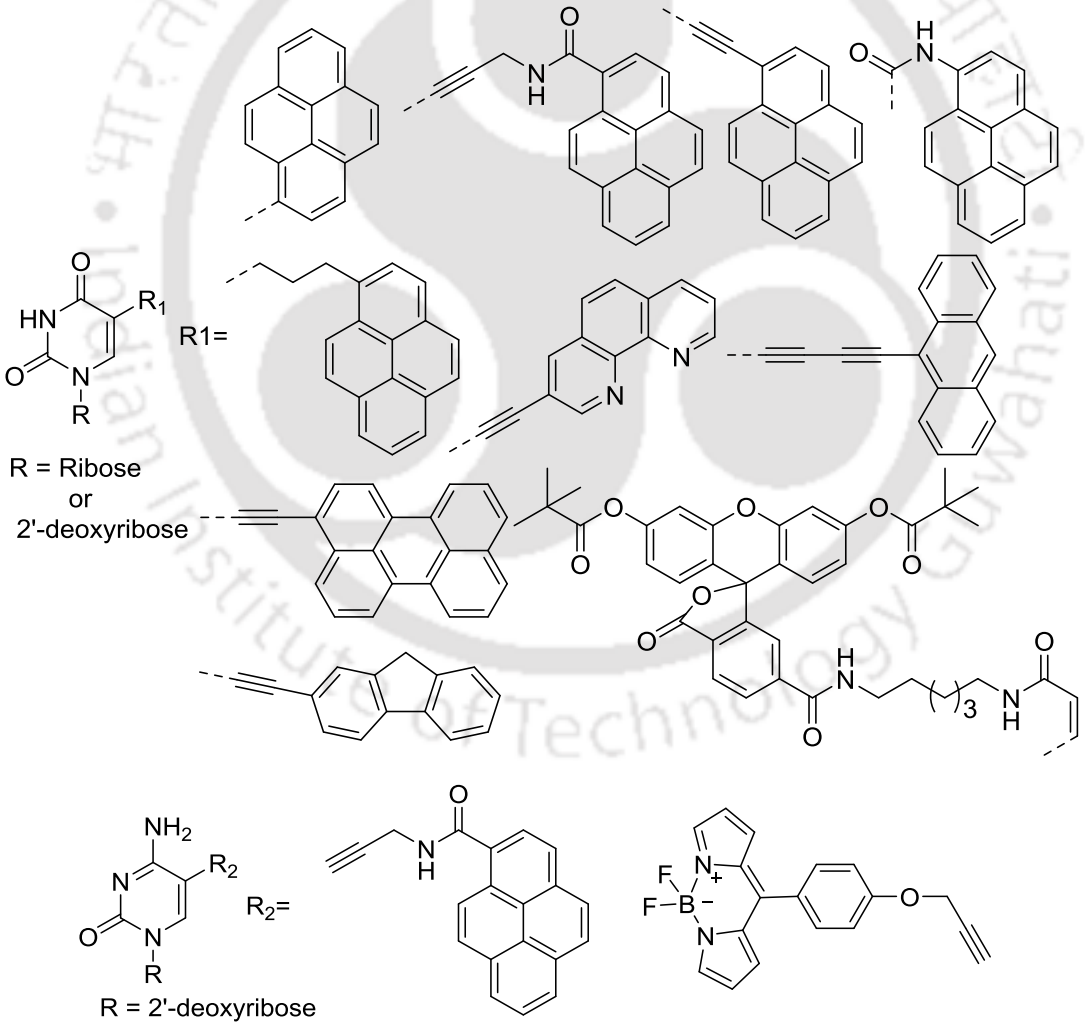


Figure I.2.2e *Fluorophore labelled nucleobases*

Thus, nucleobases substituted with aromatic rich fluorophores were incorporated into DNA, which may act as reporter group. Usually, metal coupling reactions were used to attach the nucleobases with the fluorophores.

Non-polar nucleobase analogues: Comparison of π-stacking affinities of natural DNA bases and simple aromatic hydrocarbons showed that hydrophobic, large surface area is an important factor in increasing the stacking interactions.[51-54] Such designer molecules, which lack H-bonding interactions, were found to be recognized by *polymerases* during PCR. Some non-polar aromatic hydrocarbons and heterocylcles act as DNA base analogues are given below.

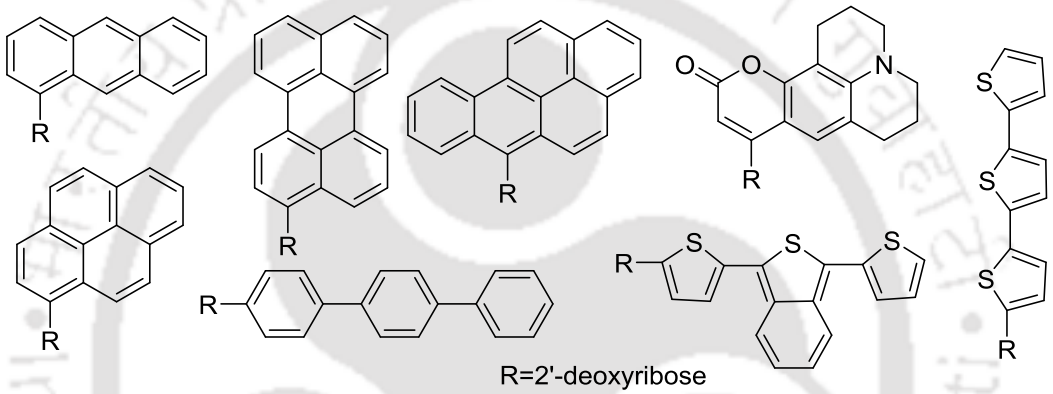


Figure I.2.2f *Non-polar DNA base analogues*

I.3 Modification in the backbone

I.3.1 Modification of the sugar moiety

Locked nucleic acid (LNA) is one of the valuable modified nucleic acids, developed by *Wengel* and has found many biological and medicinal features. A normal LNA has been synthesized by attaching 2'-oxygen and 4'-carbon of the ribose ring though a methylene linkage. The modifications at the sugar moiety introduce conformational restriction into the oligonucleotides. This conformational change in locked nucleic acids would encourage the base for high affinity hybridization.[55-58] Such locked oligonucleotides have been used as potential therapeutic agents.[59]

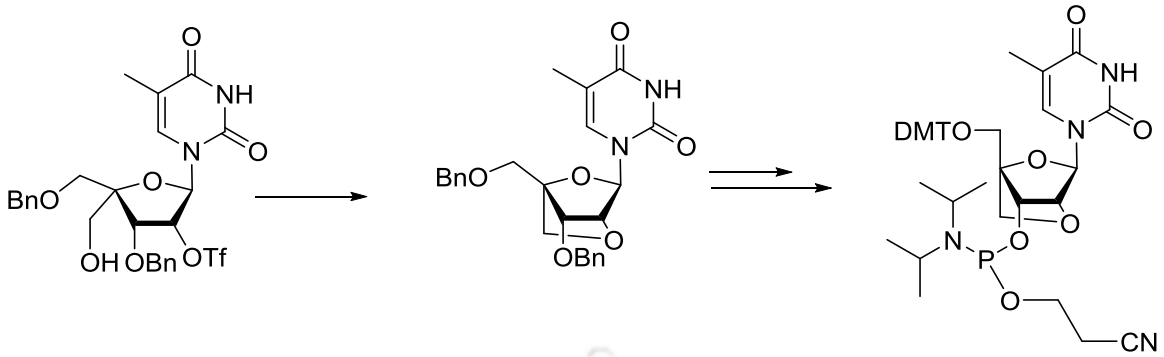


Figure I.3.1a *Scheme of LNA synthesis*

Various structural analogues of LNA mimics containing O, S and N atoms have been synthesized and studied for a range of attributes.[60-62] Most of the locked nucleic acids exhibit high binding affinity to RNA.

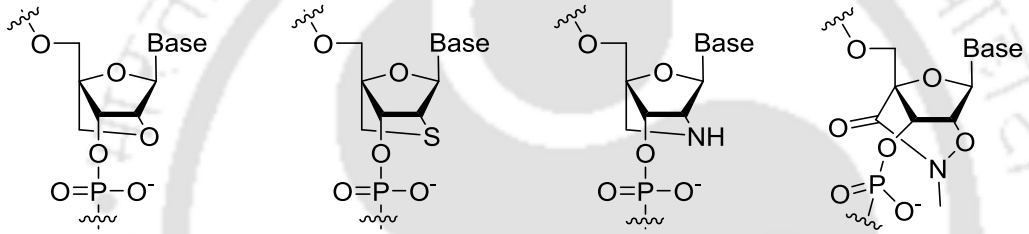


Figure I.3.1b *Modified LNAs*

The pyrene labelled LNAs were reported for high thermal affinity towards target nucleic acid containing abasic sites. It is also reported that pyrene would also stabilize the duplex due to its π-stacking with other nucleobases. A number of LNA's have been reported for improved target selectivities with various unique modifications.[63, 64]

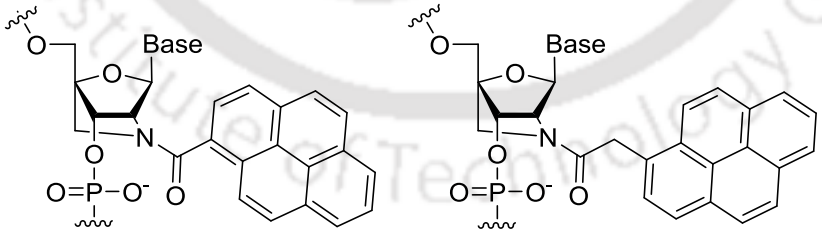


Figure I.3.1c *Labelled LNAs*

I.3.2 Peptide Nucleic Acid (PNA): Replacement of sugar-phosphate group

In DNA, the deoxyribose phosphodiester backbone is replaced by peptide to form PNA. This neutral peptide backbone affects the free rotation of the backbone. The structure of PNA is simple, consisting of repeating N-(2-aminoethyl)-glycine units linked by amide

bonds. The purine and pyrimidine bases are attached to the backbone through methylene carbonyl linkages. In 1994 *peter E. Nielsen* and co-workers showed the synthesis of peptide nucleic acid monomers containing the four natural nucleobases. These monomers were used for the preparation of mixed-sequence PNAs.[65] PNA sequences form strong duplexes with DNA at lower ionic strength, and also form very strong triplexes even at normal ionic strength. Unlike DNA, PNAs do not contain any sugar moieties or phosphate groups. The novel PNA is shown below.

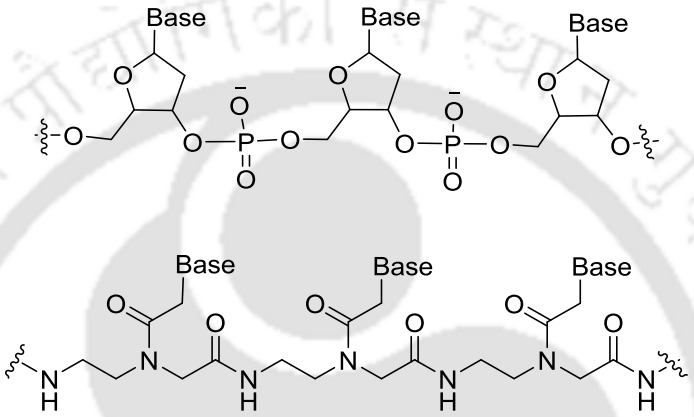


Figure I.3.2a *Structure* of *DNA and PNA*

PNA is a powerful new biomolecular tool with a wide range of important applications.[66-69] PNA mimics the behaviour of DNA and bind complementary nucleic acid strands. PNA has received great attention due to many favourable properties including chemical and thermal stability, stronger and faster binding affinity to the complementary nucleic acid, hybridization under low salt concentration, and higher specificity and sensitivity to a single mismatch as well as its ability to be nuclease stable, enabling it a cell viable probe.[70-73] PNA also obeys the *Watson-Crick* hydrogen bonding rule and has ability to form a triplex with duplex via strand invasion.[74] The above unique properties of PNA have been exploited to produce powerful bimolecular tools, antisense and antigene agents, molecular probes and biosensors.[74].

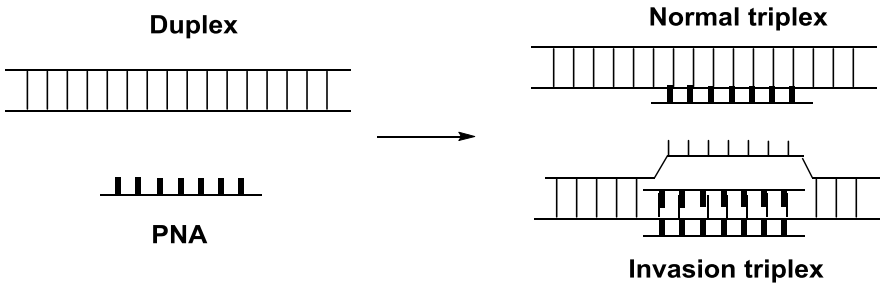


Figure I.3.2b *Pictorial representation of triplex formation of PNA*

A variety of conformational modified peptide nucleic acids have been synthesized and utilized for specific target binding with DNA strands.[75-77] Here, the backbone is converted into five or six membered cyclic ring which can produce various conformational changes and it may cause variation in binding of PNA.

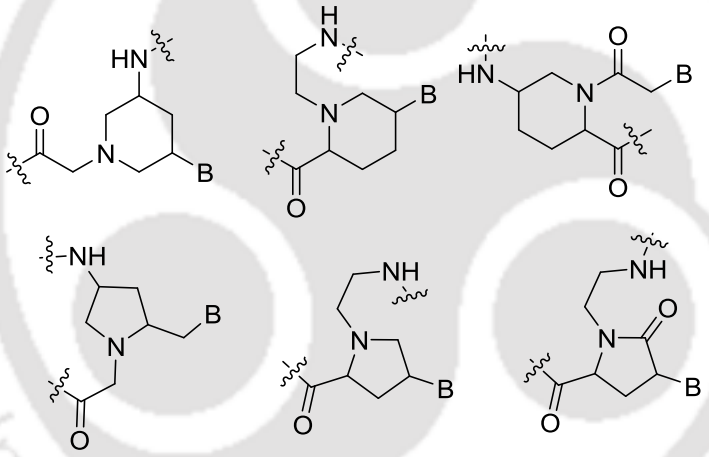


Figure I.3.2c *Modified PNAs*

The base modified PNA also have been reported by various groups where the base portion was altered without affecting its hydrogen bonding pattern.[78-81]

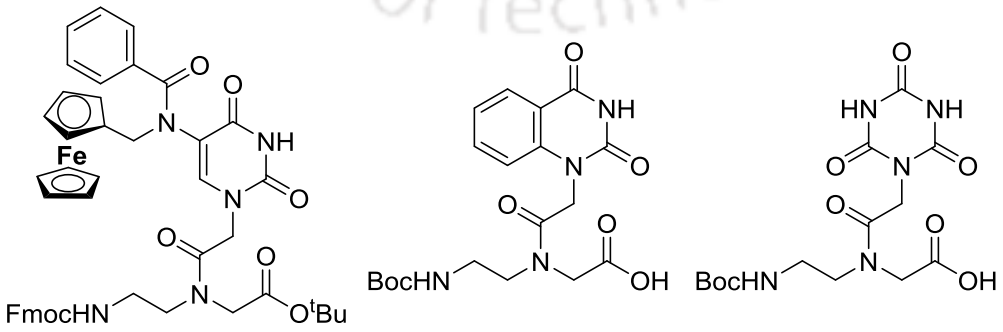


Figure I.3.2d *PNA monomers have modified nucleobases*

I.4 Role of metal ions in nucleic acids

The role of metal ions in nucleic acids is well established and the interaction between them would bring new functions to the nucleic acids. Transition metal ions have good binding affinity towards nucleic acids. Nucleobases have different metal ion affinities and the stability order is Guanine > Adenine, Cytosine > Thymine. When nucleobases are incorporated into DNA duplex, the binding towards metal ions are changed. Metal ions such as Cu^{2+}, Mn^{2+} and Pt^{2+} would prefer GC rich regions but Hg^{2+} prefers AT regions.[82,83] Metal ion binding to nucleobase residues is sequence dependent and such metal complexes may be used for gene silencing.[84, 85]

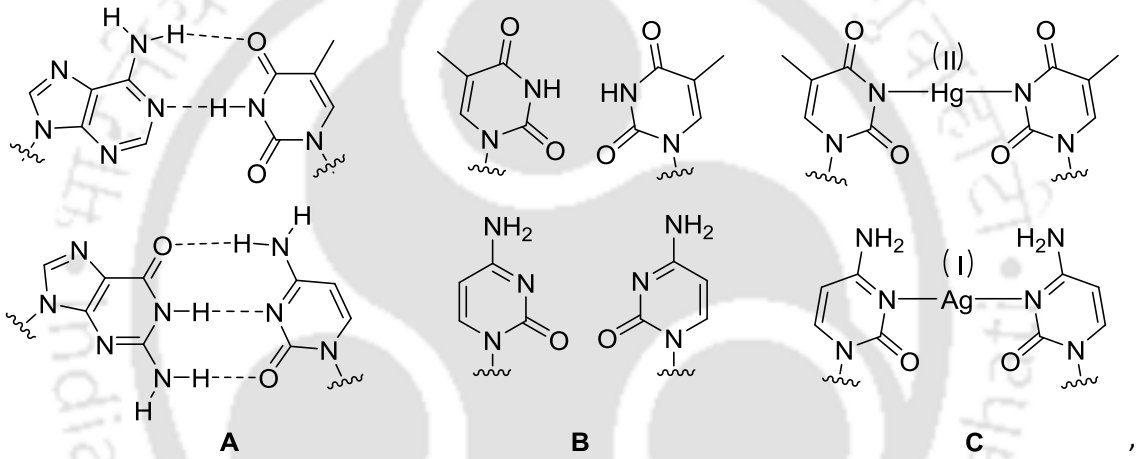


Figure I.4a *A) Natural base pairs B) Pyrimidine mis-pairs C) Metal mediated base pairs*

Metal ions in nucleic acids would form metal mediated base pairing in oligonucleotides. It is reported that Hg(II) and Ag(I) metal ions have great binding affinity towards natural nucleobases.[86] Many synthetic oligonucleotides have been developed using artificial nucleobases where the *Watson-Crick* base pair is replaced by metal-mediated base pair.[87-90]

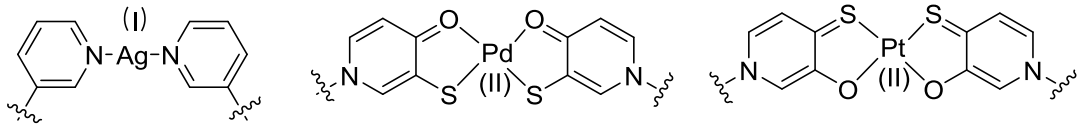


Figure I.4b *Metal mediated base pairs of artificial bases*

These metal mediated base pairs have ability to form strong and stable duplexes because the energy of the metal coordination bond is higher than hydrogen bond. The melting

temperatures of such duplexes are quite higher than natural duplex. Various applications of metal ions in nucleic acids and nucleobases have also been established such as metal mediated supramolecular assembly,[91-94] used as drugs for diseases,[95-97] and sensor applications.[98, 99]

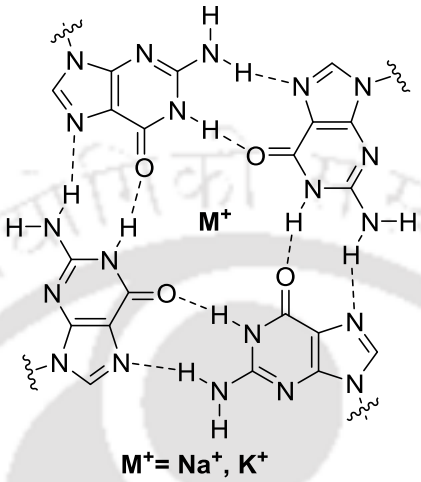
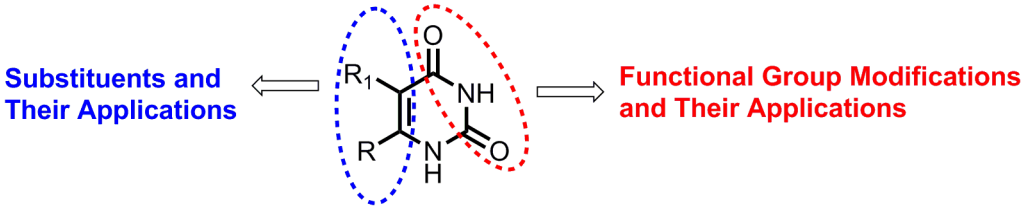


Figure I.4c *Supramolecular interactions of nucleobase with metals*

Conclusion and Objective

The above literature survey clearly shows the important of modified nucleic acids in biology. Modified pyrimidine nucleobases are playing very crucial role to bring out various properties of nucleic acids that would have numerous applications in biology as well as medicinal fields.

Our goal is to synthesize various modified pyrimidine nucleobase analogues which would have unique properties such as altering base-pair and fluorescence. And also, pyrimidine has flexible structural features to bring in various modifications through chemical reactions. The modifications are either functional group modifications or various useful molecules as substituent.



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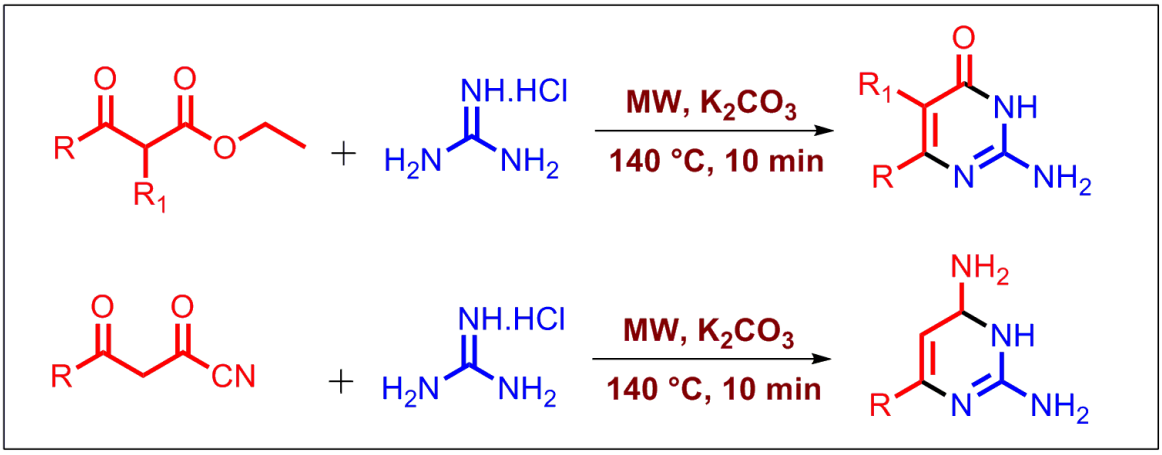
Chapter 2

Direct Synthesis of 5- and 6-substituted 2-Aminopyrimidines as Potential Non-natural Pyrimidine Nucleobase Analogues

Direct Synthesis of 5- and 6-substituted 2-Aminopyrimidines as Potential Non-natural Pyrimidine Nucleobase Analogues

Abstract

In this chapter we have shown the synthesis of 2-aminopyrimidne derivatives using microwave assisted method under solvent free condition. This method is quite efficient and environment friendly. 2-aminopyrimidines are biologically important molecules and also act as nucleobase analogues because of its structural resemblance with pyrimidine nucleobases. The base-pairing properties of synthesized 2-aminopyrimidines were studied through co-crystals. The effect of substituent on base-pairing was also studied. These molecules have ability to form strong base pair with cytosine act as base-pairing partner for cytosine instead of guanine.



II.1 Introduction

Heterocycles are essential scaffolds for drug design in medicinal fields. Pyrimidines are an interesting six membered nitrogen heterocyclic compounds, among various heterocycles, which has permanent place in organic synthesis and drug design because of its wide use. Moreover, the incorporation of biologically active substituents into pyrimidine could be possible due its structural flexibility.

Pyrimidine heterocycles have found a wide range of applications in the pharmaceutical industry as anti-bacterial, anti-viral and anti-tumor agents, as well as their applications as artificial base-pairs.[1–5] Biological activities of such heterocycles are largely due to their structural resemblance to the nucleobases or coenzymes, enabling them to act as potential inhibitors. A substantial number of 2-aminopyrimidine compounds were synthesized and various derivatives have been found to be clinically active molecules that exhibit cytotoxic, antibacterial and other kinds of inhibition properties.[6–9]

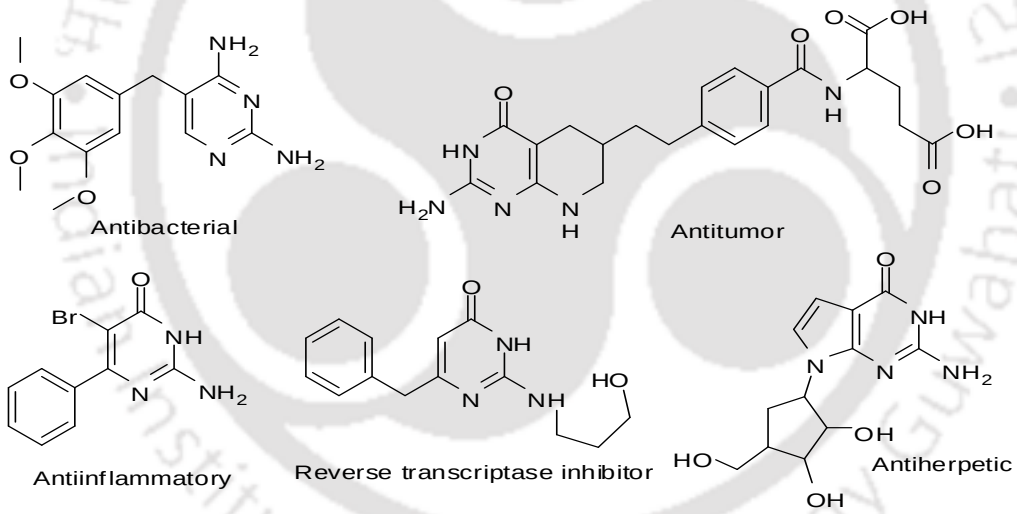


Figure II.1a *Biologically active 2-aminopyrimidines*

Pyrimidine is an essential core moiety present in nucleic acids that determines the genetic information flow in all living systems. A number of pyrimidine analogues were introduced in nucleic acids for their effective applications such as alternate base pairing and fluorescence signaling. This promotes synthesis of new functional nucleic acids which could have numerous applications in disease diagnosis, gene targeting[10] and gene silencing.[11,12]

2-aminopyrimidinone is a class of 2-aminopyrimidine derivative, also called as isocytosine that is an isomer of cytosine, with exchanged position of carbonyl and amine functional groups. It also has structural resemblance with guanine nucleobase which is bearing one more fused imidazole ring.

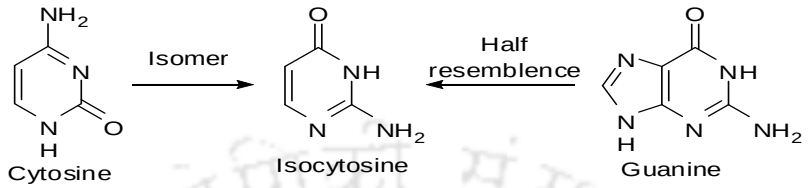


Figure II.1b

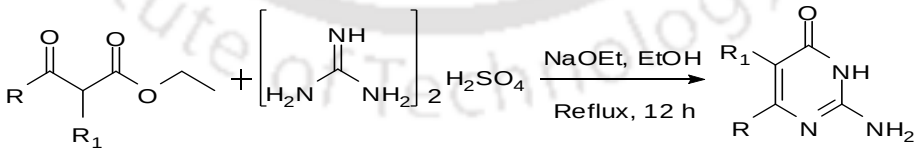
II.2 Synthesis of 2-aminopyrimidinone

In 1940 Caldwell *et al.* demonstrated the synthesis method of isocytosine using malic acid under strong acidic condition.[13] In this method, the guanidine hydrochloride was refluxed with malic acid in presence of 15% fuming sulfuric acid, followed by basic workup to yield isocytosine. Caldwell also reported that better yields of isocytosine were obtained from guanidine hydrochloride than guanidine carbonate.

Later isocytosine synthesis was further modified by chemists using beta-ketoester as precursor. Guanidine salts were heated with substituted or unsubstituted beta-ketoester in basic condition using organic solvents.

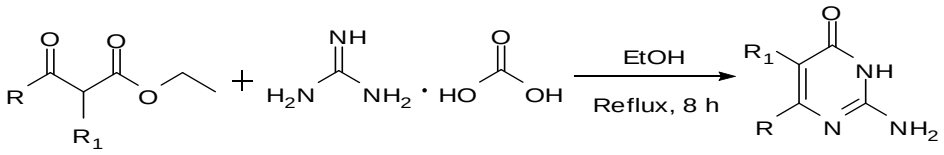
II.2.1 Reported methods

In 2000 Liliana Craciun *et al.* have shown the synthesis of 2-aminopyrimidinone through the condensation of beta-ketoester with guanidine sulfate in presence of sodium ethoxide in reflux condition.[14]



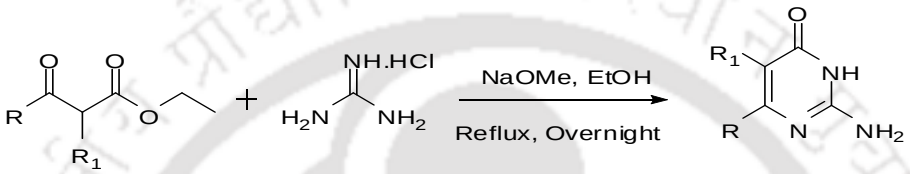
Scheme II.2.1.1

Later the synthesis was modified by Patil *et al.* using guanidine carbonate instead of guanidine sulfate.[15] Advantage of this method was absence of base. The reaction was refluxed without base because the carbonate salt itself acts as a base.



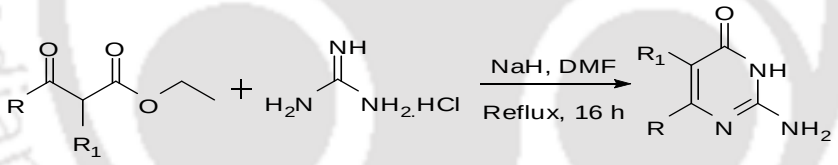
Scheme II.2.1.2

Synthesis of isocytosine was further improved by using guanidine hydrochloride. Elena *et al.*[16] showed the synthesis of 2-aminopyrimidinone using guanidine hydrochloride and beta-ketoester in presence of strong base.



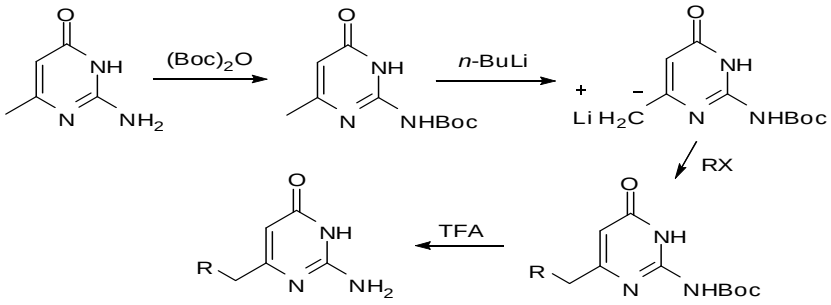
Scheme II.2.1.3

In 2012, the modified synthesis was developed by Ugarkar *et al.* that sodium hydride was used in place of sodium methoxide or sodium ethoxide, under reflux condition.[17] Dimethyl formamide was used as solvent for this reaction with long heating.



Scheme II.2.1.4

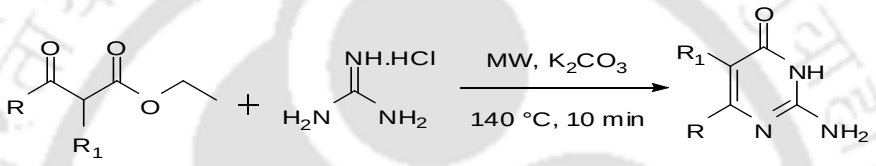
Jan Balzarini *et al.* showed introduction of various substituents at C-6 position by multistep reactions.[18] Here, 6-methyl-2-amino pyrimidinone was used as precursor to synthesize various substituted 2-aminopyrimidinone. The synthetic route of substituted pyrimidine derivatives is given below.



Scheme II.2.1.5

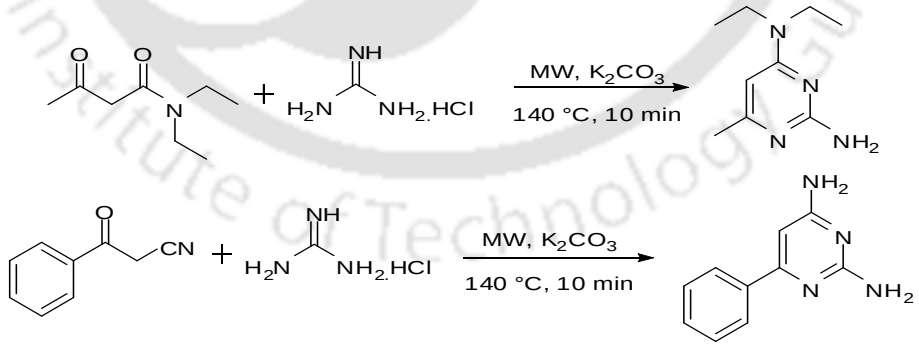
II.3 Present work

To avoid the above problems we have developed a one pot, solvent-free, synthesis of 2-aminopyrimidinones (Isocytosines) using microwave-assisted method under mild condition. A series of compounds, with various C-5 and C-6 substitutions, were synthesized in high yield, proving robustness of the present method. In modern organic synthesis, microwave method has important role to carry out various organic transformations.[19,20] Here, the substituted or unsubstituted beta-ketoester, guanidine hydrochloride and a mild base were taken in a reaction vessel and subjected to microwave irradiation for 10 minutes, without adding any solvent. These reactions were quite efficient and yields of these reactions were also impressive.[21]



Scheme II.3.1

The wide scope of the present methodology was further illustrated with synthesis of 2, 4-diaminopyrimidine derivatives, using microwave-assisted method under similar condition, because of its wide application in biology and also most of the anti-bacterial drugs consist of 2, 4-diaminopyrimidine heterocycle as core moiety. Beta-ketoamide and beta-ketocyanide were used to synthesize 2,4-diaminopyrimidines and the reactions were very facile.

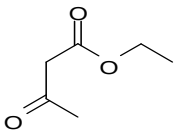
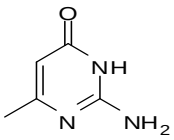
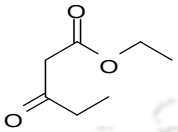
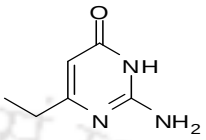
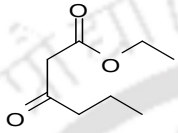
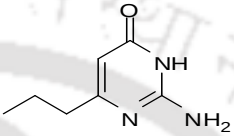
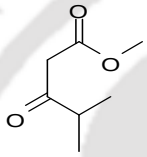
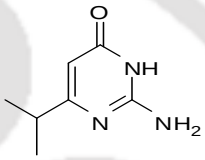
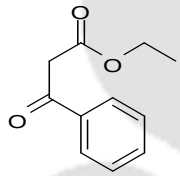
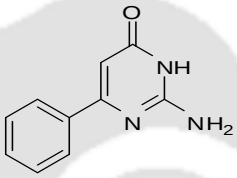
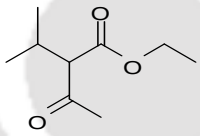
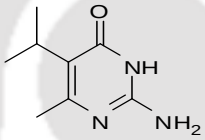
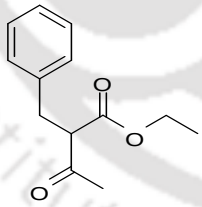
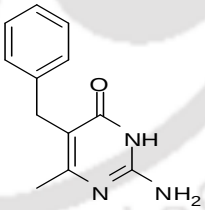
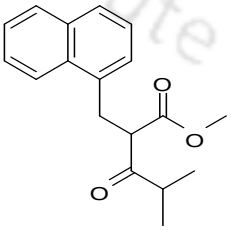
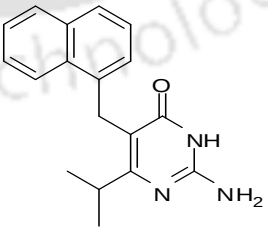
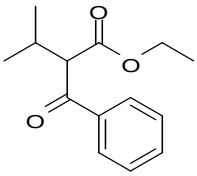
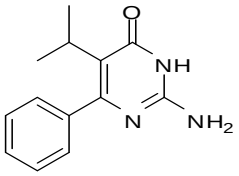


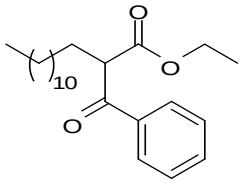
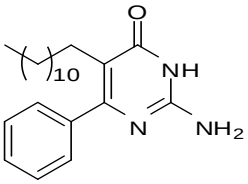
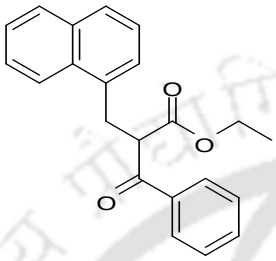
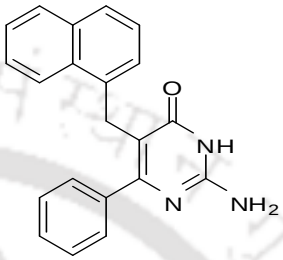
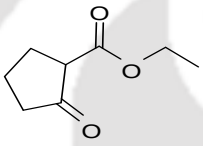
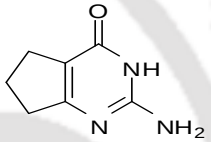
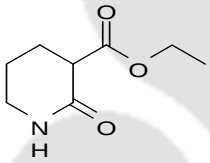
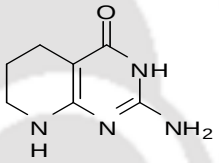
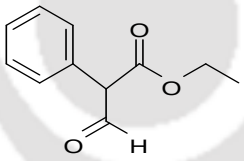
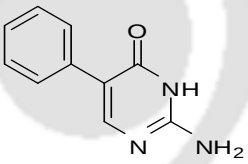
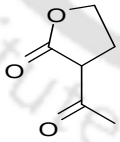
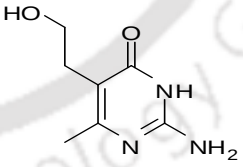
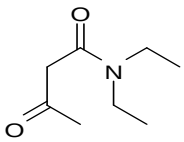
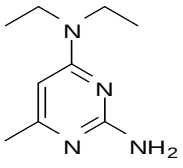
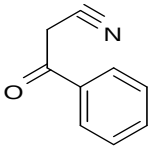
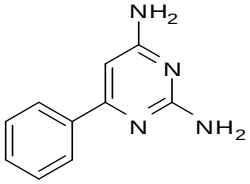
Scheme II.3.2

Advantages of the present methodology

1. Use of mild base and Solvent free
2. Reduced time
3. Large substrate scope, improved yield and Eco-friendly.

Table II.1

S. No	Substrate (**II.1-II.9**)	Product (**II.1a-II.9a**)	Yield (%)
1			85
2			82
3			84
4			79
5			82
6			76
7			73
8			67
9			72

S. No	Substrate (**II.10-II.17**)	Product (**II.10a-II.17a**)	Yield (%)
10			60
11			61
12			86
13			70
14			80
15			70
16			61
17			74

All the compounds were synthesized using *CEM Discover LabMate* microwave reactor in a closed vessel condition. Potassium carbonate was found to be a good base for all reactions and the reaction did not proceed without base even at higher temperature. To our understanding, this mild base primarily acts as a scavenger of hydrochloric acid, present in the guanidine salt. The yields of the reactions were increased when two equivalents of guanidine hydrochloride was used instead of stoichiometric ratio. Use of organic bases such as triethylamine, DBU led to lower the yield of the reaction. It is noteworthy from **Table II.1** that the reactivity of the substituted beta-ketoesters **II.6–II.11** were found to be lowered, presumably due to increased steric crowding in the transition state.

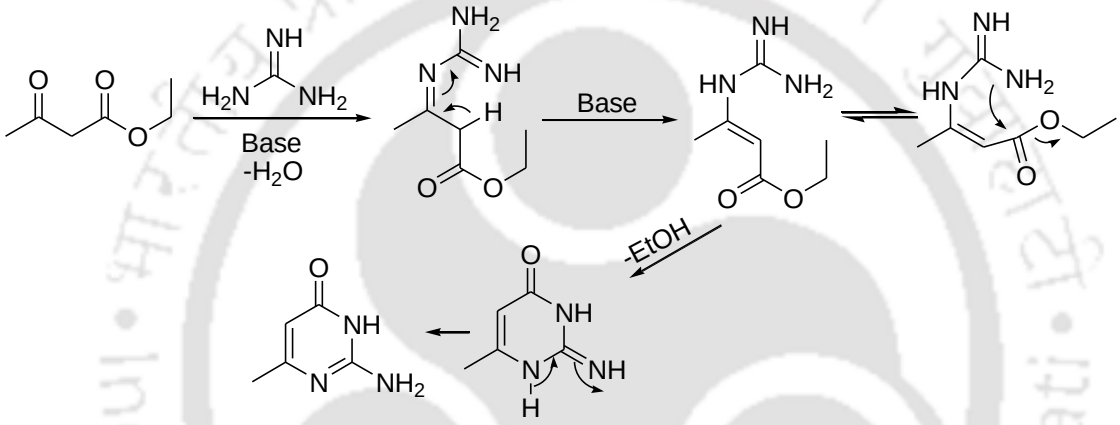


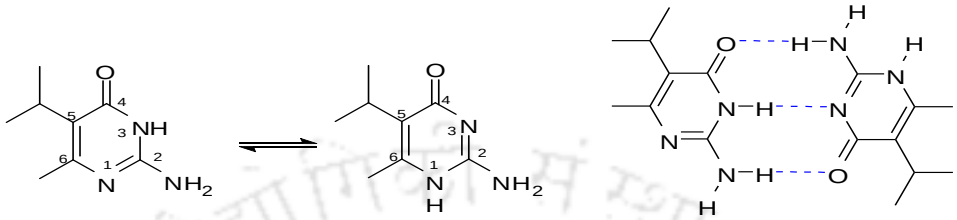
Figure II.3.1a *Proposed mechanism*

A large variety of 2-aminopyrimidines were synthesized with various substitutions at C-5 and C-6 position. Depending upon stereo and electronic factors of substituents present at C-5 and C-6 positions, the property of the molecule could be changed. We also found that not only beta-ketoesters, but also beta-amido, beta-aldehydo, and beta-cyclic esters undergo microwave reaction very rapidly with enhanced yields. The substituted beta-ketoesters were prepared by the reaction of unsubstituted beta-ketoester with alkyl halides in presence of base at 70 °C. Synthesized compounds were purified by column chromatography and confirmed by NMR and mass spectrometric techniques.

II.4 Tautomers of isocytosine

Isocytosine is an isomer of cytosine which has tendency to form two tautomers, namely, Keto-N3H and Keto-N1H tautomers in solid state with 1:1 ratio.[22,23] Several spectroscopic studies reveal that the tautomeric ratio in solution is solvent and temperature dependent.[24-28]

We have synthesized various substituted isocytosine derivatives that might have tautomeric forms mentioned above. Herein, we obtained the crystal of 6-methyl-5-isopropylisocytosine (**II.6a**) in 1:1 ratio of tautomers in methanol-water (2:1) solvent mixture which exhibits G-C type *Watson-Crick* hydrogen bonding interactions.



FigureII.4a *Tautomeric forms and hydrogen bonding pattern of* ***II.6a*** *in solid state*

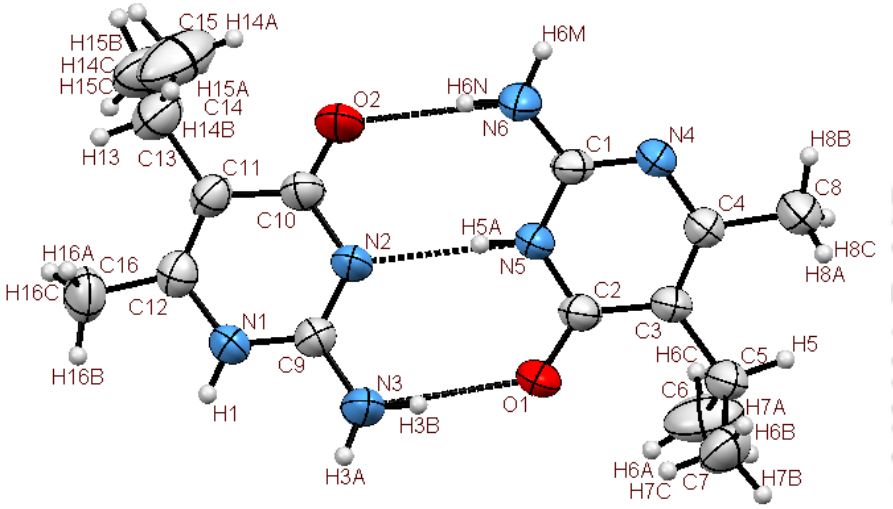


Figure II.4b *Crystal structure (ORTEP view) 5-isopropyl-6-methylisocytosine (**II.6a**) in 1:1 ratio*

II.5 Study of base-pairing properties

The tautomerism of nucleobases play an essential role in nucleic acids that could determine the selective pairing partner to form stable nucleic acid for effective incorporation.[29-32] Isocytosine is an isomer of cytosine which has tendency to form reverse *Watson–Crick* base pair with guanine leading to formation of parallel-stranded DNA helix.[33-35] Sugiyama *et al.* have demonstrated the use of oligonucleotides containing isocytosine to selectively recognize guanine as well as isoguanine, a potential oxidative lesion in DNA.[36] Moreover, C-glycosidic isocytidine was employed as triplex forming oligonucleotides whereas N-glycosidic isocytosine was reported for diagnostic assay of branched DNA.[37-39] Lippert et al.

showed the Co-crystal structure of isocytosine with cytosine proved that isocytosine is best pairing partner for cytosine in their free form.[40]

According to the pairing selectivity, keto-N3H form can pair with cytosine and keto-N1H form a base-pair with guanine through three *Watson-Crick* hydrogen bonds as mentioned below.

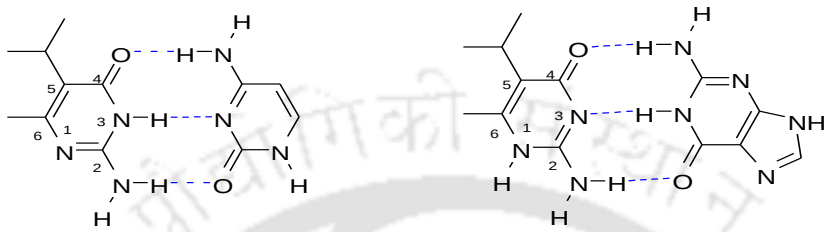
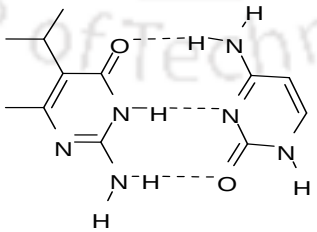


Figure II.5a *Hydrogen bonding pattern of* ***II.6a*** *tautomers with its pairing partners*

We have crystallized two co-crystals: A) Co-crystal of 5-isopropyl-6-methylisocytosine (**II.6a**) with cytosine in 1:1 ratio, and B) Co-crystal of 6-phenylisocytosine (**II.5a**) with cytosine in 1:1 ratio. The crystal structure analysis show three hydrogen bonds to form a single base pair and the bonding pattern is found to be exactly same as that of natural G-C base pair. Among various solvents, better quality co-crystals were obtained in methanol/water (2:1), using slow evaporation technique. The co-crystal structures of nucleobases in their free form are very rare and obtaining a co-crystal with *Watson-Crick* fashion is difficult to achieve.[41,42] We have attempted variety of solvents to obtain a co-crystal and finally succeeded in methanol/water solvent mixture. Water is an important medium in co-crystallization technique, and for nucleobases, it assists formation of base pair through H-bonding, facilitating the co-crystal formation.



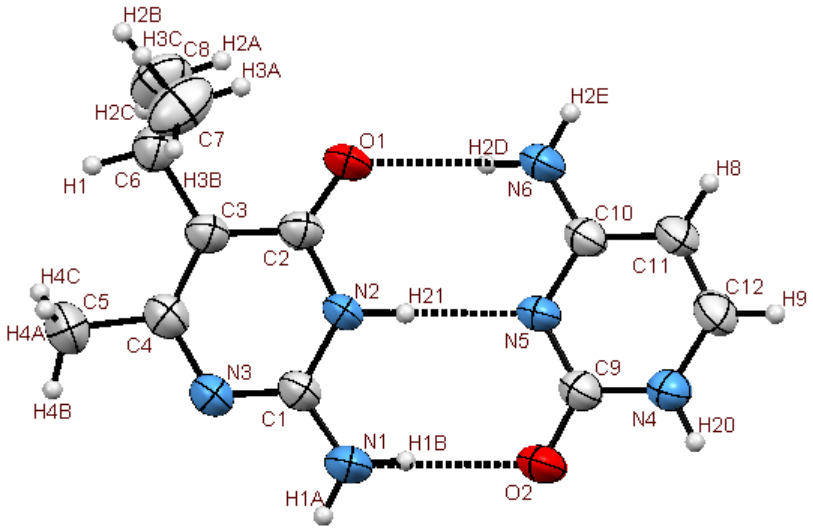
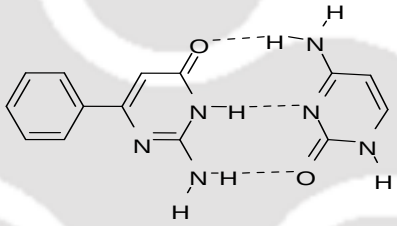


Figure II.5b *Co-crystal representation of 5-isopropyl-6-methylisocytosine- cytosine* ***(A)*** *with ORTEP view*



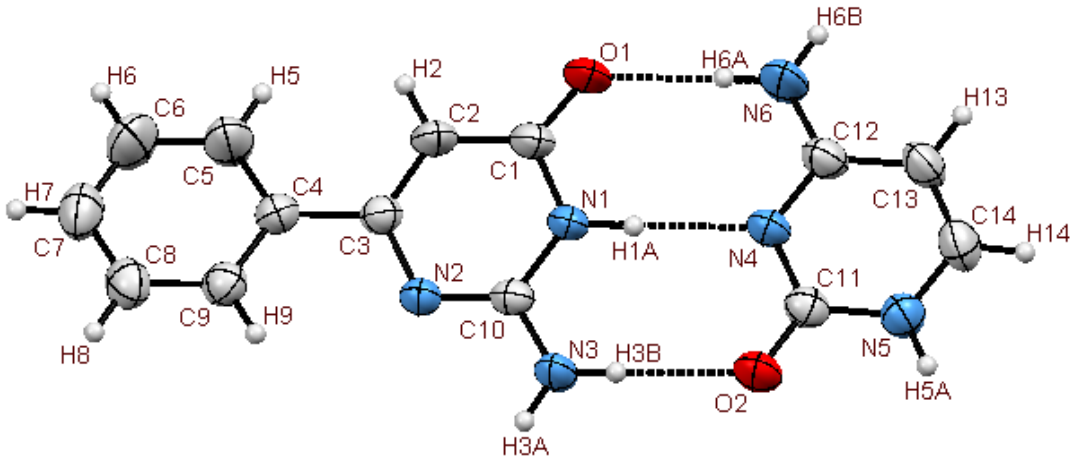


Figure II.5c *Co-crystal representation of 6-phenyl isocytosine-cytosine* ***(B)*** *with ORTEP view*

In case of co-crystal **A**, isocytosine is attached to cytosine with three distinct hydrogen bonds. These hydrogen bonds are considerably weaker than the natural G-C *Watson-Crick* pair, due to steric repulsion between isopropyl groups of isocytosine and cytosine which is present in the next layer in supramolecular structure. The planarity of hydrogen bonds was

also affected by isopropyl substitution. But in the case of co-crystal **B**, the hydrogen bonds are planar and stronger than the G-C pair due to strong π-stacking interaction of isocytosine ring with cytosine. The co-planarity of phenyl group at 6-position of isocytosine did not hamper the π-stacking interaction. Therefore 6-phenyl isocytosine could have potential applications as non-natural nucleotide and as oligonucleotide probe to selectively recognize cytosine in DNA.

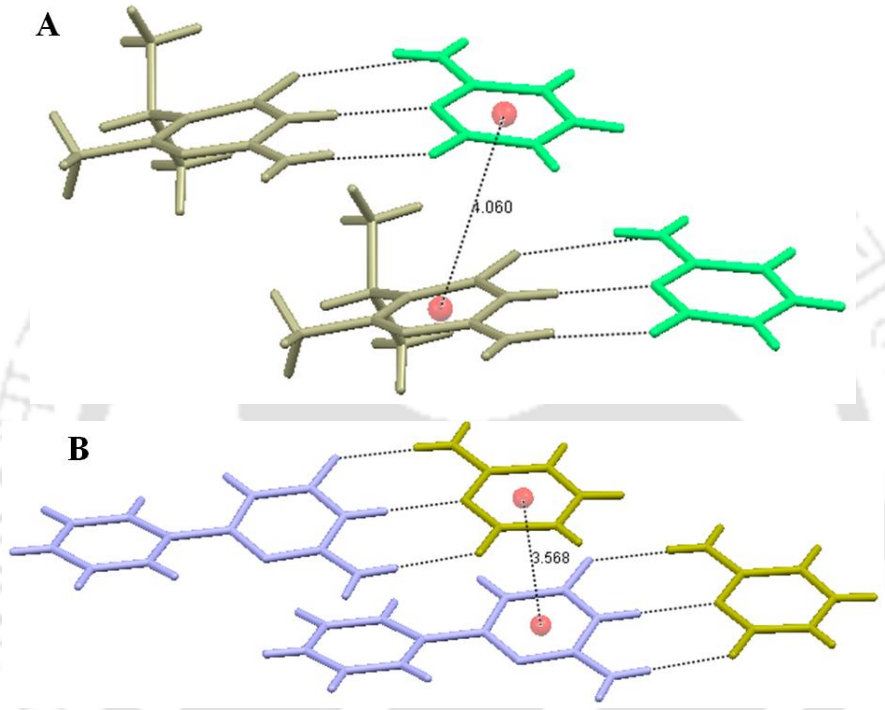


Figure II.5d *π-stacking representation of co-crystals **A** and **B***

Table II.2 ***Comparison of hydrogen bond distances of co-crystals A and B with G-C***

	Co-crystal **A** (Å)	Co-crystal **B** (Å)	Co-crystal **G-C** (Å)
(Pyr) O….H-N(Cyt)	2.93	2.81	2.91
(Pyr) N-H….N (Cyt)	2.95	2.87	2.95
(Pyr) N-H….O (Cyt)	2.91	2.90	2.86

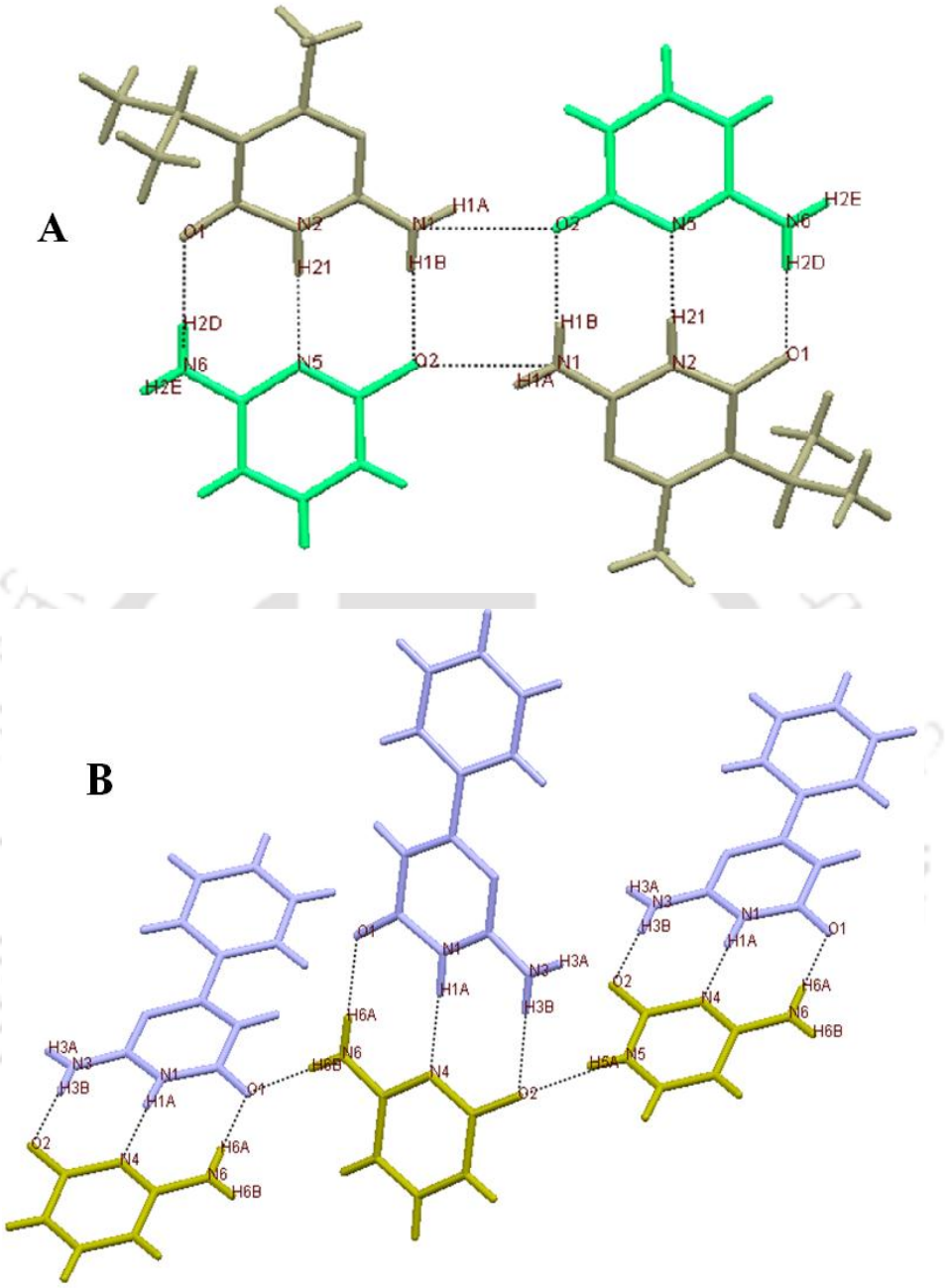


Figure II.5e *Supramolecular structures of co-crystals **A** and **B**. Top: In co crystal **A** two base pairs are almost planar to each other to form well defined polymer network without any π-stacking. Bottom: In co-crystal **B** one pair is almost perpendicular to another pair but with significant π-stacking interactions.*

We have also developed the synthesis of 2, 4-diaminopyrimidine using microwave-assisted method. The aim was to synthesize this compound, to show the base paring property of

pyrimidines. Consequently, 2, 4–diaminopyrimidine has structural resemblance with adenine which has tendency to form a base pair with thymine via three hydrogen bonds.

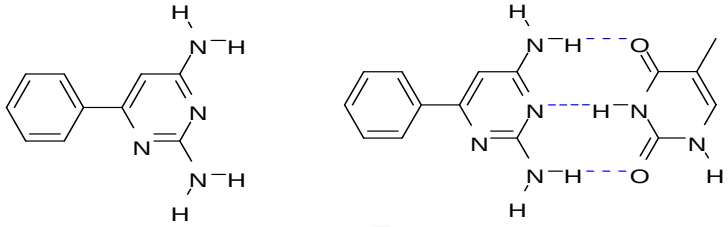


Figure II.5f *Proposed base pair interaction of* ***II.17a*** *with thymine*

The ^{1}H-NMR study shows that there is no significant interaction of **II.17a** with thymine and failed to get a co-crystal. The zig-zag orientation of compound **II.17a** in crystal structure leads to a very fine ladder type polymeric network with well-defined hydrogen bonding pattern. The polymer has consecutive N3….H-N2 bonding interactions with proper arrangements. There are no significant π-stacking interactions between the molecules.

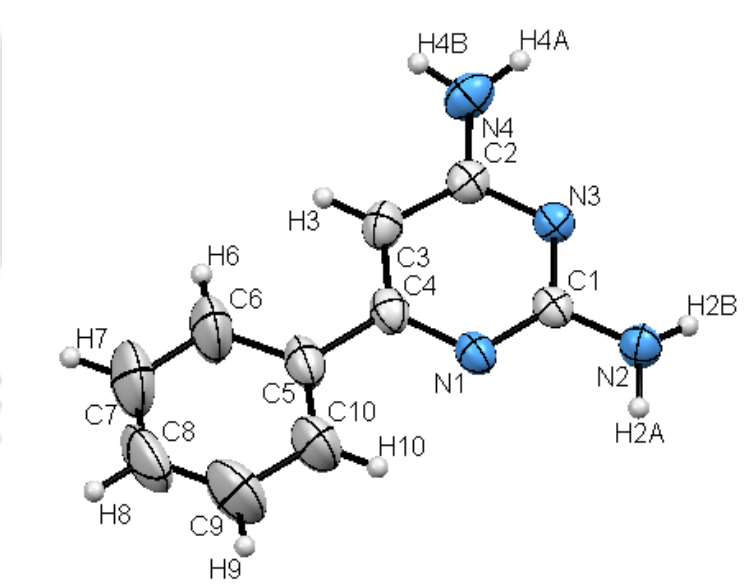


Figure II.5g *ORTEP view of* ***II.17a***

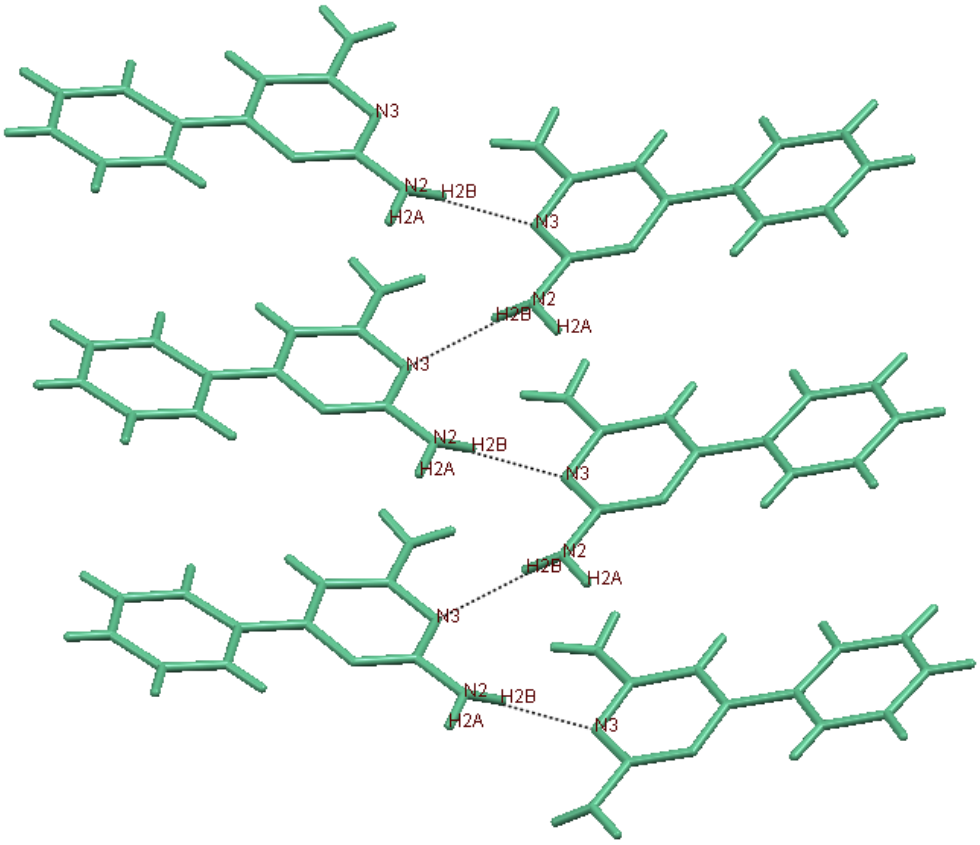


Figure II.5h *Supramolecular architecture of* ***II.17a****.*

In summary, in the present work, we have demonstrated a robust microwave-directed methodology for the synthesis of 2-aminopyrimidine derivatives as nucleobase analogues, in a single step, in absence of any solvent and harsh reagents. A library of compounds, with varying substitutions at C-5 and C-6 position of the pyrimidine, were synthesized in high yield in a very short period of reaction time. The potential applications of such derivatives as artificial base-pairing partner were evaluated by studying the co-crystals of two of the synthesized compounds (**II.5a and II.6a**) with that of cytosine. Analysis of co-crystals clearly suggests that such 2-aminopyrimidines form strong base-pair selectively with cytosine.

II.6 Experimental Section

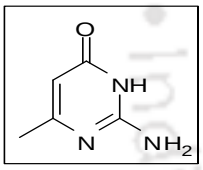
II.6.1 General Information: Chemicals used for these experiments were purchased from Merck, Spectrochem, and Sigma Aldrich and were used as such. *CEM Discover LabMate* closed vessel microwave reactor was used to carry out all reactions. All the NMR spectra

were recorded from *DRX-400 MHz Varian* and *600 MHz Bruker* NMR spectrometers using DMSO-d_6 and $CDCl_3$ as solvents. Melting points were obtained from *Buchi-B 545* instrument. HRMS was analyzed from *Agilent Q-TOF 6500* LC/MS system. All crystal data were obtained from *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structure was solved by direct method using *SHELX-97*[44,45] (University of Gottingen, Germany).

II.6.2 General Procedure: Ketoester or keto amide or keto cyanide (2 mmol), guanidine hydrochloride (4 mmol) and potassium carbonate (2 mmol) were taken in a microwave reactor vessel and was closed immediately. The vessel was subjected to microwave irradiation for 10 minutes at 140 °C. The reaction vessel was allowed to cool, and the products were isolated. The desired compounds (**II.1a-II.17a**) were further purified by column chromatography using methanol/chloroform.

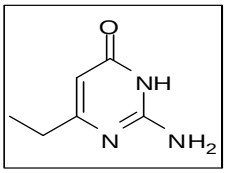
II.7 Characterization Section

2-Amino-6-methylpyrimidin-4(3*H*)-one (II.1a)



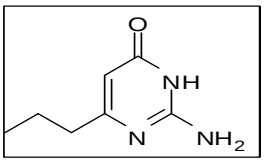
White solid, m.p: 280 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.82 (br, 1H), 6.55 (br, 2H), 5.39 (s, 1H), 1.97 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 155.91, 100.81, 23.05. HRMS (ESI): Calcd for $C_5H_7N_3O$ $[M+H]^+$ 126.0662, found 126.0662.

2-Amino-6-ethylpyrimidin-4(3*H*)-one (II.2a)



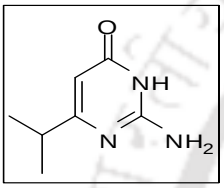
White solid, m.p: 249 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.80 (br, 1H), 6.58 (br, 2H), 5.39 (s, 1H), 2.26 (q, J = 7.2 Hz, 2H), 1.07 (t, J = 7.3 Hz, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 167.48, 156.69, 100.54, 29.60, 13.02. HRMS (ESI): Calcd for $C_6H_9N_3O$ $[M+H]^+$ 140.0818, found 140.0814.

2-Amino-6-propylpyrimidin-4(3*H*)-one (III.3a)



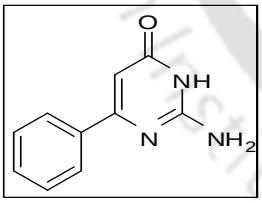
White solid, m.p: 254 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.74 (br, 1H), 6.48 (br, 2H), 5.37 (s, 1H), 2.20 (t, *J* = 7 Hz, 2H), 1.49-1.58 (m, 2H), 0.85 (t, *J* = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 155.95, 100.11, 38.17, 20.77, 13.55. HRMS (ESI): calcd for $C_7H_{11}N_3O$ $[M+H]^+$ 154.0975, found 154.0976.

2-Amino-6-isopropylpyrimidin-4(3*H*)-one (II.4a)



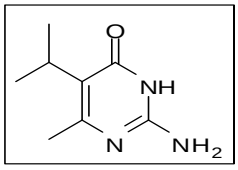
White solid, m.p: 243 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.75 (br, 1H), 6.53 (br, 2H), 5.37 (s, 1H), 2.42-2.51 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 156.11, 97.82, 34.52, 21.12. HRMS (ESI): Calcd for $C_7H_{11}N_3O$ $[M+H]^+$ 154.0975, found 154.0976.

2-Amino-6-phenylpyrimidin-4(3*H*)-one (II.5a)



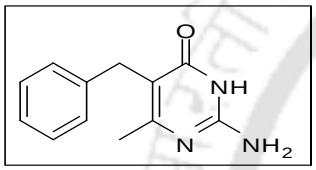
White solid, m.p: 272 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.82 (br, 1H), 7.92 (br, 2H), 7.43 (br, 3H), 6.60 (br, 2H), 6.09 (s, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 164.10, 155.95, 137.24, 130.15, 128.55, 126.81, 97.84. HRMS (ESI): Calcd for $C_{10}H_9N_3O$ $[M+H]^+$ 188.0818, found 188.0818.

2-Amino-5-isopropyl-6-methylpyrimidin-4(3*H*)-one (II.6a)



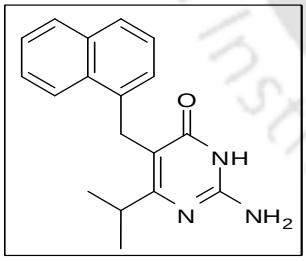
White solid, m.p: 280 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.74 (br, 1H), 6.32 (br, 2H), 2.84-2.91 (m, 1H), 2.05 (s, 3H), 1.16 (d, J = 7 Hz, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 154.29, 118.61, 27.04, 21.21, 21.00. HRMS (ESI): Calcd for $C_8H_{13}N_3O$ $[M+H]^+$ 168.1131, found 168.1125.

2-amino-5-benzyl-6-methylpyrimidin-4(3*H*)-one (7a)



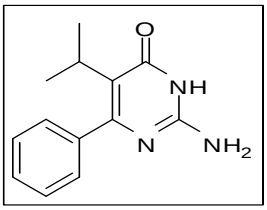
White solid, m.p: 226 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.90 (br, 1H), 7.10-7.24 (m, 5H), 6.38 (br, 2H), 3.63 (s, 2H), 2.00 (s, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 154.39, 141.83, 128.80, 128.56, 126.18, 111.14, 30.53. HRMS (ESI): Calcd for $C_{12}H_{13}N_3O$ $[M+H]^+$ 216.1131, found 216.1131.

2-Amino-6-isopropyl-5-(naphthalen-1-ylmethyl) pyrimidin-4(3*H*)-one (II.8a)



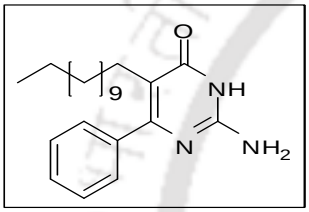
White solid, m.p: 289 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.76 (br, 1H), 8.23 (d, J = 8.72 Hz 1H), 7.91 (d, J =7.84 Hz, 1H), 7.73 (d, J = 8.7 Hz, 1H), 7.41 (t, J = 8.3 Hz, 2H), 7.22 (t, J = 7.4 Hz, 2H), 6.47 (br, 2H), 4.12 (s, 2H), 2.74 (m, 1H), 1.07 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 5.8 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$+ DMSO-d_6): δ (ppm) 155.11, 135.76, 133.24, 131.75, 128.19, 126.06, 125.44, 125.13, 123.37, 123.04, 26.32, 20.20. HRMS (ESI): Calcd for $C_{18}H_{19}N_3O$ $[M+H]^+$ 294.1601, found 294.1602.

2-Amino-5-isopropyl-6-phenylpyrimidin-4(3*H*)-one (II.9a)



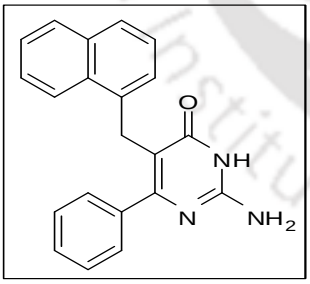
White solid, m.p: 260 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.85 (br, 1H), 7.28-7.42 (m, 5H), 6.32 (br, 2H), 2.58-2.61 (m, 1H), 1.14 (d, J = 6.9 Hz, 6H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 153.47, 128.30, 127.99, 127.77, 126.67, 116.30, 27.83, 20.45. HRMS (ESI): Calcd for $C_{13}H_{15}N_3O$ $[M+H]^+$ 230.1288, found 230.1289.

2-Amino-5-dodecyl-6-phenylpyrimidin-4(3*H*)-one (II.10a)



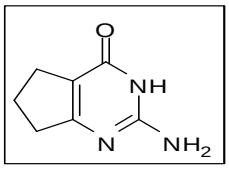
White solid, m.p: 292 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.88 (br, 1H), 7.33-7.37 (m, 5H), 6.32 (br, 2H), 2.17 (t, J = 7.6 Hz, 2H), 1.08-1.29 (m, 20H), 0.84 (t, J = 5.6 Hz, 3H). HRMS (ESI): Calcd for $C_{22}H_{33}N_3O$ $[M+H]^+$ 356.2696, found 356.2696.

2-Amino-5-(naphthalen-1-ylmethyl)-6-phenylpyrimidin-4(3*H*)-one (II.11a)



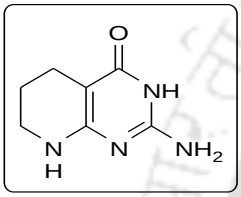
White solid, m.p: >300 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 11.00 (br, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 8 Hz, 1H), 7.47 (m, 2H), 7.35 (m, 3H), 7.25 (m, 3H), 7.08 (d, J = 6.8 Hz, 1H), 6.59 (br, 2H), 4.01 (s, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 154.01, 136.73, 133.32, 131.56, 128.53, 127.90, 127.69, 126.19, 125.94, 125.66, 125.61, 124.10, 123.40, 108.91, 28.59. HRMS (ESI): Calcd for $C_{21}H_{17}N_3O$ $[M+H]^+$ 328.1444, found 328.1443.

2-Amino-6, 7-dihydro-3H-cyclopenta[d]pyrimidin-4(5H)-one (II.12a)



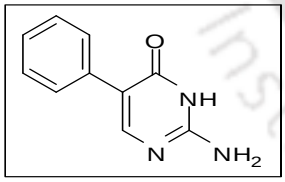
White solid, m.p: > 300 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.60 (br, 1H), 6.43 (br, 2H), 2.46-2.52 (m, 4H), 1.85 1.94 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 161.22, 158.29, 156.27, 110.84, 34.42, 26.62, 20.96. HRMS (ESI): Calcd for $C_7H_9N_3O$ $[M+H]^+$ 152.0818, found 152.0814.

2-Amino-5, 6, 7, 8-tetrahydropyrido [2, 3-d] pyrimidin-4(3*H*)-one (II.13a)



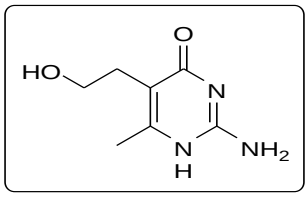
White solid, m.p: 274 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 9.72 (br, 1H), 6.21 (br, 1H), 5.94 (br, 2H), 3.10 (br, 2H), 2.20 (t, J = 6 Hz, 2H), 1.61-1.66 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 161.36, 159.68, 153.26, 82.55, 21.17, 19.17. HRMS (ESI): Calcd for $C_7H_{10}N_4O$ $[M+H]^+$ 167.0927 found 167.0928.

2-Amino-5-phenylpyrimidin-4(3*H*)-one (II.14a)



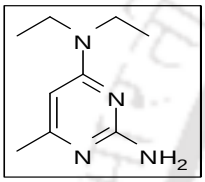
White solid, m.p: 240 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 10.84 (br, 1H), 7.12-7.28 (m, 5H), 6.34 (br, 2H), 3.49 (s, 1H). HRMS (ESI): Calcd for $C_{10}H_9N_3O$ $[M+H]^+$ 188.0818, found 188.0824.

2-Amino-5-(2-hydroxyethyl)-6-methylpyrimidin-4(1*H*)-one (II.15a)



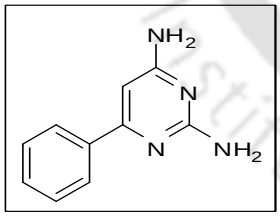
White solid, m.p: 256 °C, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 7.04 (s, 1H), 6.58 (br, 2H), 4.53 (br, 1H), 3.35 (t, J = 7.2 Hz 2H), 2.43 (t, J =7.2 Hz, 2H), 2.06 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 164.52, 158.12, 153.43, 108.34, 60.00, 28.81, 20.53. HRMS (ESI): Calcd for $C_7H_{11}N_3O_2$ $[M+H]^+$ 170.0924, found 170.0928.

N^4, N^4-Diethyl-6-methylpyrimidine-2, 4-diamine (II.16a)



White solid, m.p: 121 °C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) 6.78 (br, 2H), 5.39 (s, 1H), 2.87 (q, J = 7.2 Hz, 2H), 1.94 (s, 3H), 1.16 (t, J = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm) 164.56, 155.75, 100.46, 41.47, 22.91, 11.08. HRMS (ESI): Calcd for $C_9H_{16}N_4$ $[M+H]^+$ 181.1448, found 181.1448.

6-Phenylpyrimidine-2, 4-diamine (II.17a)



Brown solid, m.p: 157 °C, ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77 (s, 2H), 7.45 (m, 3H), 6.12 (s, 1H), 5.38 (br, 2H), 5.21 (br, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm) 164.87, 163.32, 137.93, 130.04, 128.67, 128.47, 126.95, 92.65 .HRMS (ESI): Calcd for $C_{10}H_{10}N_4$ $[M+H]^+$ 187.0978, found 187.0976.

Crystallographic data

5-Isopropyl-6-methylisocytosine/Cytosine co-crystal A	**CCDC-980516**
Chemical Formula	C12 H20 N6 O3
Formula mass	296.34
Temperature /K	296 (2)
Crystal system	Monoclinic
Space group	P21/n
a /Å	15.5249 (9)
b /Å	5.5769 (3)
c /Å	18.7323 (11)
α /°	90
β /°	112.714 (3)
γ /°	90
Unit cell Volume /Å	1496.07 (15)
Z	4
Final R1 value	0.0405
Final wR value	0.1109
Goodness of fit	0.876

6-Phenylisocytosine / Cytosine co-crystal B	**CCDC-980518**
Chemical Formula	C14 H14 N6 O2
Formula mass	298.31
Temperature /K	296 (2)
Crystal system	Monoclinic
Space group	P21/c
a /Å	12.4798 (6)
b /Å	6.8611 (5)
c /Å	17.0339 (10)
α /°	90

β /°	95.021 (5)
γ /°	90
Unit cell Volume /Å	1452.94 (15)
Z	4
Final R1 value	0.0501
Final wR value	0.0856
Goodness of fit	1.096

5-Isopropyl-6-methyl isocytosine (6) tautomeric forms CCDC-980517

Chemical Formula	C16 H28 N6 O3
Formula mass	352.44
Temperature /K	296 (2)
Crystal system	Monoclinic
Space group	C2/c
a /Å	19.3452 (7)
b /Å	13.9961 (7)
c /Å	14.4876 (6)
α /°	90
β /°	103.855 (3)
γ /°	90
Unit cell Volume /Å	3808.0 (3)
Z	8
Final R1 value	0.0438
Final wR value	0.1038
Goodness of fit	1.007

6-Phenyl pyrimidine-2,4-diamine (II.17a)

Chemical Formula	C10 H10 N4
Formula mass	186.22
Temperature /K	293 (2)
Crystal system	Orthorhombic
Space group	Pbca
a /Å	37.324 (2)
b /Å	5.1083 (3)
c /Å	9.8426 (5)
α /°	90
β /°	90
γ /°	90
Unit cell Volume /Å	1876.61 (19)
Z	8
Final R1 value	0.0729
Final wR value	0.2594
Goodness of fit	0.838

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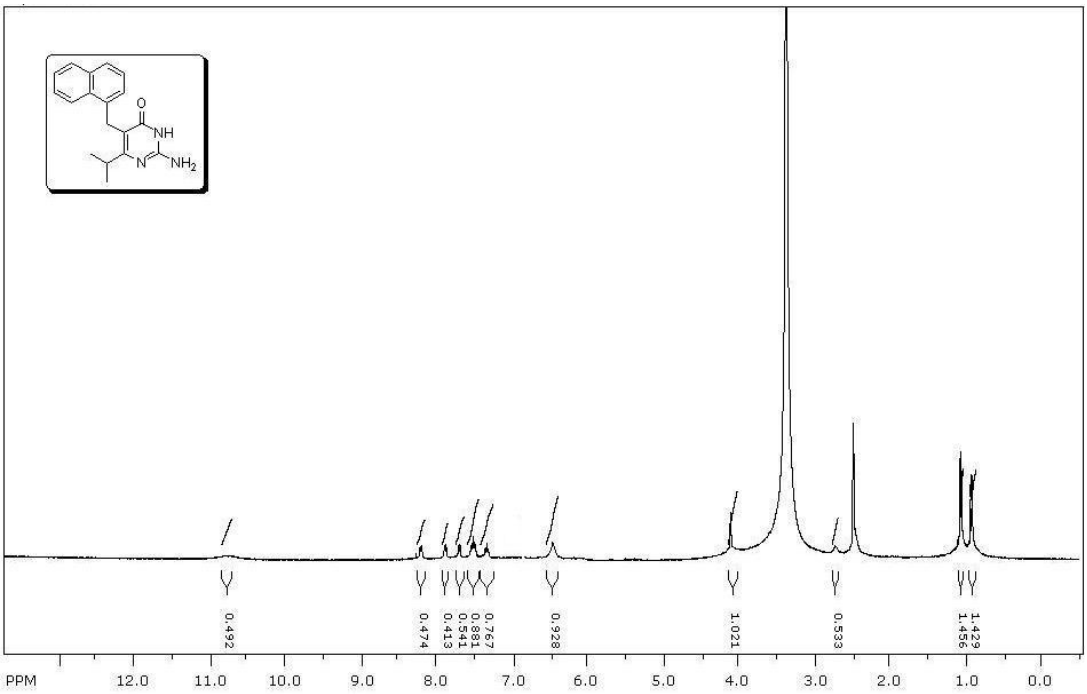
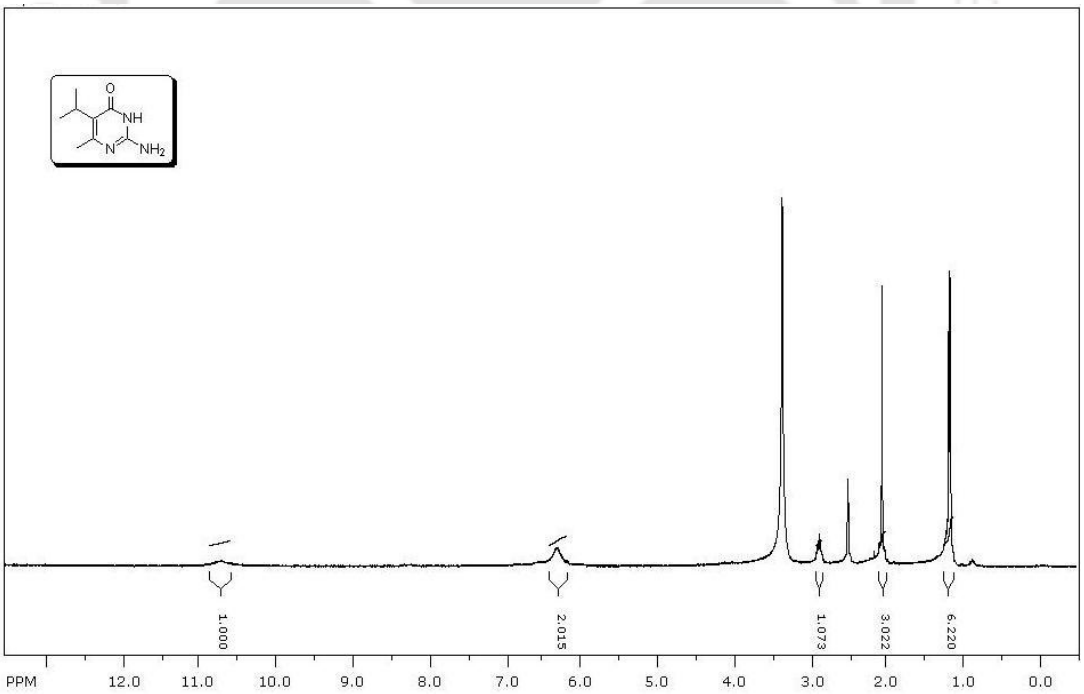
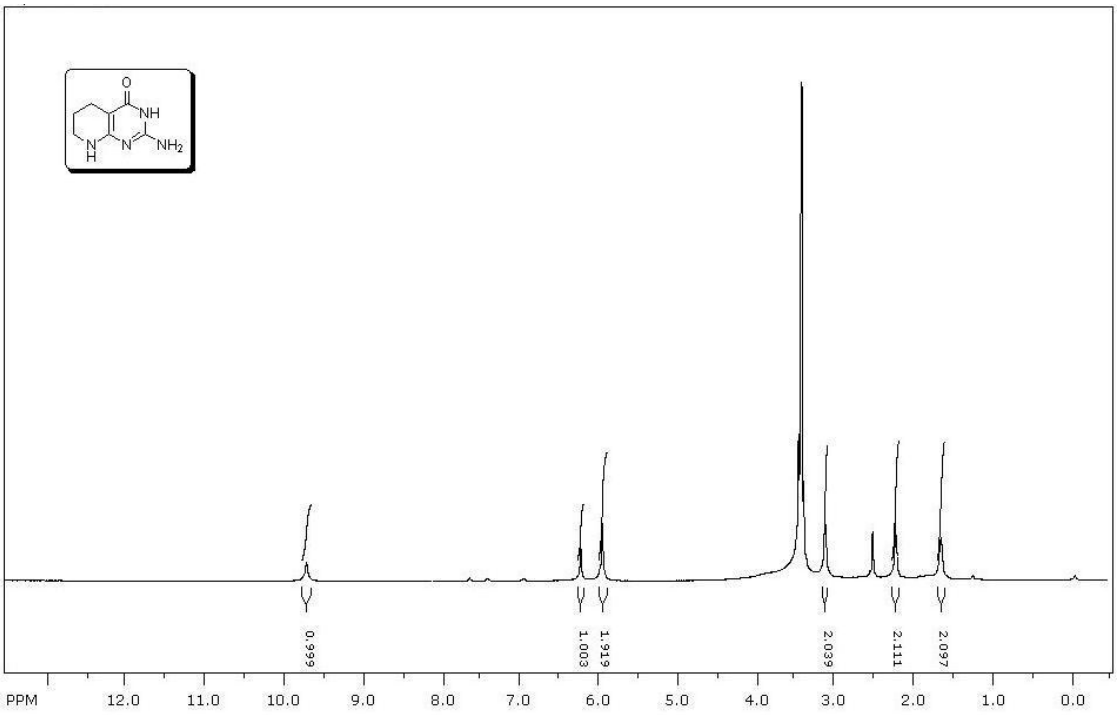
Appendix

^{1}H-NMR spectra of some representative examples






O
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N
H
N
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2.039
2.111
2.097
PPM
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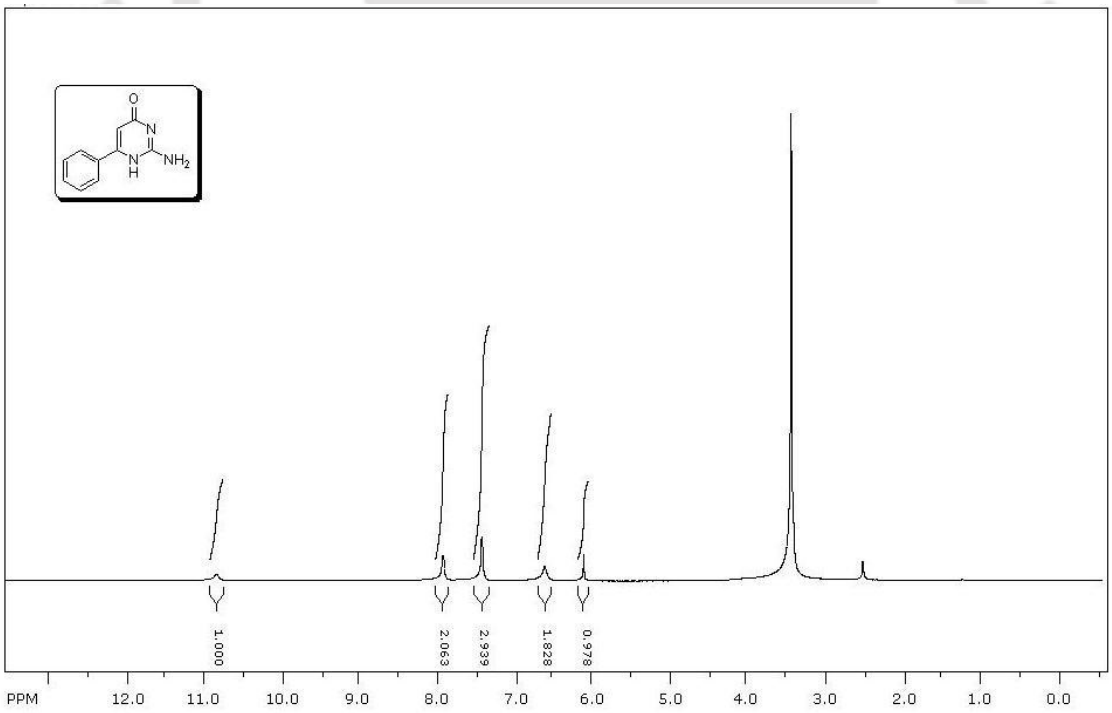
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0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H
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PPM
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11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

Hydrogen bond distances of 6-methyl-5-isopropylisocytosine-cytosine co-crystal A

D-H…A	D-H/Å	H…A/Å	D-H…A/Å	∟D-H…A/°	Symmetry
N1-H1A…O2	0.86	2.12	2.849(4)	143	-x+1/2, y-1/2, -z+1/2
N1-H1B…O2	0.86	2.06	2.919(3)	172	x-1/2, -y-1/2, z-1/2
N6-H2D…O1	0.86	2.10	2.933(3)	161	x+1/2, -y-1/2, z+1/2
N6-H2E…O1	0.86	2.08	2.918(4)	166	-x,-y,-z+1
N4-H20…O3	0.91(3)	1.90(3)	2.806(4)	176(3)	-x+1/2, y+1/2, -z+1/2
N2-H21…N5	0.94(3)	2.02(3)	2.957(4)	179(3)	x-1/2, -y-1/2, z-1/2
O3-H22…O2	0.90(4)	2.06(4)	2.944(3)	168(3)	-x+1/2, y+1/2, -z+1/2
O3-H23…N3	0.87(4)	2.01(4)	2.873(4)	176(3)	

Hydrogen bond distances of 6-phenylisocytosine-cytosine co-crystal B

D-H…A	D-H/Å	H…A/Å	D-H…A/Å	∟D-H…A/°	Symmetry
N1-H1A...N4	0.94(3)	1.94(3)	2.870(3)	177(2)	-x+1, y+1/2, -z+1/2
N3-H3A...N2	0.86	2.48	3.220(2)	144	-x+1, -y+1, -z
N3-H3B...O2	0.86	2.04	2.904(3)	178	-x+1, y+1/2, -z+1/2
N5-H5A...O2	0.86(3)	2.05(3)	2.839(3)	152(2)	-x+1, y+1/2,-z+1/2
N6-H6A...O1	0.86	1.95	2.811(3)	176	-x+1, y-1/2, -z+1/2
N6-H6B...O1	0.86	1.99	2.839(3)	172	x-1, y, z

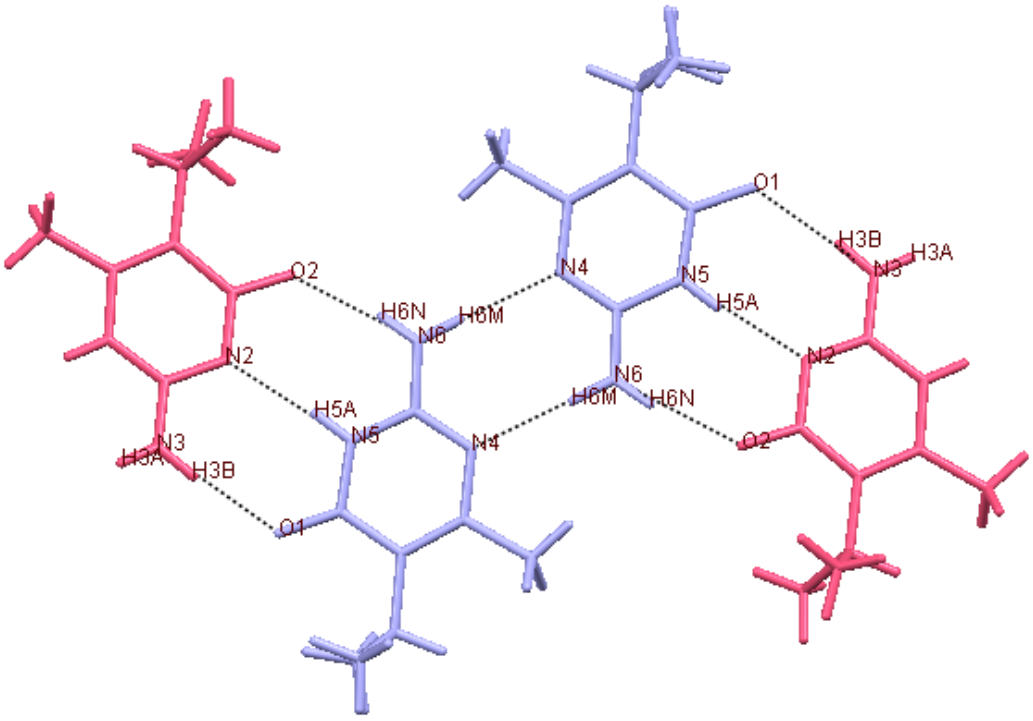


Figure A1 *Supramolecular motif of 5-isopropyl-6-methylisocytosine* ***(II.6a)*** *with three distinct hydrogen bonding pattern (O1…H-N3, N5-H…N2, N6-H…O2). The supramolecular interactions are further extended by N6-H…N4 interactions of adjacent base pairs.*

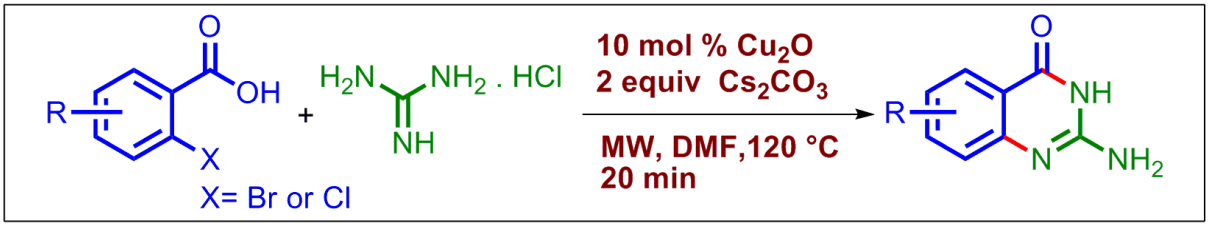
Chapter 3

Copper Catalyzed Microwave-assisted Synthesis of Ring Expanded Pyrimidine Nucleobase Analogues

Copper Catalyzed Microwave-assisted Synthesis of Ring Expanded Pyrimidine Nucleobase Analogues

Abstract

This chapter describes the synthesis of size expanded 2-aminopyrimidines as nucleobase analogues. These molecules are also biologically active molecules and were synthesized using Cu_2O catalyst in presence of base under microwave condition. This method also elucidates the synthesis of various potentially active expanded 2-aminopyrimidines with some useful modifications. The base-pairing was studied through ^{1}H-NMR titration with cytosine and it revealed that these molecules could have ability to recognize cytosine via hydrogen bonding.



III.1 Introduction

Synthesized nucleobases and a large variety of their analogues are widely applied as biological markers or pharmaceutically active compounds.[1] Modified nucleobases and nucleic acids are primarily synthesized to induce a) fluorescence signal, b) base-pair selectivity or c) pi-stacking intereactions.[2-14] Even though variety of nucleobases were synthesized with many modification, ring-expanded nucleobases play a crucial role for the expansion of genetic alphabet and DNA based sensing techniques.[15] The duplex stability of size-expanded nucleic acids is much higher than the unmodified duplex. This stability enhancement not only depends on hydrogen bonding but also depends upon the hydrophobic interactions.[16, 17]

Pyrimidine heterocycle is the basic structural unit of nucleobases which is responsible for various biological processes. It is a six-membered heterocyclic compound where 1, 3 positions are occupied by nitrogen atoms. Quinazoline is a pyrimidine heterocycle which bears an aromatic ring that can be called as ring-fused or expanded pyrimidine.

Figure III.1a

Quinazolines are important molecules in heterocyclic chemistry which are obtained in natural products such as, tryptanthrin and benzomalvin.[18] Quinazolines also possess many known medicinal applications such as antibacterial, antifungal, antitumor and antihypertensive activities.[19-28] The biological properties of quinazolines will vary with substituents and nature of the analogs. 2-aminoquinazolinone is a biologically active derivative of quinazoline which has structural resemblance with nucleobase analogues especially pyrimidines.[29] Hence, these can be called as expanded pyrimidine nucleobase analogues.

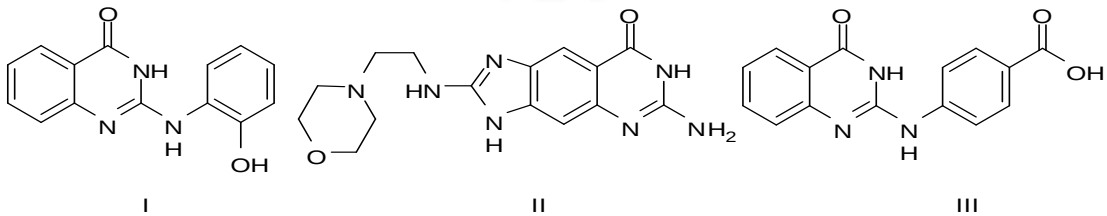


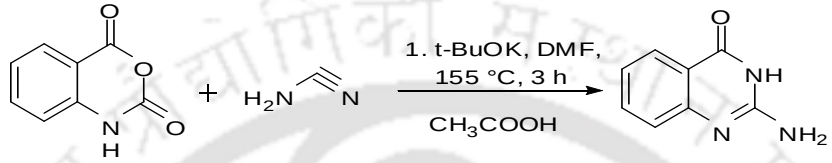
Figure III.1b *Some of the biologically active 2-aminoquinazolinones*

The above quinazoline derivatives I, II and III have antihypersensitive,[30] tRNA guanine transglycosylase inhibition,[31] and enzyme aldose reductase inhibition[32] properties, respectively.

III.2 Synthesis of 2-aminoquinazolinones

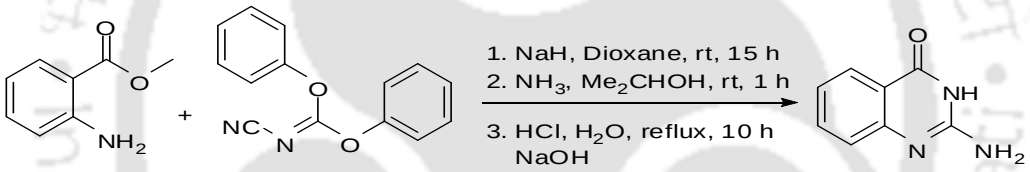
III.2.1 Existing methods

In 1987 Leonard *et al.* had shown the synthesis of benzopurines via quinazolinone as an intermediate by heating isatoic anhydride with cyanamide in presence of strong base.[33]



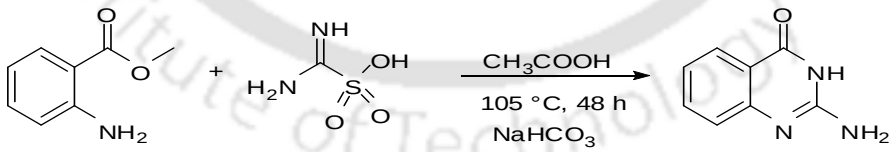
Scheme III.2.1.1

Later, Garratt *et al.* demonstrated 2-aminoquinazolinone synthesis using methyl 2-aminobenzoate in two steps. [34]



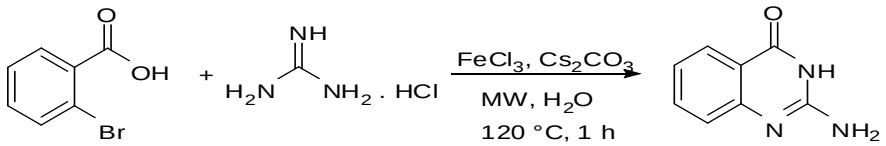
Scheme III.2.1.2

Further, 2-aminoquinazolinone was prepared by reflux of methyl 2-aminobenzoate with amidine sulphonic acid under acidic condition. This synthesis was established by Prashad *et al.* in 1998, and the major drawback of this method was low yield. [35]



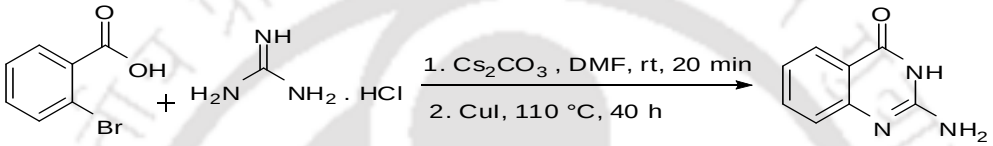
Scheme III.2.1.3

Synthesis of 2-aminoquinazolinone was further developed using metal catalysts. Zhang *et al.* used Iron (III) chloride as a catalyst and 2-bromo benzoic acid was used as precursor for the synthesis of quinazolinone under microwave condition.[36] However, the method had only very limited substrate scope.



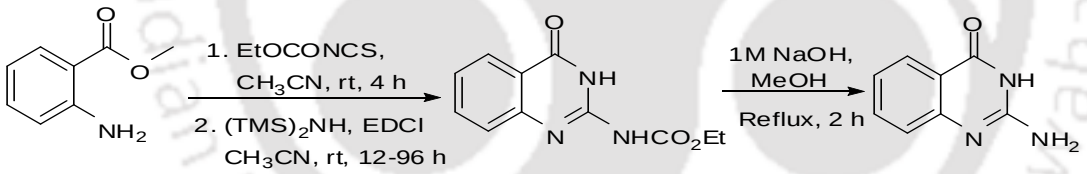
Scheme III.2.1.4

It was again modified by Huang *et al*. using copper(I) iodide as a metal catalyst under strong basic condition.[37] 2-halo benzoic acid and guanidine hydrochloride were used as starting materials for this reaction. The reaction requires long time reflux to form desired product and it was reported that guanidine hydrochloride lowers the yield of the reaction due to poor reactivity.



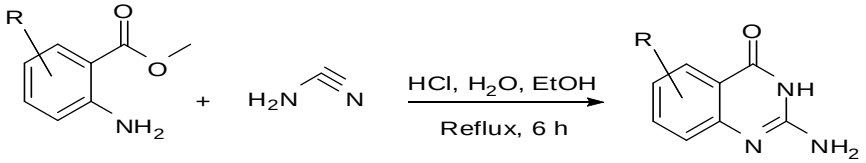
Scheme III.2.1.5

In 2011, Lecoutey *et al.* showed the multistep synthesis of 2-aminoquinazolinone using methyl 2-amino benzoate.[38] The important drawback of this method is that the reaction requires four days to complete.



Scheme III.2.1.6

The reaction was further modified and demonstrated by McGowan *et al.*[39] In this reaction, cyanamide was treated with derivatives of 2-aminobenzoate in presence of hydrochloric acid under reflux condition to yield desired 2-aminoquinazolinone derivatives.



Scheme III.2.1.7

Longer reaction time and less substrate scopes are the common problems of above all reactions.

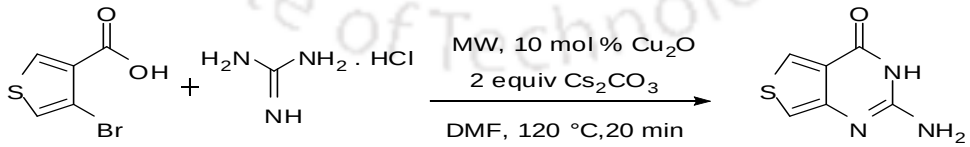
III.3 Present work

Here, we have established a novel method for the synthesis of 2-aminoquinazolinone derivatives through coupling reaction. Copper is one of the best catalysts for various coupling reactions especially cross-coupling reactions.[40-42] As far as coupling reaction is concerned, C-N bond formation has wide scope to synthesize various organic compounds by using copper sources.[43-48] Nowadays chemists have greater attention in copper catalyzed coupling reactions because of easy availability, and low cost of copper salts. Here we report a Cu (I) catalyzed microwave-promoted synthesis of modified pyrimidine nucleobase analogs in a single step. The selection of microwave method is due to its unique advantages like, eco-friendly, reduced time and higher product yields.[49]

Scheme III.3.1

As is demonstrated in **Scheme III.3.1**, the nucleobase analogues were synthesized by treating substituted or unsubstituted *ortho*-halobenzoic acid with guanidine hydrochloride in presence of copper (I) oxide (Cu_2O) under microwave irradiation. Cesium carbonate (Cs_2CO_3) was found to be a good basic source for these reactions. All the reactions were carried out using *CEM Discover labmate* microwave reactor in a closed vessel.

We have also synthesized ring expanded 2-aminopyrimidinone (**14a**) which consists of fused five membered sulphur heterocycle using the same reaction condition mentioned above (**Scheme III.3.1**).



Scheme III.3.2

The compound **14a** will not come into the category of 2-aminoquinazolinone because, the aromatic ring was replaced by sulphur heterocyclic ring, but, still it will comply with the

category of ring expanded 2-aminopyrimidinone. This molecule also maintains the planarity that 2-aminoquinazolinones usually have.

Optimization

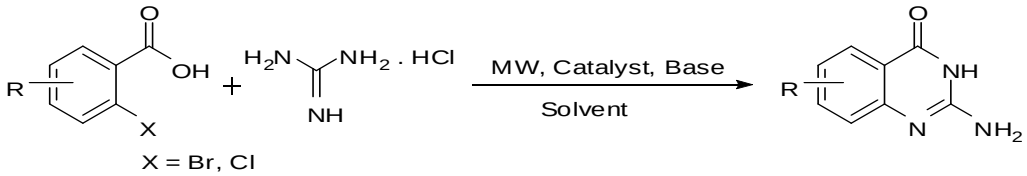
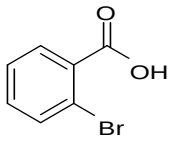
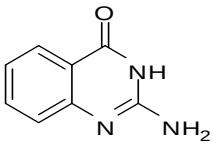
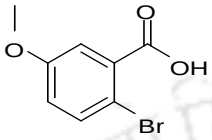
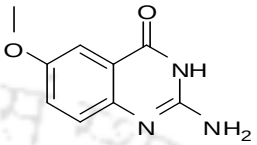
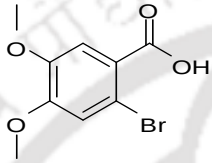
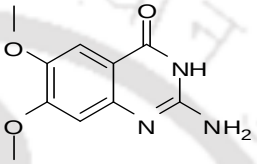
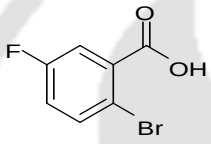
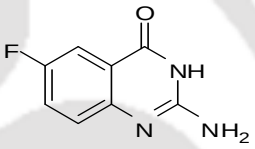
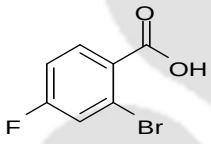
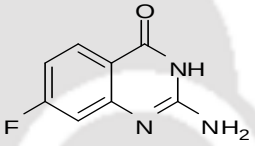
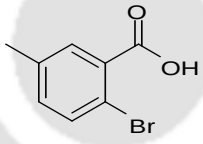
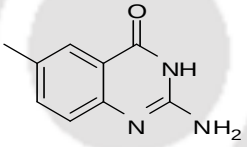
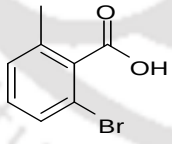
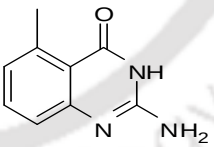
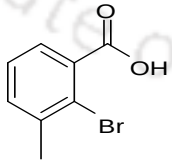
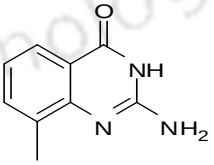
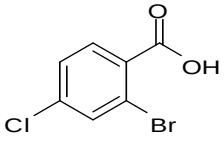
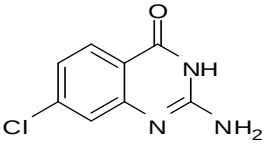


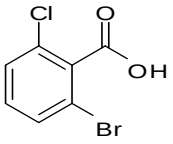
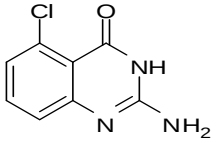
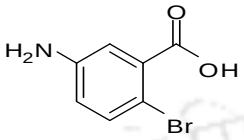
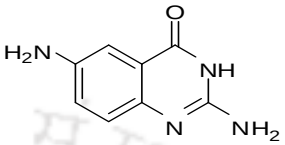
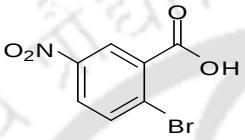
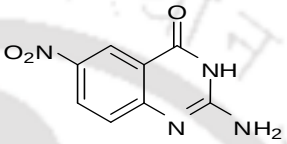
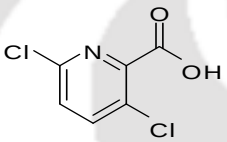
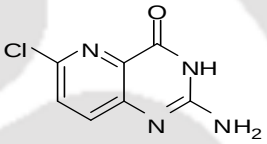
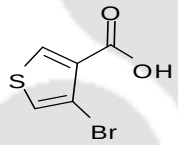
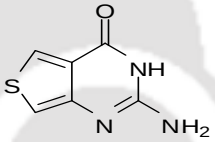
Table III.1

S. No	Catalyst	Ligand	Base	Solvent	Yield (%)
1	Cu_2O	-	Cs_2CO_3	DMF	88
2	Cu_2O	-	Cs_2CO_3	DMSO	70
3	Cu_2O	-	Cs_2CO_3	Dioxane	67
4	Cu_2O	-	Cs_2CO_3	H_2O	-
5	Cu_2O	-	Cs_2CO_3	Toluene	-
6	Cu_2O	-	K_2CO_3	DMF	42
7	Cu_2O	Proline	Cs_2CO_3	DMF	56
8	Cu_2O	Bipyridine	Cs_2CO_3	DMF	30
9	CuI	-	Cs_2CO_3	DMF	86
10	-	-	Cs_2CO_3	DMF	-
11	$ZnCl_2$	-	Cs_2CO_3	DMF	Trace
12	$Zn(OAC)_2$	-	Cs_2CO_3	DMF	Trace

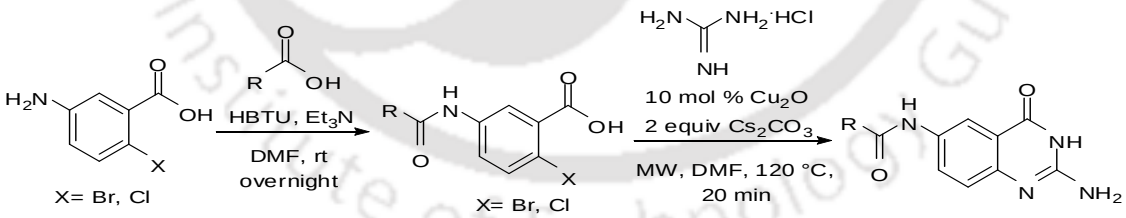
The reaction condition was optimized by screening solvents, catalyst as well as the basic source. Both Cu_2O and CuI were proved to be good catalyst, whereas, N, N-dimethyl formamide (DMF) was found to be the best solvent. Use of ligand, such as, proline or bipyridine, which is commonly used in copper-catalyzed coupling reactions, was found to reduce the yield of the reactions. Only trace amount of desired product was separated when zinc salts were used as catalysts instead of Cu (I). Control experiment with only Cs_2CO_3, in absence of the Cu (I) catalyst, failed to yield any product. Dimethyl sulphoxide (DMSO) also gave moderate yield during optimization of compound **1a**. We have not used any organic base such as triethylamine, DBU, and DIPEA due to their hazardousness and cytotoxicity.

Table III.2

S. No	Substrate (1-9)	Product (1a-9a)	Yield (%)
1			88
2			70
3			79
4			71
5			65
6			80
7			74
8			79
9			63

S. No	Substrate (10-14)	Product (10a-14a)	Yield (%)
10			67
11			52
12			39
13			55
14			52

Here, the beauty of substrate **11** is the free amine group which is prone to bring in various useful molecules through amine-acid coupling reaction using a coupling reagent.



Scheme III.3.3

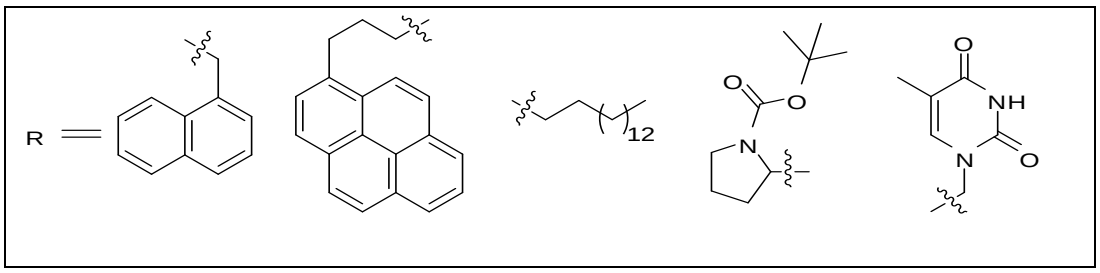
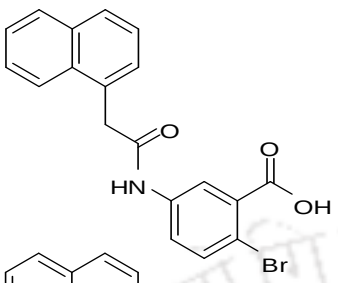
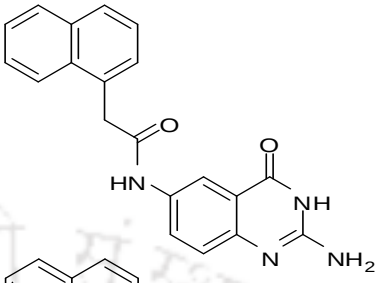
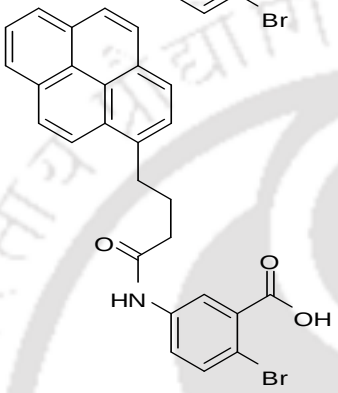
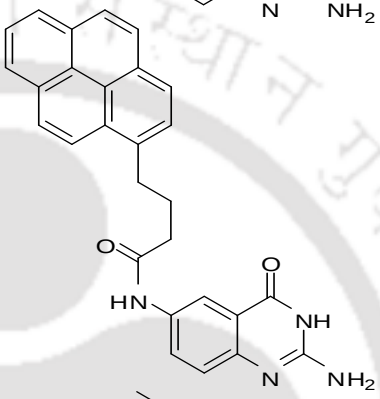
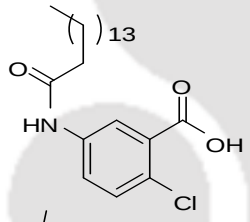
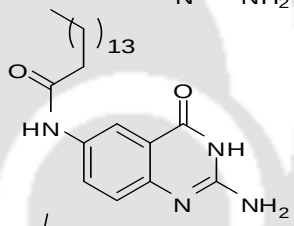
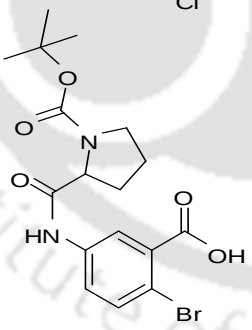
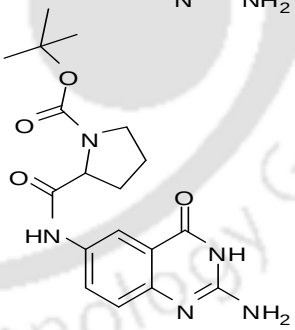
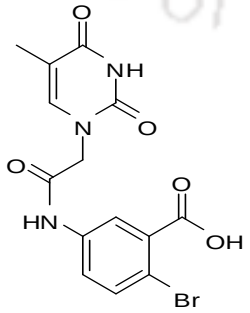
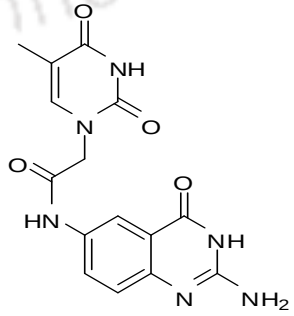


Table III.3

S. No	Substrate (15-19)	Product (15a-19a)	Yield (%)
15			63
16			59
17			69
18			70
19			61

As can be evident from **Table III.2**, a large variety of substituted aromatic carboxylic acids were used as substrates. According to literature reports, compound **1a** was mostly synthesized from 2-halo benzoic acid.[36,37] Other compounds **2a-10a** and **14a** were previously synthesized from different precursors such as, 2-aminobenzoates, by conventional methods which require long reaction hours, multiple reagents and ligands.[39,50-52] Compounds **11a-13a** were also reported to be synthesized from other precursors.[53] In the present method, use of *ortho*-halobenzoic acids as substrates were found to improve the yields under mild condition and without any ligand. However, compound **12a** resulted lower yield, due to its poor reactivity.

We have also synthesized the compounds **15a-19a** (**Table III.3**), which has been never reported before. Moreover, these compounds are relatively large molecules containing useful moieties, such as, naphthalene and pyrene (**14a** and **15a**, respectively), which could function as fluorescence probes. Amino acid residue (**18a**) or even a thymine nucleobase, through an amide spacer (**19a**) were also synthesized. Thus, the present methodology could be a robust and yet, an easier way to synthesize a spectrum of 2-aminoquinazolinone analogues.

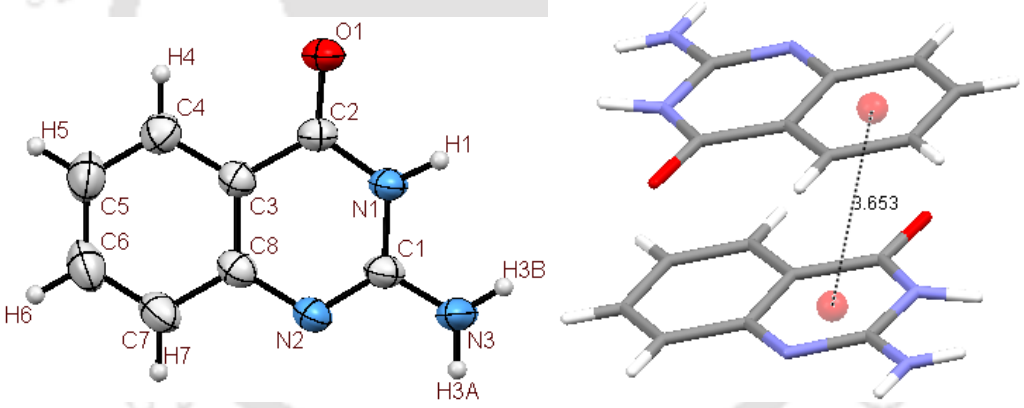


Figure III.3a *Crystal structure (ORTEP view and pi-stacking) of* ***1a***

III.4 Proposed reaction mechanism

The proposed mechanistic pathway of such reactions is well known.[37,54,55] As demonstrated in **Fig III.4a**, Cu (I) forms an oxygen coordinated complex (**A**) with *ortho*-halo-benzoic acid (**1**) under basic condition followed by formation of Cu (III) complex (**B**) through oxidative addition. The coordination of guanidine with complex **B** leads to C-N bond formation (**C**) between the aromatic ring and the guanidine moiety. The final compound (**1a**) is obtained by a condensation reaction of $-NH_2$ with carboxylic acid group, leading to the cyclized product (**1a**). During this

process, Cu (III) is reduced and eliminated as Cu (I) halo complex (CuX) which continues the catalytic cycle.

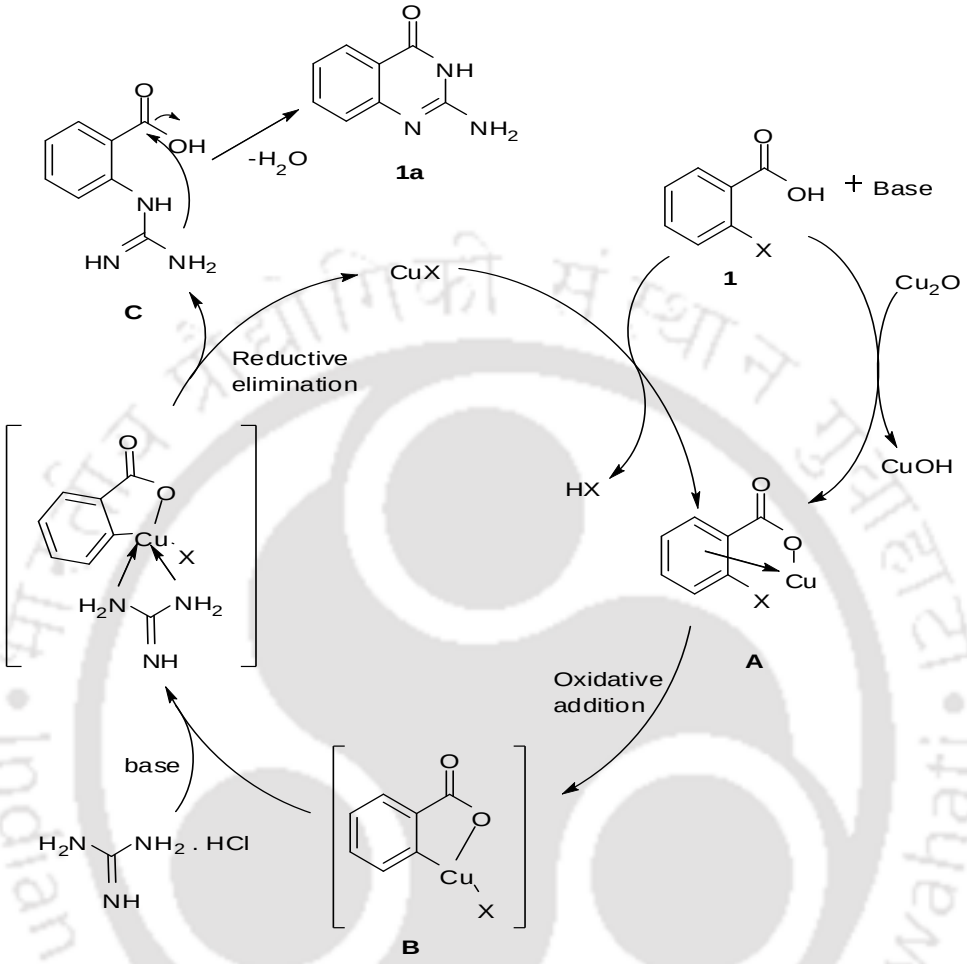


Figure III.4a *Proposed mechanism*

III.5 Base-pairing study of 2-aminoquinazolinone

It is well known that isocytosine is a structural isomer of cytosine, which can form strong duplex with guanine in parallel strand DNA.[56] Unique co-crystals of isocytosine with cytosine in 1:1 ratio were obtained by Lippert *et al.* followed by our group that clearly showed isocytosine could be an effective base-pairing partner for cytosine, instead of guanine.[57,58] 2-aminoquinazolinones have same structural unit of isocytosine with expanded aromatic ring. Therefore, such compounds might have potential as artificial nucleobases to base-pair with cytosine.

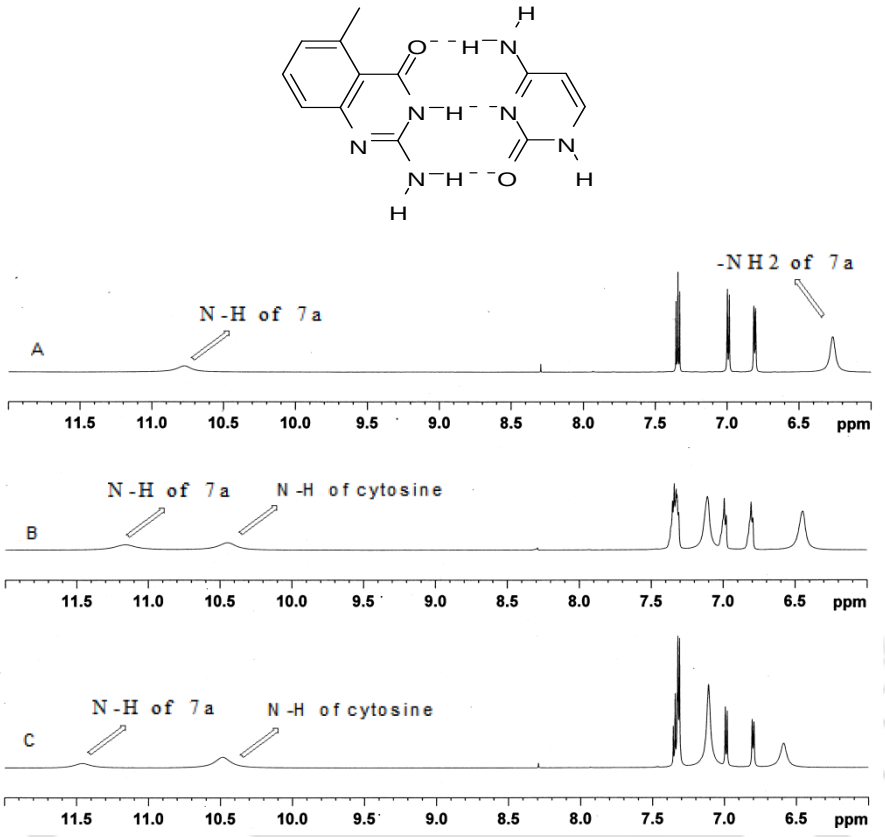


Figure III.5a *^{1}H-NMR titration of **7a** with cytosine. **A)** Only **7a**. **B)** After addition of 1equiv of cytosine. **C)** After addition of 2 equiv. of cytosine*

Compound **7a** was chosen to study its base-pairing ability against the nucleobases using proton NMR. A careful ^{1}H-NMR titration was performed by gradual addition of cytosine in a solution of **7a**, prepared in DMSO-d_6. The peak for N3-H of **7a** was monitored during addition of cytosine. The down field shift of N3-H proton clearly indicates that there is a strong hydrogen-bonding interaction between **7a** and cytosine. A shift of -NH_2 of **7a** was also observed. On the other hand cytosine N1-H was not involved in any kind of interaction with **7a**. ^{1}H-NMR titration of **7a** with other nucleobases (adenine and thymine) showed no significant interactions.

In the present chapter, we have demonstrated a Cu (I) catalyzed methodology for C-N bond formation in a microwave-directed process which does not require any additional ligand. A series of 2-aminoquinazoline analogues, which are an important class of natural products and biologically active pharmaceutical agents, have been synthesized with high yield. The derivatives of 2-aminoquinazoline have also been reported as size-expanded nucleobase analogues. The diversity of the method was explained by synthesis of compounds containing

various markers such as, fluorophores, amino acid residues or a tethered thymine nucleobase. Preliminary base-pairing studies, conducted by ^{1}H-NMR titration reveals that such compounds could act as non-natural nucleobase probes and may have potential applications in nucleic acids.

III.6 Experimental Section

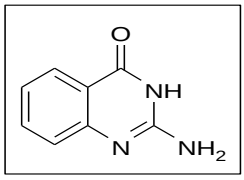
III.6.1 General Information: All the chemicals were purchased from Sigma Aldrich, Alfa Aeser, Spectrochem and were used directly without any further purification. *CEM Discover Labmate* closed vessel microwave reactor was used for all the reactions. All the NMR spectra were recorded using *Bruker-600 MHz* spectrometer using DMSO-d_6 and $CDCl_3$ as reference solvents. Crystal data was obtained from *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structure was solved by direct method using *SHELX-97*[59,60] (University of Gottingen, Germany). HRMS analyses were carried out by *Agilent Q-TOF 6500 LC/MS* instrument.

III.6.2 General Procedure: (1a-14a): 2-halo benzoic acid (1 equiv), Guanidine hydrochloride (2 equiv), Cs_2CO_3 (2 equiv), Cu_2O (10 mol%, 0.1 equiv) were taken in a microwave reactor vessel and 1mL of dry DMF was added carefully. The reaction vessel was closed and exposed to microwave irradiation about 120 °C for 20 minutes. Stirring was maintained during the reaction and the vessel was cooled after the reaction. Initially the product formation was confirmed by thin layer chromatography. The purification of products was carried out by column chromatography using methanol/chloroform.

III.6.3 General Procedure for 15-19: 5-amino-2-halo benzoic acid (1 equiv), acid (1 equiv), HBTU (1.2 equiv), were taken in a round bottom flask and 3 mL of dry DMF containing Et_3N (1.3 equiv.) was added. The reaction mixture was stirred for 12 h under inert atmosphere. The product was confirmed by thin layer chromatography, and purified by column chromatography using methanol/chloroform solvent mixture.

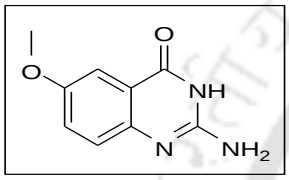
III.7 Characterization Section

2-Aminoquinazolin-4(3*H*)-one (1a)



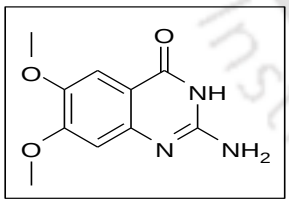
White solid, Yield: 88%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.04 (br, 1H), 7.87 (d, 1H, *J* = 7.2 Hz), 7.54 (t, 1H, *J* = 7.2 Hz), 7.18 (d, 1H, *J* = 7.8 Hz), 7.09 (t, 1H, *J* = 7.2 Hz), 6.43 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 133.99, 125.91, 121.50, 117.16. FTIR (KBr): ν/cm^{-1} 3256.3, 3056.6, 2924.2, 2851.4, 1728.1, 1704.3, 1672.0, 1617.6, 1444.6, 1405.5, 1301.2, 1141.7, 757.23 HRMS (ESI): calculated for $C_8H_7N_3O$ $(M+H)^+$ 162.0662, found 162.0672. Crystal data: Formula: C8 H7 N3 O, M: 161.17; Monoclinic; C2/c; a = 14.8913 (6) Å; b = 15.8185 (6) Å; c = 7.2225 (2) Å; α = 90°; β =109.353(3)°; γ = 90°; V = 1605.18 (10): Z = 8; R1 = 0.062: wR2 = 0.1762; S = 1.002.

2-Amino-6-methoxyquinazolin-4(3*H*)-one (2a)



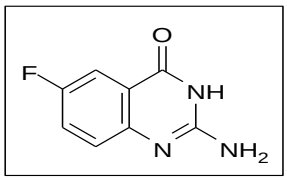
White solid, Yield: 70%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 7.30 (s, 1H), 7.20 (m, 2H), 6.718 (s, 1H), 6.368 (s, 2H), 3.776 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 162.52, 154.45, 150.91, 143.96, 124.50, 123.71, 117.36, 106.37, 55.39. FTIR (KBr): ν/cm^{-1} 3400.3, 3150.2, 2924.4, 1680.0, 1650.2, 1618.5, 1568.2, 1486.0, 1365.5. HRMS (ESI): calculated for $C_9H_9N_3O_2$ $(M+H)^+$ 192.0768, found 192.0774.

2-Amino-6, 7-dimethoxyquinazolin-4(3*H*)-one (3a)



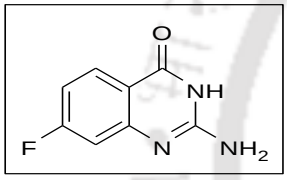
White solid, Yield: 79%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.80 (s, 1H), 7.23 (s, 1H), 6.66 (s, 1H), 6.11 (s, 2H), 3.81 (s, 3H), 3.76 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 162.21, 154.73, 151.43, 145.18, 109.30, 105.76, 104.90, 55.58, 55.53. FTIR (KBr): ν/cm^{-1} 3354.92, 3092.66, 2926.29, 2828.68, 1685.13, 1645.60, 1545.53, 1489.21, 1438.07, 1382.50, 1278.43, 1212.82, 1162.40. HRMS (ESI): calculated for $C_{10}H_{11}N_3O_3$ $(M+H)^+$ 222.0873, found 222.0872.

2-Amino-6-fluoroquinazolin-4(3*H*)-one (4a)



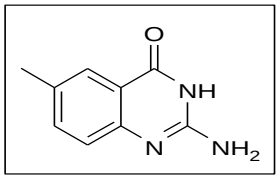
White solid, Yield: 71%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.17 (br, 1H), 7.54 (d, 1H, *J* = 9 Hz), 7.45 (td, 1H, *J* = 9 Hz), 7.24 (dd, 1H, *J* = 9 Hz), 6.44 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 162.27, 157.89, 156.31, 151.84, 125.60, 122.45 (d), 117.65 (d), 110.37 (d). FTIR (KBr): ν/cm^{-1} 3404.98, 3169.18, 2924.38, 2852.70, 1689.35, 1656.31, 1622.90, 1574.67, 1481.82, 1399.29, 1351.13, 1249.49, 1138.30. HRMS (ESI): calculated for $C_8H_6FN_3O$ $(M+H)^+$ 180.0568, found 180.0568.

2-Amino-7-fluoroquinazolin-4(3*H*)-one (5a)



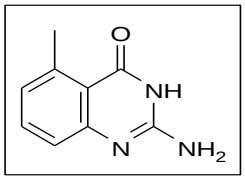
White solid, Yield: 65%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.15 (br, 1H), 7.91 (t, 1H, *J* = 7.8 Hz), 6.92 (m, 2H), 6.58 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 166.83, 165.18, 161.67, 152.84, 128.8 (d), 114.11, 109.7 (d), 108.4 (d). FTIR (KBr): ν/cm^{-1} 3472.3, 3288.8, 3128.4, 2925.3, 2855.9, 1686.9, 1630.7, 1602.3, 1452.4, 1385.6. HRMS (ESI): calculated for $C_8H_6FN_3O$ $(M+H)^+$ 180.0568, found 180.0568.

2-amino-6-methylquinazolin-4(3*H*)-one (6a)



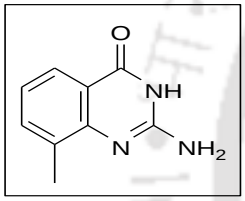
White solid, Yield: 80%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 7.67 (s, 1H), 7.38 (dd, 1H, *J* = 8.4 Hz), 7.11 (d, 1H, *J* = 8.4 Hz), 6.37 (s, 2H), 2.31 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.02, 151.99, 135.72, 131.12, 125.70, 123.42, 117.23, 20.79. FTIR (KBr): ν/cm^{-1} 3405.2, 2924.1, 2853.8, 1683.4, 1651.7, 1617.5, 1577.3, 1485.9, HRMS (ESI): calculated for $C_9H_9N_3O$ $(M+H)^+$ 176.0818, found 176.0818.

2-Amino-5-methylquinazolin-4(3*H*)-one (7a)



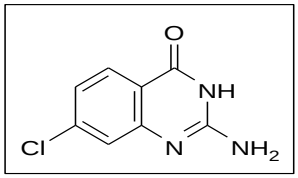
White solid, Yield: 74%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.83 (br, 1H), 7.360 (t, 1H, *J* = 7.2 Hz), 7.01 (d, 1H, *J* = 8.4 Hz), 6.82 (d, 1H, *J* = 7.2 Hz), 6.330 (br, 2H), 2.65 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.35, 162.96, 151.65, 139.79, 133.03, 124.18, 121.85, 115.59, 22.47. FTIR (KBr): ν/cm^{-1} 3433.63, 3115.46, 2924.39, 2853.55, 1681.65, 1654.39, 1628.33, 1599.52, 1522.98, 1474.83, 1376.69, 1309.75. HRMS (ESI): calculated for $C_9H_9N_3O$ $(M+H)^+$ 176.0818, found 176.0818.

2-Amino-8-methylquinazolin-4(3*H*)-one (8a)



White solid, Yield: 79%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.00 (s, 1H), 7.73 (d, 1H, *J* = 7.8 Hz), 7.42 (d, 1H, *J* = 7.2 Hz), 6.98 (t, 1H, *J* = 7.2 Hz), 6.44 (br, 2H), 2.35 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.12, 151.30, 134.31, 123.59, 120.93, 116.86, 17.58. FTIR (KBr): ν/cm^{-1} 3386.75, 3162.26, 2923.27, 2853.05, 1652.52, 1625.10, 1605.32, 1552.96, 1523.90, 1477.12, 1453.22, 1344.09, 1202.98. HRMS (ESI): calculated for $C_9H_9N_3O$ $(M+H)^+$ 176.0818, found 176.0818.

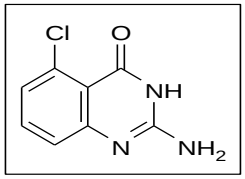
2-Amino-7-chlorolquinazolin-4(3*H*)-one (9a)



White solid, Yield: 63%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.06 (s, 1H), 7.85 (d, 1H, *J* = 8.4 Hz), 7.19 (d, 1H, *J* = 1.8 Hz), 7.10 (dd, 1H, *J* = 8.4 Hz), 6.56 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 166.50, 165.97, 152.82, 138.78, 127.97, 121.61, 115.95, 79.17. FTIR (KBr): ν/cm^{-1} 3405.86, 3164.41, 2924.20, 2853.33, 1655.20, 1634.02, 1602.37, 1550.95,

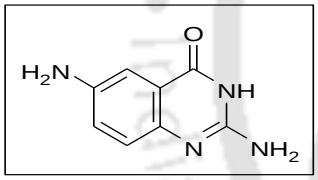
1467.69, 1452.66, 1403.49, 1336.73, 1163.53. HRMS (ESI): calculated for $C_8H_6ClN_3O$ $(M+H)^+$ 196.0272, found 196.0272.

2-Amino-5-chlorolquinazolin-4(3*H*)-one (10a)



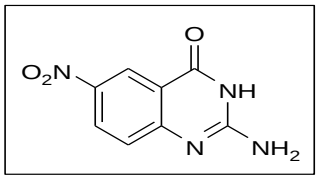
White solid, Yield: 67%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.98 (s, 1H), 7.44 (t, 1H, *J* = 8.4 Hz), 7.11 (d, 1H, *J* = 8.4 Hz), 7.05 (d, 1H, *J* = 7.8 Hz), 6.44 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 160.19, 154.26, 151.96, 133.74, 132.53, 123.83 (d), 113.92. FTIR (KBr): ν/cm^{-1} 3363.34, 3126.42, 2921.43, 2851.74, 1656.86, 1604.21, 1585.99, 1570.68, 1505.71, 1466.32, 1449.39, 1393.63, 1308.93, 1101.14. HRMS (ESI): calculated for $C_8H_6ClN_3O$ $(M+H)^+$ 196.0272, found 196.0280.

2, 6-Diaminoquinazolin-4(3*H*)-one (11a)



Brown solid, Yield: 52%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.76 (br, 1H), 7.04 (d, 1H, *J* = 2.4 Hz), 6.65 (d, 1H, *J* = 8.4 Hz), 6.90 (dd, 1H, *J* = 8.4 Hz), 5.98 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 143.76, 122.80, 117.83, 107.48. FTIR (KBr): ν/cm^{-1} 3406.43, 3178.28, 2924.20, 2848.86, 1654.47, 1621.88, 1560.22, 1525.19, 1484.72, 1356.54. HRMS (ESI): calculated for $C_8H_8N_4O$ $(M+H)^+$ 177.0771, found 177.0771.

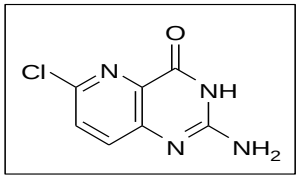
2-Amino-6-nitroquinazolin-4(3*H*)-one (12a)



Yellow solid, Yield: 39%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.51 (br, 1H), 8.62 (s, 1H), 8.30 (dd, 1H, *J* = 6.6 Hz), 7.28 (d, 1H, *J* = 8.4 Hz), 6.62 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-

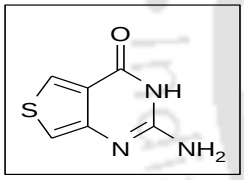
d_6): δ (ppm) 166.46, 165.93, 154.38, 140.61, 128.28, 122.63, 116.29. FTIR (KBr): ν/cm^{-1} 3416.2, 2924.6, 2853.9, 1662.4, 1582.4, 1522.5, 1414.0, 1353.1. HRMS (ESI): calculated for $C_8H_6N_4O_3$ $(M+H)^+$ 207.0513, found 207.0505.

2-amino-6-chloropyrido [3, 2-*d*] pyrimidin-4(3*H*)-one (13a)



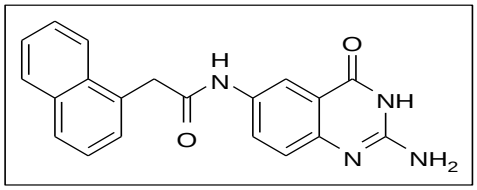
White solid, Yield: 55%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.37 (s, 1H), 7.62 (d, 1H, *J* = 8.7 Hz), 7.58 (d, 1H, *J* = 8.5 Hz), 6.68 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6+TFA): δ (ppm) 159.68, 158.68, 148.07, 137.19, 131.92, 130.64. FTIR (KBr): ν/cm^{-1} 3353.73, 3054.96, 2924.10, 1680.70, 1646.78, 1586.59, 1540.12, 1488.76, 1462.29, 1422.83, 1133.83. HRMS (ESI): calculated for $C_7H_5ClN_4O$ $(M+H)^+$ 197.0225, found 197.0231.

2-Aminothieno [3, 4-*d*] pyrimidin-4(3*H*)-one (14a)



Pale yellow solid, Yield: 52%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.52 (br, 1H), 8.23 (dd, 1H, *J* = 3 Hz), 6.95 (d, 1H, *J* = 3 Hz), 6.08 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 158.95, 150.91, 133.23, 127.73, 127.24, 127.09, 123.77, 108.41. FTIR (KBr): ν/cm^{-1} 3434.13, 3382.71, 3178.33, 2924.18, 2853.37, 1691.03, 1654.73, 1628.29, 1546.92. HRMS (ESI): calculated for $C_6H_5N_3OS$ $(M+H)^+$ 168.0226, found 168.0225.

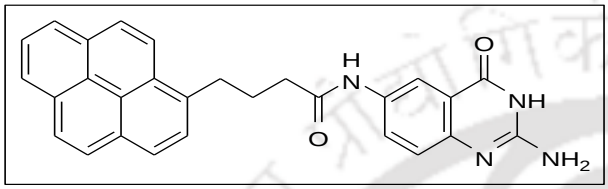
N-(2-amino-4-oxo-3, 4-dihydroquinazolin-6-yl)-2-(naphthalene-1-yl) acetamide (15a)



White solid, Yield: 63%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.94 (br, 1H), 10.41 (s, 1H), 8.19 (s, 1H), 8.15 (d, 1H, *J* = 8.4 Hz), 7.93 (d, 2H, *J* = 8.4 Hz), 7.84 (d, 1H, *J* = 8.4 Hz),

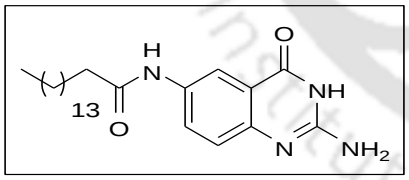
7.75 (d, 1H, *J* = 7.8 Hz), 7.54 (m, 4H), 7.15 (d, 1H, *J* = 8.4 Hz), 6.31 (br, 2H), 4.14 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 168.76, 166.57, 165.84, 157.99, 133.32, 132.43, 131.96, 128.36, 127.80, 127.16, 126.35, 126.04, 125.63, 125.49, 124.17, 117.03, 115.32, 40.56. FTIR (KBr): ν/cm^{-1} 3389.40, 2923.49, 2851.59, 1659.95, 1606.57, 1558.99, 1490.42, 1396.77, 1376.56. HRMS (ESI): calculated for $C_{20}H_{16}N_4O_2$ $(M+H)^+$ 345.1346, found 345.1353.

N-(2-amino-4-oxo-3, 4-dihydroquinazolin-6-yl)-4-(pyren-1-yl) butanamide (16a)



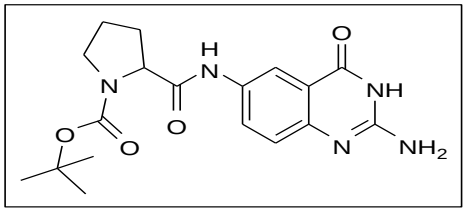
Pale yellow solid, Yield: 59%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.93 (br, 1H), 9.98 (s, 1H), 8.42 (d, 1H, *J* = 9.6 Hz), 8.27 (t, 2H, *J* = 7.2 Hz), 8.23 (t, 2H, *J* = 7.2 Hz), 8.13 (dd, 2H, *J* = 9 Hz), 8.05 (t, 1H, *J* = 7.2 Hz), 7.97 (d, 1H, *J* = 7.8 Hz), 7.72 (dd, 1H, *J* = 6.6 Hz), 6.27 (br, 2H), 3.39 (t, 2H, *J* = 7.8 Hz), 2.47 (t, 2H, *J* = 7.2 Hz), 2.12 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 171.11, 166.85, 136.83, 133.87, 131.25, 130.79, 129.70, 128.56, 127.92, 127.81, 127.62, 126.87, 126.49, 125.32, 125.29, 125.16, 124.62, 124.52, 123.85, 117.39, 115.63, 36.21, 32.57, 27.66. FTIR (KBr): ν/cm^{-1} 3412.94, 3171.77, 2924.35, 1669.77, 1544.99, 1469.57, 1384.33, 1262.51, 1104.49. HRMS (ESI): calculated for $C_{28}H_{22}N_4O_2$ $(M+H)^+$ 447.1816, found 447.1852.

N-(2-amino-4-oxo-3, 4-dihydroquinazolin-6-yl)palmitamide (17a)



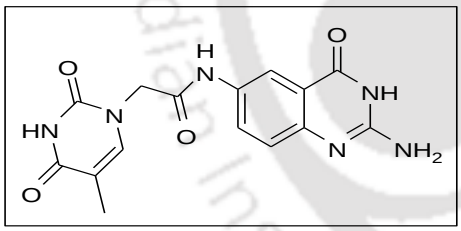
Grey solid, Yield: 69%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.01 (s, 1H), 9.93 (s, 1H), 8.19 (s, 1H), 7.71 (d, 1H, *J* = 7.8 Hz), 7.12 (d, 1H, *J* = 8.4 Hz), 6.31 (br, 2H), 2.27 (t, 2H, *J* = 7.2 Hz), 1.57 (d, 2H, *J* = 6.6 Hz), 1.22 (m, 24H), 0.84 (t, 3H, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 170.95, 126.17, 123.55, 36.28, 31.22, 28.98, 28.94, 28.86, 28.73, 28.64, 28.60, 25.10, 22.02, 13.86. FTIR (KBr): ν/cm^{-1} 3432.20, 3324.81, 2920.11, 2849.94, 1676.04, 1651.78, 1607.44, 1549.31, 1514.26, 1481.94, 1382.00, 1069.75. HRMS (ESI): calculated for $C_{24}H_{38}N_4O_2$ $(M+H)^+$ 415.3068, found 415.3067.

***Tert*-butyl 2-(2-amino-4-oxo-3, 4-dihydroquinazolin-6-ylcarbamoyl) pyrrolidine-1-carboxylate (18a)**



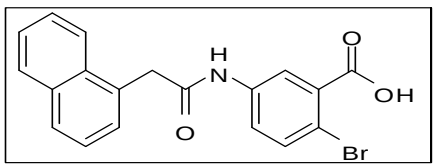
White solid, Yield: 70%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.02 (s, 1H), 8.20 (dd, 1H), 7.74 (dd, 1H, *J* = 8.4 Hz), 7.16 (dd, 1H, *J* = 8.4 Hz), 6.39 (br, 2H), 4.2 (m, 1H), 3.43 (m, 2H), 3.32 (m, 2H), 2.49 (br, 1H), 2.19 (m, 1H), 1.89 (m, 2H), 1.78 (m, 1H), 1.39 (s, 3H), 1.25 (s, 6H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 171.29, 170.84, 162.65, 153.62, 153.17, 151.44, 133.26, 126.52, 126.38, 123.54, 117.0, 115.58, 115.43, 78.65, 78.47, 60.41, 60.04, 46.75, 46.55, 35.77, 31.00, 30.76, 30.65, 30.21, 28.14, 27.94, 23.96, 23.39. FTIR (KBr): ν/cm^{-1} 3399.33, 2924.06, 2848.86, 1663.59, 1640.63, 1537.63, 1485.58, 1384.55, 1162.56, 1017.37. HRMS (ESI): calculated for $C_{18}H_{23}N_5O_4$ $(M+H)^+$ 374.1823, found 374.1823.

N-(2-amino-4-oxo-3, 4-dihydroquinazolin-6-yl)-2-(5-methyl-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetamide (19a)



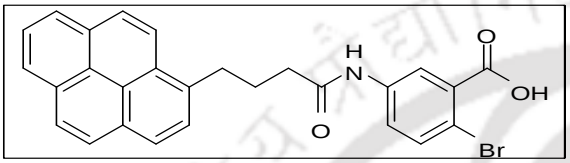
Black solid, Yield: 61%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.32 (s, 1H), 10.57 (s, 1H), 8.19 (s, 1H), 7.73 (d, 1H, *J* = 7.8 Hz), 7.53 (s, 1H), 7.19 (s, 2H), 6.65 (br, 2H), 4.52 (s, 2H), 1.76 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 165.50, 164.45, 158.37, 151.10, 142.46, 126.19, 115.41, 107.97, 49.91, 11.89. HRMS (ESI): calculated for $C_{15}H_{14}N_6O_4$ $(M+H)^+$ 343.1149, found 343.1152

2-Bromo-5-(2-(naphthalen-1-yl) acetamido) benzoic acid (15)



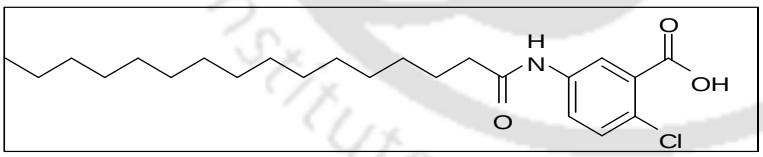
White solid, Yield: 66%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.40 (br, 1H), 10.59 (s, 1H), 8.09 (t, 1H, *J* = 7.8 Hz), 7.93 (d, 1H, *J* = 8.4 Hz), 7.85 (d, 1H, *J* = 7.2 Hz), 7.63 (t, 1H, *J* = 9 Hz), 7.53 (m, 3H), 7.48 (t, 2H, *J* = 7.2 Hz), 4.16 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.48, 166.99, 138.61, 134.16, 133.62, 133.33, 132.01, 131.93, 128.42, 127.90, 127.31, 126.13, 125.68, 125.50, 124.09, 122.71, 120.90, 112.99, 40.61. HRMS (ESI): calculated for $C_{19}H_{14}BrNO_3$ $(M+H)^+$ 384.0230, found 384.0216.

2-Bromo-5-(4-(pyren-1-yl)butanamido) benzoic acid (16)



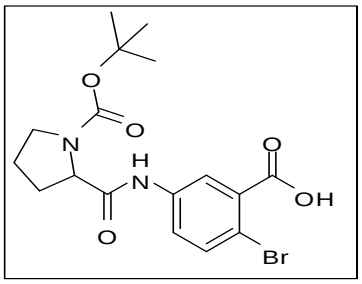
White solid, Yield: 60%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 13.60 (br, 1H), 10.22 (s, 1H), 8.41 (d, 1H, *J* = 9 Hz), 8.27 (t, 2H, *J* = 7.2 Hz), 8.22 (t, 2H, *J* = 7.8 Hz), 8.13 (dd, 2H, *J* = 9 Hz), 8.05 (t, 2H, *J* = 7.8 Hz), 7.97 (t, 2H, *J* = 9 Hz), 7.71 (d, 1H, *J* = 7.2 Hz), 7.65 (m, 2H), 7.54 (t, 1H, *J* = 7.8 Hz), 7.41 (t, 1H, *J* = 7.2 Hz), 3.38 (t, 2H, *J* = 7.8 Hz), 2.47 (t, 2H, *J* = 7.2 Hz), 2.11 (m, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 167.08, 136.34, 134.07, 133.54, 130.40, 129.34, 128.17, 127.56, 127.43, 127.25, 126.52, 126.13, 124.94, 124.79, 124.23, 124.13, 123.45, 122.73, 120.85, 35.88, 32.08, 27.02. HRMS (ESI): calculated for $C_{27}H_{20}BrNO_3$ $(M+H)^+$ 486.0699, found 486.0442.

2-Chloro-5-palmitamidobenzoic acid (17)



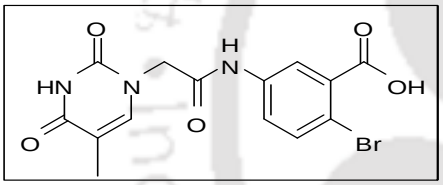
White solid, Yield: 72%. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 8.41 (d, 1H, *J* = 8.4 Hz), 8.01 (d, 1H, *J* = 8.4 Hz), 7.77 (t, 1H, *J* = 7.8 Hz), 7.56 (t, 1H, *J* = 7.8 Hz), 3.11 (t, 2H, *J* = 7.2 Hz), 1.83 (m, 2H), 1.42 (m, 2H), 1.25 (m, 22H), 0.87 (t, 3H, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, $CDCl_3$ + DMSO-d_6): δ (ppm) 14.09, 22.62, 25.54, 28.98, 29.18, 29.28, 29.39, 219.61, 31.85, 37.31, 123.54, 126.77, 128.75, 131.15, 133.06, 167.23, 172.33. HRMS (ESI): calculated for $C_{23}H_{36}ClNO_3$ $(M+H)^+$ 410.2456, found 410.2448.

2-Bromo-5-(1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxamido) benzoic acid (18)



Grey solid, Yield: 50%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.78 (s, 1H), 10.19 (s, 1H), 7.84 (s, 1H), 7.58 (d, 1H, *J* = 7.8 Hz), 7.52 (d, 1H, *J* = 7.8 Hz), 4.19 (d, 1H, *J* = 7.2 Hz), 3.33 (m, 2H), 1.90 (m, 4H), 1.39 (s, 3H), 1.28 (s, 6H). HRMS (ESI): calculated for $C_{17}H_{21}BrN_2O_5$ $(M+H)^+$ 413.0707, found 413.0710.

2-Bromo-5-(2-(5-methyl-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetamido) benzoic acid (19)



White solid, Yield: 68%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.35 (s, 1H), 10.52 (s, 1H), 7.92 (d, 1H, *J* = 1.8 Hz), 7.58 (d, 1H, *J* = 9 Hz), 7.55 (d, 1H, *J* = 9 Hz), 7.50 (s, 1H), 4.50 (s, 2H), 1.76 (s, 3H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.88, 167.96, 166.16, 164.47, 151.14, 142.10, 137.89, 133.86, 121.76, 120.50, 113.10, 108.08, 50.05, 11.91. HRMS (ESI): calculated for $C_{14}H_{12}BrN_3O_5$ $(M+H)^+$ 382.0033, found 382.0030.

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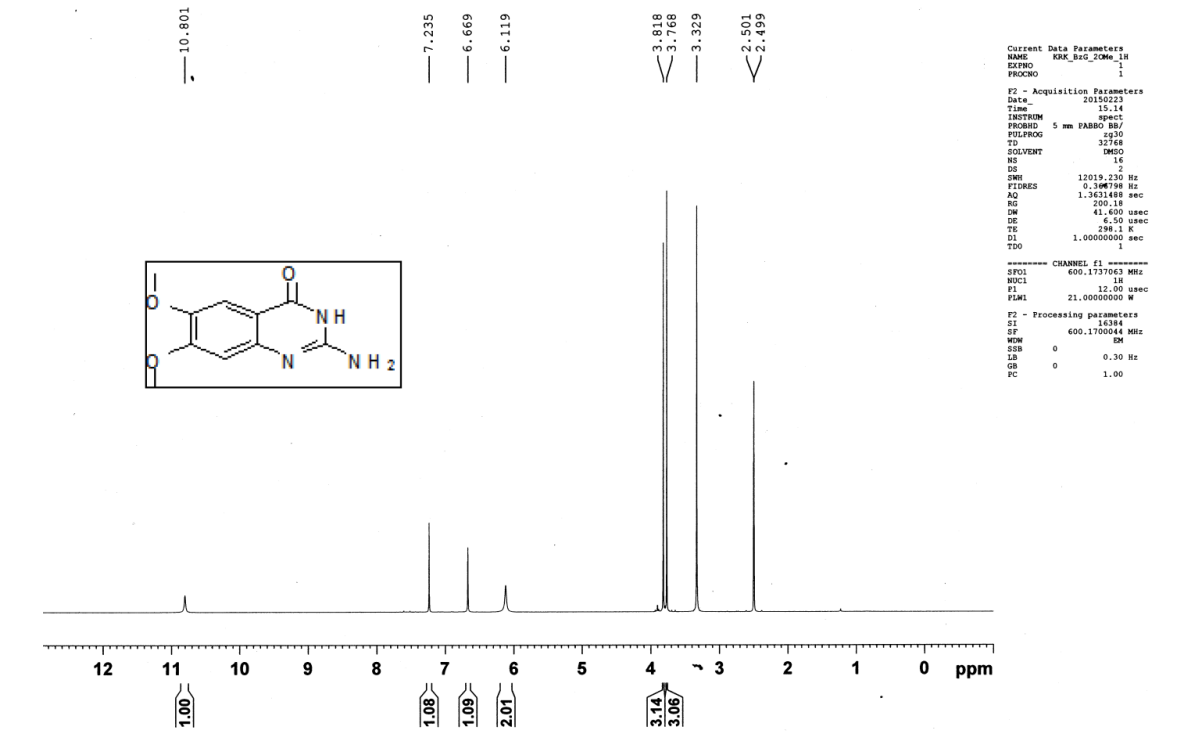
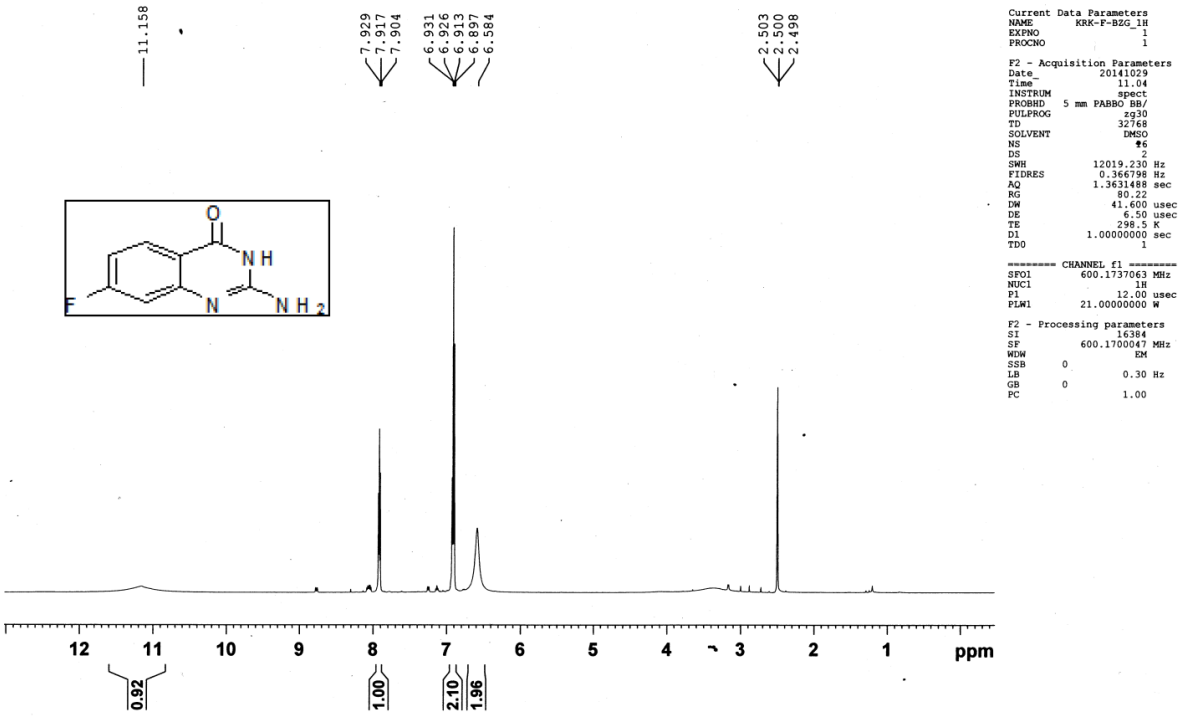
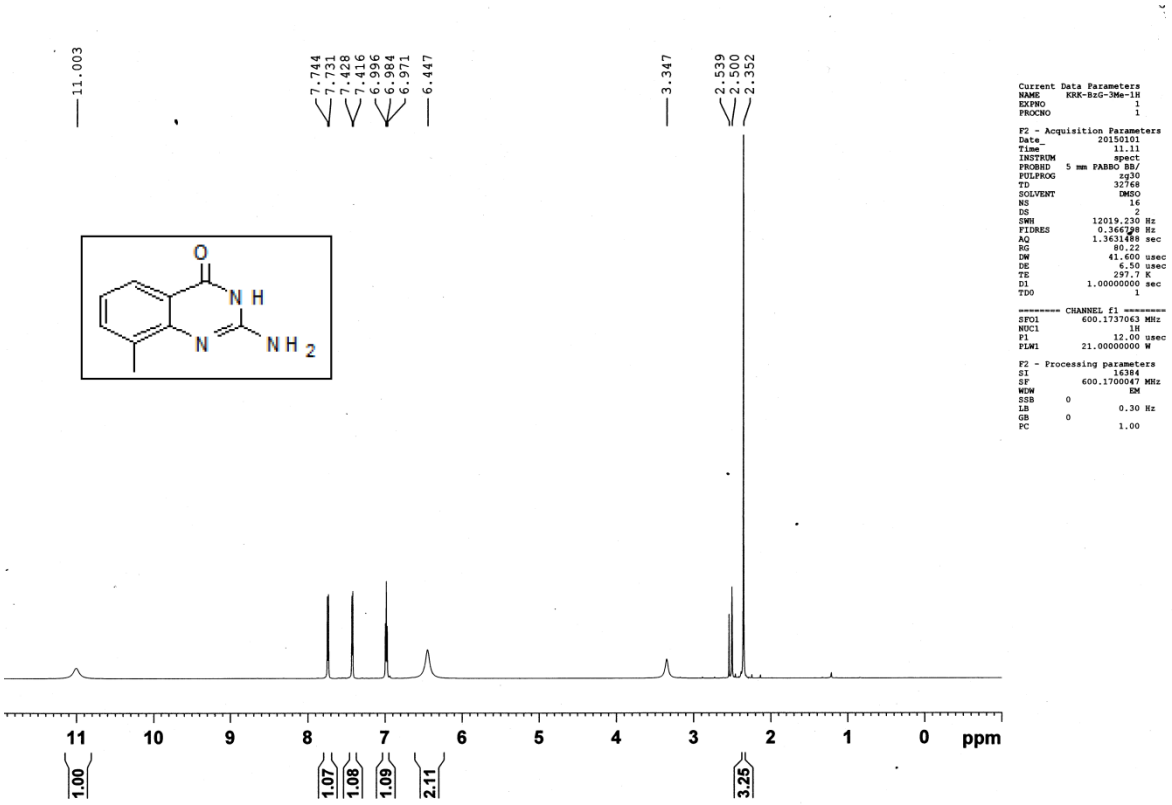
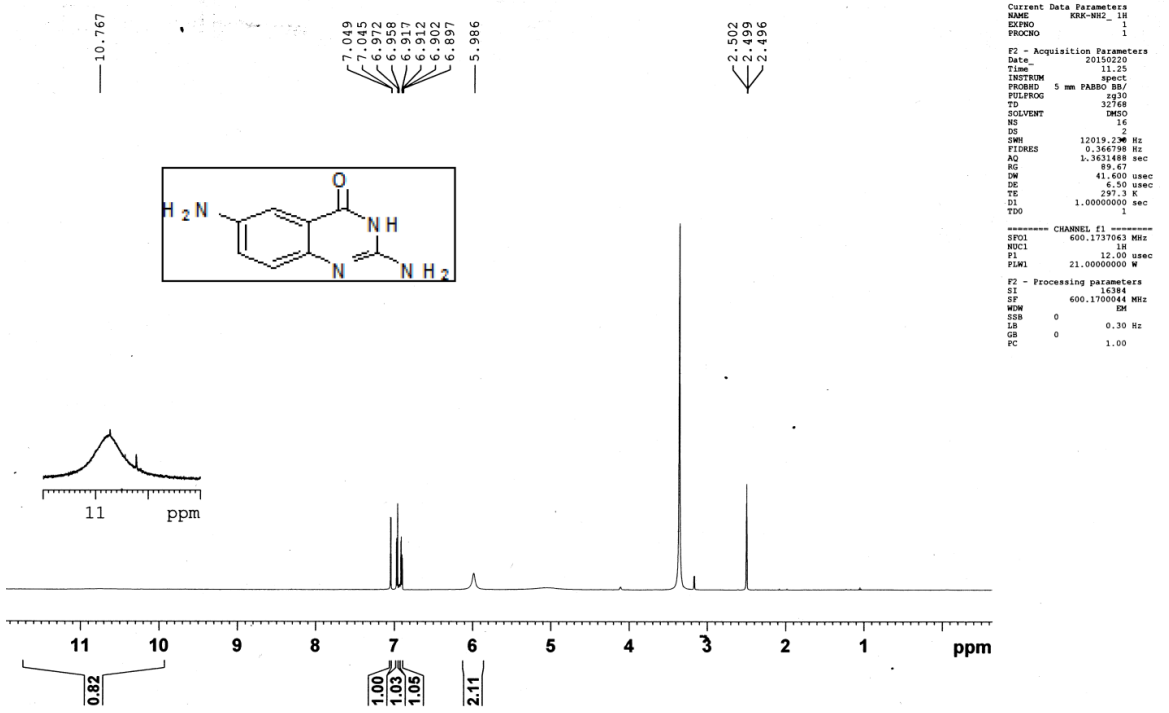
Appendix

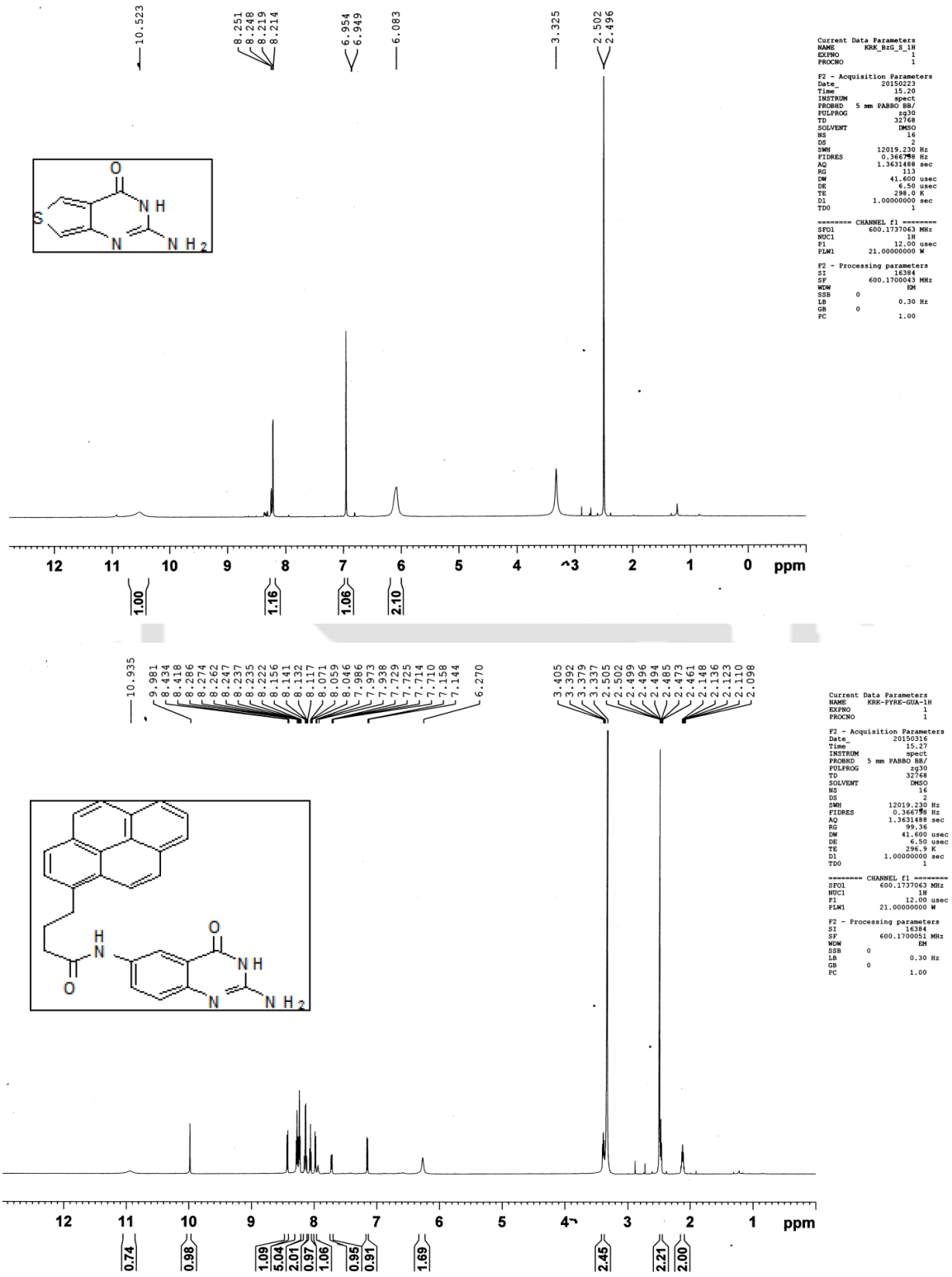
^{1}H-NMR spectra of some representative examples







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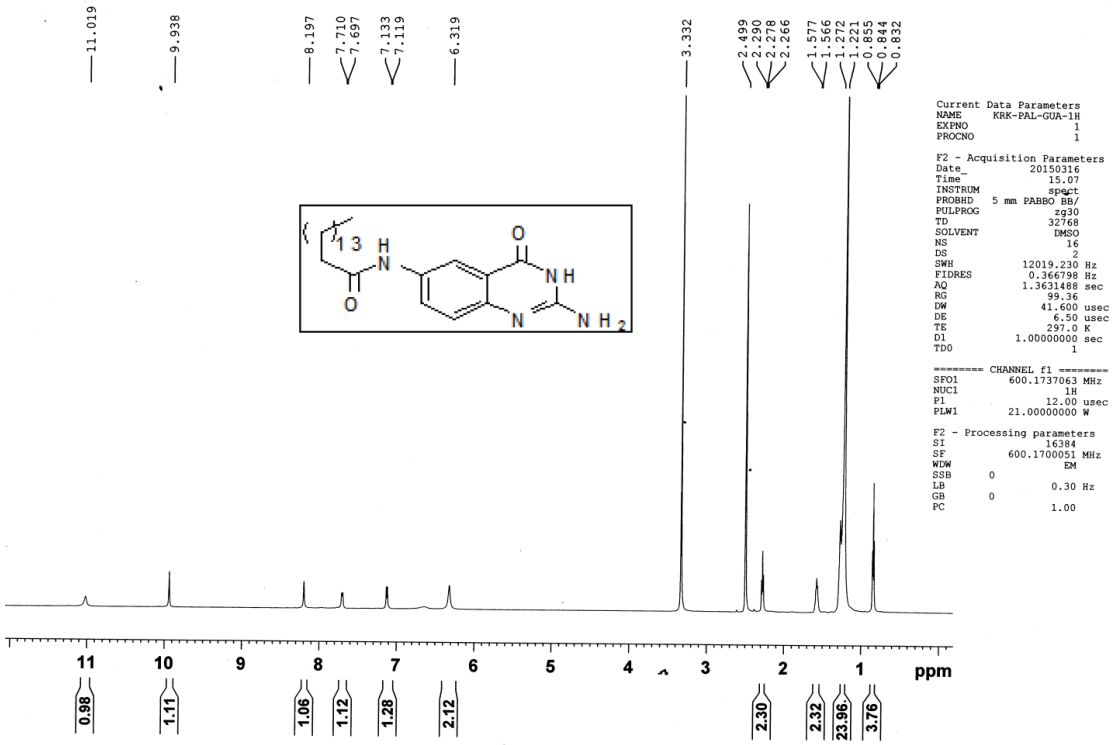
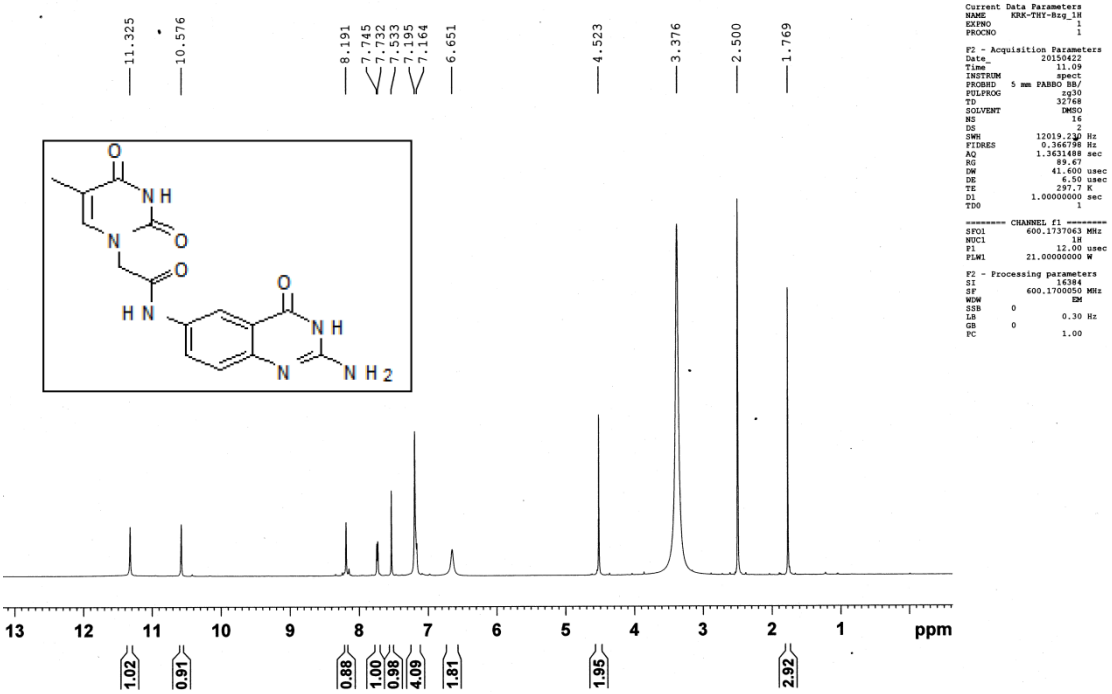




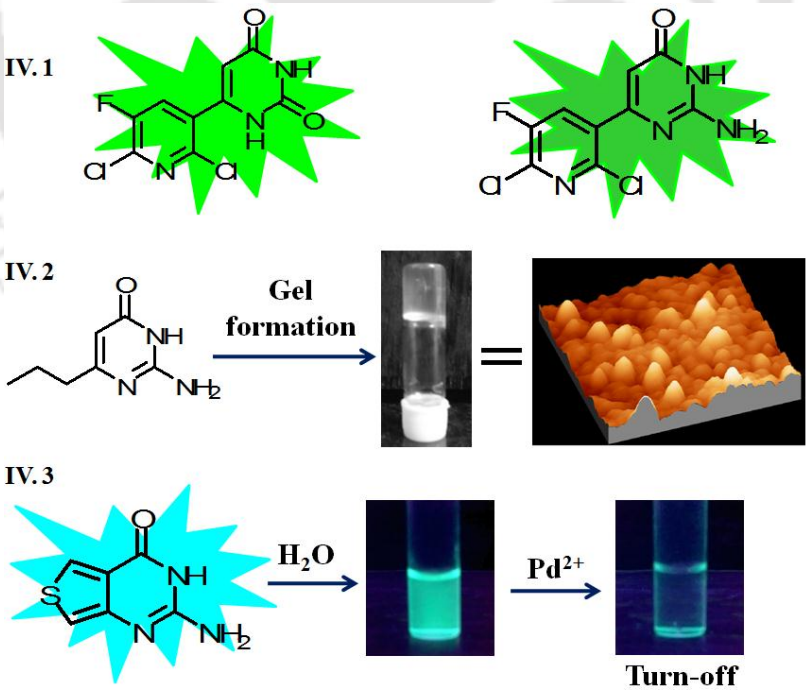
Chapter 4

Design and Synthesis of Pyrimidine and 2-Aminopyrimidine Nucleobases as Molecular Probes

Design and Synthesis of Pyrimidine and 2-Aminopyrimidine Nucleobases as Molecular Probes

Abstract

Based on the properties of nucleobase analogues, this chapter divided into three parts, (1) Fluorescence active nucleobases, (2) Gel-forming nucleobase and (3) Selective metal ion sensing using modified nucleobase analogues. The first part describes one pot microwave-assisted synthesis of C-6 substituted fluorescence active pyrimidine and 2-aminopyrimidine nucleobase analogues. The photophysical properties of active nucleobases (6-PU, 6-PiC), were explored by absorption and fluorescence techniques. Moreover, the crystal structures and base pairing properties were also explored. The second part, we report the gel property of low molecular weight 2-aminopyrimidine derivative in organic solvents. Effect of substituents on gelation and their crystal structures were also explored. Further, base-pairing were justified by gelation-degelation process and ^{1}H-NMR titration with cytosine. The third part discusses the selective detection of Pd^{2+} ion using 2-aminopyrimidine nucleobase analogue via "turn-off" quenching method by fluorescence spectroscopy.



IV.1 Fluorescence Active Nucleobases

IV.1.1 Introduction

Photophysical property of a molecule occurs when it absorbs energy from light sources that produces electronic transitions between discrete energy levels bound in the molecule. Such a transition occurs when absorbed energy equals to difference between initial and excited energy states of a molecule. Fluorescence is an important photophysical phenomenon where a molecule absorbs photons of light that causes excitation of electron and return to its ground state by rapid emission of light photons. The emission wavelength is always higher than the excitation.

Fluorescence spectroscopy is one of the reliable, sensitive and informative analytical techniques, with dominant role in modern research. Fluorescence based molecular tools are widely used in organic chemistry[1] and explore the inherent activities of biomolecules such as nucleic acids, proteins.[2] Fluorescence based bioimaging technique have also been used in cellular studies.[3,4]

Nucleic acids are important genetic component which involves storage and transfer of genetic information in living organisms. Modified nucleic acids have been synthesized that mimic the behavior of natural nucleic acids to understand various mechanisms.[5-7] Fluorescence is one of the tools that assist to investigate the properties and functions of modified nucleic acids. DNA based biosensors are applied in nucleic acids for recognition, gene targeting and DNA repair processes.[8-13]

Usually, such reporter nucleic acids are synthesized mostly from fluorescent active nucleobases which are labeled by a chromophore.[14,15] There are numerous modified pyrimidine nucleobase which were employed to study the nucleic acids (**Chapter 1**). The nucleic acid fluorescent probes should have the following requirement: 1) specificity and affinity towards target nucleic acids 2) minimum cytotoxicity, 3) stable in biological environment and 4) good cellular penetration.

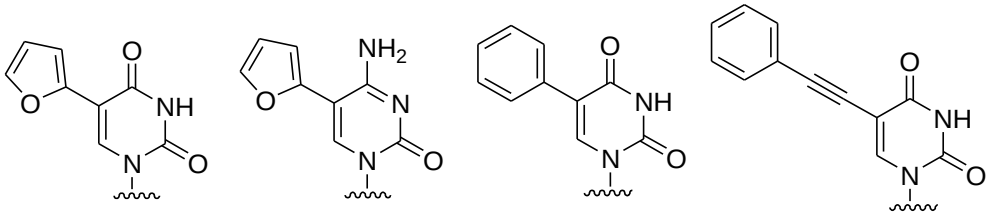
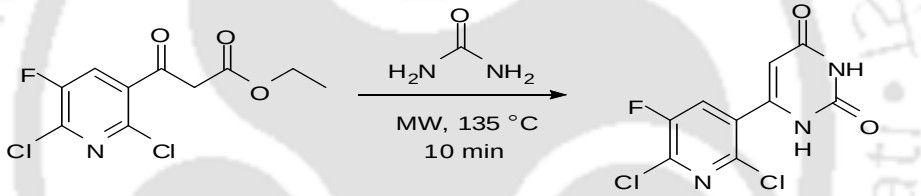


Figure IV. 1.1a *Examples of some fluorescence active pyrimidine nucleobase analogues*

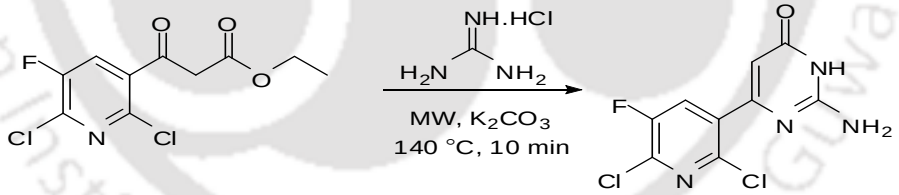
IV.1.2 Present Work

A large number of C-5 substituted fluorescence nucleobases have been reported whereas, C-6 substituted pyrimidine nucleobases are rarely reported in literatures.[16,17] In the present work we have developed a new class of pyrimidine nucleobase that is, in itself, fluorescence and at the same time retain its base-pairing properties. Such nucleobases could really be utilized both as reporter unit as well as target-specific detection systems.

For the synthesis, pyridine substituted beta-ketoester was treated with either urea or guanidine hydrochloride, yielding the corresponding C-6 pyridine substituted uracil (6-PU) (**Scheme IV.1.2.1**) or isocytosine (6-PiC) (**Scheme IV.1.2.2**) nucleobase analogues, respectively. The C-6 substituted fluorescence nucleobases are rare. Control experiment confirmed that the pyridine moiety in itself is non-fluorescent. Fluorescence signal is obtained only when pyridine ring is conjugated to the isocytosine or uracil nucleobases, leading to extended π-conjugation.



Scheme IV.1.2.1



Scheme IV.1.2.2

These two molecules could act as nucleobase analogues which are inherently fluorescence active. Usually in nucleic acids, the fluorescence active chromophore moieties are attached to the nucleobases in order to introduce the fluorescence signal. In the present work, the synthesized nucleobase analogues are highly emissive in nature without any fluorescence tag. Such kinds of nucleobases could have numerous applications in nucleic acids chemistry.

Table: IV.1 UV-Visible absorption of 6-PU and 6-PiC in various solvents

Solvents	6-PU (λ_{Max}: *nm*)	6-PiC (λ_{Max}: *nm*)
Water	281	285
Tris buffer (pH-7.2)	282	284
Chloroform	286	287, 315
DMSO	280	284, 316
THF	284	285, 317
Ethyl acetate	281	284, 312
Methanol	280	283, 309
DMF	281	283, 310
Acetonitrile	281	281

The photophysical properties of the synthesized nucleobase analogues were thoroughly studied in this work. UV-vis spectra of 6-PU and 6-PiC were measured in different solvents, as shown in **Table IV.1**. Absorbance maxima of 6-PU and 6-PiU were 286 and 285 nm respectively.

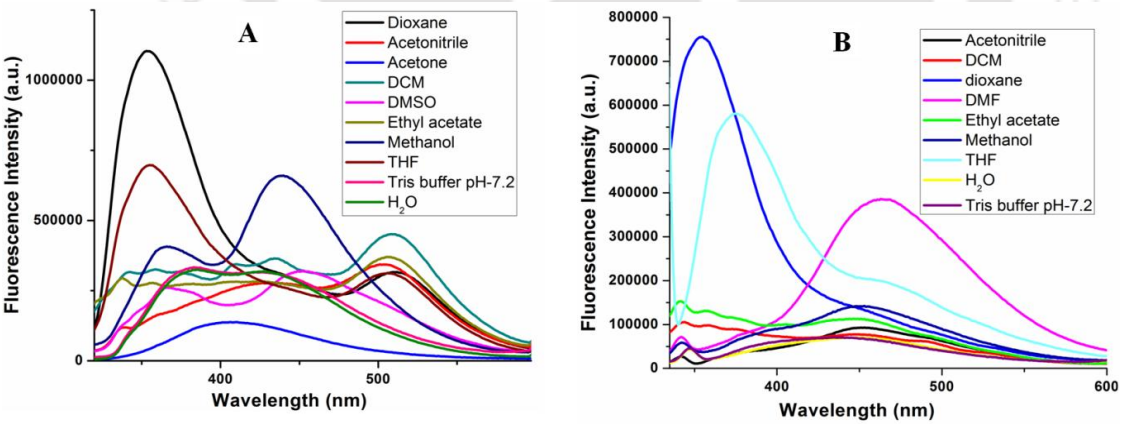


Figure IV.1.2a *Fluorescence spectra of 6-PU (20 µM), λ_{ex}: 305 nm (**A**) and 6-PiC (20 µM), λ_{ex}: 310 nm (**B**) in various solvents*

The fluorescence of both the pyrimidine analogues were measured in various solvents, as depicted in **Figure IV.1.2a**. The emission maximum and minimum of 6-PU was found to be

515 and 355 nm respectively. 6-PiU shows emission maximum at 460 nm and emission minimum at 350 nm. In polar solvents , the emission was shifted to higher wavelength (red shift) and the non-polar solvents showed blue shift. The polar solvents have high dipole moment that increases the solute polarity, leading to emission at higher wavelength. The intensity of both the analogues were quenched in polar solvents such as DMSO, water, and methanol. The quenching is primarily due to hydrogen bonding of solvent-solute molecules or aggregation of solute molecules in polar solvents.[18-20]

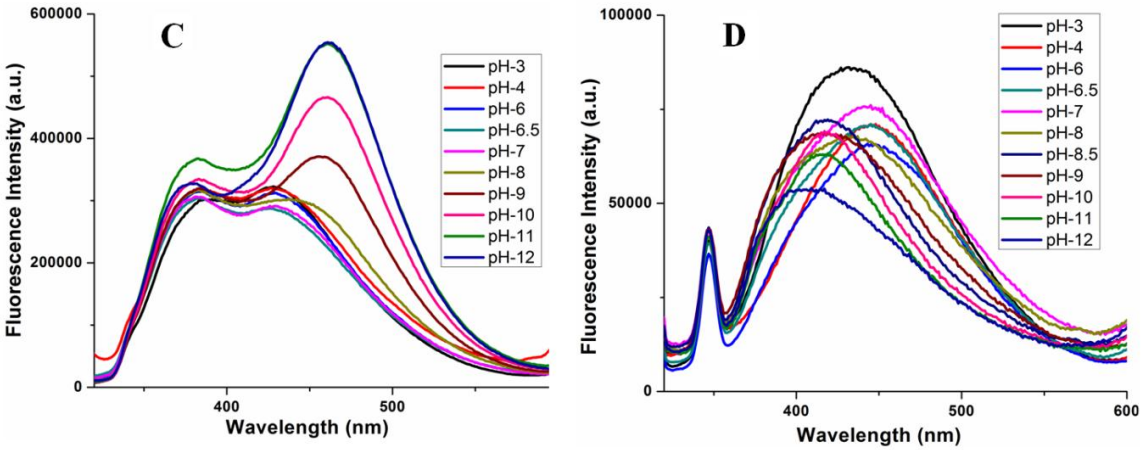


Figure IV.1.2b *pH-dependent fluorescence spectra of 6-PU (20 µM) (**C**) and 6-PiC (10 µM) (**D**); λ_{ex}: 305 nm for (6-PU) and λ_{ex}: 310 nm for (6-PiC).*

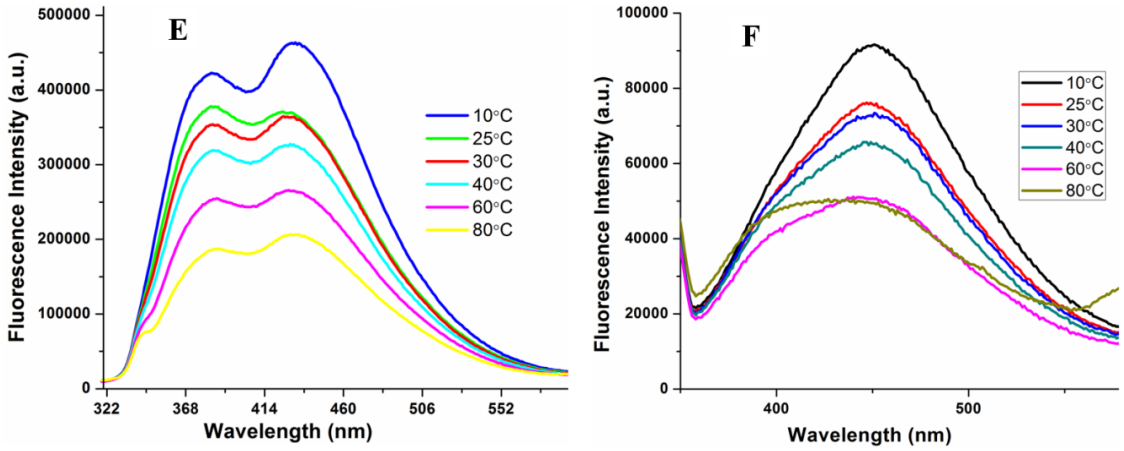


Figure IV.1.2c *Temperature dependent fluorescence spectra of 6-PU (20 µM) (**E**) and 6-PiC (10 µM) (**F**) in water; λ_{ex}: 305 nm for (6-PU) and λ_{ex}: 310 nm for (6-PiC).*

At higher pH, as in **Figure IV.1.2b**, the red shift of 6-PU (**C**) with increased intensity indicates that de-protonation of 6-PU enhances electrons mobility around the nucleobase. Quenching occurred at lower pH, is due to protonation of nucleobase which reduces extended conjugation. But in the case of 6-PiC, at higher pH, the emission spectra was shifted to blue region and causes red shift at lower pH. Gradual increase of temperature (**Figure IV.1.2c**) reduces the fluorescence of both the nucleobase analogues as they undergo decomposition.[21]

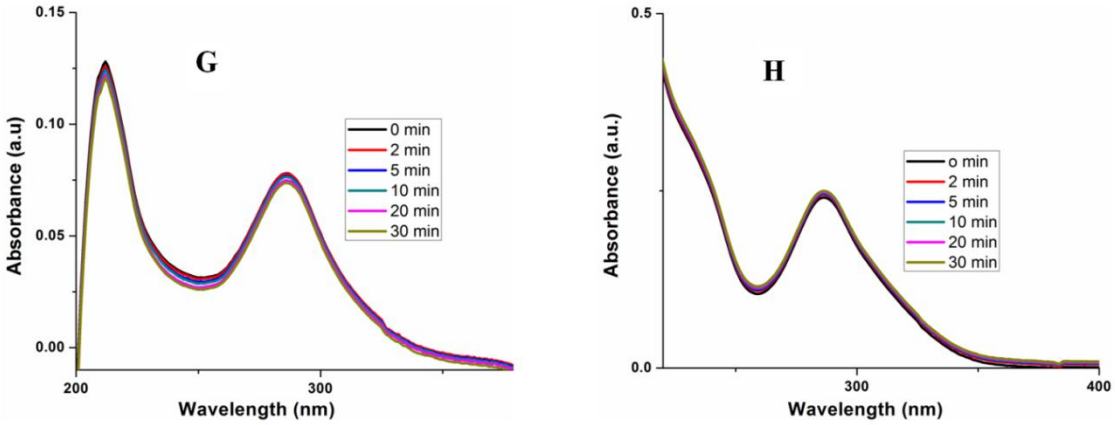


Figure IV.1.2d *Time dependent UV spectra of 6-PU (20 µM) (**G**) and 6-PiC (40 µM) (**H**)*

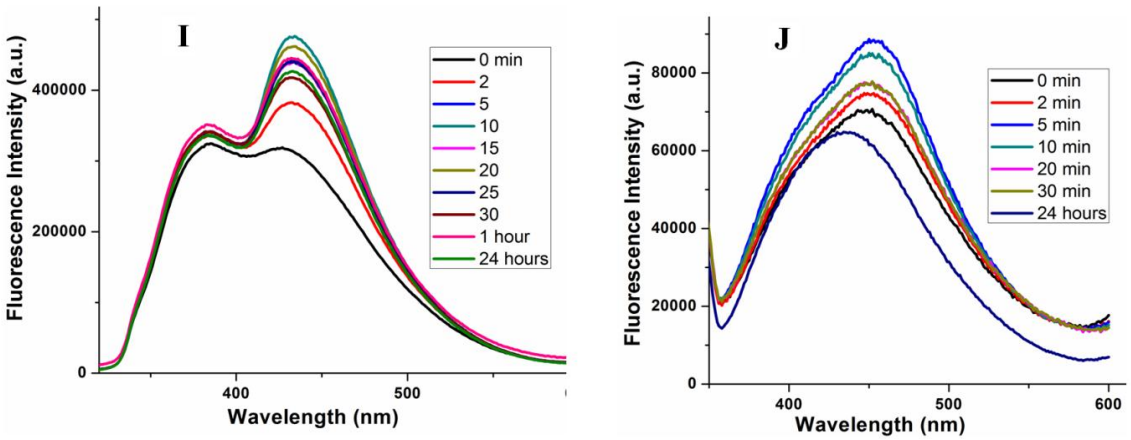


Figure IV.1.2e *Time dependent fluorescence spectra of 6-PU (20 µM) (**I**) and 6-PiC (10 µM) (**J**) in water λ_{ex}: 305 nm for (6-PU) and λ_{ex}: 310 nm for (6-PiC)*

Solubility and aggregation issues may affect the spectra of fluorescence active compounds resulting change in emission wavelength or intensity. Such effects were observed when we have measured the fluorescence in water. Variation in the spectral intensity was found in

both nucleobase analogues for time dependent measurement in water (**Figure IV.1.2d** and **Figure IV.1.2e)**. 6-PiC shows gradual blue shift along with quenching over time, probably owing to aggregation and hydrogen bonding effects.[21]

The crystal structure of 6-PU was obtained from ethanol by slow evaporation method. The crystal structure shows that two heterocyclic rings are tilted from one another. A significant amount of interaction was observed between chlorine of pyridine ring and N2-H of uracil ring (**Figure IV.1.2f)**. The bulky nature of chlorine atom forces the pyridine ring out of plane. This is further confirmed by N-substituted 6-PU. Two rings were positioned perpendicular to each other (**Figure IV.1.2g)**. But since these two heterocyclic rings act as biphenyl system and are lying on the same plane in solution phase that increases the electron delocalization within the molecule, results the fluorescence emission. We have failed to obtain any crystal structure of 6-PiC.

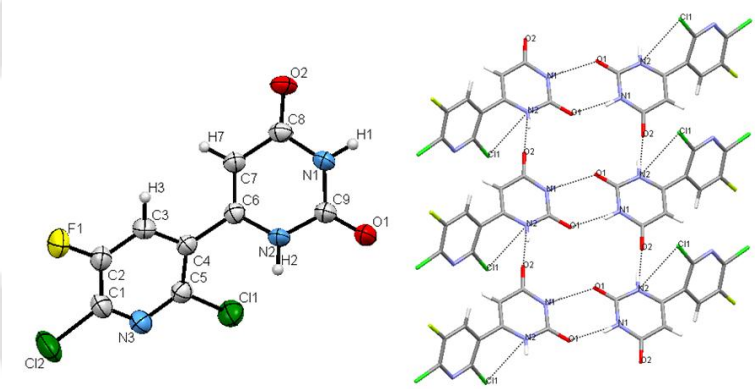


Figure IV.1.2f *ORTEP and Supramolecular assembly of 6-PU*

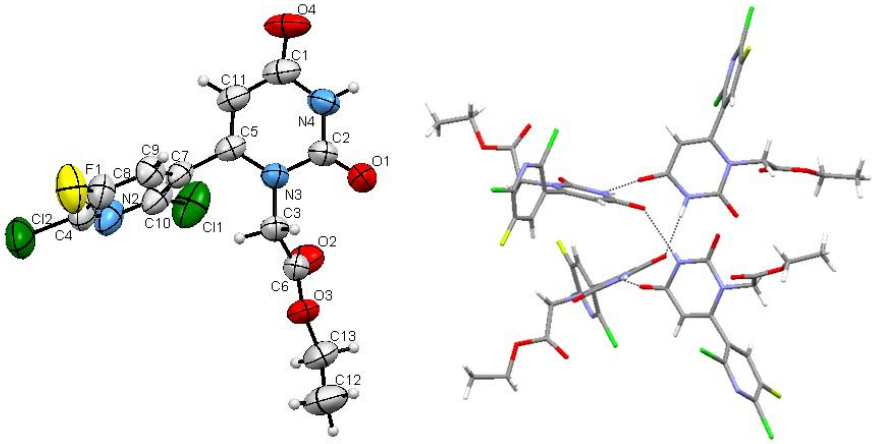


Figure IV.1.2g *ORTEP and Polymeric structure of N-substituted 6-PU*

IV.1.3 Base pairing study

It is already known that Uracil or thymine forms a base-pair with adenine[22] whereas, isocytosine forms a base-pair with cytosine.[23,24] We have tried to establish the base pairing interactions of 6-PU and 6-PiC in solid and solution phase. Both the molecules did not form any co-crystals with their counterparts. Proton NMR titration of 6-PU does not show any interaction with adenine but 6-PiC does with cytosine. The proton NMR spectrum of 6-PiC was recorded after every addition of cytosine (**Figure IV.1.3a)**. During each addition of cytosine, a downfield shift of N3-H proton (11 ppm) of isocytosine was observed, indicating clear H-bonding interaction with cytosine. The chemical shift of N1-H proton (10.4 ppm) of cytosine remained unchanged. This evidently shows that N1-H of cytosine is not involved in any kind of bonding interactions with isocytosine. Significant shift of $-NH_2$ protons of isocytosine and cytosine was also found during the experiment. So we can assume that these interactions could be same as that of natural Guanine -Cytosine base pair.

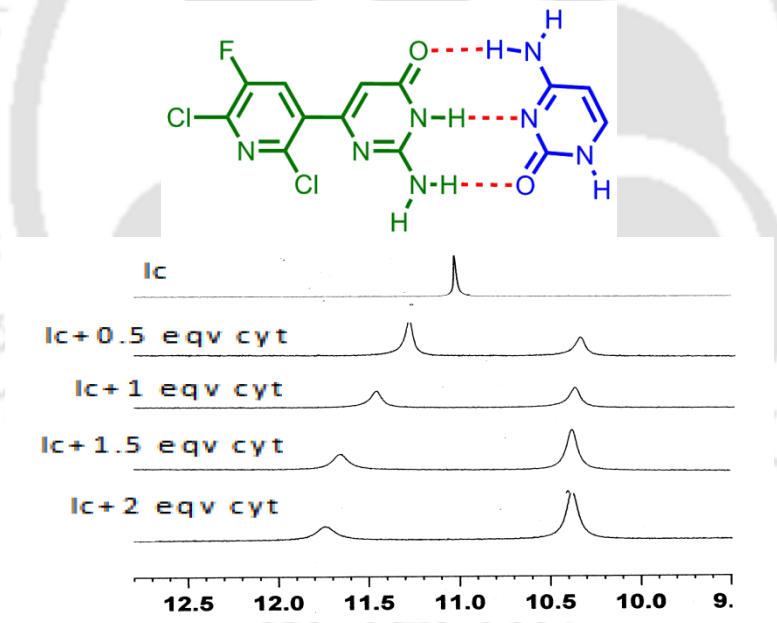


Figure IV.1.3a *^{1}H-NMR titration of 6-PiC with cytosine in DMSO-d_6 (IC=6-PiC, cyt=cytosine)*

^{1}H-NMR titration was also performed against adenine, and thymine which showed no major shift of peaks, indicating that adenine and thymine did not have significant pairing interactions with the synthesized isocytosine. In case of guanine we are unable to show the NMR study due to poor solubility of guanine in all solvents.

In conclusion, we have synthesized a new class of fluorescence active nucleobase analogues, using microwave assisted method under solvent free condition. The photophysical properties were studied in various conditions using different solvents. The crystal structure and base-pairing property were also explored. The studies result preliminary evidence that such nucleobase derivatives retain their base-pairing abilities while exhibiting fluorescence signals.

IV.1.4 Experimental Section

IV.1.4.1 General Information: All the chemicals were purchased from Sigma Aldrich, Spectrochem and were used directly without any further purification. *CEM Discover Labmate* closed vessel microwave reactor was used for all the reactions. Fluorescence spectra were carried out on a *FluoroMax-4 Spectrofluorometer-Horiba Scientific*. All the NMR spectra were recorded using *Bruker-600 MHz* spectrometer using DMSO-d_6 as reference solvent. The structure was solved by direct method using *SHELX-97*[25,26](University of Gottingen, Germany). HRMS analyses were carried out by *Agilent Q-TOF 6500 LC/MS* instrument.

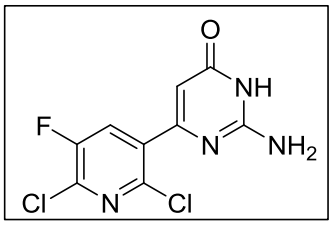
IV.1.4.2 Procedure for 6-PU: Beta-ketoester (1 mmol), urea (1.5 mmol) were taken in a microwave reactor vessel and was closed immediately. The vessel was subjected to microwave irradiation for 10 minutes at 135 $^{\circ}$C. The reaction vessel was allowed to cool, and the product was isolated. The desired compound was further purified by column chromatography using ethylacetate /hexane.

N-substituted 6-PU: 6-PU (1 mmol) and K_2CO_3 (1 mmol) were taken in a round bottom flask under inert atmosphere. Dry DMF (2 mL) was added to the reaction mixture and it was stirred for five minutes. Ethyl 2-bromoacetate (1 mmol) was added dropwise and the reaction mixture was stirred for 3 hours at room temperature. The product was confirmed by thin layer chromatography and purified by column chromatography using ethylacetate/hexane.

IV.1.4.3 Procedure for 6-PiC: Beta-ketoester (1 mmol), guanidine hydrochloride (2 mmol) and potassium carbonate (1 mmol) were taken in a microwave reactor vessel and was closed immediately. The vessel was subjected to microwave irradiation for 10 minutes at 140 $^{\circ}$C. The reaction vessel was allowed to cool, and the product was isolated. The desired compound was further purified by column chromatography using methanol/chloroform.

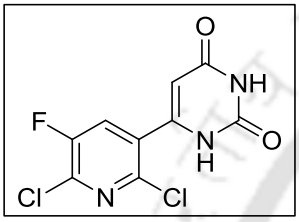
IV.1.5 Characterization Section

2-amino-6-(2, 6-dichloro-5-fluoropyridin-3-yl) pyrimidin-4(3*H*)-one (6-PiC)



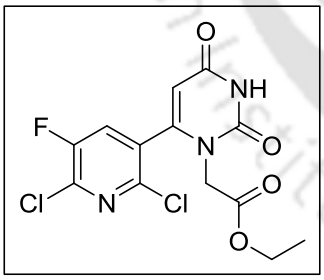
Pale Yellow solid, Yield: 43%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.06 (s, 1H), 8.18 (d, 1H, 8.4Hz), 6.78 (br, 2H), 5.84 (s, 1H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 159.09, 153.85, 152.13, 140.29, 135.28, 127.88, 102.44. HRMS (ESI): calculated for $C_9H_5Cl_2FN_4O$ $(M+H)^+$ 274.9897, found 274.9891.

6-(2, 6-dichloro-5-fluoropyridin-3-yl) pyrimidine-2, 4(1*H*, 3*H*)-dione (6-PU)



Yellow solid, Yield: 30%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.31 (s, 1H), 11.18 (s, 1H), 8.36 (d, 1H, J=7.8 Hz), 5.69 (s, 1H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.56, 152.39, 151.12, 149.86, 147.28, 141.65, 129.45, 129.30, 102.12. HRMS (ESI): calculated for $C_9H_5Cl_2FN_3O$ $(M+H)^+$ 275.9737, found 275.9740. Crystal data: Formula: C9 H4 Cl2 F N3 O2; M: 276.05; Monoclinic; P 21/c; a =7.9422 (5) Å; b = 19.8462 (13) Å; c= 6.7573 (4) Å; α = 90°; β = 99.656(4)°; γ = 90°; V = 1050.01 (11): Z = 4; R1 = 0.0468: wR2 = 0.1197; S = 0.885

Ethyl 2-(6-(2, 6-dichloro-5-fluoropyridin-3-yl)-3, 4-dihydro-2, 4-dioxopyrimidin-1(2*H*)-yl) acetate (N-substituted 6-PU)



Yellow Solid, Yield: 41%. ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 9.46 (s, 1H), 7.60 (d, 1H, J=6.4 Hz), 5.69 (s 1H), 4.94 (d, 1H, J=17.7 Hz), 4.14 (q, 2H, J=7 Hz), 3.7 (d, 1H, J=17.7 Hz), 1.21 (t, 3H, J=6.9 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 167.81, 161.9, 154.88, 153.13, 150.94, 149.27, 142.41, 128.38, 128.24, 105.52, 62.71, 46.06, 14.18. HRMS (ESI): calculated for $C_{13}H_{10}Cl_2FN_3O_4$ $(M+H)^+$ 362.0105, found 362.0107. Crystal data: Formula: C13 H10 Cl2 F N3 O4; M: 362.14; Tetragonal; P-4 21/c; a =19.9722 (6) Å; b = 19.9722 (6) Å; c= 7.8582 (6) Å; α = 90°; β = 90°; γ = 90°; V = 3134.6 (3): Z = 8; R1 = 0.0480: wR2 = 0.1318; S = 0.914

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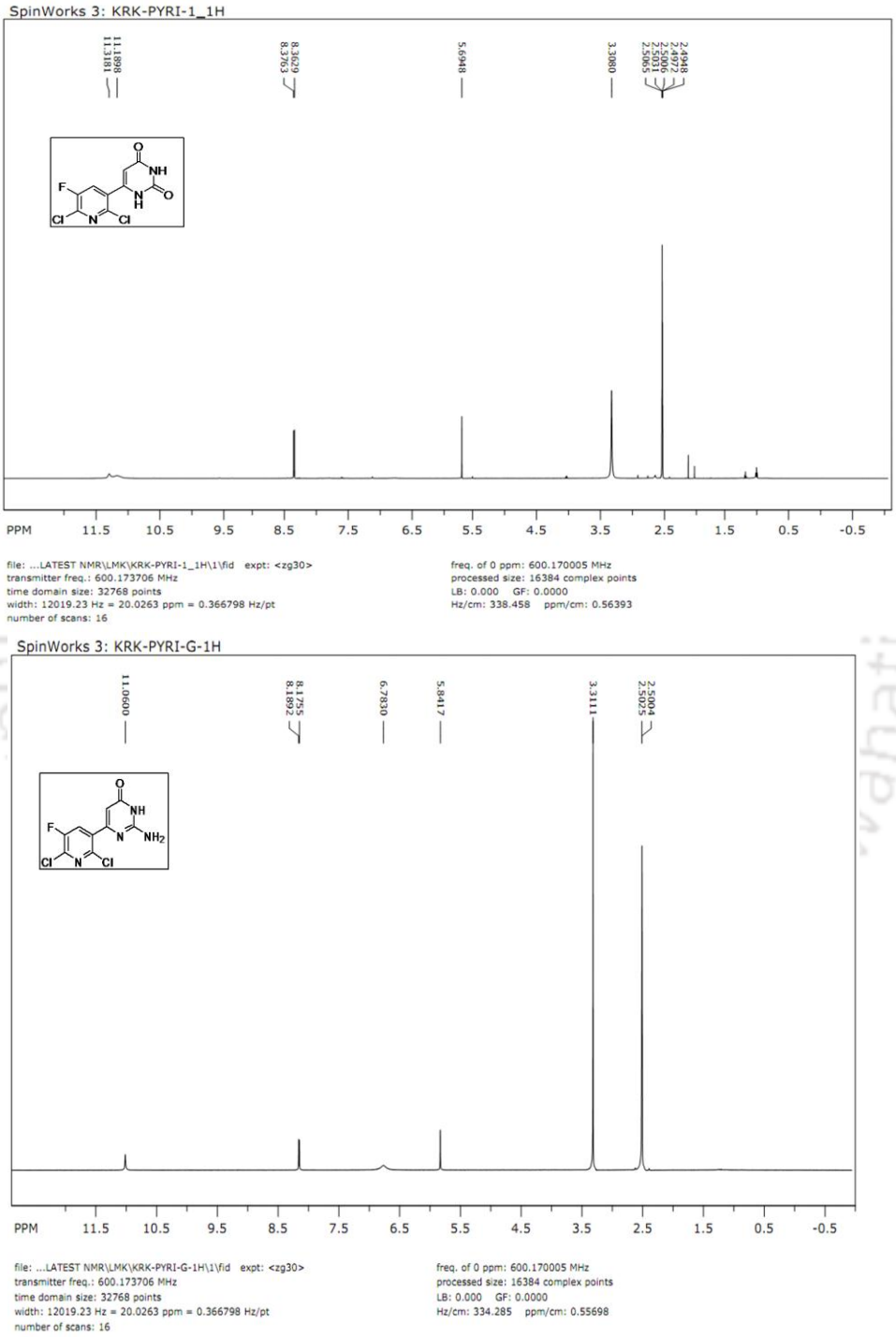
Appendix

^{1}H-NMR spectra of 6-PU, 6-PiC



IV.2 Gel-forming Nucleobases as Selective Probe

IV.2.1 Introduction

Gelation is a unique property of a organic or inorganic molecule which can be defined as the self-assembly of a small gelator molecule in an appropriate solvent *via* physical or chemical interactions, such as, hydrogen bonds, π-stacking, *van der* Waals interaction and covalent interactions.[1] In other words, gel is defined as organization or association of monomeric molecule into complex higher order structures. These structures may be fibrous, tubular or helical.[2-4] Solvent is an essential factor that can drive the gelation process of a molecule. There are two types of gels i) hydrogel: contains high amount of water ii) organogel: gel formation in organic solvents.

Low molecular weight gelators have numerous applications[5-8] including sensors[9,10] and biomaterials.[11,12] Molecular recognition have also been achieved by gelators which contains biomolecules.[13] Nucleobases, peptides are the fundamental building blocks of the biomacromolecules which evolve in life. The nucleobase derived gelators have been reported for significant biological applications such as base pairing and metal recognition.[14-16] A number of nucleobase derived gelators have been established in organic and aqueous phase[17-19] and their molecular assembly, recognition or base-pairing were studied through hydrogen bonding interactions which is the unique as well as essential feature of nucleobases.[20]

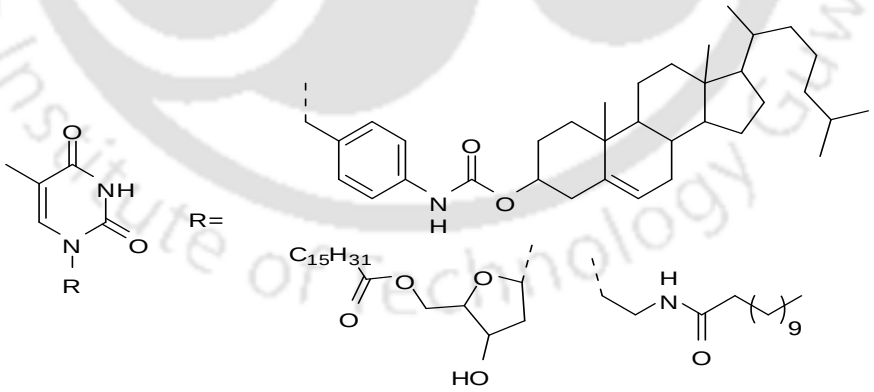


Figure IV.2.1a *Examples of organogelators containing nucleobases*

Usually, nucleobases are not gelator molecules and the introduction of known gelator or polymeric molecules including cholesteral, aminoacids and long alkyl chains would bring gelation property into the nucleobases.[21-24]

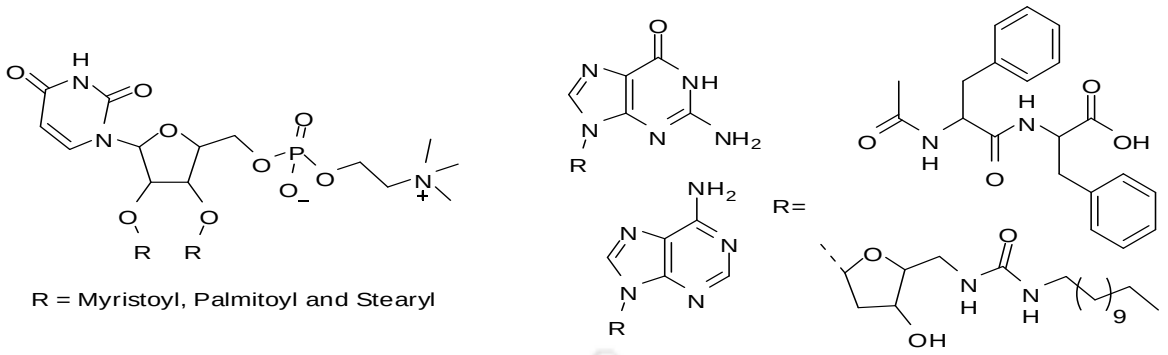


Figure IV.2.1b *Examples of hydrogels*

Molecular Recognition of Nucleobases

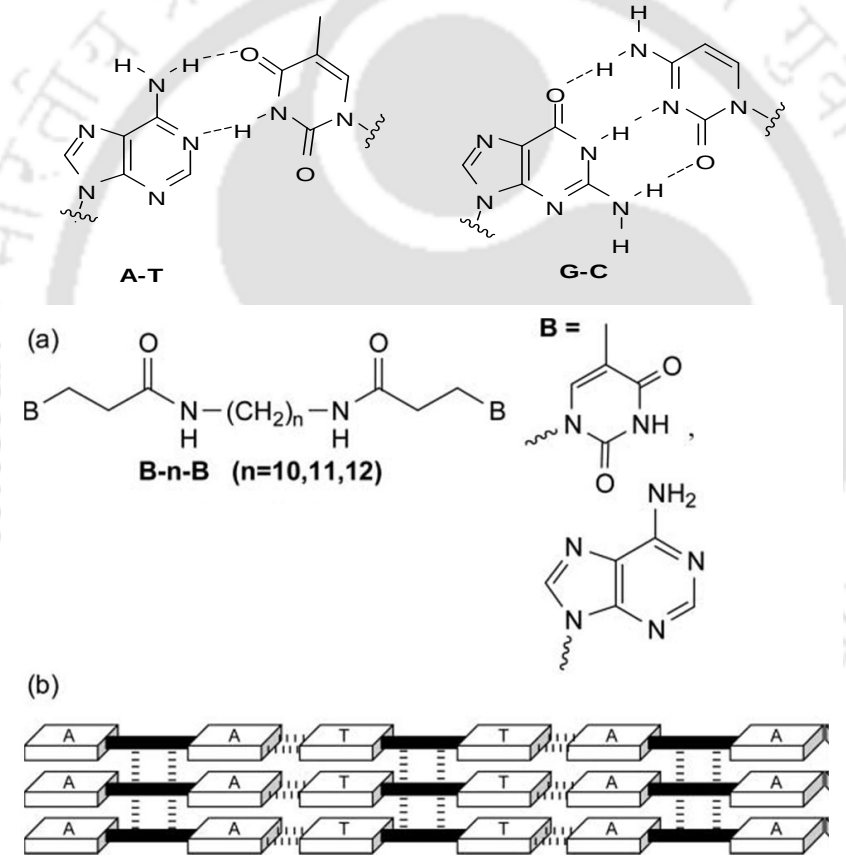
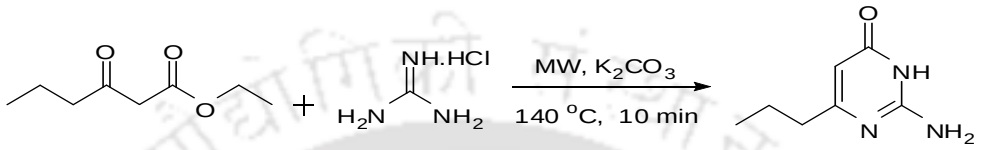


Figure IV.2.1c *Hydrogen bond mediated supramoleculer assembly (molecular recognition)*

IV.2.2 Present work

Here, we are reporting the smallest nucleobase analogue which has special ability to form gel in organic solvents without having any functional gelator molecules, as mentioned above. The 2-aminopyrimidine (6-propylisocytosine) analogue is synthesized by microwave assisted method using ethyl butyryl acetate (beta-ketoester) under solvent free basic condition,[25] described in **chapter II**.



Scheme IV.2.2.1

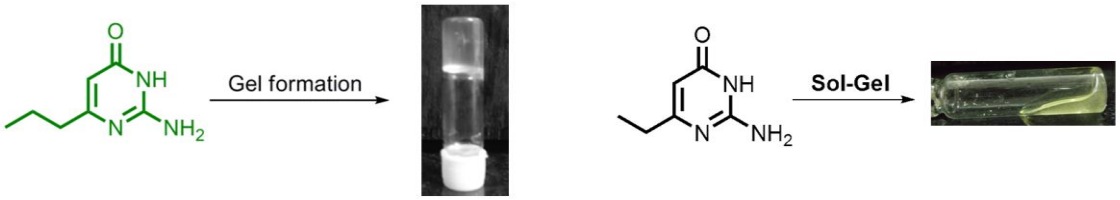


Figure IV.2.2.1a *Gel representation of 6-propyl and 6-ethylisocytosine*

Table IV.2.1

Solvents	Critical gelation concentration of 6-propylisocytosine in wt %
Dimethyl sulphoxide	2.5 wt%
Dimethyl formamide	2.5 wt%
Ethanol	2.0 wt%
Methanol	no gelation
Chloroform	insoluble
Tetrahydrofuran	insoluble
1, 4-dioxane	insoluble
Water	insoluble
Dimethyl sulphoxide-Water (9:1)	2.5 wt%
Ethanol-Water (7:3)	2.0 wt%

Gelation property of 6-propylisocytosine was studied in different organic solvents and water. The molecule was found to have propensity to form gels in organic solvents

(organogel) such as DMSO, ethanol, and DMF, but gel formation did not occur in water due to poor solubility. The gelation concentration was also examined in various solvents and mixture of solvents.

Organogels have diverse advantages such as moisture insensitive, resistant to microbial contamination, and improve skin penetration.[26] Organogel also acts as bioactive agents that improve the drug delivery process.[27]

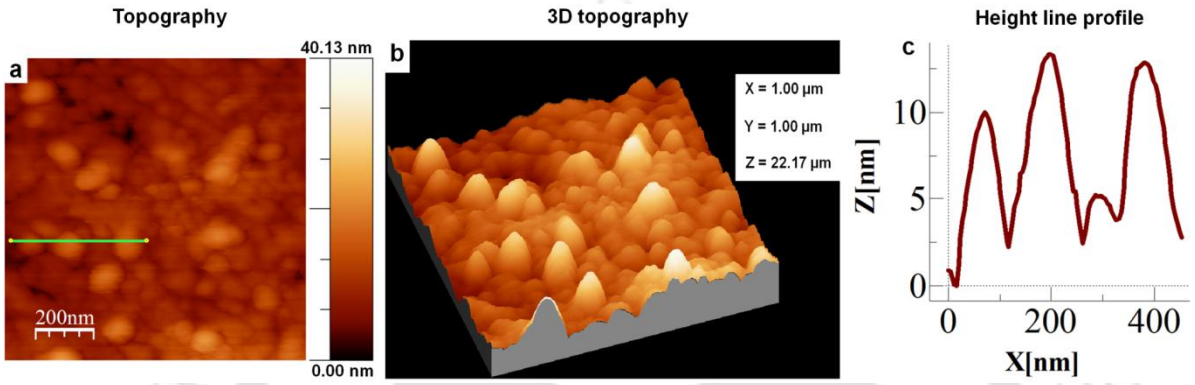


Figure IV.2.2.1b *AFM image of 6-propylisocytosine (20µM in EtOH) X: Diameter of a particle, Z: Height of a particle*

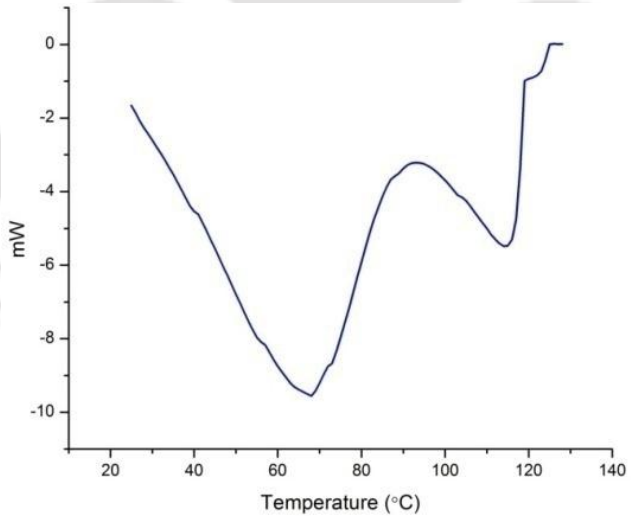


Figure IV.2.2.1c *DSC experiment of 6-propylisocytosine (2 wt% in EtOH). Melting point: 67 °C (DSC) and 64 °C (dropping ball method)*

IV.2.3 Influence of structural factors on gelation

Organogel, the focus of this chapter, which is formed by a molecule in predominantly organic phase, can be categorized in two, based on their hydrogen bonding capability. a) hydrogen bond forming organogelators that consist of amino acids, amides and urea moieties b) non-hydrogen bonded organogelators. 6-propylisocytosine consists of cyclic amide that may induce the gel-formation through hydrogen bonding. In order to understand the structural requirements (position of substitution as well as chain length) for such gel-formation, varoius C-5, C-6 substituted (alkyl, aryl) isocytosines were synthesized and their crystal structures were explored (**Figure IV.2.3a**).

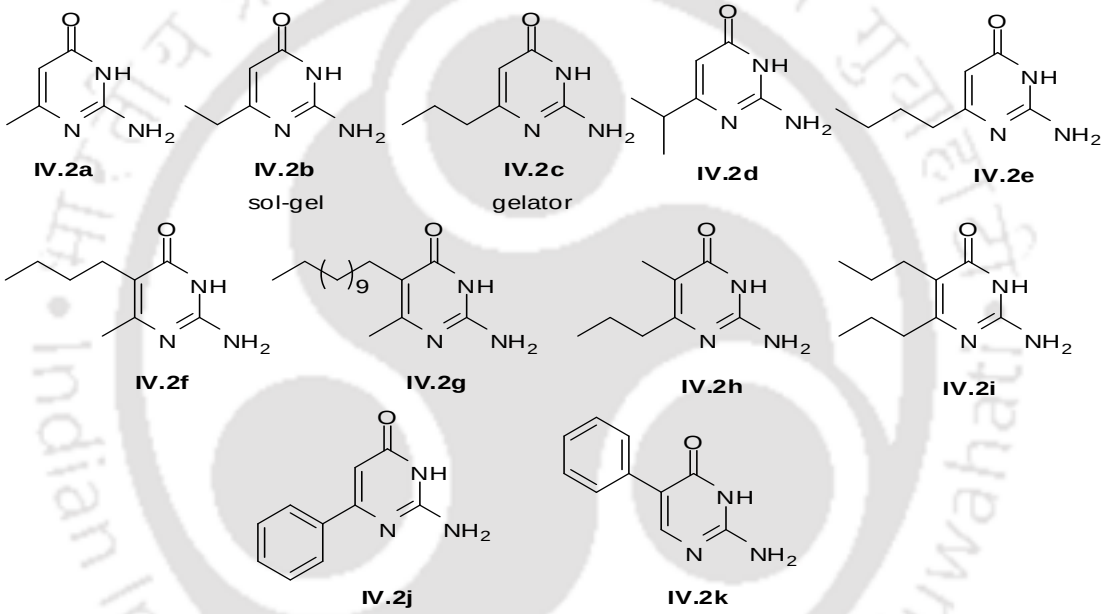


Figure IV.2.3a *Substituted isocytosines*

All the compounds were synthesized according to **Scheme IV.2.2.1**. All of the compounds were extensively explored to test their gel-forming abilities. Compound **IV.2a** did not show any gel characteristics. Compound **IV.2b,** which contains an ethyl substitution at C-6 position, was found to form a sol-gel in DMSO (2.5 wt %). Only 6-propylisocytosine **(IV.2c),** as demonstrated in **Table IV.2.1**, was showing gel-formation in several organic solvents. Even after long trial, we were not able to see any gelation from any of the other compounds. This may be due to the fact that only 6-propylisocytosine has the critical hydrophobicity as well as pi-stacking, to form the organogel (further established by crystal data analysis).

In order to establish why only two compounds (**IV.2b** and **IV.2c**) have gelation abilities and not the others, despite having similar alkyl chains, the crystal structures of the compounds were analyzed. This might reveal the supramolecular self-assembly pattern, although in solid phase, which is an essential criteria for gelation. The crystal structures of compounds **IV.2b, IV.2c** obtained from DMSO, show well defined hydrogen bonding pattern to form polymeric structures. But when moving from methyl to propyl substituted isocytosines, the π-stacking forces as well as hydrophobic nature were found to increase gradually. The molecules also undergo self-pairing with one another in a precise manner. For the gelator **IV.2c,** crystal structure clearly showed a well-organized self-assembled (**Figure IV.2.3b and Figure IV.2.3c**) molecular architecture, with the propyl groups on the same plane (suggesting adequate hydrophobic interaction) and a strong π-stacking between the heterocyclic rings.

Table IV.2.2

Molecule	π-Stacking distances (Å)
6-Methylisocytosine (IV.2a)	3.950
6-Ethylisocytosine (IV.2b)	3.510
6-Propylisocytosine (IV.2c)	3.441
5-butyl-6-methylisocytosine (IV.2f)	3.606

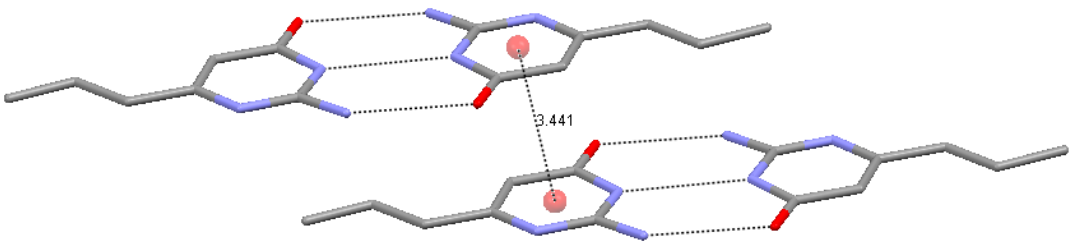


Figure IV.2.3b *π-stacking representation of 6-propylisocytosine (**IV.2c**)*

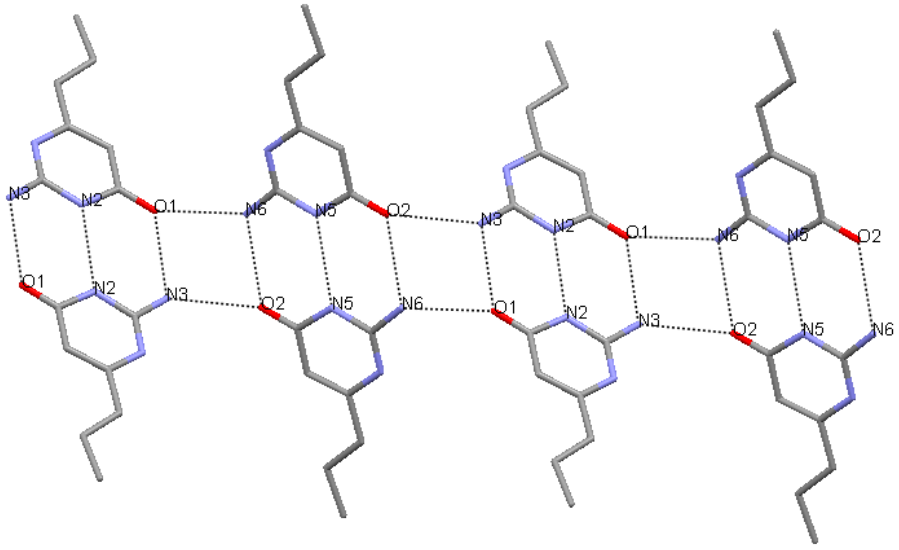


Figure IV.2.3c *Supramolecular assembly of 6-propylisocytosine (hydrogens were omitted for clarity)*

But, in the case of 6-ethylisocytosine (**IV.2b)** the two ethyl groups of a self-pair are leaning in different planes with well ordered supramolecular structures via hydrogen bonding (**Figure IV.2.3d**). The orientation of alkyl moieties did not affect the π-stacking much and the molecule has ability to form slight sol-gel in dimethyl sulphoxide. Compound **IV.2a** containing a C-6 methyl group, was also showing a nicely stacked, layered molecular architecture. The lack of gel formation might be, therefore, due to its lack of hydrophobic nature (**Figure IV.2.3e**).

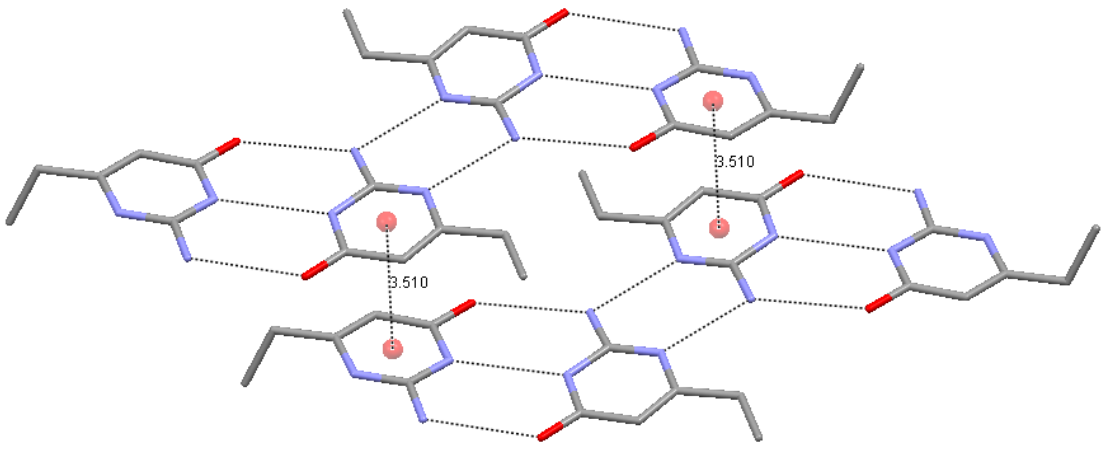


Figure IV.2.3d *Representation of π-stacking and polymeric assembly of 6-ethylisocytosine* ***(IV.2b)***

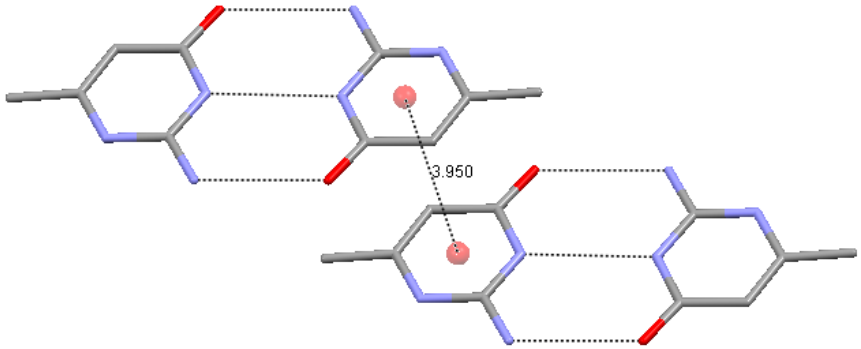


Figure IV.2.3e *Representation of π-stacking of 6-methylisocytosine* ***(IV.2a)***

We have anticipated that 6-butylisocytosine (**IV.2e**) should have the ability to yield gel because of its higher hydrophobicity than corresponding 6-propyl analogue. But the gelation did not take place in any solvents. Interestingly, the crystal structure also revealed that even though it is forming a polymeric network, no π-stacking was found (**Figure IV.2.3f**). Moreover, it clearly showed that the butyl groups are oriented in different directions that strongly disrupt hydrophobic interactions, thereby preventing gelation.

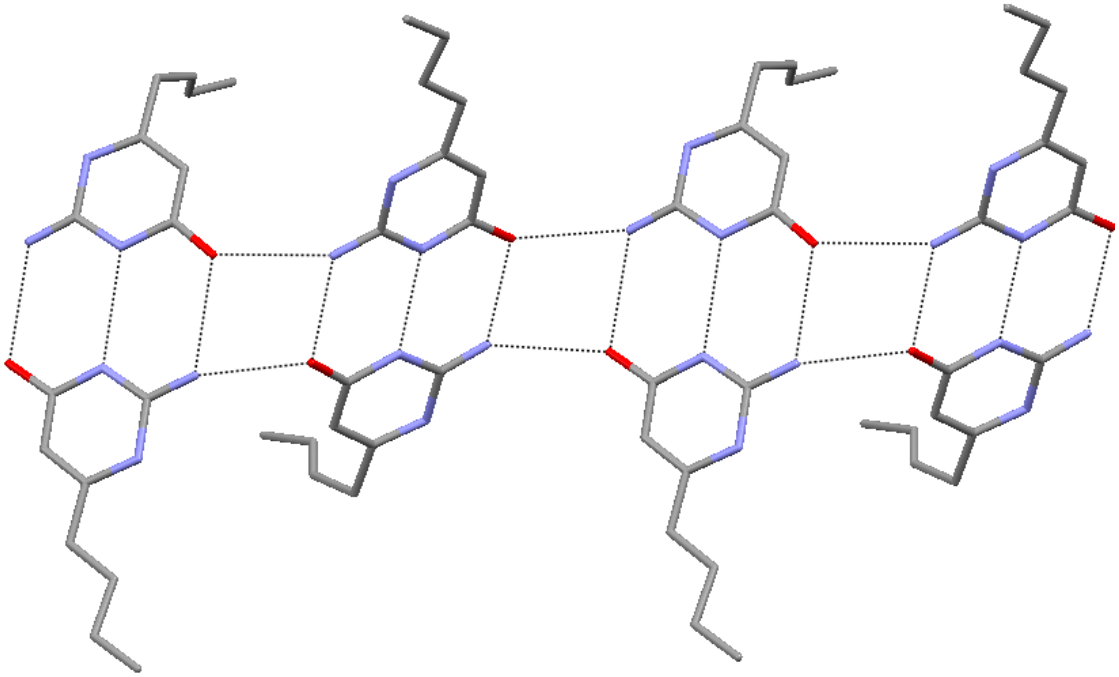


Figure IV.2.3f *Self-paired orientation of 6-butylisocytosine* ***(IV.2e)***

The gelation of 6-propylisocytosine was affected when a methyl group was inserted at C-5 position (**IV.2h**). The same was found for **IV.2i**, when a propyl group was introduced at C-5 position. From the results it could be postulated that presence of a C-5 substituent perturbs

self-assembly, thereby gel-formation. This was further supported by 5-butyl (**Figure IV.2.3g)**, 5-dodecyl substituted isocytosines (**IV.2f, IV.2g**) mentioned in **Figure IV.2.3a**.

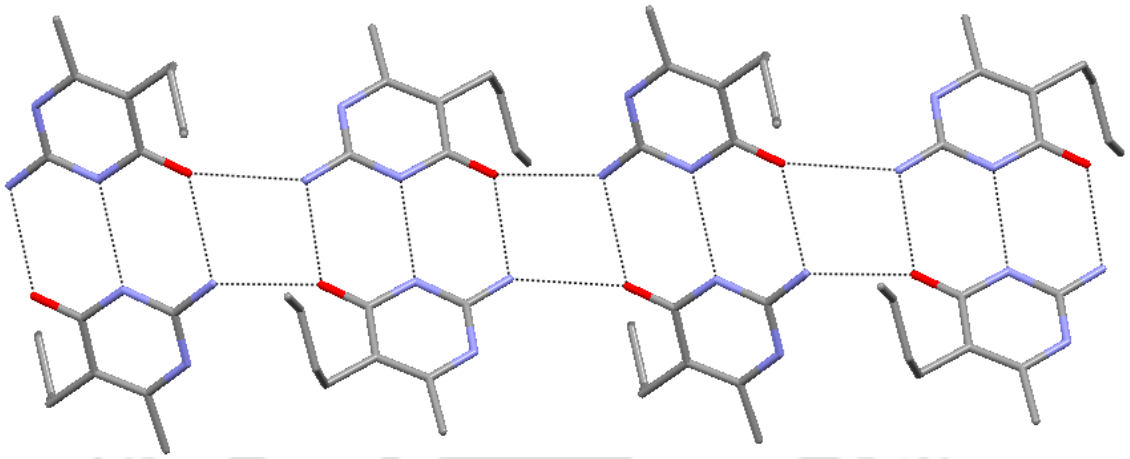


Figure IV.2.3g *Crystal structure orientation of* ***IV.2f***

IV.2.4 Cytosine detection using gelation process

6-propylisocytosine is a good nucleobase analogue that belongs to 2-aminopyrimidinone category and acts as counterpart for recognition of guanine or isoguanine strands in nucleic acids.[28,29] Recently our group showed that isocytosine can be a base pairing partner for cytosine in their free form through crystal structures and proton NMR studies.[26]

Here, we have explored another important property of 6-propylisocytosine *via* gelation technique. In this report, we demonstrate that this unique molecule could be utilized as a probe for selective recognition and detection of cytosine through gelation and de-gelation. The experiment, as depicted in **Figure IV.2.4a**, demonstrates that the gel-form of 6-propylisocytosine undergoes selective de-gelation upon addition of cytosine nucleobase.

6-propylisocytosine (**IV.2c**), dissolved in Dimethyl sulphoxide, was taken in two separate glass vials. Solution of cytosine was added to one of the vials in a 1:1 ratio. The two vials were kept side by side for gelation, under identical condition at room temperature. The vial containing only compound **IV.2c** rapidly forms strong gel, while the vial containing 1:1 equimolar mixture of compound **IV.2c** and cytosine (base-pairing partner) failed to produce any gel, even after days. In another experiment, the vial containing gel of **IV.2c** was treated with equivalent amount of cytosine. The gel started melting over time and finally became liquid.

The gelation network of gelator **IV.2c** was affected by strong base-pairing with cytosine through hydrogen bonding interactions. This was further confirmed by ^{1}H-NMR titration. Addition of other natural nucleobases, such as, thymine and adenine were found to have no effect on the gel.

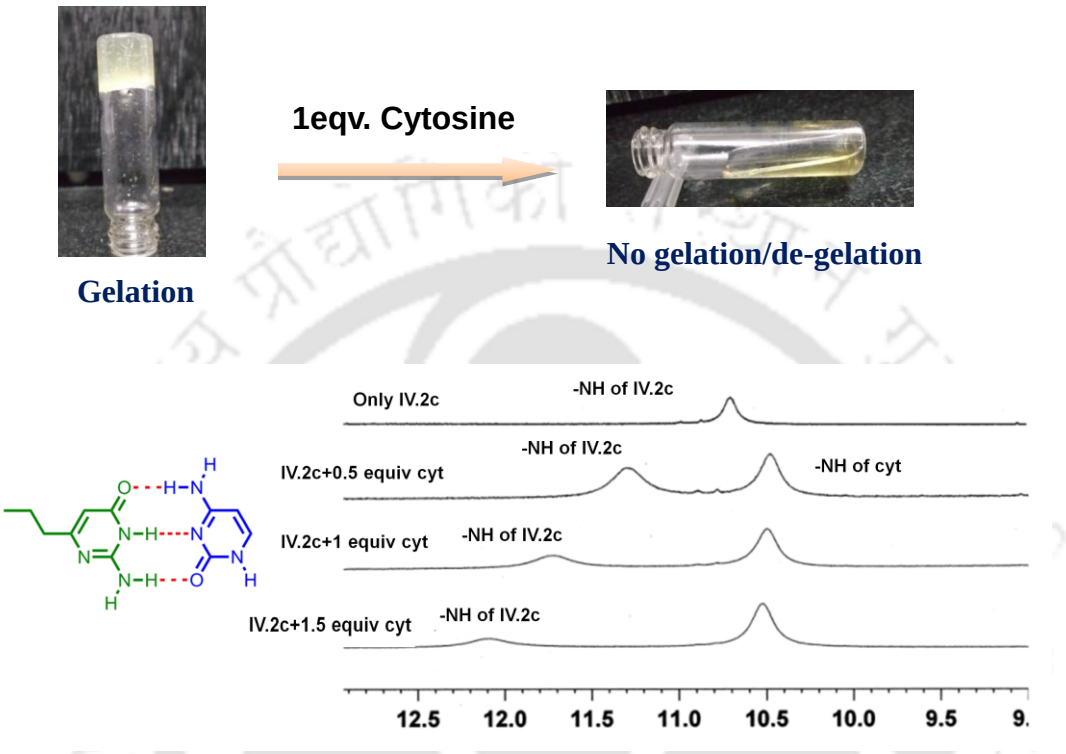


Figure IV.2.4a *Base-pairing of 6-propylisocytosine using gelation and ^{1}H-NMR titration with cytosine*

In conclusion, we have synthesized, characterized isocytosine derivatives and explore the gelation property of 6-propylisocytosine. The unique structural factors of various substituted isocytosines were explained by crystal structures and clearly show the effect of substituent on gelation process. The base-pairing property was also studied via ^{1}H-NMR titration and gelation process.

IV.2.5 Experimental Section

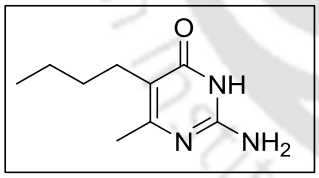
IV.2.5.1 General Information: All the chemicals were purchased from Sigma Aldrich, Alfa Aeser, Spectrochem and were used directly without any further purification. *CEM Discover Labmate* closed vessel microwave reactor was used for all the reactions. All the NMR spectra were recorded using *Bruker-600MHz* spectrometer using DMSO-d_6 as reference

solvent. Atomic force microscopy (AFM) was recorded on an *Agilent instrument, model 5500 series* with noncontact mode. DSC experiment was recorded using METTLER TOLEDO (Model No: DSC 821) instrument. All crystal data were obtained from *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structure was solved by direct method using *SHELX-97*[30,31] (University of Gottingen, Germany). HRMS analyses were carried out by *Agilent Q-TOF 6500 LC/MS* instrument.

IV.2.5.2 General Procedure: Beta-ketoester (1 mmol), guanidine hydrochloride (2 mmol) and potassium carbonate (1 mmol) were taken in a microwave reactor vessel and was closed immediately. The vessel was subjected to microwave irradiation for 10 minutes at 140 °C. The reaction vessel was allowed to cool, and the products were isolated. The desired compounds were further purified by column chromatography using methanol/chloroform.

IV.2.5.3 Sample Preparation: for AFM, 20 µM solution of 6-propylisocytosine was prepared in ethanol. Then, 20 µL of this solution was placed on clean glass plate and dried for AFM analysis. DSC experiment was carried out using 2 wt% of 6-propylisocytosine in ethanol (gel concentration). 1 mg of the solution was placed in heating furnace and heated upto 140°C. For dropping ball method, a small iron ball was placed on the gel and the gel was heated on a water bath.

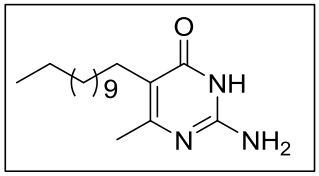
IV.2.6 Characterization section:



2-amino-5-butyl-6-methylpyrimidin-4(3*H*)-one (IV.2f)

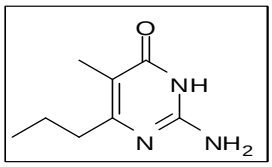
White solid, Yield: 70%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.17 (br, 1H), 6.58 (s, 2H), 2.23 (t, 2H, J = 7.2 Hz), 2.02 (s, 3H), 1.28 (m, 4H), 0.86 (t, 3H, J = 7.2Hz).^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 163.95, 153.20, 111.50, 30.92, 24.31, 22.15, 20.30, 13.90. HRMS (ESI): calculated for $C_9H_{15}N_3O$ $(M+H)^+$ 182.1288, found 182.1280.

2-amino-5-dodecyl-6-methylpyrimidin-4(3*H*)-one (IV.2g)



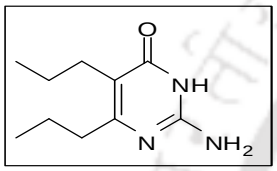
White solid, Yield: 65%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.72 (br, 1H), 6.25 (s, 2H), 2.22 (t, 2H, J = 7.2 Hz), 2.01(s, 3H), 1.29 (br, 20H), 0.84 (t, 3H, J = 7.2 Hz). HRMS ESI): calculated for $C_{17}H_{31}N_3O$ $(M+H)^+$ 294.254, found 294.2565.

2-amino-5-methyl-6-propylpyrimidin-4(3*H*)-one (IV.2h)



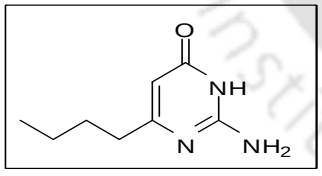
White solid, Yield: 76%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.69 (br, 1H), 6.19 (br, 2H), 2.30 (t, 2H, *J* = 7.2 Hz), 1.77 (s, 3H), 1.54 (m, 2H), 0.88 (t, 3H, *J* = 7.2 Hz).^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 153.06, 106.28, 79.15, 36.01, 20.69, 13.55, 9.89. HRMS (ESI): calculated for $C_8H_{13}N_3O$ $(M+H)^+$ 168.1131, found 168.1125.

2-amino-5, 6-dipropylpyrimidin-4(3*H*)-one (IV.2i)



White solid, Yield: 74%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.81 (br, 1H), 6.30 (br, 2H), 2.29 (t, 2H, *J* = 7.2 Hz), 2.23 (m, 2H), 1.55 (m, 2H), 1.35 (m, 2H), 0.89 (t, 3H, *J* = 7.2 Hz), 0.86 (t, 3H, *J* = 7.2 Hz).^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 153.17, 111.31, 26.48, 22.39, 21.32, 13.95, 13.86. HRMS (ESI): calculated for $C_{10}H_{17}N_3O$ $(M+H)^+$ 196.1444, found 196.1437.

2-amino-6-butylpyrimidin-4(3*H*)-one (IV.2e)



White solid, Yield: 81%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.86 (br, 1H), 6.54 (br, 2H), 5.35 (s, 1H), 2.22 (t, 2H, *J* = 7.2 Hz), 1.50 (quint, 2H, *J* = 7.2 Hz), 1.28 (sext, 2H, *J* = 7.2 Hz), 0.86 (t, 3H, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 155.59, 138.51, 99.56, 29.64, 21.73, 13.71. HRMS (ESI): calculated for $C_8H_{13}N_3O$ $(M+H)^+$ 168.1131, found 168.1138. Crystal data: Formula: C16 H26 N6 O2; M: 334.43; Monoclinic; P2/n; a =14.0065 (14) Å; b = 9.1330 (14) Å; c= 14.4248 (19) Å; α = 90°; β = 96.655(11)°; γ = 90°; V = 1832.8 (4): Z = 4; R1 = 0.1504: wR2 = 0.4078; S = 1.477.

Synthesis and characterization of other isocytosines were already mentioned in chapter 2.

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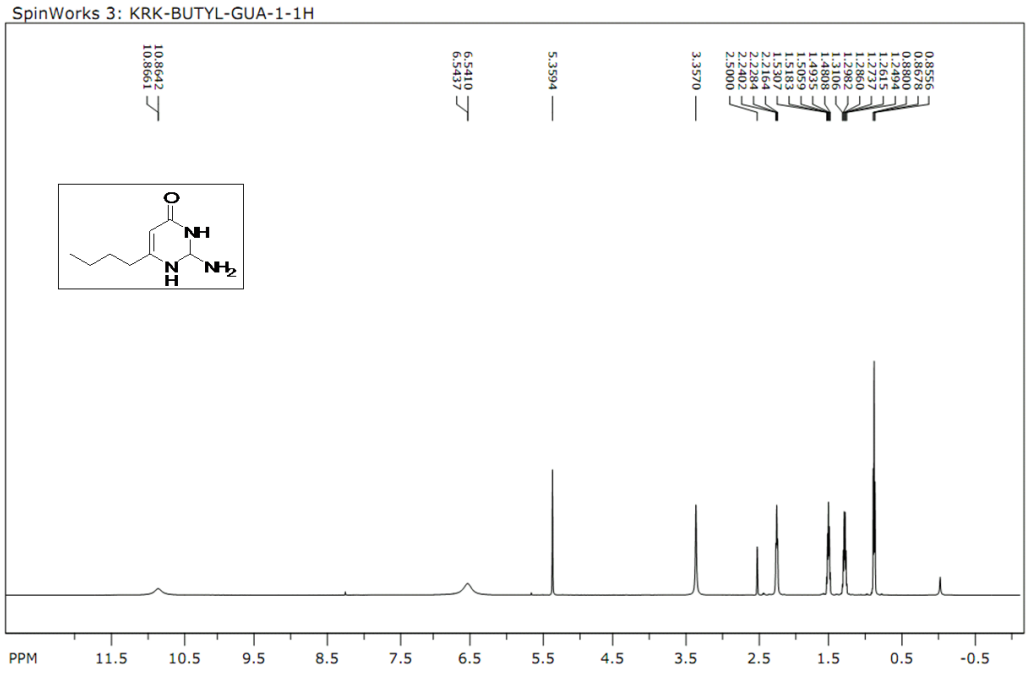
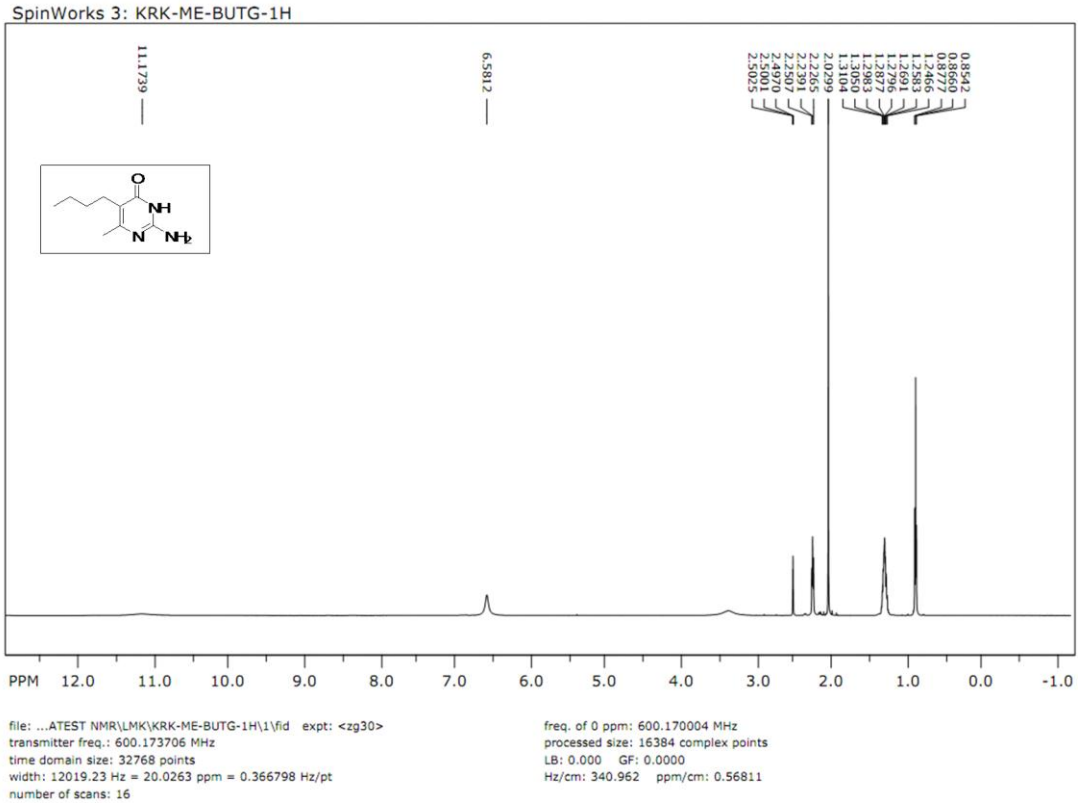
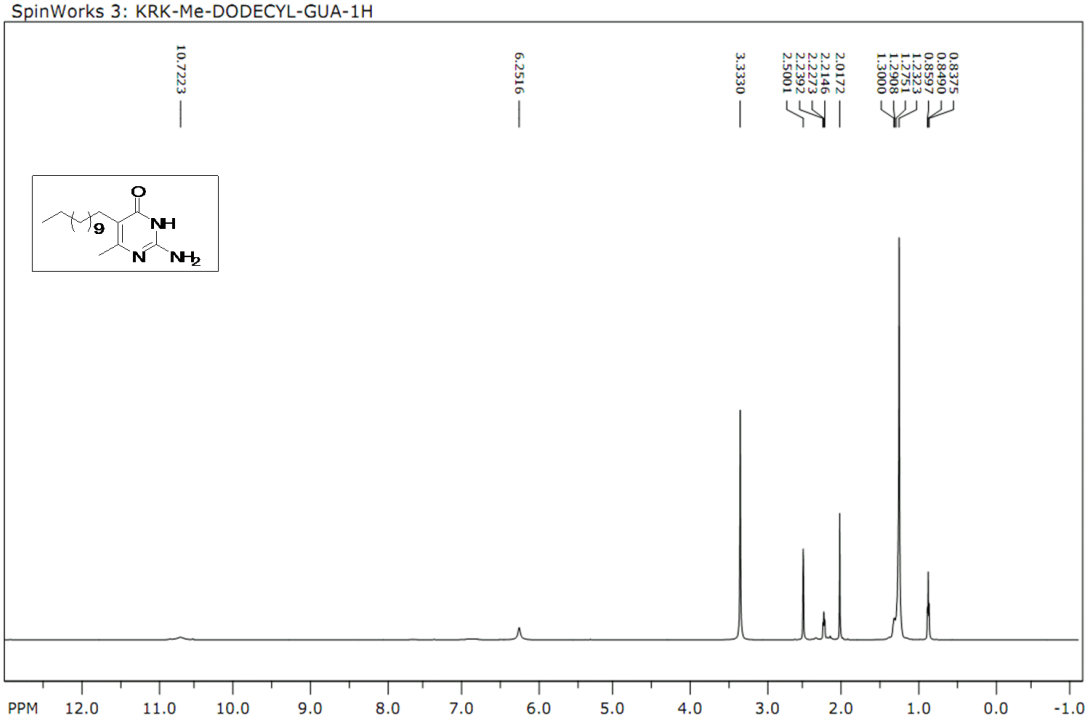
Appendix

^{1}H-NMR spectra of isocytosine derivatives





SpinWorks 3: KRK-Me-DODECYL-GUA-1H



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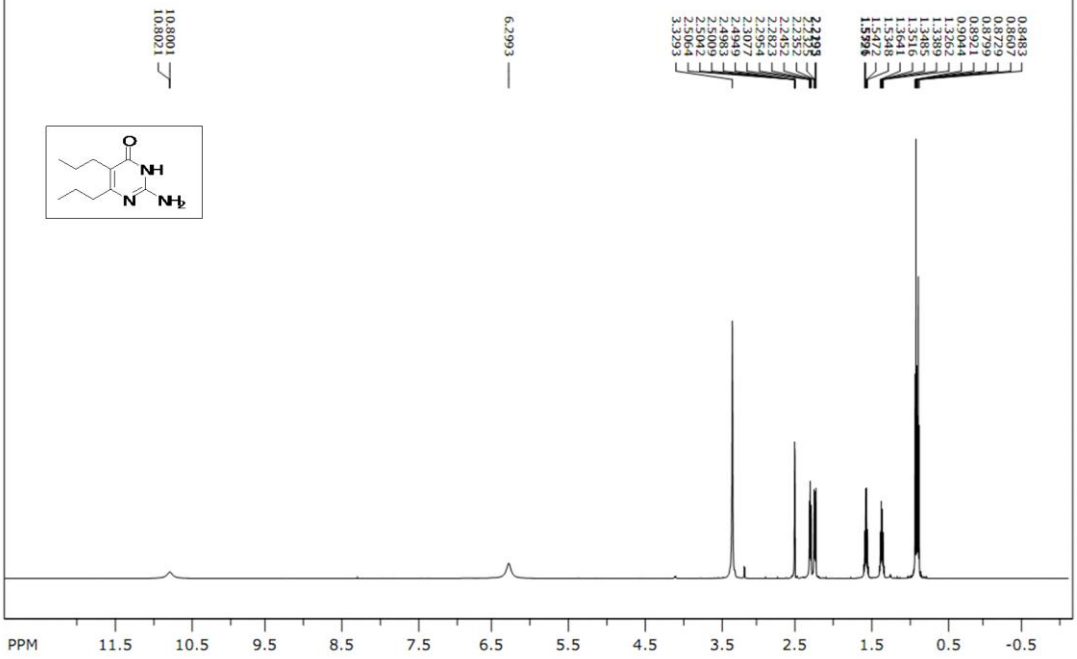
time domain size: 32768 points
width: 12019.23 Hz = 20.0263 ppm = 0.366798 Hz/pt
number of scans: 16

freq. of 0 ppm: 600.170005 MHz
processed size: 16384 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 340.545 ppm/cm: 0.56741

SpinWorks 3: KRK-PR-PR-G-1H



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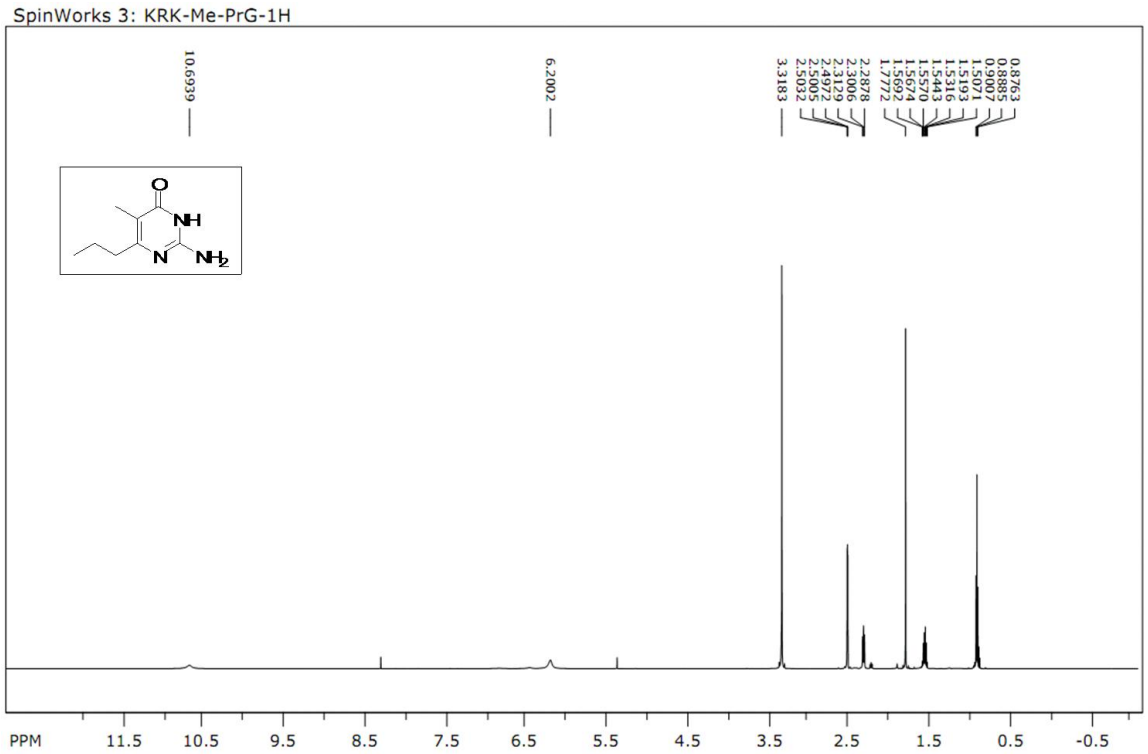
time domain size: 32768 points
width: 12019.23 Hz = 20.0263 ppm = 0.366798 Hz/pt
number of scans: 16

freq. of 0 ppm: 600.170005 MHz
processed size: 16384 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 339.293 ppm/cm: 0.56532



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width: 12019.23 Hz = 20.0263 ppm = 0.366798 Hz/pt
number of scans: 16

freq. of 0 ppm: 600.170005 MHz
processed size: 16384 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 338.458 ppm/cm: 0.56393

Crystal data of IV.2a

Chemical Formula	C5 H7 N3 O
Formula mass	125.14
Temperature /K	296 (2)
Crystal system	Triclinic
Space group	P-1
a /Å	6.9320(9)
b /Å	6.9409(8)
c /Å	14.1319(19)
α /˚	76.026(12)
β /˚	76.064(9)
γ /˚	66.078(8)
Unit cell Volume /Å	595.26(13)
Z	4
Final R1 value	0.0550
Final wR value	0.1795
Goodness of fit	1.071

Crystal data of IV.2b

Chemical Formula	2(C6 H9 N3 O) H2 O
Formula mass	296.34
Temperature /K	296 (2)
Crystal system	Monoclinic
Space group	P2 (1)/c
a /Å	6.856(18)
b /Å	17.55(5)
c /Å	13.28(4)
α /°	90
β /°	96.33(2)
γ /°	90
Unit cell Volume /Å	1588(7)
Z	4
Final R1 value	0.0470
Final wR value	0.1742
Goodness of fit	0.642

Crystal data of IV.2c

Chemical Formula	C14 H22 N6 O4
Formula mass	338.38
Temperature /K	296 (2)
Crystal system	Triclinic
Space group	P-1
a /Å	7.0581 (9)
b /Å	11.2236 (15)
c /Å	13.3296 (19)
α /°	105.302 (9)
β /°	104.687 (9)
γ /°	102.913 (8)

Unit cell Volume /Å	936.3(2)
Z	5
Final R1 value	0.1127
Final wR value	0.2346
Goodness of fit	1.228

Crystal data of IV.2f

Chemical Formula	C9 H15 N3 O
Formula mass	181.24
Temperature /K	293 (2)
Crystal system	Monoclinic
Space group	C2/c
a /Å	6.9873(3)
b /Å	20.9502(17)
c /Å	14.2153(7)
α /°	90
β /°	98.145(4)
γ /°	90
Unit cell Volume /Å	2059.9(2)
Z	8
Final R1 value	0.0579
Final wR value	0.1843
Goodness of fit	1.042

IV.3 Selective Metal ion (Pd^{2+}) Sensing using Modified Nucleobase Analogue

IV.3.1 Introduction

Using selective probes, metal ions can be detected qualitatively as well as quantitatively from environment.[1,2] Various chemosensors have been developed for metal ions based on their selectivity and sensitivity.[3,4] Palladium is an important metal among transition metals which has significant role in chemistry. It is widely used in electronic, automobile and jewellery industries as well as in medicinal fields.[5-7] It also acts as catalyst for various organic reactions such as Buchwald-Hartwig, Heck, Sonogashira, Negishi and Suzuki coupling reactions.[8-11] The release of palladium from above processes lead to various biological and environmental issues. Moreover, Pd (II) catalysts are heavily used in fine chemicals and pharmaceutical industries, and if not properly removed, could contaminate the end products and drugs. Palladium (II) chloride is more toxic among its various forms.[12] Due to the better binding ability of Pd^{2+} with amino acids containing thiol group and various biomolecules or biomacromolecules (DNA, Proteins), it may affect the cellular functions.[13] Threshold level of palladium in drugs is 5-10 ppm.[14] To avoid health and environment issues, it is necessary to detect palladium species using simple and reliable methods with high substrate selectivity.

Usually palladium detection was carried out by some conventional methods such as atomic absorption spectrophotometry, ion-coupled plasma emission mass spectrometry etc. The major drawbacks of these techniques are expensive, time consuming, pre-sample treatments and requirement of trained individuals.[15-17] On the other hand, optical methods, particularly fluorescence is the best way to detect various metal ions with very high selectivity and sensitivity using fluorescence sensors.

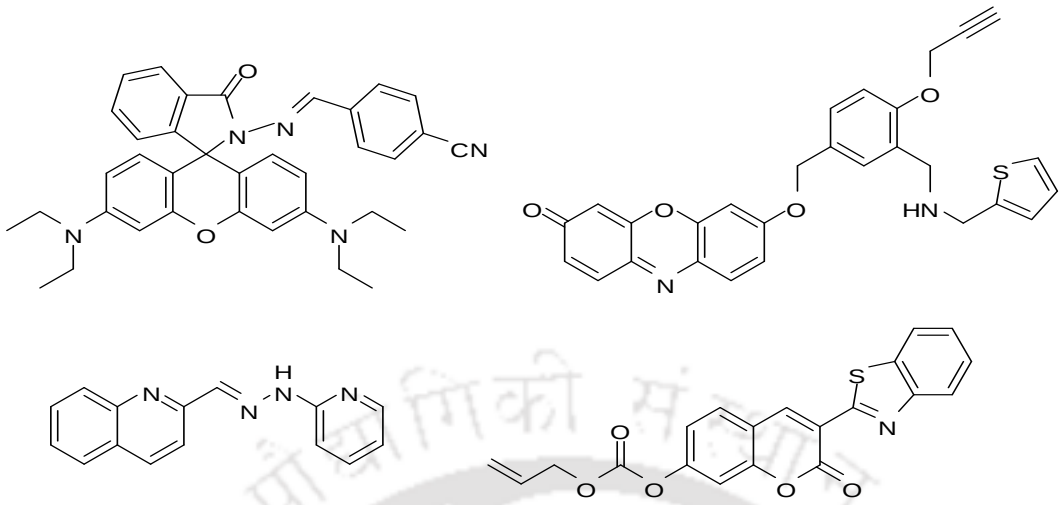


Figure IV.3.1a *Examples of* ***Pd***$^{2+}$ *"turn-on" sensors*

Usually, chemosensors have two types of detection process, namely, fluorescence enhancement (turn-on process), fluorescence quenching (turn-off process). A number of turn -on processes have been reported for Pd^{2+} detection with several chemosensors.[18-30] and it is widely used in bioimaging techniques.[31-33] A limited number of turn-off processes of Pd^{2+} were established (**Figure IV.3.1b**) using fluorescence sensors where the fluorescence was quenched during addition of palladium species.[34-36]

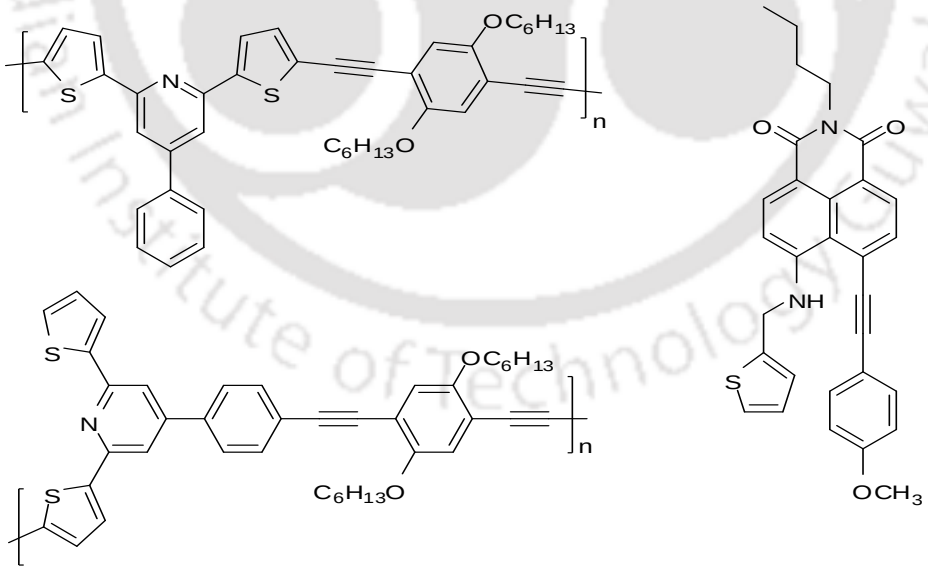
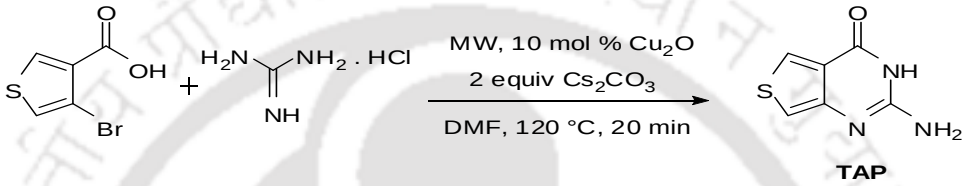


Figure IV.3.1b *Examples of* ***Pd***$^{2+}$ *"turn-off" sensors*

IV.3.2 Present Work

Here, we have developed a fused isocytosine heterocycle (**TAP, Scheme IV.3.2.1**) to act as fluorescence sensor for Pd^{2+}. The compound was synthesized by Cu (I) catalyzed coupling reaction mentioned in chapter III. 4-bromothiophene-3-carboxylic acid is treated with guanidine hydrochloride in presence of Copper (I) oxide under microwave irradiation, yielded 2-aminopyrimidine (TAP) containing the thiophene moiety. The final compound is highly fluorescent which was already reported by Tor *et al.* and acts as a nucleobase analogue.



Scheme IV.3.2.1

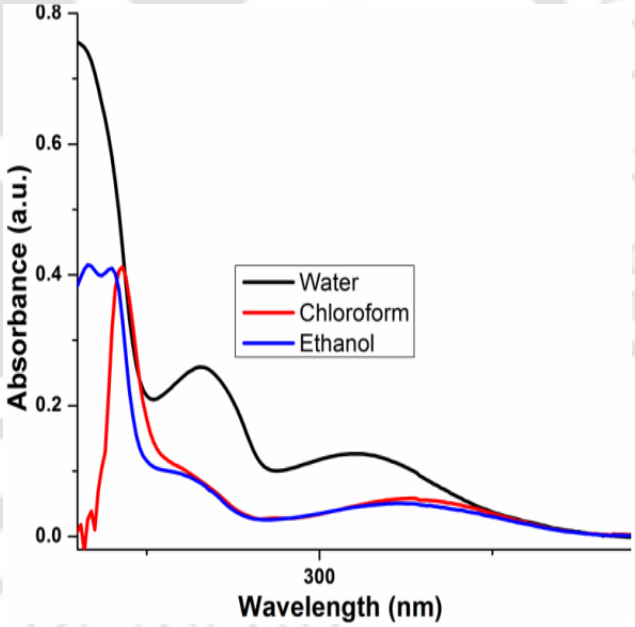


Figure IV.3.2a *UV-vis spectra of TAP in different solvents*

The photophysical studies of TAP were carried out using UV-visible and fluorescence techniques. Two major bands were observed in UV-visible spectroscopy around 260 nm and around 320 nm. The absorption may vary in few nanometers depending upon the nature of solvents (polar or non-polar). The fluorescence emission of TAP was measured in various solvents with the excitation at 330 nm, as presented in **Figure IV.3.2b**.

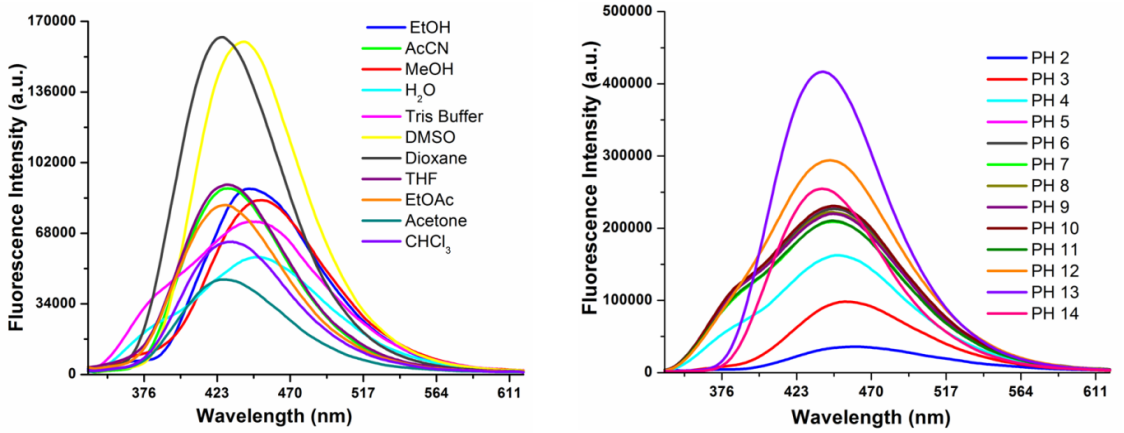


Figure IV.3.2b *Fluorescence spectra of 2-aminopyrimidine (TAP) Left: in different solvents (10 µM) (λ_{ex}: 330 nm), Right: in different pH range (20 µM) (λ_{ex}: 330 nm)*

The fluorescence emission of TAP in polar solvents such as water, DMSO, methanol, and tris-buffer was shifted to longer wavelength (red shift). But in non-polar solvents the emission was shifted towards blue region (shorter wavelength). Different pH-dependent experiments were also conducted and resulted that the fluorescence was quenched at lower pH (acidic), owing to the restriction of electrons mobility (**Figure IV.3.2b**). At higher pH, the fluorescence was enhanced since the anionic nature of the molecule increases.

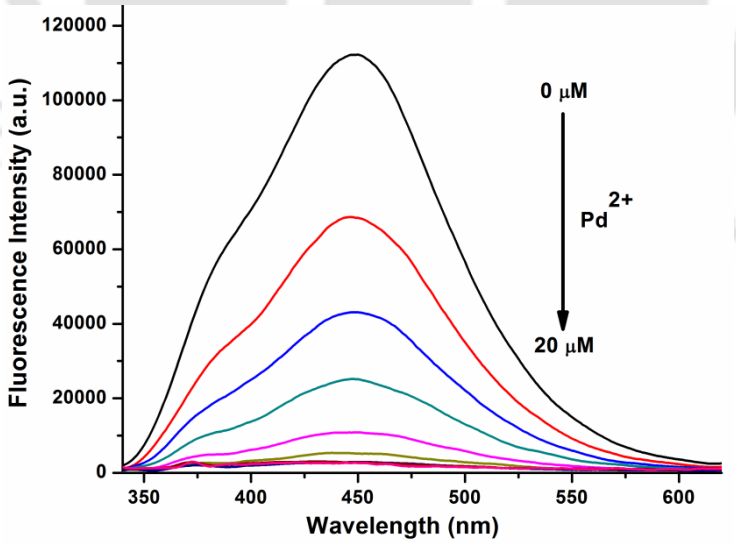


Figure IV.3.2c *Fluorescence titration of 2-aminopyrimidine (TAP) (10 µM) with varying concentration of Pd^{2+} ion (0-20 µM) in aqueous medium (water) at pH 6.8 (λ_{ex}: 330 nm)*

We have also studied the metal ion sensing property of TAP with various metal salts in aqueous solution (**Figure IV.3.2c** and **Figure IV.3.2e**). We observed that TAP selectively binds with Pd^{2+} among various metal ions (Hg^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Cr^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, and Cd^{2+}). During addition of aqueous solution of Pd^{2+} ion with TAP, the fluorescence was quenched gradually, owing to the strong complex formation of Pd^{2+} ion with TAP.

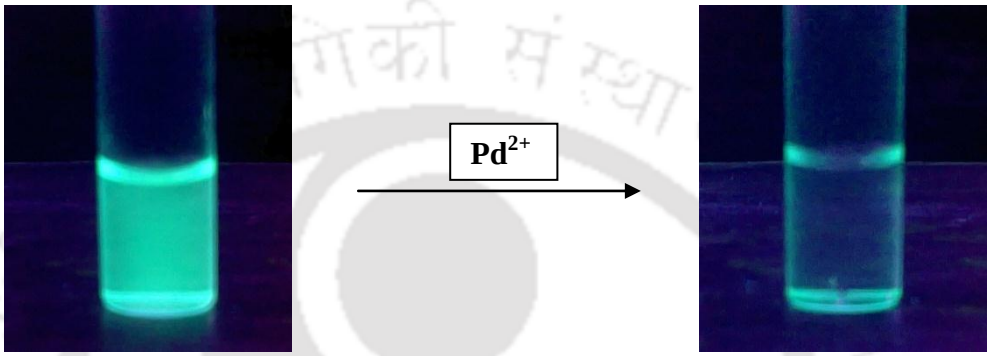


Figure IV.*3.2d* *Pictorial representation of* ***Pd^{2+}*** *detection:* ***a)*** *before* ***b)*** *after the addition of metal ion*

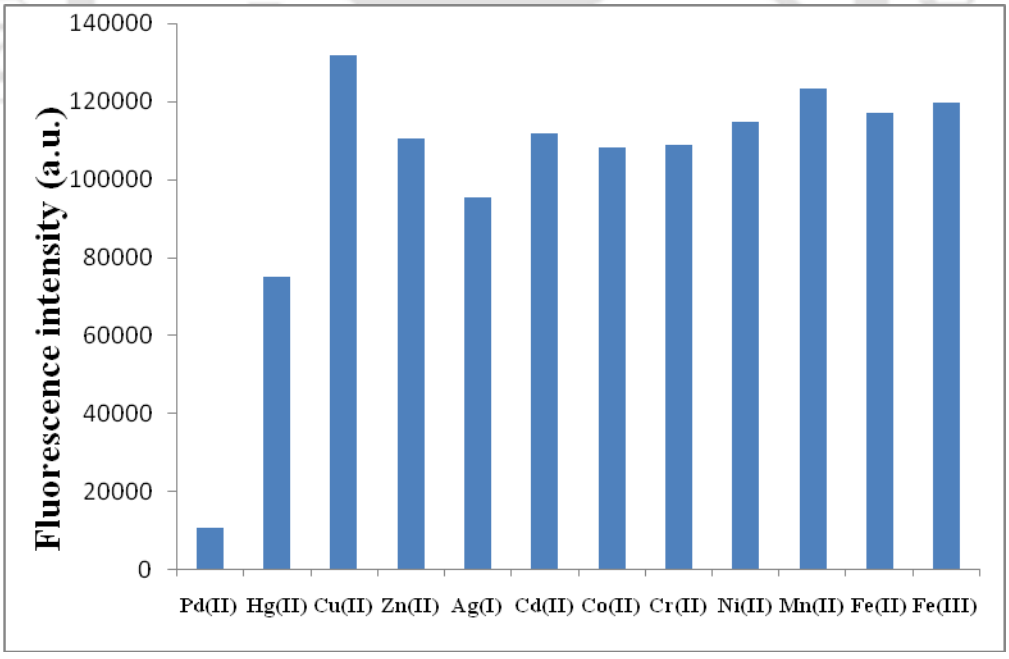


Figure IV.3.2e A *comparison diagram of fluorescence sensing property of TAP with various metal ions*

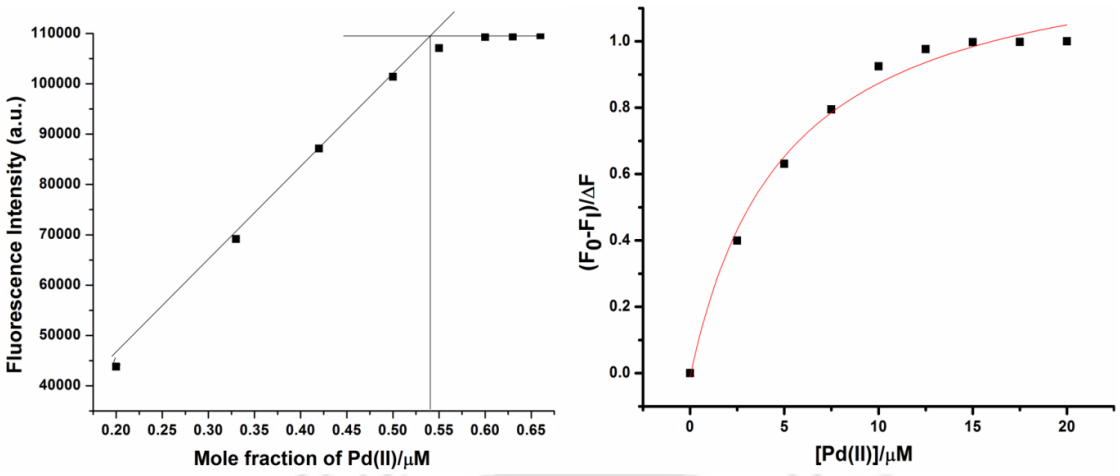


Figure IV.3.2f *Plots of binding ratio (Job's plot) and binding constant*

Pd^{2+} Sensing study of TAP in different solvents (DMSO, THF, Dioxane) and mixture of solvents were also performed. These studies did not show any significant interaction of Pd^{2+} with TAP. The binding of Pd^{2+} was clearly visible in aqueous solution, especially, water at pH 6.8. All metal ions were titrated in aqueous medium at pH 6.8. Use of buffer solutions such as tris-buffer and HEPES buffer did not reveal any binding consequence of metal ions.

Since attempt to obtain crystal of the complex failed, the mole fraction of metal ion was plotted against the fluorescence intensity (Job's plot), to figure out stoichiometry and the binding parameters of the complex. The plot suggested that the binding ratio of metal ion: TAP was 1:1. *i.e.* one metal ion (Pd^{2+}) is binding with one molecule of TAP. The binding constant of metal- TAP complex was calculated to be 4.9751. Both the binding ratio and binding constant were calculated using following set of equations:

Binding ratio measurement

F_0-F

F_0 = Fluorescence intensity of TAP before addition of metal ion

F_l = Fluorescence intensity during each addition of metal ion (2.5 μM, 5 μM etc)

Mole fraction = Z/ (Z+Y)

Z = Metal ion concentration during each addition

Y = Concentration of TAP

Binding constant measurement

$F_0-F_l = \Delta F[X/(X+K_D)]+C$

F_0 = Fluorescence intensity of fluorophore before addition of metal ion

F_1 = Fluorescence intensity after each addition of metal ion (2.5 µM, 5 µM etc)

$\Delta F = F_0 - F$ where F = Fluorescence intensity after final addition of metal ion

X = Metal ion Concentration during each addition

K_D = Binding constant

C = Constant (usually 0)

In conclusion, we have shown the synthesis of fluorescence active 2-aminopyrimidine (TAP) using Cu (I) catalyzed microwave assisted method. Selective metal ion (Pd^{2+}) detection of 2-aminopyrimidine was established by fluorescence quenching titration in aqueous medium. The binding ratio and binding constant were also calculated.

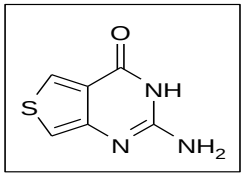
IV.3.3 Experimental Section

IV.3.3.1 General Information: All the chemicals were purchased from Sigma Aldrich, Alfa Aeser, Spectrochem and were used directly without any further purification. *CEM Discover Labmate* closed vessel microwave reactor was used for the reaction. Fluorescence spectra were carried out on a *FluoroMax-4 Spectrofluorometer-Horiba Scientific*. NMR spectra were recorded in *Bruker-600 MHz* spectrometer using DMSO-d_6 as reference solvent. HRMS analyses were carried out by *Agilent Q-TOF 6500 LC/MS* instrument. Absorbance was measured using *UV-Visible Perkin-Elmer spectrophotometer*. Metal chlorides and metal perchlorate salts were used for the titrations.

IV.3.3.2 General Procedure: 4-bromothiophene-3-carboxylic acid (1 equiv), Guanidine hydrochloride (2 equiv), Cs_2CO_3 (2 equiv), Cu_2O (10 mol %, 0.1 equiv) were taken in a microwave reactor vessel and 1mL of dry DMF was added carefully. The reaction vessel was closed and exposed to microwave irradiation about 120 °C for 20 minutes. Stirring was maintained during the reaction and the vessel was cooled after the reaction. Initially the product formation was confirmed by thin layer chromatography. The purification of product was carried out by column chromatography using methanol/chloroform solvents mixture. NMR and Mass techniques were used for final confirmation of compound.

IV.3.4 Characterization section

2-aminothieno [3, 4-*d*] pyrimidin-4(3*H*)-one (TAP)



Pale yellow solid, Yield: 52%. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 10.52 (br, 1H), 8.23 (dd, 1H, J = 3 Hz), 6.95 (d, 1H, J = 3 Hz), 6.08 (br, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 158.95, 150.91, 133.23, 127.24, 123.77, 108.41. FTIR (KBr): ν/cm^{-1} 3434.13, 3382.71, 3178.33, 2924.18, 2853.37, 1691.03, 1654.73, 1628.29, 1546.92. HRMS (ESI): calculated for $C_6H_5N_3OS$ $(M+H)^+$ 168.0226, found 168.0225.

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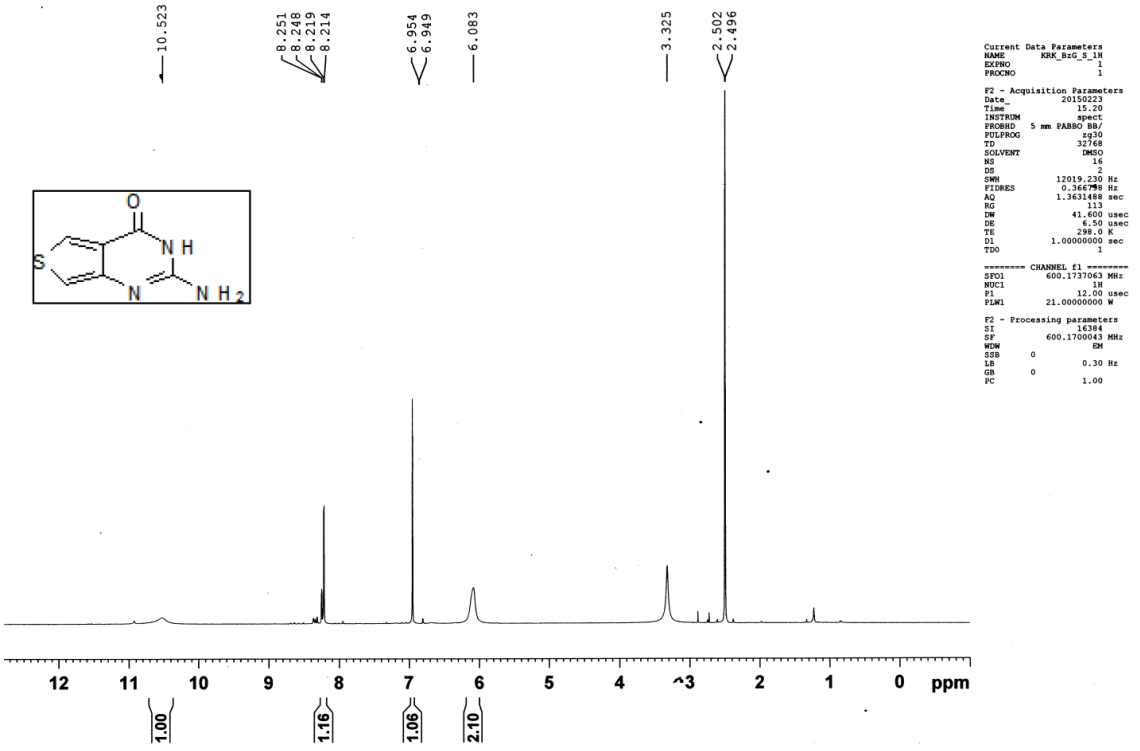
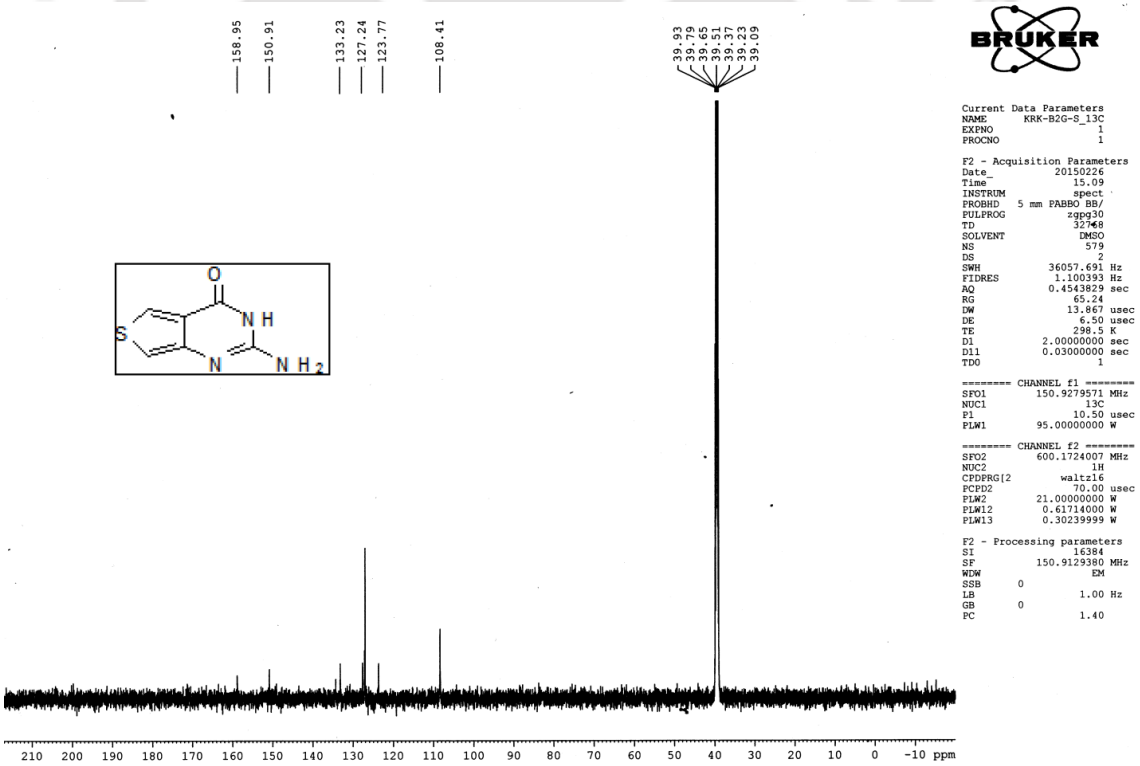
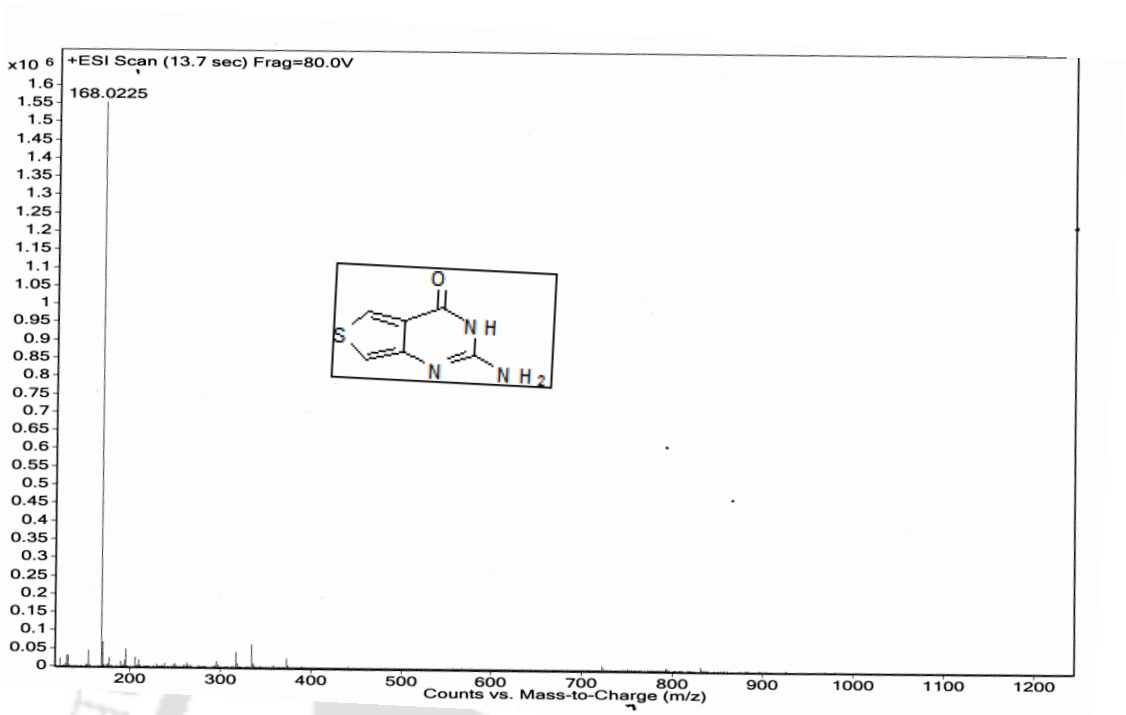
Appendix

NMR and Mass Spectra of TAP







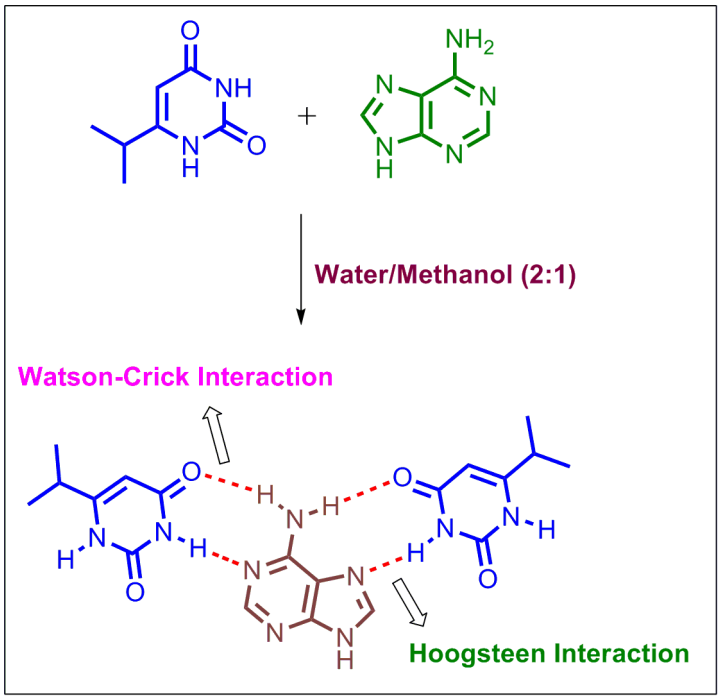
Chapter 5

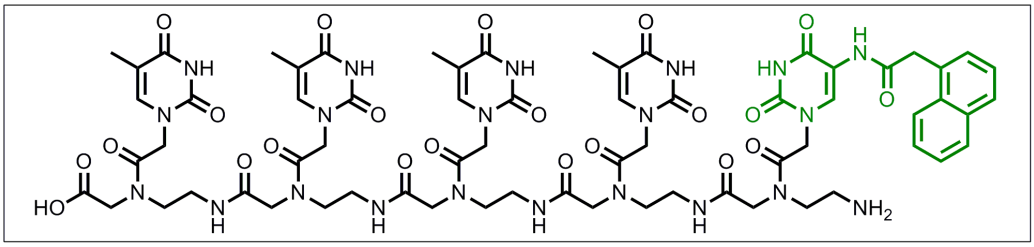
Synthesis of Pyrimidines for Artificial Base-pairing and Peptide Nucleic Acid

Synthesis of Pyrimidines for Artificial Base-pairing and Peptide Nucleic Acid

Abstract

In this chapter we have shown the synthesis of modified uracil nucleobase analogues using microwave and coupling reactions. The formation of tri-base pair from free nucleobase is rare. The tri-base-pair of 6-isopropyl uracil-adenine was achieved by co-crystal structure. Such a nucleobase might have potential application in nucleic acids. We have also demonstrated the synthesis of peptide nucleic acids using solid phase peptide synthesis. Peptide nucleic acid containing modified uracil was synthesized and characterized.





V.1 Introduction of Base-pairing

The double helical structure of naturally occurring DNA is largely attributed to Watson–Crick base pairing interactions, along with pi-stacking forces.[1,2] Usually, adenine forms a H-bonded complex with thymine, whereas guanine forms a base-pair with cytosine. Hoogsteen base-pairs were also found in nature, where the formation of a triple helix was more probable.[3–5] The formation of such triple helices could be important to selectively modulate gene expression, by controlling gene transcription. Hoogsteen first reported a crystal structure of a 1:1 complex between1-methylthymine and 9-methyladenine in which N7 and C6-NH_2 of adenine form an unusual complex with the thymine moiety.[6] Later on, a number of crystal structures, NMR and melting temperature studies of the short oligonucleotides were reported to elucidate various Hoogsteen interactions, [3,5,7–9] including RNA–protein complexes[10] and G-quadruplex.[11]

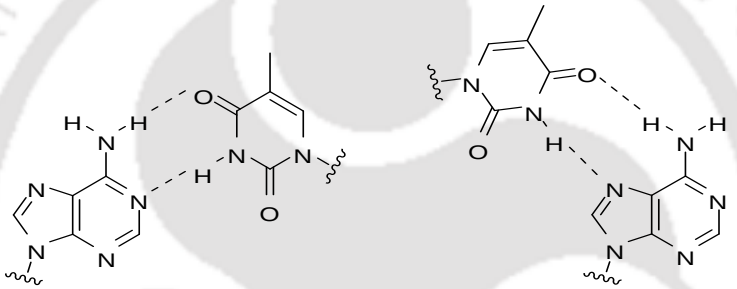


Figure V.1a *Watson-Crick and Hoogsteen H-bonding interactions of Adenine-Thymine*

Chemically modified oligonucleotides, such as peptide nucleic acid, efficiently form a triple helix involving both *Watson–Crick* and *Hoogsteen* interactions.[13–16] *Hoogsteen* interactions were also demonstrated with complementary nucleobases stabilized by metal-ion coordinations.[17,18] However, there is rare evidence from the co-crystal structure of the free nucleobases to illustrate such complex formation involving both Watson–Crick and Hoogsteen interactions. The co-crystal structure from the free nucleobases is indeed very difficult to achieve, primarily due to the poor solubility of the nucleobases in organic solvents. Recently, Chandrasekhar and coworkers reported the crystal structure of a complex, in which one molecule of thymine is flanked by two molecules of adenine (A: T: A), at low temperature.[19] The complex was reported to be formed due to *quasi-Watson–Crick* and *Hoogsteen* base pairing. The important drawback of this base-pair was that the formation of quasi-Watson-Crick H-bonding through thymine N1H. These interactions are not possible in DNA because, hydrogen at thymine N1 position is not available in DNA.

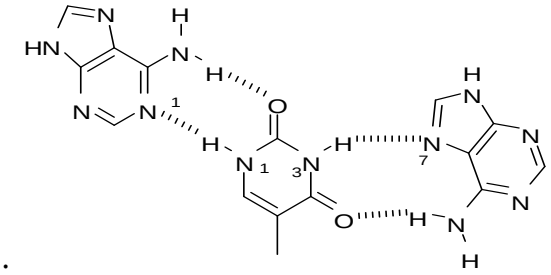
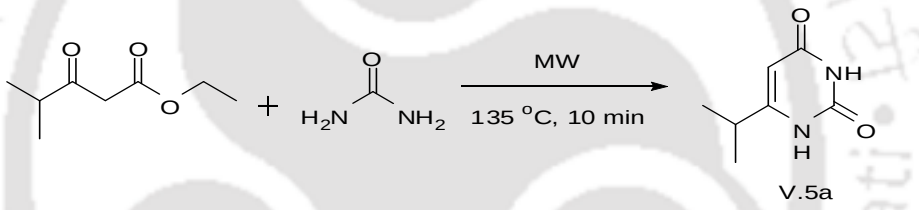


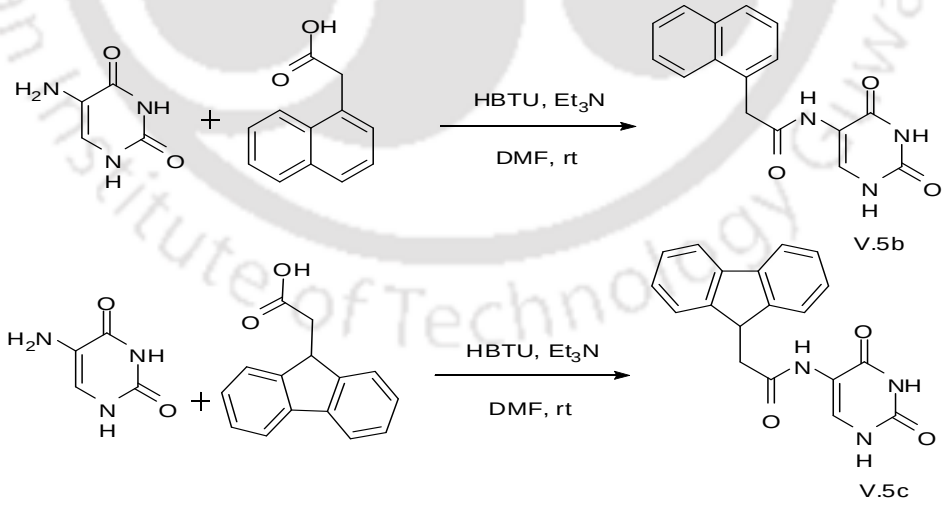
Figure V.1b *Quasi-Watson-Crick and Hoogsteen interaction between Adenine-Thymine*

V.1.1 Present Work

We have already shown the Synthesis of unnatural pyrimidine nucleobases using microwave assisted method.[20] We anticipated that such a modified nucleobase may have different properties such as alter the base pairing, better π-stacking etc. Here we present the synthesis of some modified uracil nucleobase analogues through microwave and coupling reactions.



Scheme V.1.1.1



Scheme V.1.1.2

Our prime focus was to show the base–pairing property of nucleobases using co-crystallization technique. The co-crystallization of above nucleobases was carried out

with adenine as counter part by slow evaporation method using combination of various solvents.

V.1.2 Co-crystallization Process

V.1.2.1 Co-crystal of 6-isopropyluracil-adenine

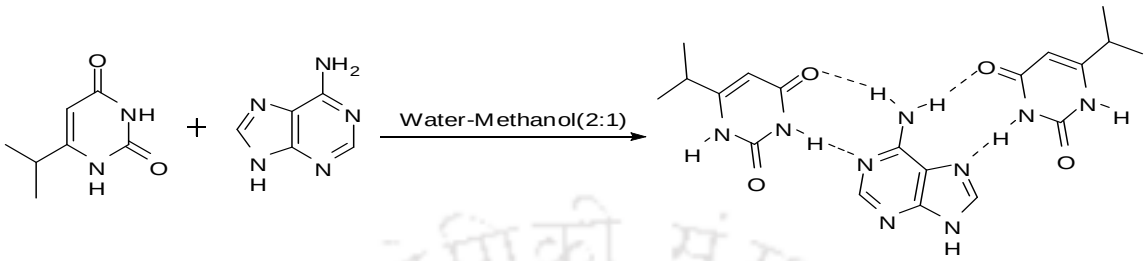


Figure V.1.2.1a

Here, we present a *Watson–Crick* and *Hoogsteen* tri-base pairing co-crystal structure (ipU:A:ipU) formed by adenine and 6-isopropyluracil **V.5a** (ipU)[21-23] in a 1:2 ratio. 6-Isopropyluracil was used to replace thymine, since the 6-isopropyl substituent can render a higher hydrophobic property compared to the methyl group on thymine. The co-crystal was obtained when equimolar quantities of 6-isopropyluracil were mixed with adenine in water–methanol (2:1 v/v), followed by slow evaporation of the solvents at ambient temperature. One molecule of the adenine nucleobase was found to be flanked by two molecules of the uracil derivative, forming a ipU:A:ipU tri-base pair. Careful crystal structure analysis showed that part of the adenine is hybridized to a 6-isopropyluracil, through *Watson–Crick* base-pairing. The other part of the adenine is bound to a second pyrimidine base via *Hoogsteen* interactions.

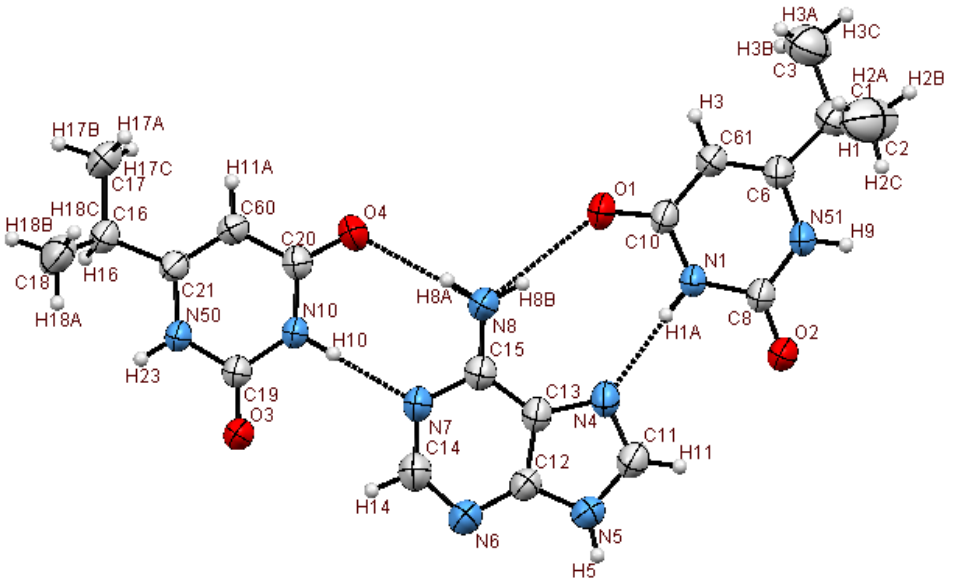


Figure V.1.2.1b *ORTEP diagram of Co-crystal*

To the best of our knowledge, such a co-crystal structure, involving both *Watson–Crick* and *Hoogsteen* interactions from the free nucleobases has not been reported. Interestingly, although the N1 position of the pyrimidine and the N9 position of the adenine (where a sugar moiety is attached in DNA or RNA) were not protected, they did not participate in forming the 'tri-base pairing' structure. The structure of the complex is in good agreement with the reported X-ray diffraction studies obtained from short oligonucleotides. Hecke *et al*. demonstrated the crystal structure of an undecamer oligonucleotide sequence, which forms a T: A: T triple helix via *Watson–Crick–Hoogsteen* base pairing. The observation of such a co-crystal could be important in developing modified oligonucleotides and molecular architectures.[24, 25]

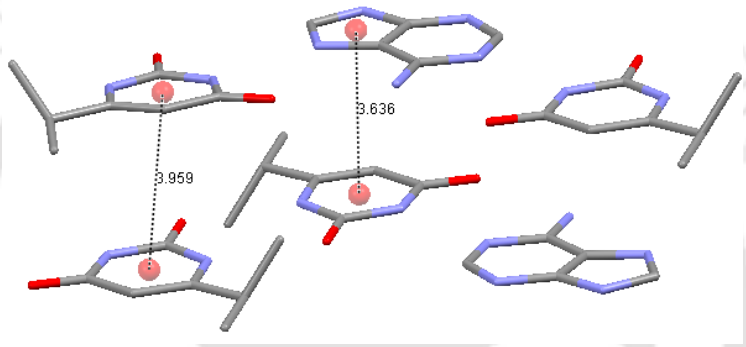


Figure V.1.2.1c *π-stacking representation of co-crystal*

The structure demonstrates the formation of strong Watson–Crick base pairing where N10–H10 and C20–O4 of a ipU are strongly H-bonded to N7 and N8–H8A of the adenine, respectively. Hoogsteen base paring could be observed, involving N4 of the imidazole ring and the amine group (N8–H8B) of the adenine forming strong H-bonds with a second ipU molecule (N1–H1A and C10-O1, respectively). Thus, the amine group of the adenine is involved in two kinds of hydrogen bonding, which has also been observed in DNA triple helix structures. Four water molecules were found to be present in the unit cell, participating in H-bond formation with N6 and N5–H5 of adenine, along with O4 and O1 of the two pyrimidine bases .Water molecules were also found to act as a 'bridge' between two layers in the supramolecular arrangement of the complex. The supramolecular architecture, as depicted in **Fig V.1.2.1d**, shows the formation of planar layers interconnected by the water molecules. As is evident, the water molecules create a pentagonal void space between the layers in the supramolecular assembly. Two types of pi-stacking interactions were also observed in the crystal lattice. Relatively strong π-

stacking (3.636 Å) occurred between the pyrimidine base and the five-membered ring of the adenine. Another pi-stacking (3.959 Å) was observed involving two ipU molecules.

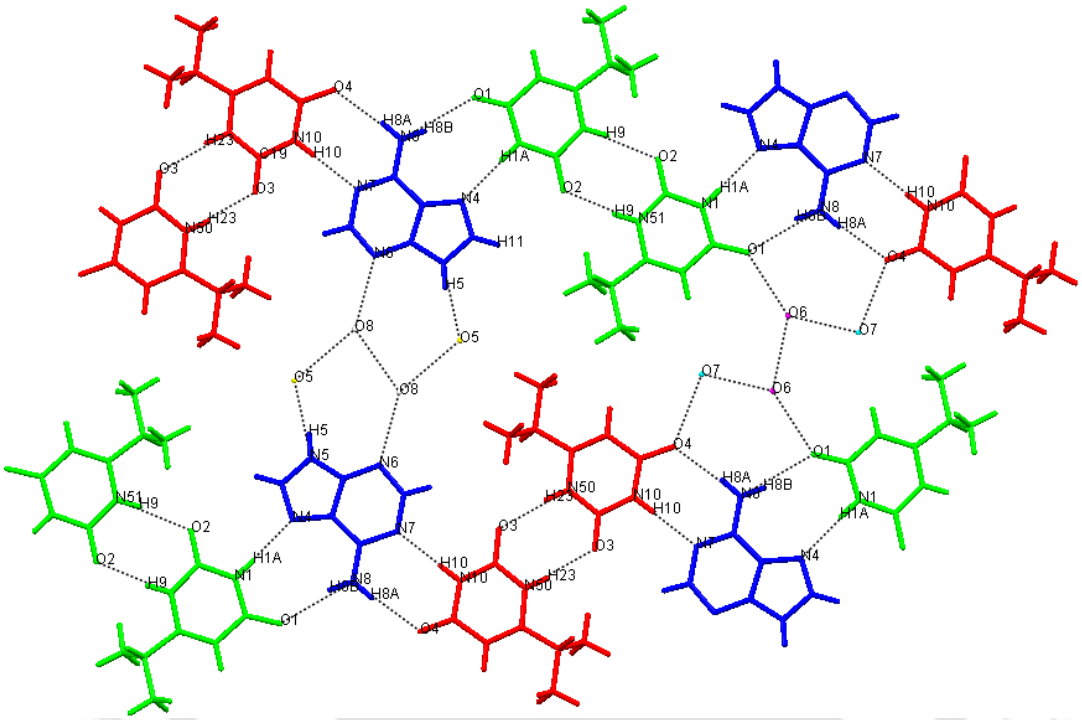


Figure V.1.2.1d *Water molecules assisted supramolecular assembly of co-crystal (colors are indicating different symmetry of molecules)*

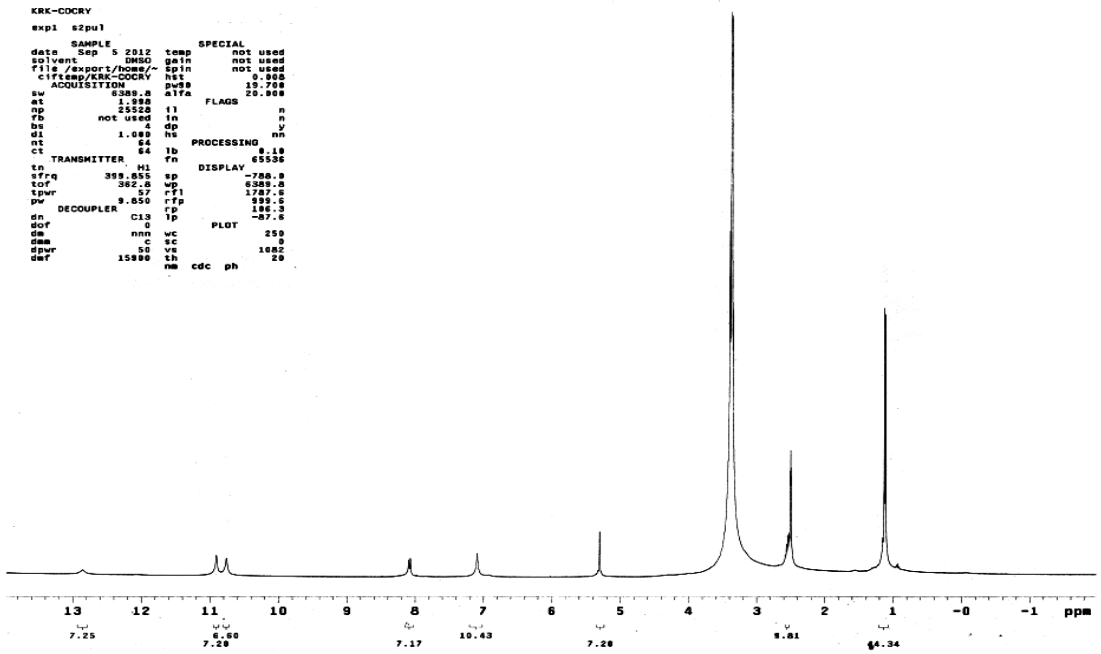


Figure V.1.2.1e *Proton NMR Spectra of co-crystal in DMSO-d_6*

In addition to the X-ray diffraction study, we also analyzed the solution-phase ^{1}H-NMR spectrum, after dissolving the co-crystal in DMSO-d_6. The ^{1}H-NMR clearly proves the

presence of adenine, as well as ipU. Interestingly, the deshielding effects by hydrogen-bond formation were not observed on the chemical shift of those protons involved in *Watson–Crick* and *Hoogsteen* interactions in the co-crystal structure, possibly due to disruption of the H-bonds in DMSO-d_6 solution.[26,27]

Table V.1 (Bond distances of 6-isopropyluracil-adenine co-crystal)

D-H…A	D-H/Å	H…A/Å	D-H…A/Å	∟D-H…A/°	Symmetry
N8-H8A…O4	0.86	2.11	2.962(4)	173	1-x, 1-y, 1-z
N8-H8B…O1	0.86	2.12	2.956(5)	165	-1+x, y, z
N10-H10…N7	0.86	1.97	2.827(4)	176	1-x, 1-y, 1-z
N50-H23…O3	0.86	1.95	2.801(4)	171	1-x, 1-y, 1-z
N1-H1A…N4	0.86	2.02	2.874(5)	173	1+x, y, z
N51-H9…O2	0.86	2.02	2.867(5)	166	1-x,-y, 1-z
N5-H5…O5	0.86	1.94	2.782(4)	165	x,-1+y, z
C2-H2C…N51	0.96	2.60	2.974(6)	104	
C2-H2C…N51	0.96	2.59	3.410(6)	144	1-x, Y, 1-z

V.1.2.2 Co-crystal of 6-isopropyluracil–Urea

We have also demonstrated the co-crystal structure of 6-isopropyluracil with urea in 2:1 ratio. Here two molecules of 6-isopropyluracil are flanked with one molecule of urea with two distinct H-bonding. The pattern of H-bonding is not in a proper manner but still we can suggest that our molecule, 6-isopropyluracil has tendency to form co-crystal via two different H-bonding.

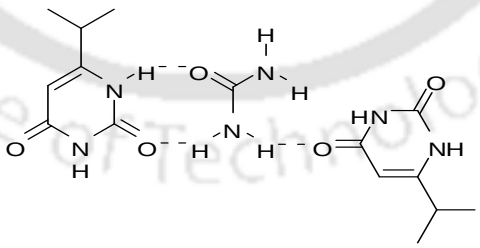


Figure V.1.2.2a *Co-crystal of 6-isopropyluracil-urea*

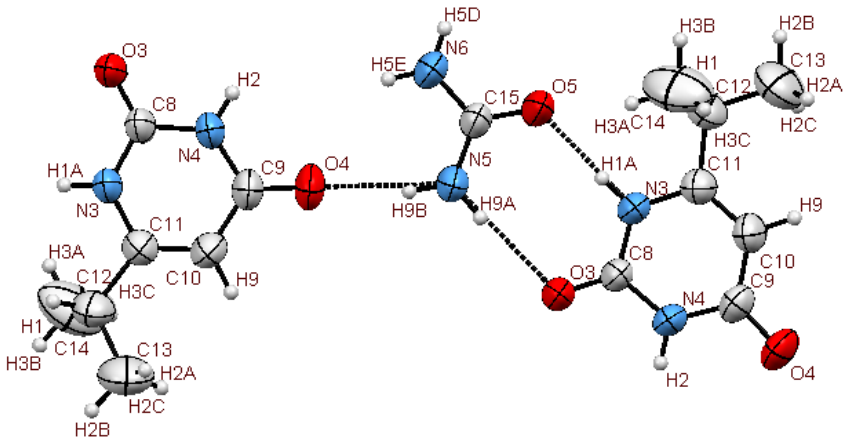


Figure V.1.2.2b *ORTEP diagram of 6-isopropyluracil-urea co-crystal (2:1 ratio)*

This Co-crystal was obtained in ethanol-water (2:0.5, v/v) at room temperature. Water is found to be an important solvent for co-crystallization of nucleobases because; it assists the interaction between molecules through H-bond 'breaking' and 'making' process. Such a Co-crystal will have strong H-bonding interaction between molecules with well-ordered crystalline network.

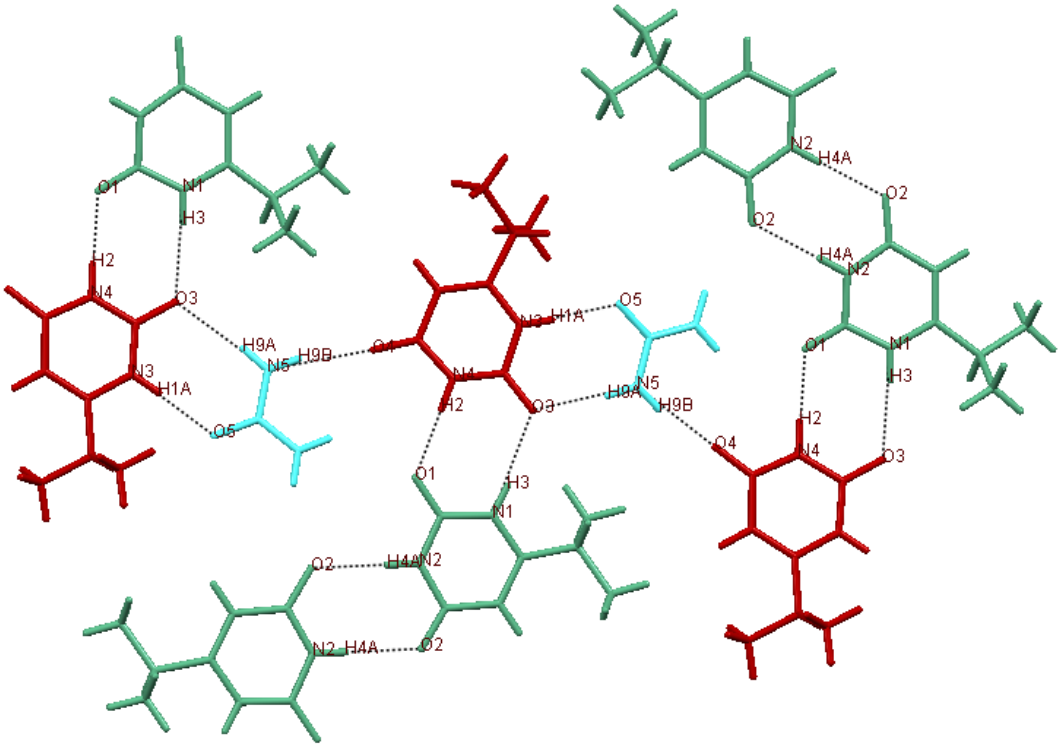


Figure V.1.2.2c *Supramolecular orientation of co-crystal (colors are indicating different symmetry of molecules)*

Molecules **V.5b** and **V.5c** were also used for co-crystallization technique with adenine to get co-crystals. But, we found that there are no significant interactions between the

molecules. We obtained a single crystal of **V.5c** in dimethyl sulphoxide as solvent. The molecule, **V.5c** is new and never reported before.

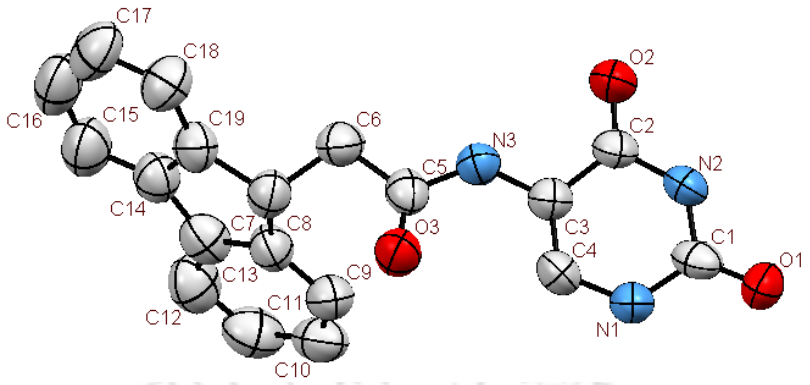


Figure V.1.2.2d *ORTEP diagram of* ***V.5c*** *(hydrogens were omitted for clarity)*

The aim of this molecule synthesis is to form a base-pair with adenine through O2, N2 atoms or O2, N3 atoms with *Watson-Crick* or *Hoogsteen* fashion. But it did not form any co-crystal. The supramolecular structure of **V.5c** shows that all the heteroatoms are involved in H-bonding network except O2, N3. This might be the reason that it was not able form co-crystal with adenine. The fluorene rings are almost perpendicular to the uracil ring assisting proper three dimensional networks.

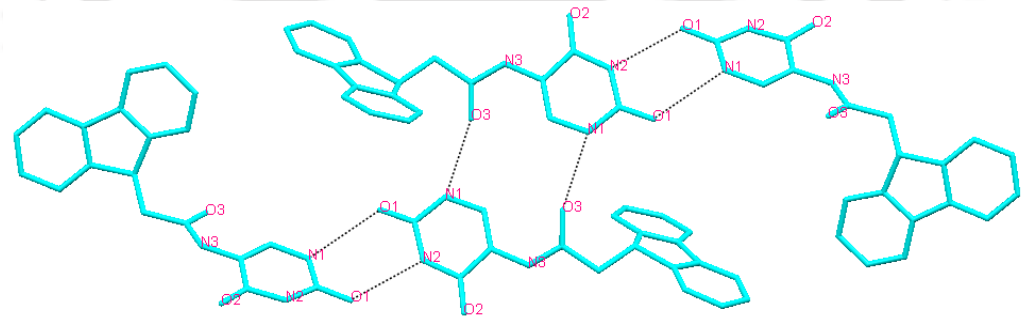


Figure V.1.2.2e *Polymeric structure of* ***V.5c*** *(hydrogens were omitted for clarity)*

V.2 Peptide Nucleic Acids and Their Application

Peptide nucleic acid (PNA) is a modified nucleic acid introduced by Nielsen *et al.*, which has amide (N-(2-aminoethyl) glycine unit) backbone instead of sugar and phosphate. This was designed to mimic oligonucleotide binding through Hoogsteen base pairing.[28] The first computing model of PNA was proposed using TAT triplex.[29] PNAs also obey Watson-Crick H-bonding pattern (rules) for target specific binding to DNA and RNA.[30,31]

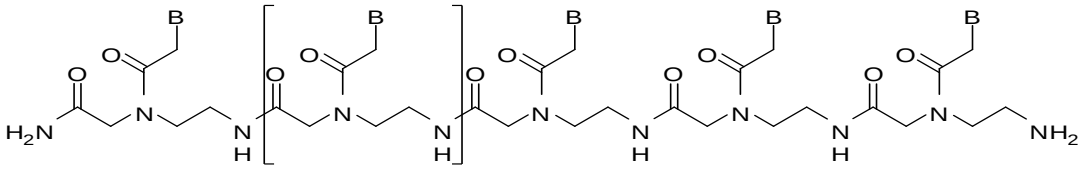


Figure V.2a *Schematic presentation of peptide nucleic acid*

The strong binding affinity order of PNA are, PNA-PNA> PNA-DNA> DNA-DNA.[32] PNA can also form a triplex with its complementary strands.[33,34] Owing to the outstanding thermal, chemical stabilities and hybridization properties with DNAs and RNAs, peptide nucleic acids have considerable interest in medicinal chemistry as well as biology.[35-39]

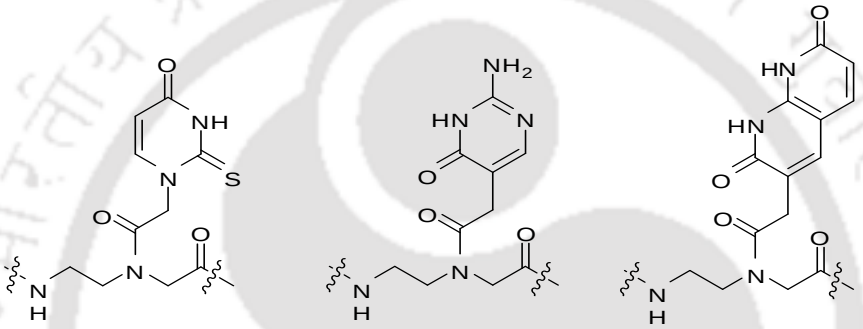


Figure V.2b *Peptide nucleic acids with simple modified nucleobases*[40-42]

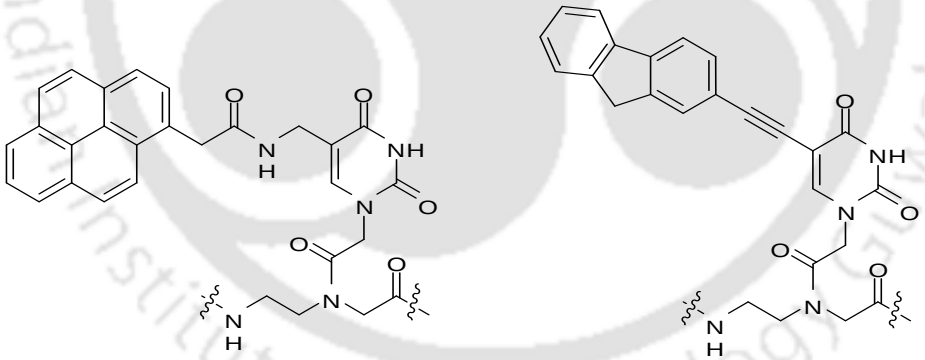


Figure V.2c *Peptide nucleic acids with fluorophore attached nucleobase*[43,44]

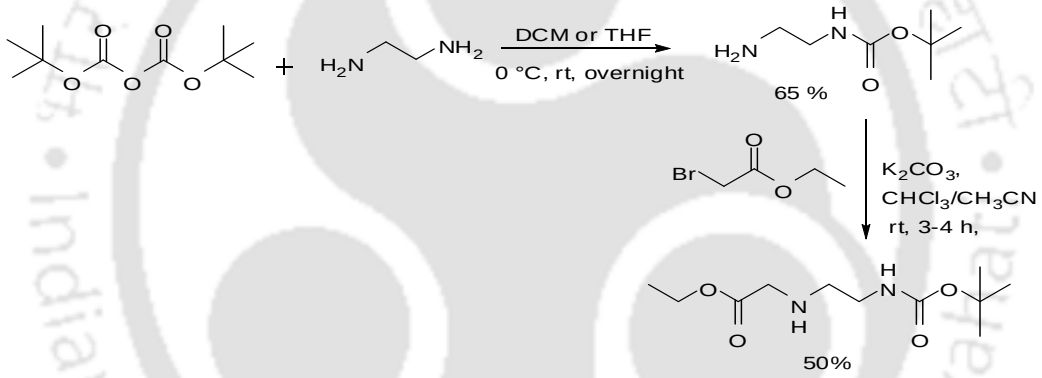
We are looking forward to synthesize peptide nucleic acid with nucleobase modification because base-modified peptide nucleic acids have vital role in various fields. The important property of these modified nucleobases is to alter or reorganize or improve the noncovalent (H-bonding, pi-stacking) forces which are stabilizing the duplex DNA. Usually aromatic compounds have very good pi-stacking property when they are in face-to-face contact. It is due to the planarity and pi-electrons mobility of the aromatic ring. Therefore, aromatic rich nucleobases to the oligonucleotides would be a way to develop stable artificial oligonuclotide probes.

V.2.1 Synthesis of PNA

Usually the PNA is synthesized by solid phase peptide synthesis via Fmoc or Boc chemistry. Here we are demonstrating the synthesis of nucleobase modified peptide nucleic acid using unknown modified nucleobase (**V.5b**). The main advantage of introduction of naphthalene moiety into nucleobase is, to increase the electron density and stacking property. This would lead to synthesize the PNA oligomer with good hybridization property. PNA monomer of naphthalene modified nucleobase was synthesised by the following route given below.

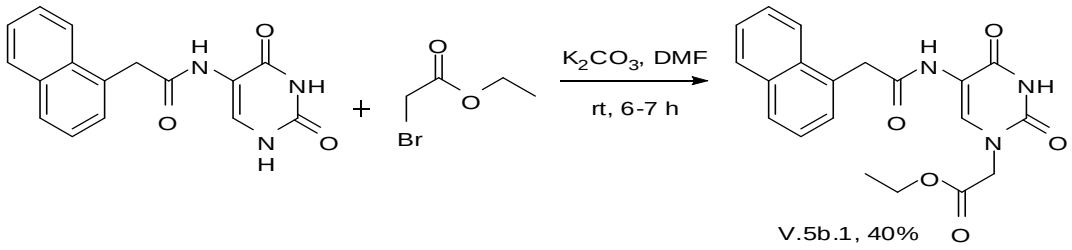
Synthesis of PNA monomer

peptide nucleic acid was synthesized using standard protocol mentioned in the literatures.[45-51] Treatment of boc-anhydride with ethylene diamine yields monoprotected ethylene diamine which was further reacted with ethylbromoacetate under basic condition gave peptide backbone with moderate yield (**Scheme V.2.1.1)**.



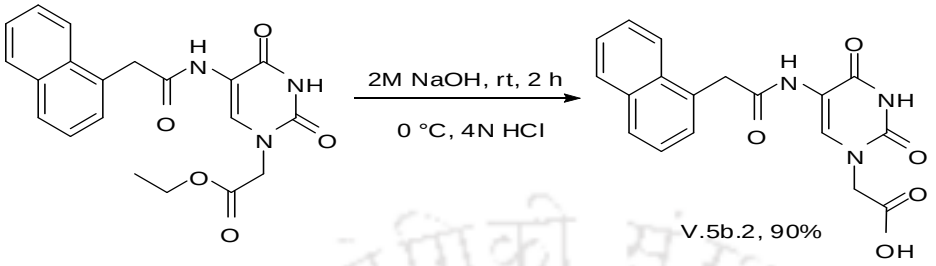
Scheme V.2.1.1(backbone synthesis)

The modified nucleobase is synthesized by acid-amine coupling reaction as mentioned in **Scheme V.1.1.2**. Modified nucleobase is first treated with 2-bromo ethylacetate in presence of mild base to give desired N-alkylated product (**Scheme V.2.1.2)**, which is further hydrolysed with sodium hydroxide yields corresponding acid (**Scheme V.2.1.3)**.



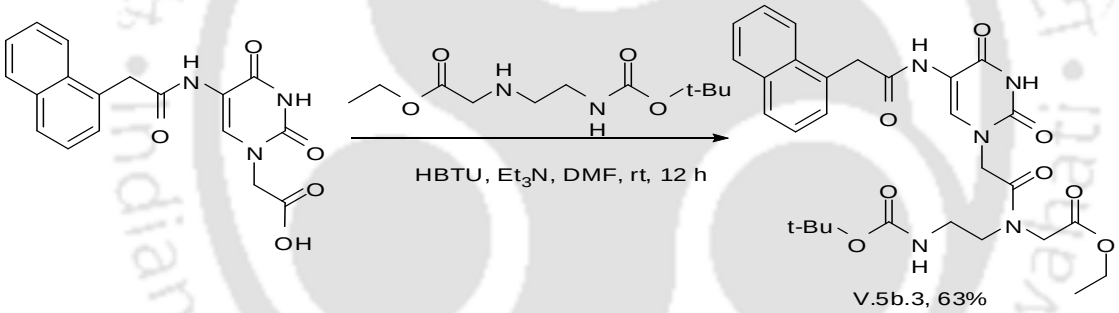
Scheme V.2.1.2 (N1-esterification)

The important step of this synthesis is to introduce the ester in N1 position (sugar is attached in DNA) of modified uracil. Because, there are three N-H positions are active towards 2-bromo ethylacetate under basic condition leads to various products (only one product is shown).

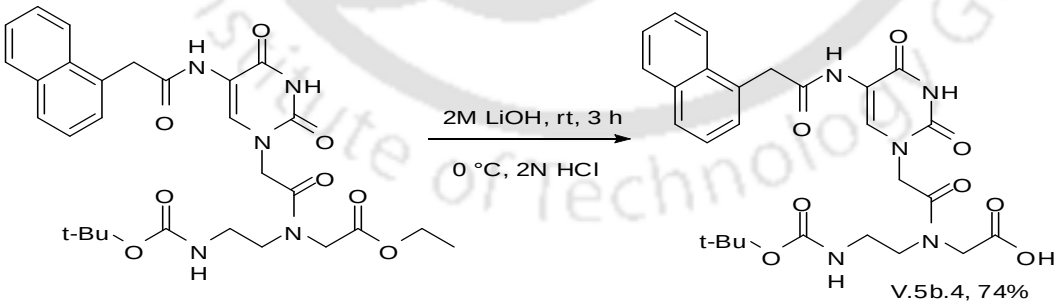


Scheme V.2.1.3 (hydrolysis)

The acid is coupled with backbone using a standard coupling reagent (HBTU) in presence of organic base (triethylamine) gives PNA monomer with ester backbone (**Scheme V.2.1.4)**. The base (LiOH) hydrolysis of this monomer ester gives the corresponding N-terminal boc protected monomer acid (**Scheme V.2.1.5).**



Scheme V.2.1.4 (backbone coupling)

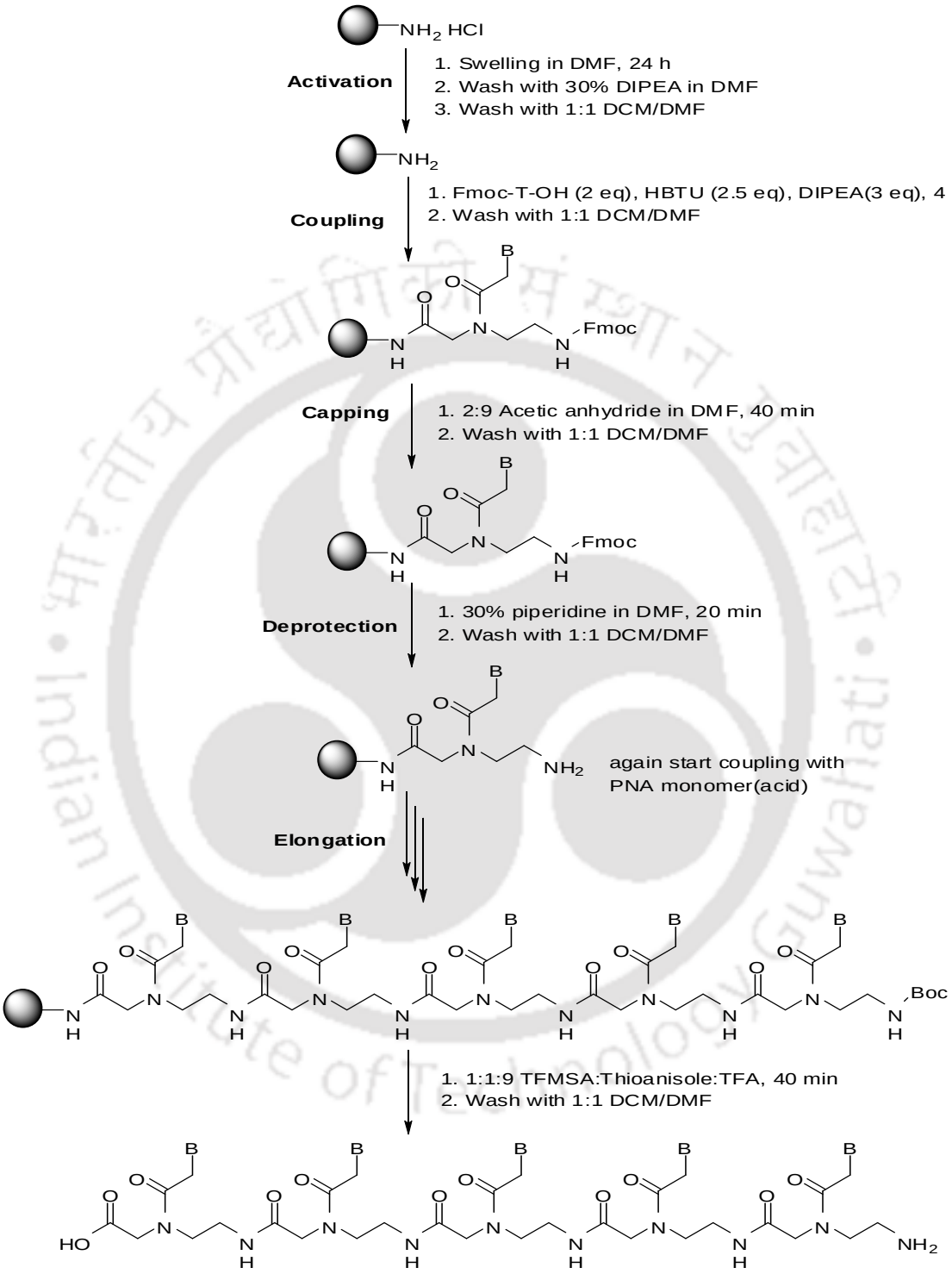


Scheme V.2.1.5 (hydrolysis)

Synthesis of PNA oligomer

During solid phase- synthesis, the carboxylic acid group of PNA monomer is attached to amine group of solid support (resin) to synthesize the PNA oligomer. PNA oligomer was

synthesized by existing protocol using (methy-benzhydryl)amine hydrochloride Polystyrene resin as solid support.[52]



Scheme V.2.1.6 *Schematic presentation of solid phase peptide synthesis*

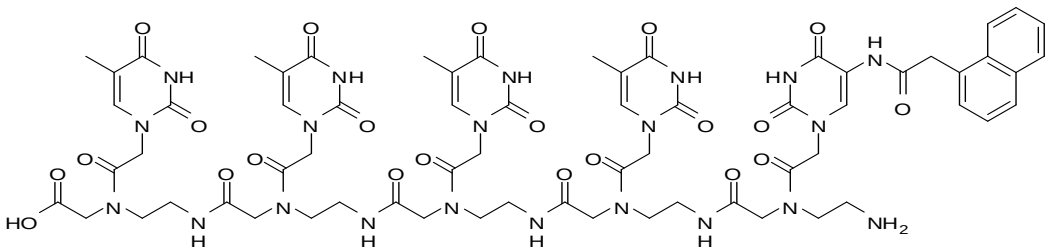


Figure V.2.1a *Peptide nucleic acid (pentamer)*

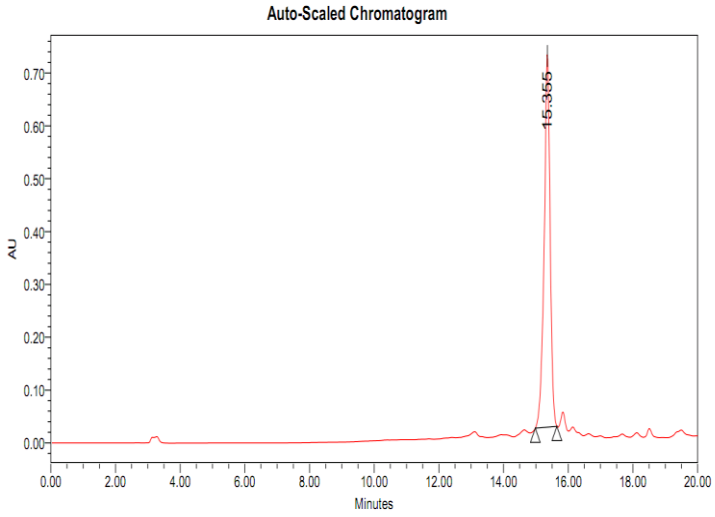


Figure V.2.1b *HPLC Chromatogram of pentamer*

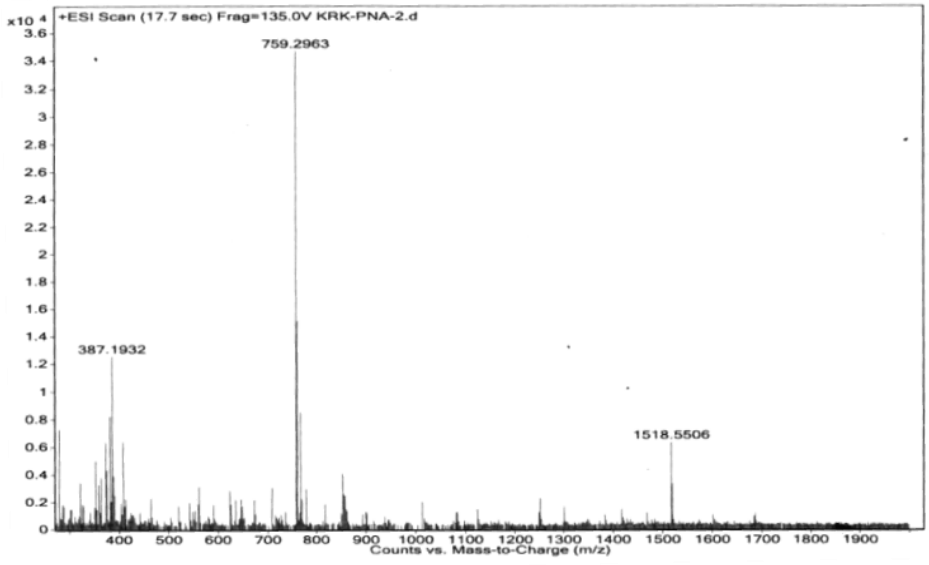


Figure V.2.1c *Mass spectra of pentamer MS (ESI): M+H* $^{+}$ *1518.5506, ((M/2)+H)* $^{+}$ *759.2963*

In conclusion, we have synthesized and demonstrated the pairing property of modified nucleobases with adenine through co-crystallization process. Such type nucleobases might have potential applications in nuleic acid chemistry. We are representing the

synthesis of modified nucleic acid using standard solid phase peptide synthesis method. The oligomer (pentamer) of nucleic acid having modified nucleobase (naphthalene attached uracil) was successfully synthesized, purified (HPLC), and characterized (MS ESI).

V.3 Experimental Section

V.3.1 General Information: Chemicals were purchased from reputed pharmaceuticals and were used without any further purification. Microwave-directed synthesis were carried out in a *Anton Paar Synthos 3000* closed vessel microwave reactor at 600 W at about 135 °C. NMR was recorded from *400 MHz (Varian) and 600 MHz (Bruker) spectrometers* using DMSO-d_6 $CDCl_3$ as solvents. X-Ray data was obtained from a *Bruker SMART APEX* equipped with a CCD area detector using Mo. The structure was solved by direct method using *SHELX-97*[52,53] (University of Gottingen, Germany). Mass spectra were analyzed from *Agilent Q-TOF 6500 LC/MS* system. HPLC analysis was carried out with an Ascentis C-18 analytical column (5 µm, 250 × 4.6 mm) coupled to a UV-visible detector. HPLC grade solvents were used for HPLC and Mass analyses. Oligomer was synthesized by using Polypropylene plastic syringes equipped with porous polypropylene disc at the bottom (5 mL).

V.3.2 General Procedure of 6-isopropyluracil (V.5a): Methylisobutyryl acetate (1 mmol) was taken in a reactor vessel and mixed thoroughly with urea (1.5 mmol). The reaction vessel was closed immediately and irradiated with microwave radiation at 135 °C for 7 minutes. The reaction mixture was cooled and the compound was purified by column chromatography. **Yield:** 79%.

V.3.3 Synthesis (6-isopropyluracil) (V.5a) using $BF_3.Et_2O$ as Lewis acid: Methylisobutyryl acetate (1 mmol) was taken in a reactor vessel with $BF_3.Et_2O$ (1 mmol), and mixed thoroughly with urea (1.5 mmol). The reaction vessel was closed immediately and irradiated with microwave radiation at 135 °C for 3 minutes. The reaction mixture was cooled and the compound was further purified by column chromatography. **Yield:** 87%

V.3.4 Synthesis of V.5b and V.5c: 5-aminouracil (1 mmol), HBTU (1.1 mmol), acid (1 mmol) were taken in a two neck round bottom flask and closed immediately. 2 mL of dry DMF containing triethylamine (1.3 mmol) was added to the reaction mixture. The reaction mixture was stirred for 12 hours under inert atmosphere. The product was

confirmed by thin layer chromatography and purified by column chromatography using methanol-chloroform solvent mixture (1:9).

V.3.5 Backbone synthesis: backbone ester was synthesized from standard protocols mentioned in the literatures.[43-46]

V.3.6 Synthesis of V.5b.1 (Scheme V.2.1.2): naphthalene attached nucleobase (**5b**) (1 mmol), K_2CO_3 (1 mmol), were taken in 50 mL round bottom flask with 5ml of dry DMF. Ethyl bromoacetate (1 mmol) in 2 mL DMF was added dropwise into the round bottom flask under inert atmosphere. Stirring was continued for 7 hours at room temperature. Product formation was confirmed by thin layer chromatography. 30 mL of chloroform was added to the reaction mixture and the reaction mixture was extracted with water. Organic layer was dried over sodium sulphate and the solvent was removed by rotary evaporation. Finally the compound was purified by column chromatography using ethyl acetate/ hexane solvent mixture. Yield 40%

V.3.7 Synthesis of V.5b.2 (Scheme V.2.1.3): Compound **V.5b.1** (1 mmol) was taken in a 25 mL round bottom flask and 3 mL of 2M NaOH was added. The reaction mixture was stirred for 2 hours at room temperature. The consumption of **V.5b.1** was confirmed by thin layer chromatography. The reaction mixture was cooled to 0 °C and acidified with 4N HCl up to pH-3. The precipitate was filtered, washed with cold water (3 times) and dried. Yield 90%

V.3.8 Synthesis of V.5b.3 (Scheme V.2.1.4): Compound **V.5b.2** (1 mmol), backbone (1.1 mmol), HBTU (1.1 mmol), were taken in a 50mL round bottom flask and 5ml of DMF containing Et_3N (1.3 mmol) was added dropwise. The reaction mixture was stirred for 12 hours under inert atmosphere. The reaction was monitored by thin layer chromatography. 20 mL of water was added to the reaction mixture and the product was extracted with 30 mL chloroform (2 times).the solvent was removed using rotary evaporation and the final compound V.5b.3 was purified by column chromatography (5% methanol/chloroform). Yield 63%

V.3.9 Synthesis of V.5b.4 (Scheme V.2.1.5): Compound **V.5b.3** (0.5 mmol) was taken in 25 mL round bottom flask and 2 mL methanol was added to dissolve 5b.3. 1mL of 2M LiOH solution was added to the reaction mixture and stirred for 3 hours at room temperature. The consumption of **V.5b.3** was monitored by thin layer chromatography. The reaction mixture was cooled to 0 °C and acidified with 2N HCl up to pH 3-4. The precipitate was filtered, washed and dried to yield compound **V.5b.4**. Yield 74%

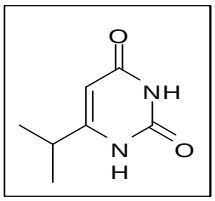
V.3.10 Solid phase peptide Synthesis

Synthesis of Oligomer: PNA oligomer was prepared by Fmoc/Boc approach with MBHA resin using standard protocol. The resin (0.038 mmol, 25 mg) was taken in a bed plastic syringe and suspended with 2 mL of DMF for 12 h. Solvent was removed and the resin was washed with 30% DIPEA (2 × 2 mL, 10 min each) and 1:1 DCM/DMF solvent mixture (2 × 2 mL).` The first coupling reaction between resin and Fmoc PNA monomer (2 equiv) was initiated by using HBTU (2.5 equiv), and DIPEA (3 equiv) in presence 1:1 DCM/ DMF mixture. The reaction was monitored by Kaiser's test. After 3.5 h the reaction was found to be completed. The coupling reaction was repeated and washed with 1:1 DCM/ DMF in case of incomplete acylation. Capping was performed after the first coupling using 30% acetic anhydride and washed with 1:1 DCM/ DMF. Deprotection of Fmoc was carried out using 3 mL of 30% piperidine in DMF for 20 minutes (2 times) and washed with 1:1 DCM/ DMF gave free amine. It was confirmed by kaiser's test.

By using above resin coupled free amine, the second coupling was performed with Fmoc PNA monomer, HBTU and DIPEA. The above processes (coupling, washing, deprotection, Kaiser's test) were repeated to achieve the full sequence of oligomer. The resin was dried after the completion of desired oligomer sequence. A freshly prepared solution of TFMSA/Thioanisole/TFA (1:1:9) was added to the resin and suspended for 30 minutes. The solution was removed by filtration and the filtrate was diluted with diethyl ether gave white precipitate. The precipitate was separated by centrifugation and purified using HPLC yielded PNA oligomer.

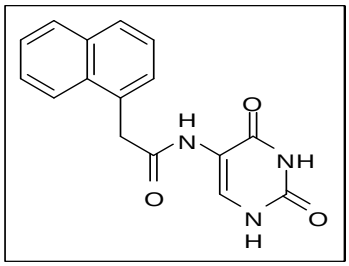
V.4 Characterization Section

6-isopropylpyrimidine-2, 4(1*H*, 3*H*)-dione (V.5a)



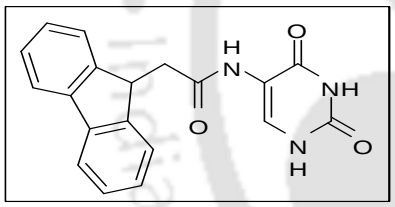
White solid, ^{1}H-NMR DMSO-d_6 (400 MHz): δ (ppm) 10.90 (br, 1H), 10.75 (br, 1H), 5.30 (s, 1H), 2.53 (m, 1H), 1.12 (d, 6H, J = 6.9 Hz).

N-(1, 2, 3, 4-tetrahydro-2, 4-dioxopyrimidin-5-yl)-2-(naphthalene-1-yl) acetamide (V.5b)



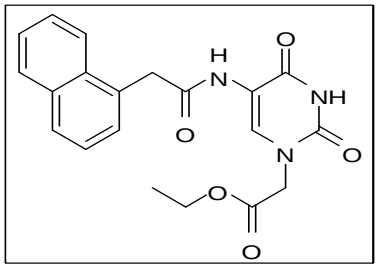
White solid, Yield: 67%, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.46 (br, 1H), 10.60 (br, 1H), 9.42 (s, 1H), 8.01 (d, 1H, *J* = 7.8 Hz), 7.91 (d, 1H, *J* = 7.8 Hz), 7.81 (d, 1H, *J* = 7.8 Hz), 7.50 (m, 4H), 4.21 (s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.53, 160.68, 149.81, 133.36, 131.98, 129.03, 128.38, 127.95, 127.23, 126.06, 125.68, 124.29, 121.96, 113.37, 39.92. MS (ESI): calculated for $C_{16}H_{13}N_3O_3$ $(M-H)^-$ 294.0884, found 294.2420.

2-(9H-fluoren-9-yl)-N-(1, 2, 3, 4-tetrahydro-2, 4-dioxopyrimidin-5-yl) acetamide (V.5c)



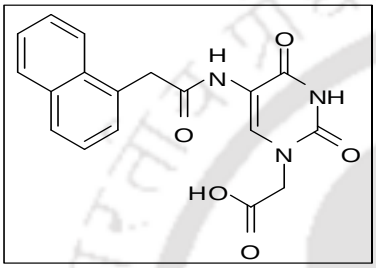
White solid, Yield: 60%, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm)11.46 (s, 1H), 10.73 (s,1H), 9.31 (s, 1H), 8.21 (s, 1H), 7.87 (d, 2H, *J* = 7.8 Hz), 7.45 (d, 2H, *J* = 7.8 Hz), 7.37 (t, 2H, *J* = 7.2 Hz), 7.29 (t, 2H, *J* = 7.2 Hz), 4.37 (t, 1H, *J* = 7.2 Hz), 2.85 (d, 2H, *J* = 7.2 Hz). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 170.44, 160.70, 149.73, 146.57, 140.14, 130.06, 127.26, 127.14, 124.60, 119.99, 113.17, 43.53. MS (ESI): calculated for $C_{19}H_{15}N_3O_3$ $(M+H)^+$ 334.1186, found 334.1201.

Ethyl 2-(5-(2-(naphthalene-1-yl) acetamido-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetate (V.5b.1)



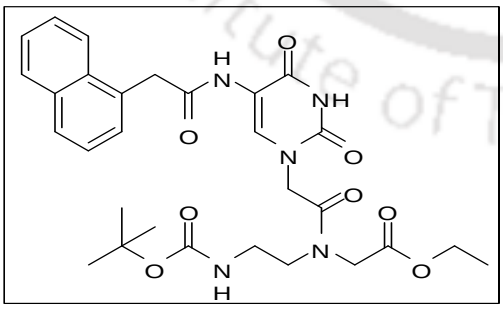
White solid, Yield: 40%, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.83 (s, 1H), 9.51 (s, 1H), 8.33 (s,1H), 8.12 (d, 1H, *J* = 7.8 Hz), 7.92 (d, 1H, *J* = 7.8 Hz), 7.83 (d, 1H, *J* = 8.4 Hz), 7.50 (m, 4H), 4.49 (s, 2H), 4.22 (s, 2H), 4.11 (q, 2H, *J* = 7.2 Hz), 1.16 (t, 3H, J = 7.2Hz).^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.47, 168.13, 160.27, 149.26, 133.75, 133.36, 132.44, 131.96, 128.34, 127.86, 127.32, 126.01, 125.65, 125.50, 124.36, 113.61, 79.17, 61.07, 49.00, 13.97. MS (ESI): calculated for $C_{20}H_{19}N_3O_5$ $(M+H)^+$ 382.1397, found 382.0451.

2-(5-(2-(naphthalene-1-yl) acetamido-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetic acid (V.5b.2)



White solid, Yield: 90%, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.74(s, 1H), 9.47 (s, 1H), 8.27 (s, 1H), 8.12 (d, 1H, *J* = 7.8 Hz), 7.92(d, 1H, *J* = 7.8 Hz), 7.82 (d, 1H, *J* = 7.8 Hz), 7.50(m, 4H), 4.39(s, 2H), 4.21(s, 2H). ^{13}C-NMR (150 MHz, DMSO-d_6): δ (ppm) 169.47, 160.30, 149.32, 134.28, 133.34, 132.42, 131.95, 128.33, 127.85, 127.21, 126.02, 125.64, 125.49, 124.33, 113.34, 49.15, 40.03. MS (ESI): calculated for $C_{18}H_{15}N_3O_5$ $(M+Na)^+$ 376.0904, found 375.9540.

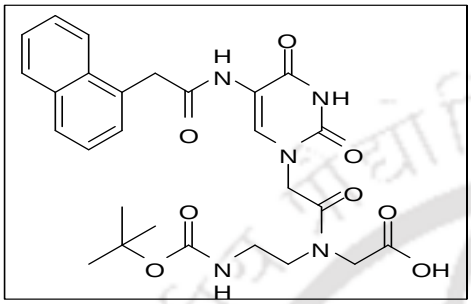
Ethyl 2-(N-(2-(*tert*-butoxycarbonylamino) ethyl)-2-(5-(2-(naphthalene-1-yl) acetamido)-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetamido) acetate (V.5b.3)



White solid, Yield: 63%, ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 8.38 (s, 1H), 7.94 (d, 1H, *J* = 7.2 Hz), 7.84 (d, 1H, *J* = 8.4 Hz), 7.79 (d, 2H, *J* = 6.6 Hz), 7.49 (q, 2H, *J* = 6.6 Hz), 7.41 (br, 2H), 5.56 (s, 1H), 4.52 (s, 2H), 4.14 (q, 2H, *J* = 7.2 Hz), 4.10 (s, 2H), 3.97 (s, 2H), 3.42 (t, 2H, *J* = 4.2 Hz), 3.24 (t, 2H, *J* = 4.2 Hz), 1.40 (s, 9H), 1.23 (t, 3H, *J* = 7.2

Hz). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 169.37, 169.14, 167.20, 159.94, 156.34, 149.25, 134.16, 132.22, 131.71, 130.58, 129.06, 128.80, 128.42, 126.97, 125.83, 123.84, 114.87, 80.19, 62.46, 61.85, 50.55, 49.26, 48.94, 41.96, 38.99, 28.55, 28.53, 14.26. MS (ESI): calculated for $C_{29}H_{35}N_5O_8$ $(M+H)^+$ 604.2378, found 604.4077.

2-(N-(2-(*tert*-butoxycarbonylamino) ethyl)-2-(5-(2-(naphthalene-1-yl) acetamido)-2, 4-dioxo-3, 4-dihydropyrimidin-1(2*H*)-yl) acetamido) acetic acid (V.5b.4)



White solid, Yield: 74%, ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 11.73 (s, 1H), 9.47(s, 1H), 8.17 (s, 1H), 8.12 (t, 2H, *J* = 7.2 Hz), 7.91 (d, 2H, *J* = 7.8 Hz), 7.82 (d, 2H, *J* = 7.8 Hz). 6.88 (q, 1H, *J* = 5.4 Hz), 4.66 (s, 1H), 4.21 (s, 2H), 3.91 (s, 1H), 3.26 (t, 1H, *J* = 7.2 Hz), 3.11 (d, 2H, *J* = 7.8 Hz), 2.97 (d, 1H, *J* = 5.4 Hz), 1.33 (s, 3H), 1.30 (s, 6H). MS (ESI): calculated for $C_{29}H_{31}N_5O_8$ $(M+H)^+$ 554.2245, found 554.2350.

Crystallographic data of 6-isopropyl uracil–Adenine co-crystal

Chemical Formula	$2(C_7H_{10}N_2O_2)\ C_5H_5N_5\ O_4$
Formula mass	507.48
Temperature /K	296 (2)
Crystal system	Triclinic
Space group	*P*-1
a /Å	10.8173 (19)
b /Å	11.0856 (19)
c /Å	13.235 (2)
α /°	89.971 (10)
β /°	69.525 (10)
γ /°	63.586 (11)
Unit cell Volume /Å	1308.7 (4)
Z	2
Radiation type	MoKα
μ/mm^{-1}	0.102
Final R1 value *(I>2σ(I))*	0.0645
Final wR1 value *(I>2σ(I))*	0.1749
Final R1 value (all data)	0.0890
Final wR value (all data)	0.1892
Goodness of fit	0.892

Crystallographic data of 6-isopropyluracil–urea co-crystal

Chemical Formula	$2(C_7H_{10}N_2O_2)\ CH_4N_2O$
Formula mass	368.40
Temperature /K	296 (2)
Crystal system	Monoclinic
Space group	*P*21/n
a /Å	8.7755 (3)
b /Å	19.3258 (7)
c /Å	11.7186 (5)
α /°	90
β /°	105.998 (2)

γ /°	90
Unit cell Volume /Å	1910.43 (13)
Z	4
Radiation type	MoKα
μ/mm^{-1}	0.098
Final R1 value *(I>2σ(I))*	0.0523
Final wR1 value *(I>2σ(I))*	0.1670
Final R1 value (all data)	0.0694
Final wR value (all data)	0.1790
Goodness of fit	1.206

Crystallographic data of 2-(9H-fluoren-9-yl)-N-(1, 2, 3, 4-tetrahydro-2, 4-dioxopyrimidin-5-yl) acetamide (V.5c)

Chemical Formula	$C_{19}H_{15}N_3O_3$
Formula mass	333.34
Temperature /K	296 (2)
Crystal system	Orthorhombic
Space group	Pbca
a /Å	6.3310(3)
b /Å	17.5705(8)
c /Å	28.8590(14)
α /°	90
β /°	90
γ /°	90
Unit cell Volume /Å	3210.2(3)
Z	8
Radiation type	MoKα
μ/mm^{-1}	0.096
Final R1 value *(I>2σ(I))*	0.0689
Final wR1 value *(I>2σ(I))*	0.1366
Final R1 value (all data)	0.1833
Final wR value (all data)	0.1850
Goodness of fit	0.706

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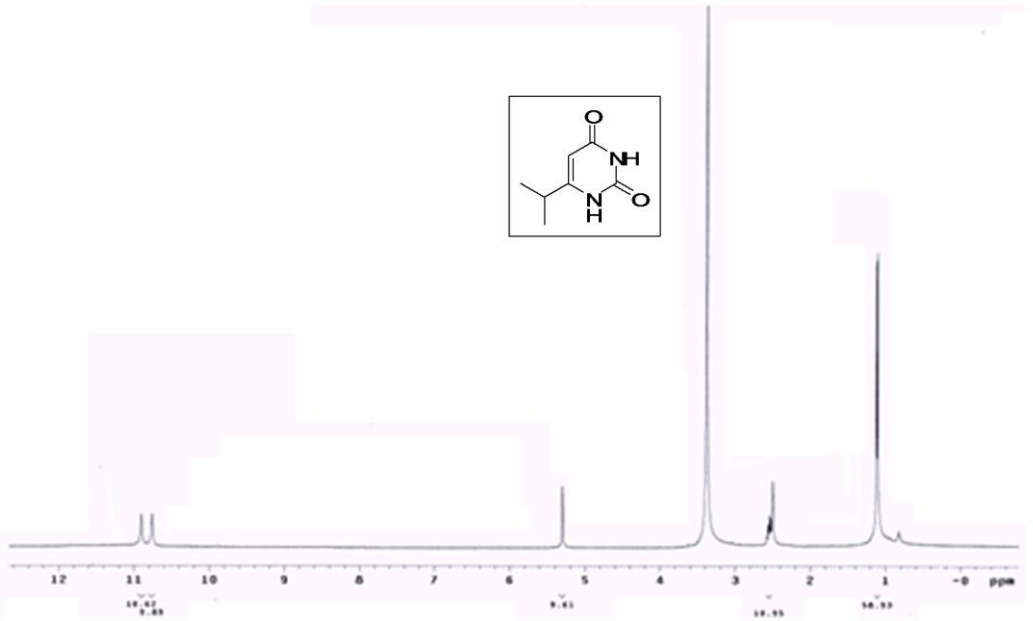
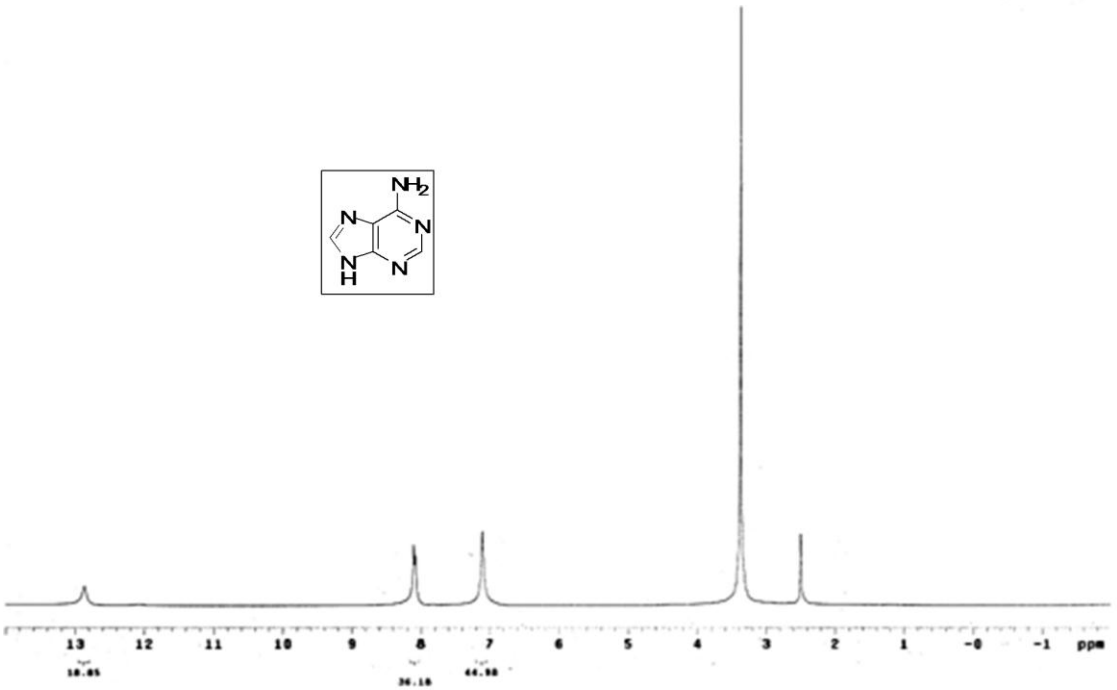
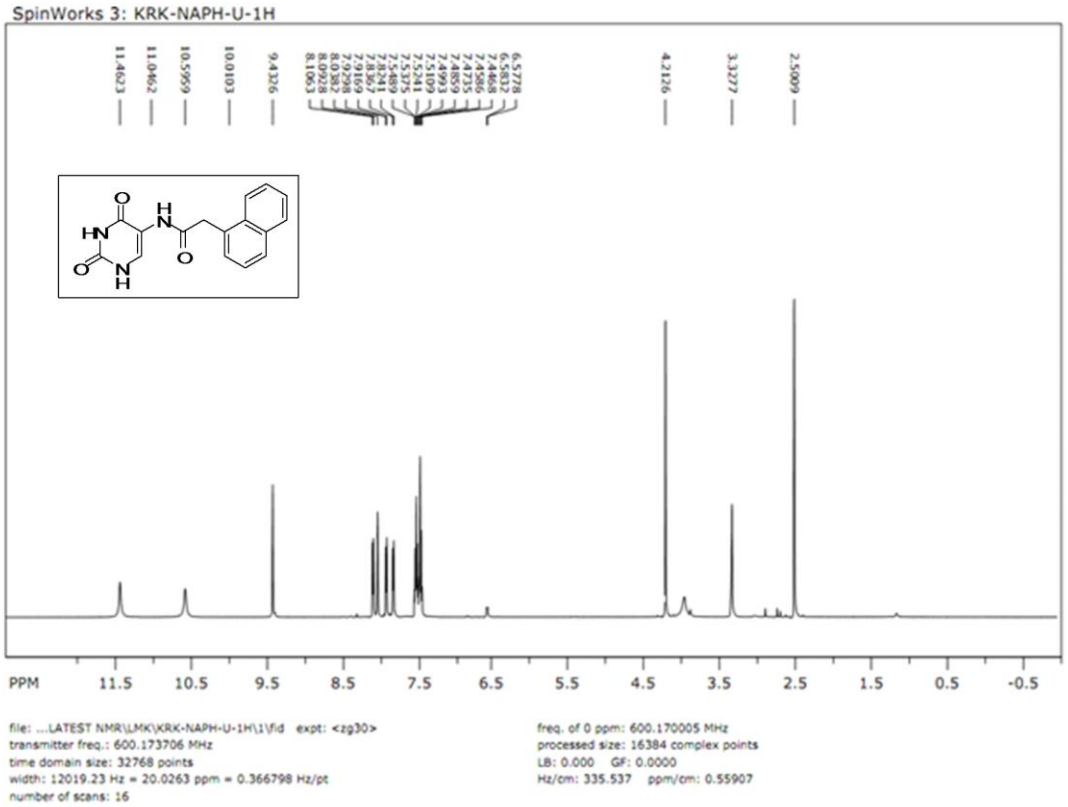
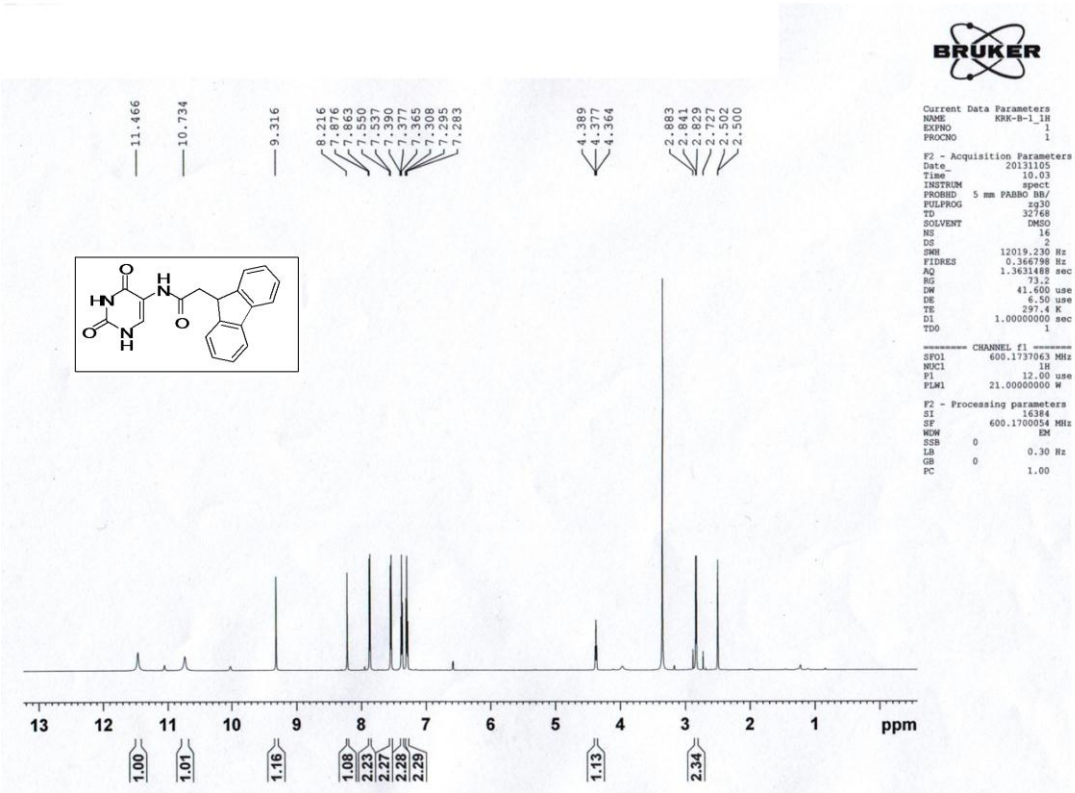
Appendix

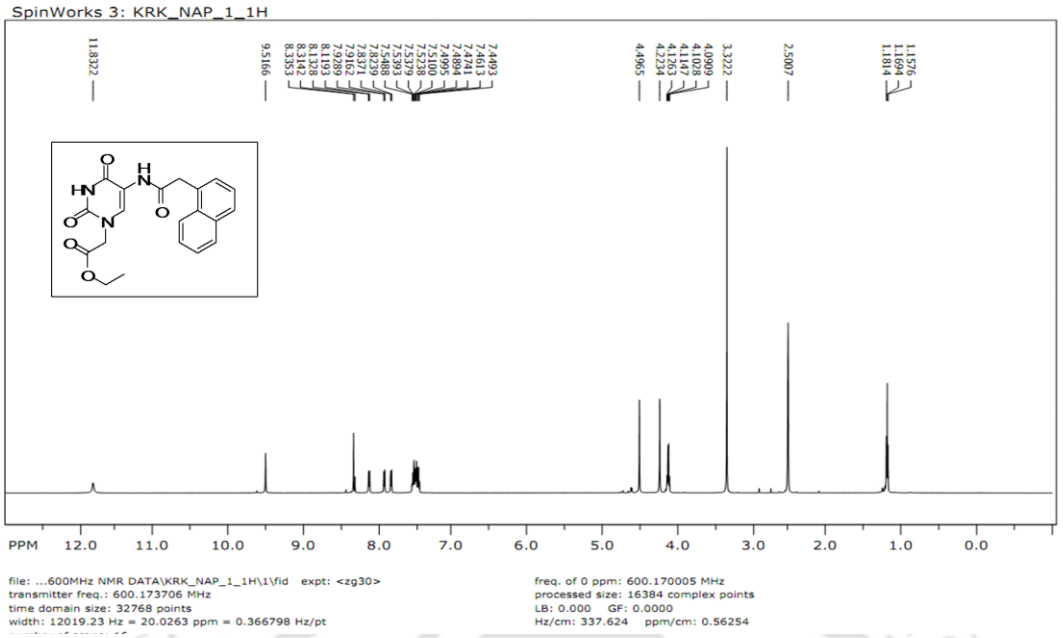
^{1}H-NMR spectra of compounds

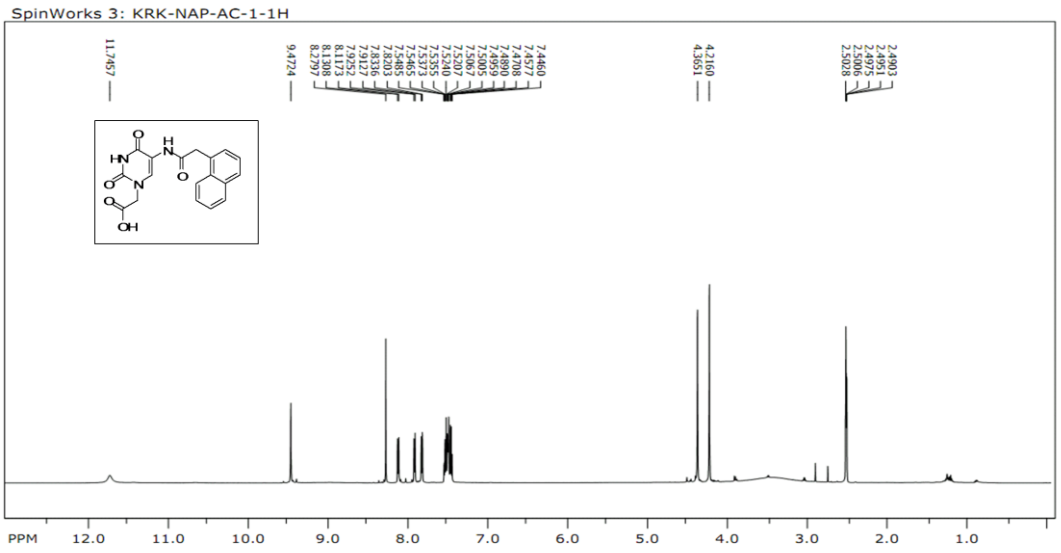


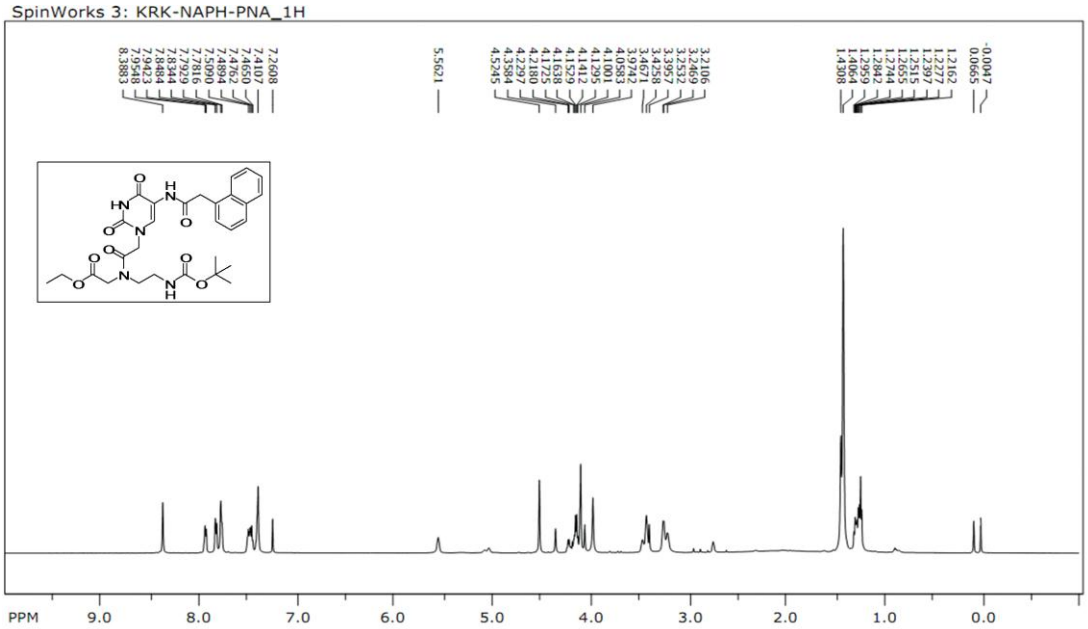


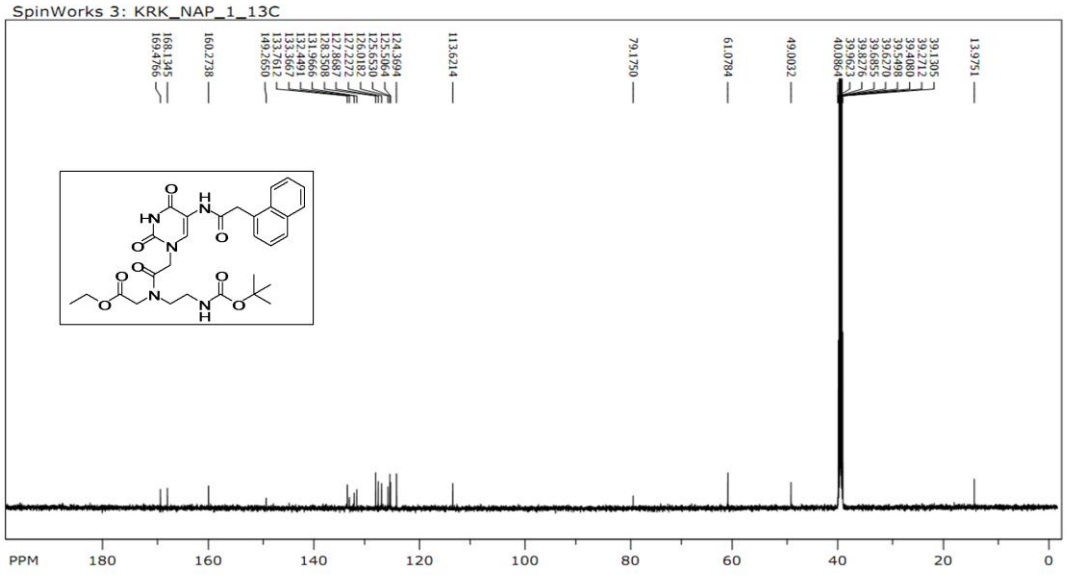












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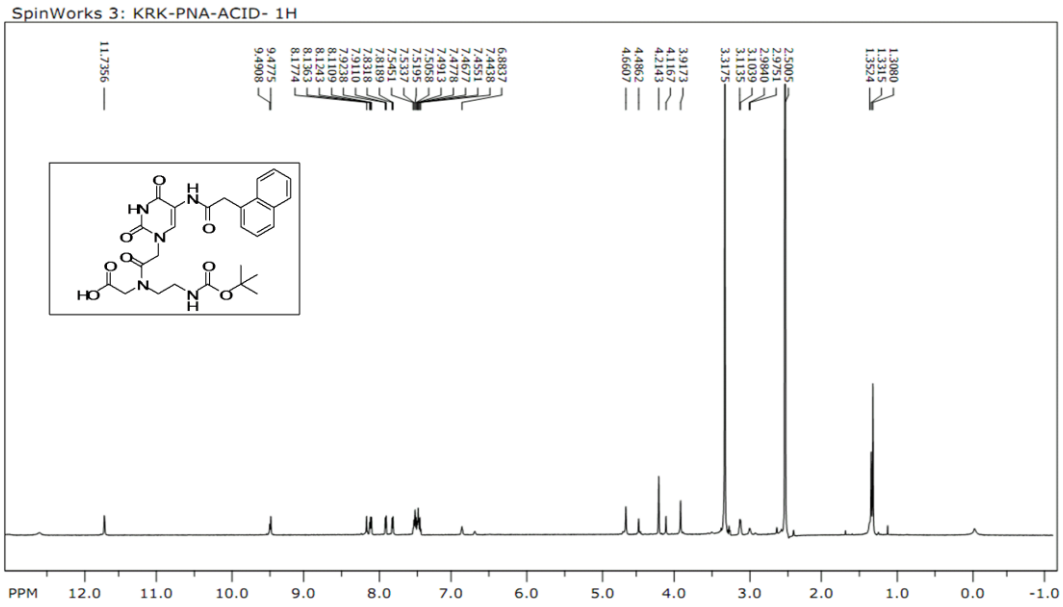
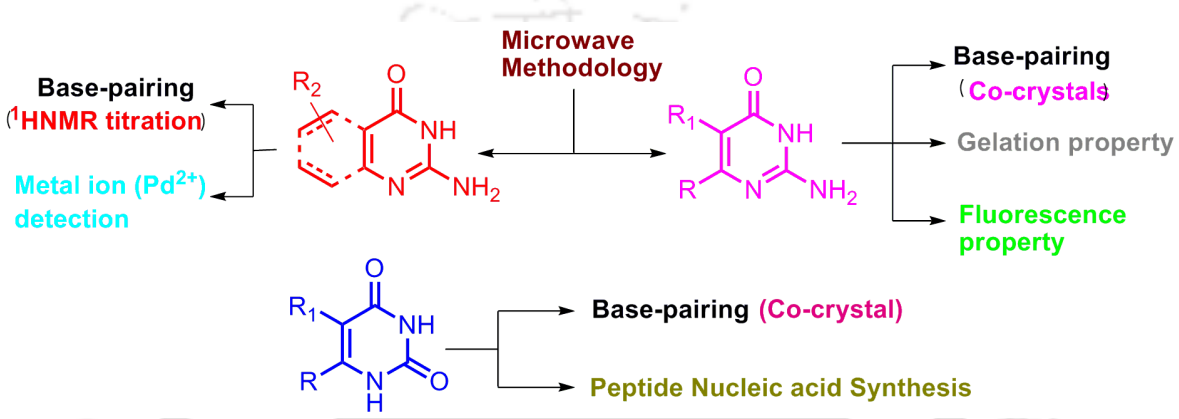


Conclusion and Thesis overview

In this thesis we have shown the synthesis of pyrimidine nucleobase analogues using microwave assisted method in short time. We have explored the base pairing interactions through co-crystal and NMR studies. The unique properties of modified nucleobases such as fluorescence property, metal ion sensing ability and gel property were also efficiently studied by various techniques (UV, fluorescence, XRD, AFM, and DSC). The peptide nucleic acid synthesis was demonstrated successfully using solid phase peptide synthesis.



Publications

1. Burgula, L. N.; **Radhakrishnan, K.;** Kundu, L. M. Synthesis of modified uracil and cytosine nucleobases using a microwave-assisted method. *Tetrahedron Lett*. **2012,** *53*, 2639-2642.

2. **Radhakrishnan, K.;** Burgula, L. N.; Kundu, L. M. Watson-Crick and Hoogsteen tri-base pairing: A co-crystal structure of a 2:1 complex of 6-isopropyluracil and adenine nucleobases. *RSC Adv*. **2013,** *3*, 7282-7284.

3. **Radhakrishnan, K.;** Sharma, N.; Kundu, L. M. Direct synthesis of 5- and 6-substituted 2-aminopyrimidines as potential non-natural nucleobase analogues. *RSC Adv*. **2014,** *4*, 15087-15090.

4. **Radhakrishnan, K.;** Das, S.; Kundu, L. M. A ligand-free microwave-assisted methodology of Cu (I) catalysed C-N bond formation for the synthesis of size-expanded nucleobase analogues. (submitted).

Conferences and Workshops

1. National Conference on Frontiers in Chemical Sciences. Dec 2010, IIT Guwahati.
2. National Conference on Frontiers in Chemical Sciences. Dec 2012, IIT Guwahati.
3. National Conference on Frontiers in Chemical Sciences. Dec 2014, IIT Guwahati
4. International Symposium on Bio-Organic Chemistry. Jan 2015, IISER Pune.