

**DESIGN, SYNTHESIS AND STUDIES ON THE
PHOTOPHYSICAL PROPERTIES OF UNNATURAL
C-TRIAZOLYL, ARYL FUSED N-TRIAZOLYL
NUCLEOSIDES AND HYBRIDISATION
ACCOMPAYNING FRET EVENT IN ANOTHER SET
OF CHIMERIC DNA DUPLEXES**

A

*Dissertation Submitted to the
Indian Institute of Technology Guwahati
As Partial Fulfillment for the Award of Degree of*

*Doctor of Philosophy
in Chemistry
by*

**Suman Kalyan Das
Roll No. 11612228**



**Department of Chemistry
Indian Institute of Technology Guwahati
Guwahati 781039
February 2017**



Dedicated to

My

Parents

INDIAN INSTITUTE OF TECHNOLOGY, GUWAHATI
Department of Chemistry



DECLARATION

I do hereby declare that the research work embodied in this thesis entitled
“Design, Synthesis and Studies on the Photophysical Properties of Unnatural C-Triazolyl, Aryl Fused N-triazolyl Nucleosides and Hybridization Accompanying FRET Event in Another Set of Chimeric DNA Duplexes” has been carried out by me under the supervision of **Dr. Subhendu Sekhar Bag** in the Department of Chemistry, Indian Institute of Technology Guwahati, India.

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

IIT Guwahati
February, 2017.

Suman Kalyan Das

Dr. Subhendu Sekhar Bag, Ph.D.
Associate Professor
Department of Chemistry
Indian Institute of Technology
Guwahati -781039
Assam, INDIA



Ph: +91-361-258-2324 (O)

Ph: +91-361-258-4324 (R)

Fax: +91-361-258-2349

E-mail: ssbag75@yahoo.co.in
ssbag75@iitg.ernet.in

CERTIFICATE

This is to certify that the research work presented in this thesis entitled
*“Design, Synthesis and Studies on the Photophysical Properties of
Unnatural C-Triazolyl, Aryl Fused N-triazolyl Nucleosides and
Hybridization Accompanying FRET Event in Another Set of Chimeric
DNA Duplexes”* is an authentic record of the results obtained from the
research work carried out by **Mr. Suman Kalyan Das** under my supervision in
the Department of Chemistry, Indian Institute of technology Guwahati, India.
This work is original and has not been submitted elsewhere for a degree or
award.

IIT Guwahati
February, 2017

Dr. Subhendu Sekhar Bag
(Thesis Supervisor)

ACKNOWLEDGEMENT

It is with high regards and profound respect that I express a deep sense of sincere gratitude to my supervisor **Dr. Subhendu Sekhar Bag** for his stimulating guidance, precious constructive suggestions and decisive insights during the entire course of my research work.

I would like to thank my Doctoral Committee members, **Prof. Tharmalingam Punniyamurthy** (chairman), **Dr. Chandan Mukherjee** (member) and **Dr. Biplab Bose** (member) for their intellectual input, encouragement, valuable suggestions and comments during the entire course of my research work.

I wish to thank my lab mates Rajen da, Sangita Di, Subhashis da, Afsana, Manoj, Suranjan, and Hiranya for their cooperation, support and pleasant company throughout my research work. Without their help it would have been impossible to complete my research work.

I would like to acknowledge Harikrishna, Samir, Himansu da and Babulal da who have indirectly helped me during the entire course of my research work. Special thanks to Babulal da for his help in collecting XRD data. Thanks to all of my friends, juniors and seniors whom I met during my past five years of life for their help.

Finally, I owe success to my parents (Uday Sankar Das and Nayantara Das) who have been a constant source of inspiration to carry out my career. I wish to thank them for giving me the freedom to pursue a career path of my choice and their constant support and encouragement in realizing my dreams.

I would like to acknowledge the Department of Chemistry, IIT Guwahati and Council of Scientific & Industrial Research (CSIR) for giving me the opportunity to carry out my research work.

Suman Kalyan Das

Suman Kalyan Das

Present Address:

C/O: Dr. Subhendu Sekhar Bag
Department of Chemistry
Indian Institute of Technology Guwahati
Guwahati – 781039, Assam, India
Phone: +91 361 2582324
E mail: sk.das@iitg.ernet.in
sumanchem1989@gmail.com

Permanent Address:

Vill – Dwarhatta
P. O. – Dwarhatta
Dist. – Hooghly
Pin – 712403
West Bengal, India
Mobile: +91 8011923694

Area of Interest

Design and synthesis of unnatural nucleosides for chemical and biochemical application.

Education:

- | | |
|------|---|
| 2017 | Ph. D. [Thesis submitted (February)] |
| 2011 | Master of Science (<i>in Organic Chemistry</i>)
University of Calcutta, West Bengal |
| 2009 | Bachelor of Science (<i>Chemistry Hons.</i>)
University of Calcutta, West Bengal |

Honors/Awards:

- ❖ Junior Research Fellowship and Eligibility for Lectureship (**CSIR-JRF-NET**), Dec– 2010, awarded by Council of Scientific & Industrial Research and University Grants Commission, India.

List of Publications

1. •Hybridization Accompanying FRET Event in Labeled Natural Nucleoside-Unnatural Nucleoside Containing Chimeric DNA Duplexes • Bag*, Subhendu Sekhar; **Das, Suman Kalyan**; Pradhan, Monoj Kumar; Jana, Subhashis • *J. Photochem. Photobiol., B: Biology* **2016**, 162, 669-673.
2. •Donor/acceptor chromophores-decorated triazolyl unnatural nucleosides: synthesis, photophysical properties and study of interaction with BSA • Bag*,

Subhendu Sekhar; Talukdar, Sangita; **Das, Suman Kalyan**; Pradhan, Manoj Kumar; Mukherjee, Soumen • *Org. Biomol. Chem.* **2016**, *14*, 5088-5108.

3. • **Design and Synthesis of Triazolyl-Donor/Acceptor Unnatural Nucleotides and Oligonucleotides** • Bag*, Subhendu Sekhar; Talukdar, Sangita; **Das, Suman K** • *Current Protocols in Nucleic Acids Chemistry* **2014**, *58*:1.32.1-1.32.27.
4. • **Design, Synthesis and Photophysical Property of a Doubly Widened Fused-Triazolyl-Phenanthrene Unnatural Nucleoside** • Bag*, Subhendu Sekhar; **Das, Suman Kalyan**; *Chemistry select* **2017**, *2*, 3577 – 3583.
5. • **Fused Triazolyl-Quinolinone Nucleoside as Widened DNA Base with Two Possible H-Bonds: Design, Synthesis, Photophysical Property and Theoretical Study** • Bag*, Subhendu Sekhar; **Das, Suman Kalyan** (Communicated).
6. • **Triazolyl-Donor/Acceptor Chomophores Decorated C-Unnatural Nucleosides: Synthesis, and Photophysical Properties** • Bag*, Subhendu Sekhar; **Das, Suman Kalyan** (Communicated).

Article 2 and 3 are not included in the thesis.



ABSTRACT

DNA is responsible for encoding the complex information necessary for life. However, it is striking to consider that nucleic acids accomplish these tasks using only four nucleobases. It was the question why nucleic acids use only four bases to have only two pair of base-pairs that motivated scientists to redesign the genetic alphabets of DNA and RNA with possible enhanced functional ability for several novel biotechnological and material science applications. The first work on the concept of orthogonal base pairing between isoG and isoC by Professor Alex Rich in 1962 greatly inspired Prof. Steven A. Benner in the late 1980's, to expand the genetic alphabet from four to six letters. Orthogonal H-bonding complementarity was the main strategy adopted by Benner's group. An increasing amount of research works has resulted in the acceleration of progress towards expanding the genetic alphabets via the synthesis of N-as well as C-nucleosides.

Later on, Kool et al., have considered the creation of non-H-bonding unnatural nucleobase surrogates that opened a new dimension in the design of hydrophobic unnatural DNA base analogues. Thus, the expansion of the genetic alphabets by Kool group has dramatically improved the functional potential of DNA. Much efforts have been put forth to develop non-natural, stable, hydrophobic nucleosides both with C-and N-glycosidic linkages. Many of such hydrophobic base pairs were recognized by polymerase efficiently creating an increased numbers of genetic alphabets. Recently developed size expanded DNA bases as well as widened DNA by Kool et al. again has impacted greatly in the field of design of more of such hydrophobic as well as H-bonded nucleosides.

Because of lack of fluorescence photophysical property and harnessing for the newer and efficient nucleosides, the design of unnatural DNA base pairs with tuned charge transfer/photophysical properties is currently an attractive research field towards the development of nucleic acid based diagnostics and sensing materials. Toward this end, several unnatural C- and N-nucleosides have appeared in the literature for the development of functional nucleic acids. However, the rational design of widened non-hydrogen bonding as well as H-bonding and simple hydrophobic nucleobases is a challenging task.

Therefore, this thesis entitled **“DESIGN, SYNTHESIS AND STUDIES ON THE PHOTOPHYSICAL PROPERTIES OF UNNATURAL C-TRIAZOLYL, ARYL FUSED N-TRIAZOLYL NUCLEOSIDES AND HYBRIDISATION ACCOMPAYNING FRET EVENT IN ANOTHER SET OF CHIMERIC DNA DUPLEXES”** includes **5 chapters**.

Chapter 1: Application of Unnatural C- and N-Nucleosides: A Review.

Chapter 2: Synthesis, Photophysical Property of Triazolyl C-nucleoside and Study of Interaction with Protein BSA.

Chapter 3: Design, Synthesis and Study of Photophysical Properties of Fused Triazolyl Phenanthrene Nucleoside.

Chapter 4: Design, Synthesis and Photophysical Properties of Fused Triazolyl 2-Quinolinone Nucleoside.

Chapter 5: Hybridization Accompanying FRET Event in Labeled Natural Nucleoside-Unnatural Nucleoside Containing Chimeric DNA Duplexes.

In short **Chapter 1** is a review on the synthesis of unnatural C-nucleosides. **Chapter 2** deals with the cobalt catalyzed conversion of 1'- α -ethynyl sugar to 1'- β -ethynyl sugar followed by the synthesis of C-triazolyl- β -nucleosides ${}^{\text{CT}}\text{BD}_{\text{o}}/\text{Ac}$ and their photophysical study. In **Chapter 3**, the synthesis of an angularly expanded compact hydrophobic Fused triazolyl phenanthrene unnatural nucleoside **FTPhen** and its photophysical properties are described. In addition, its DNA duplex stabilization within a trimeric DNA duplex against all the natural nucleobases was explored theoretically. **Chapter 4** explains the synthesis of an angularly expanded fused triazolyl nucleoside **FTQuon** which retains a donor and an acceptor face to make hydrogen bonds with cytosine and thymine. Theoretical calculation suggested that this angularly expanded fused triazolyl nucleoside can act as a complementary nucleoside against cytosine and thymine. **Chapter 5** illustrates FRET event within a chimeric DNA duplex involving triazolyl phenanthrene labelled unnatural nucleoside ${}^{\text{TPhen}}\text{BD}_{\text{o}}$ as a FRET donor and oxopyrene ${}^{\text{OxoPy}}\text{U}$ or perylene ${}^{\text{Per}}\text{U}$ labelled natural nucleoside as a FRET acceptor.

Chapter 1: Application of Unnatural C- and N-Nucleosides: A Review

The revolutionary idea of Prof. Alex Rich to expand the genetic alphabet put a new insight towards exploring synthetic protocol of unnatural nucleoside creation. He discovered isoG and isoC which are the isosteres of G and C and both of them have the same ability to replicate Watson-Crick hydrogen bonds framework. Prof. Rich's idea for the expansion of DNA and RNA with new functionalities was then extended and successfully executed by Prof. Steven A. Benner. These unnatural isosteres were used in successful transcription with the involvement of ribosome into a protein with nonstandard amino acid which unwrapped a new era of genetic engineering involving the artificial creation of genetic alphabet. As all the natural nucleosides of DNA and RNA are N-nucleosides, so it was first started to explore the synthesis of N-nucleosides. Nonetheless most of the discovered synthetic protocols suffered from several drawbacks. The problem of α -anomerisation was solved by acid catalyzed α to β anomeric conversion. But the acid catalyzed anomeric conversion step suffers because of the labile anomeric C-N glycosidic bond cleavage. Apart from that N-glycosidic bond can also be cleaved enzymatically. To avoid such problem, the concept of C-nucleosides was introduced. C-nucleoside is much more stable than N-nucleoside and there are a lot of use of C-nucleosides in medicinal chemistry and chemical biology. Pseudouridine and Showdomycin are the naturally occurring C-nucleosides which are therapeutically active. There are two ways through which the C-nucleoside can be synthesized. One is the construction of nucleobase upon sugar and the another is the construction of sugar upon nucleobase. The first one is the frequently used concept includes the use of Wittig type reaction, cycloaddition etc. The second one includes a number of synthetic steps through which a precursor would be converted into desired sugar with the expected conformation. The addition of organometallic reagents like Grignard, organocadmium, organocopper added as a foreign

substance to a modified sugar moiety with an anomeric labile leaving group, is the other strategy to assist the construction of unnatural C-nucleoside (**Figure A1**).

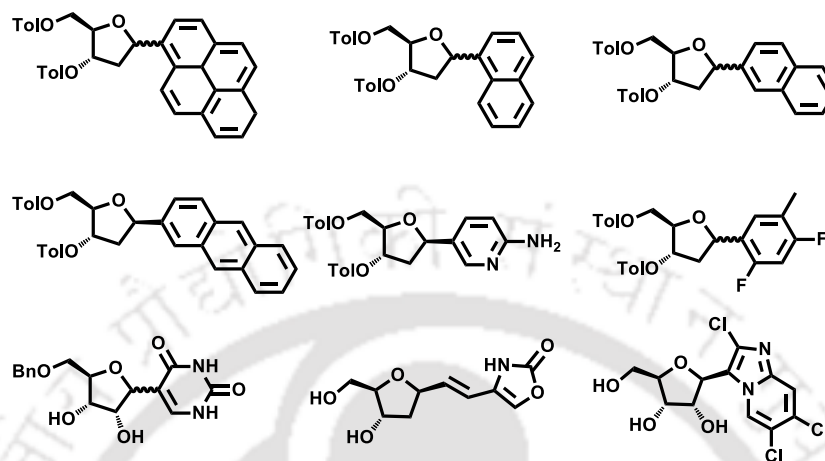


Figure A1: Examples of synthesized unnatural C-nucleoside.

Chapter 2: Synthesis, Photophysical Property of Triazolyl C-nucleoside and Study of Interaction with Protein BSA.

There are lot of methodologies available to synthesize C-nucleoside. Most of the protocols ended up giving α -anomer as a major product. So the formation of β -anomer is a major challenging area in the construction of C-nucleoside. We have chosen β -1'-ethynylriboside as a precursor to synthesize C-nucleoside. Although there are few protocols available to generate β -1'-ethynylriboside through the addition of ethynyl magnesium Grignard to the Hoffer's chloro sugar but all these protocols generate α -1'-ethynylriboside as a major product and the yield of β -1'-ethynylriboside is very low. Moreover, the α and β anomer of ethynylriboside are so close to each other that they are mostly inseparable in column chromatography. So these two problems were overcome by using $\text{Co}_2(\text{CO})_8$ catalyzed intramolecular Nicholas reaction. Cobalt complexation followed by oxidation produced β -1'-ethynyl riboside as a major product. 1,3-diaxial interaction is the driving force for the α to β anomeric conversion. After that Cu(I)- Et_3N or Cu(II)-TBTA catalyzed click reaction between different donor/acceptor azide and β -1'-ethynylriboside generated unnatural triazolyl donor-acceptor aromatic C-nucleosides (${}^{\text{cT}}\text{B}_{\text{Do/Ac}}$) within 30 minutes.

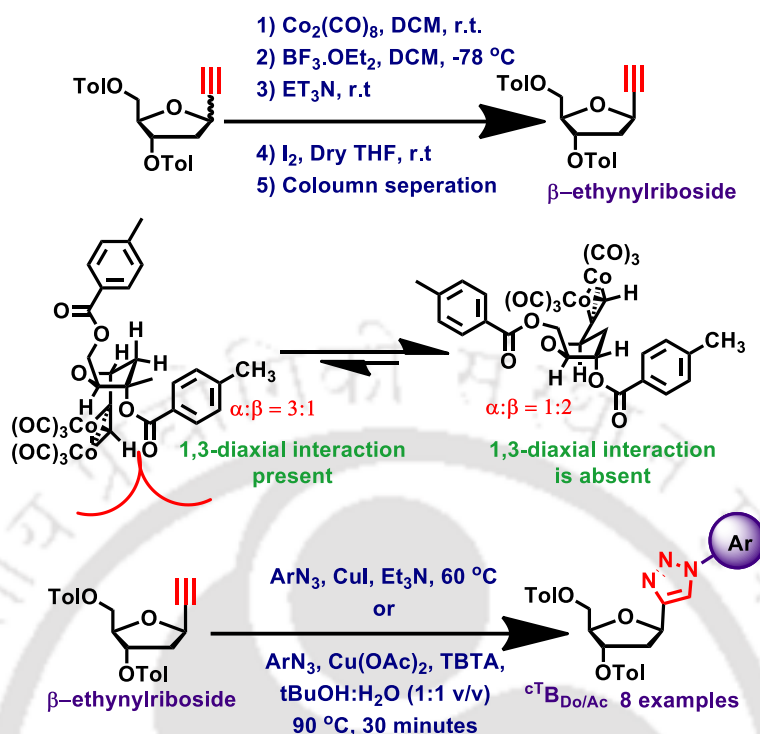


Figure A2: Schematic representation of the synthesis of β -ethynyl riboside and C-triazolyl ${}^{\text{c}}\text{TBD}_{\text{D}/\text{Ac}}$.

X-ray crystal structure of ${}^{\text{c}}\text{TDMB}_{\text{D}_0}$ suggested that the C-triazolyl nucleosides synthesized from β -1'-ethynyl riboside are β -riboside. Crystal structure of ${}^{\text{c}}\text{TDMB}_{\text{D}_0}$ also showed that the puckered conformation of sugar is C2'-endo/C3'-exo. Photophysical studies of these nucleosides revealed that unnatural C-nucleoside ${}^{\text{c}}\text{TAnth}_{\text{D}_0}$ and ${}^{\text{c}}\text{TPy}_{\text{D}_0}$ can act as a nucleoside based molecular probe. Dioxane-water titration revealed the highly solvatochromic nature of the unnatural C-nucleoside ${}^{\text{c}}\text{TDMB}_{\text{D}_0}$ and ${}^{\text{c}}\text{TBMA}_{\text{B}_{\text{Ac}}}$. Photophysical study of unnatural C-nucleoside ${}^{\text{c}}\text{TAnth}_{\text{D}_0}$ and ${}^{\text{c}}\text{TPy}_{\text{D}_0}$ with BSA suggested that the triazolyl anthracene and triazolyl pyrene probe entered into the hydrophobic pocket of BSA which was also confirmed from the docking study (**Figure A2**).

Chapter 3: Design, Synthesis and Photophysical Property of a Doubly Widened Fused- Triazolyl-Phenanthrene Unnatural Nucleoside.

We report herein the design and synthesis of a novel fused triazolyl phenanthrene nucleoside **FTPhen** as a new generation of doubly widened hydrophobic unnatural nucleobase surrogate. A retrosynthetic analysis suggested a click chemistry between β -azidosugar and 2-(iodoethynyl)-1,1'-biphenyl followed by Pd-catalysed C-C bond formation as the two key reactions to achieve our target nucleoside.

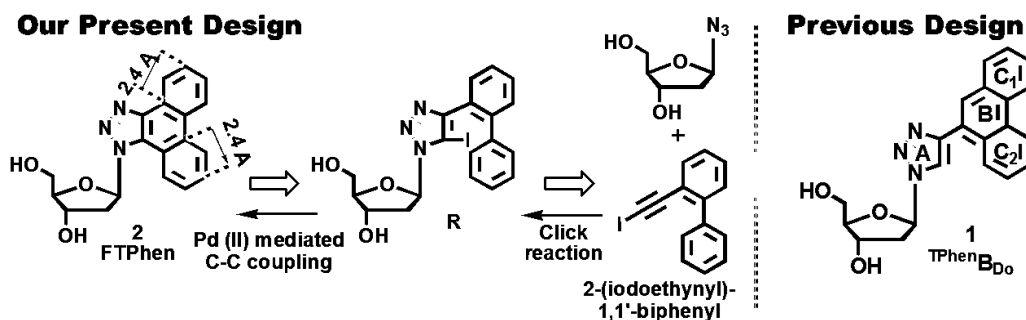


Figure A3: (a) The chronological approaches for the design of our present base, **FTPhe** from our earlier reported base, **TphenBDo**. (b) The chemical structures of **dA**, **dG**, **d(aza)G** and Kool's **dyA** bases.

FTPhe showed interesting photophysical property with chrysene type emission. Slightly blue shifted solvatochromicity was exhibited by the nucleoside as the solvent polarity increases. Molecular dynamics simulation using Schrodinger Macromodel revealed that regardless of the opposite natural base, all the DNA duplex containing the designed nucleoside, **FTPhe**, showed right-handed B-form helix with all residues having anti glycosidic bond and C2'-endo/C3'-exo sugar conformations. Helical rise values 3.3–3.5 Å are close to those observed in B-form natural DNA (3.4 Å), with slight deviations at and near the purine bases. A theoretical (DFT) study was carried out to know the stacking propensity in the context of DNA which revealed a good stabilizing property of the designed base via pairing with natural bases in a trimeric DNA duplex if incorporated (**Figure A3**).

Chapter 4: Design, synthesis and photophysical properties of Fused triazolyl 2-Quinolinone nucleoside.

This chapter deals with the synthesis of fused triazolyl 2-Quinolinone (**FTQuon**) nucleoside. **FTQuon** is an angular hydrophobic expansion of 8-azaguanosine. It is designed by replacing one hydrogen bond of 8-azaguanosine with a benzene ring. The synthesis of **FTQuon** was carried out by using click chemistry. The two precursors, β -1'-ethynylribose and N-(p-tolyl)propiolamide were allowed to react in click reaction condition where Cu(I) catalysed insitu ring cyclisation followed by subsequent benzyl and toluoyl deprotection led the desired **FTQuon**. β -configuration of **FTQuon** was established from NOESY spectra.

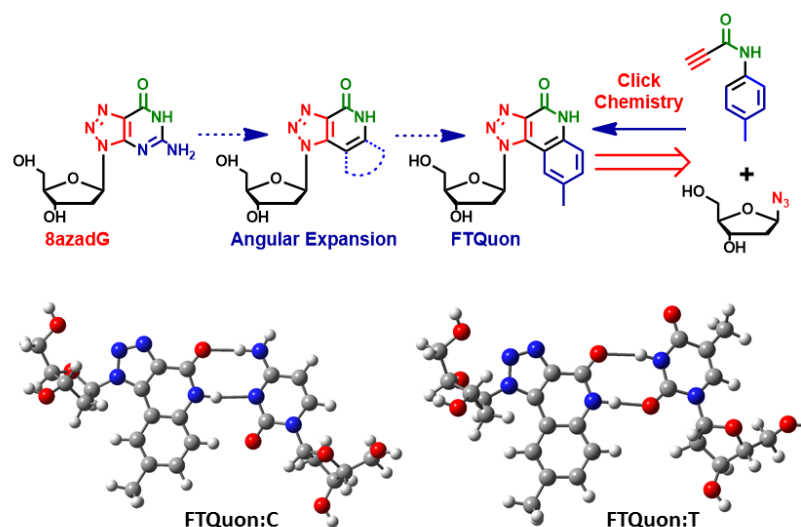


Figure A4: Design and synthesis of **FTQuon**. Theoretically established hydrogen bonds formed between **FTQuon:T** and **FTQuon:C** base pair.

Theoretical study suggested that **FTQuon** can form two hydrogen bonds with both cytosine and thymine. The interaction energy of the ONIOM optimised geometry of the trimeric duplex **AFTQuonA:TXT** ($X = T$ and C) and the distance of formed hydrogen bonds of **FTQuon** with cytosine and thymine within trimeric duplex suggested that **FTQuon** can be capable to act as a complementary base of both cytosine and thymine. Moreover, the interaction energy of **FTQuon:C** base pair is although lower than **G:C** pair but higher than **A:T** pair where **A:T** and **FTQuon:C** both pair have two hydrogen bonds. The higher interaction energy of **FTQuon:C** rather than **A:T** might be attributed by the angular hydrophobic expansion. Apart from that **FTQuon** showed interesting photophysical properties. Dioxane-water titration suggested a solvatochromic photophysical property of the base. Photophysical study and docking study both suggested its interaction with the hydrophobic pocket of BSA (**Figure A4**).

Chapter 5: Hybridization Accompanying FRET Event in Labeled Natural Nucleoside-Unnatural Nucleoside Containing Chimeric DNA Duplexes

Förster resonance energy transfer (FRET) is a highly efficient strategy in illuminating the structures, structural changes and dynamics of DNA, proteins and other biomolecules and thus is being widely utilized in studying such phenomena, in designing molecular/biomolecular probes for monitoring the hybridization event of two single stranded DNA to form duplex, in gene detection and in many other sensory applications in chemistry, biology and material sciences. Moreover, FRET can give information about the positional status of chromophores within the associated biomolecules with much more accuracy than other methods can yield.

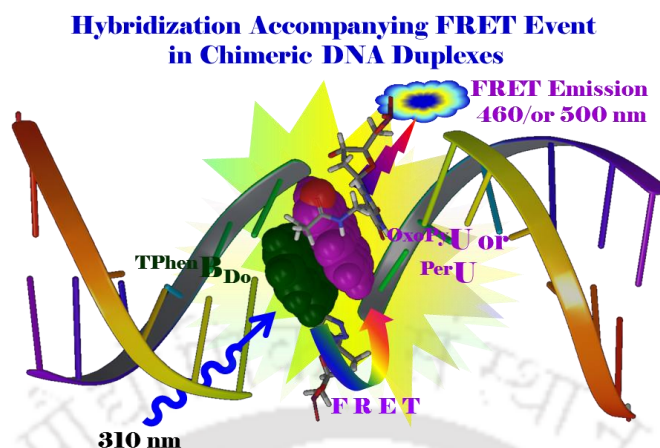


Figure A5: Hybridisation accompanying FRET event in chimeric DNA duplex involving TPhenBDo as a FRET donor and PerU or OxoPyU as a FRET acceptor.

Toward this end, we want to report here the ability of fluorescent unnatural nucleoside, triazolyphenanthrene (TPhenBDo) to show FRET interaction upon hybridization with fluorescently labeled natural nucleosides, OxoPyU or PerU , forming two stable chimeric DNA duplexes. The pairing selectivity and the thermal duplex stability of the chimeric duplexes are higher than any of the duplexes with natural nucleoside formed. The hybridization results in a Förster resonance energy transfer (FRET) from donor triazolyphenanthrene of TPhenBDo to acceptor oxopyrene of OxoPyU and/or to perylene chromophore of PerU , respectively, in two chimeric DNA duplexes. Therefore, we have established the FRET process in two chimeric DNA duplexes wherein a fluorescently labeled natural nucleoside (OxoPyU or PerU) paired against an unnatural nucleoside (TPhenBDo) without sacrificing the duplex stability and B-DNA conformation. The hybridization accompanying FRET event in these classes of interacting fluorophores is new. Moreover, there is no report of such designed system of chimeric DNA duplex. Our observed phenomenon and the design can potentially be exploited in designing more of such efficient FRET pairs for useful application in the detection and analysis of biomolecular interactions and in material science application (**Figure A5**).

List of Abbreviations

A	Adenine
Ac	Acyl
Ar	Aryl
Abs	Absorbance
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
Bn	Benzyl
B3LYP	Becke, three-parameter, Lee-Yang-Parr
BDF	Base-discriminating fluorescent
C	Cytosine
$cT_{B_{Do}/Ac}$	C-triazolyl donor/acceptor nucleoside
$cTNDB_{Do}$	C-triazolyl N,N-dimethyl benzene donor nucleoside
$cTDMB_{Do}$	C-triazolyl dimethoxy benzene donor nucleoside
$cTNap_{Do}$	C-triazolyl naphthalene donor nucleoside
$cTAnth_{Do}$	C-triazolyl anthracene donor nucleoside
$cTPy_{Do}$	C-triazolyl pyrene donor nucleoside
$cTNB_{Ac}$	C-triazolyl nitrobenzene acceptor nucleoside
$cTBMA_{Ac}$	C-triazolyl methyl acetate acceptor nucleoside
CD	Circular Dichroism
CT	Charge Transfer
Cu	Copper
CuI	Copper iodide
Cyhex	Cyclohexane
CHCl ₃	Chloroform
CuSO ₄	Copper sulfate
CH ₃ CN	Acetonitrile

Diox	1,4-Dioxane
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DFT	Density functional theory
DCM	Dichloromethane
DIPEA	N,N-Diisopropyl ethyl amine
DMAP	N, N-Dimethylamino pyridine
Et ₃ N	Triethylamine
EtOAc	Ethylacetate
EtOH	Ethanol
eV	Electron volt
EDC.HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide-hydrochloride
Eqv.	Equivalent
Fl	Fluorescence
FTPhen	Fused triazolyl phenanthrene nucleoside
FTQuon	Fused Triazolyl 2-Quinolinone Nucleoside
FRET	Fluorescence Resonance Energy Transfer
G	Guanine
GNPs	Gold nano particles
HOBT	1-hydroxy-benzotriazole
HRMS	High Resolution Mass Spectroscopy
HOMO	Highest Occupied Molecular Orbital
ICT	Intramolecular Charge Transfer
IR	Infrared Spectroscopy
KOH	Potassium hydroxide

K ₂ CO ₃	Potassium carbonate
k	Rate Constant
LUMO	Lowest Unoccupied Molecular Orbital
Max	Maxima
MeOH	Methanol
mM	Mili molar
m.p.	Melting point
MALDI-ToF	Matrix Assisted Laser Desorption Ionization-Time of Flight
Na	Sodium
Na ₂ CO ₃	Sodium carbonate
NMR	Nuclear Magnetic Resonance
nM	Nano molar
nm	Nanometer
ODN	Oligodeoxyribonucleotide
ORTEP	Oak Ridge Thermal Ellipsoid Plot
OLED	Organic light emitting diodes
O ₂	Oxygen
OxoPyU	Oxopyrene uridine nucleoside
Py	Pyrene
Per	Perylene
PerU	Perylene uridine nucleoside
PNA	Protein nucleic acid
PCR	Polymerase chain reaction
PPh ₃	Triphenyl phosphine
Pd	Palladium
Ph	Phenyl
ppm	Parts per million

r.t.	Room temperature
RNA	Ribonucleic acid
SNP	Single Nucleotide Polymorphism
ss	Single strand
T	Thymine
TBAF	Tetrabutylammonium fluoride
TFE	Trifluoroethanol
TMV	Tobacco mosaic virus
TLC	Thin layer chromatography
THF	Tetrahydrofuran
TDDFT	Time dependent density functional theory
TEA	Triethyl amine
TFA	Trifluoroacetic Acid
T_m	Thermal melting temperature of DNA
TMS	Trimethylsilyl
TLC	Thin Layer Chromatography
UV	Ultra violet
μM	Micro molar
Wt	Water
Φ	Quantum Yield
ϵ	Molar extinction co-efficient
τ	Decay time
$\text{\AA}$	Angstrom (10^{-8}cm)
$\tilde{\nu}$	Wave Number
λ	Wave Length
λ_{max}^{abs}	Absorption maxima
λ_{max}^{fl}	Fluorescence maxima

Δf	Solvent polarity parameter
μ_e	Excited state dipole moment
μ_g	Ground state dipole moment

NMR Data

δ	Chemical shift in NMR
s	singlet
d	doublet
t	triplet
q	quartet
m	multiplet
bs	broad singlet
dd	double doublet
dt	doublet of triplet
ddd	doublet of doublet of doublet
J	coupling constant in Hz

CONTENTS

Page No.

CHAPTER 1: APPLICATION OF UNNATURAL C- AND N-NUCLEOSIDES: A REVIEW 1-46

1.1. Introduction	1-2
1.2. Top down and Bottom up approach: A journey towards extension of genetic alphabet	3-5
1.3. Introduction of C-nucleoside	5-6
1.4. The need of C-Nucleosides in the area of chemical biology	6-8
1.4.1. Extension of genetic alphabet	6-7
1.4.2. Additional applications of artificially synthesized C-nucleoside in chemical biology	7-8
1.5. Role of C-nucleoside in Medicinal Chemistry	8-12
1.5.1. Naturally occurring C-nucleoside	8
1.5.2. Mechanism and biological activity of C-nucleoside	8-9
1.5.2.1. Nucleolytic enzymes inhibitors	9-11
1.5.2.2. Oxidoreductase inhibitor	11-12
1.6. Synthesis of artificial C-nucleoside	13-34
1.6.1. Construction and modification of aglycon upon carbohydrate	13-25
1.6.1.1. Modification through the insertion of nitrile group followed by heterocyclizations	13-18
1.6.1.2. Modification through Wittig type reaction	18-20
1.6.1.3. Modification through cycloaddition	20-25
1.6.2. Construction of ribose ring upon aglycon	25-26
1.6.3. Use of organometallic reagents in construction of C-nucleoside analogues	27-33
1.6.3.1. Organolithium and organomagnesium mediated nucleophilic attack to the anomeric centre	27-28

1.6.3.2.Triarylaluminum mediated nucleophilic coupling with 1,2-dihydrofuran	28-29
1.6.3.3.Nucleophilic organometallic addition to sugar halogenose	29-30
1.6.3.4.Heck mediated C-nucleoside formation	30-32
1.6.3.5.Lewis acid mediated C-nucleoside formation	32-33
1.6.4. Nucleoside modification	33-34
1.7. Conclusion	34
1.8. Reference	35-46

CHAPTER 2: SYNTHESIS, PHOTOPHYSICAL PROPERTY OF TRIAZOLYL C-NUCLEOSIDE AND STUDY OF INTERACTION WITH PROTEIN BSA

2.1. Introduction	47-48
2.2. Click chemistry in the modification of nucleosides	48-54
2.3. Synthesis of 1-ethynylriboside	55-56
2.4. Synthesis of different C-nucleoside analogues starting from 1-ethynyl riboside	57-62
2.5. Nicholas reaction	62-64
2.6. Intramolecular Nicholas reaction and β -selectivity	64-66
2.7. Background	67
2.8. Objective	67-68
2.9. Results and discussion	68-88
2.9.1. Synthesis of β -1-ethynylriboside via intramolecular Nicholas reaction and the synthesis of triazolyl nucleoside derivatives.	68-70
2.9.2. Structural elucidation by Single crystal analysis	71
2.9.3. 1,3-diaxial interaction triggering 2.84 α to 2.84 β conversion	72
2.9.4. Study of photophysical properties	72-80

2.9.5. Theoretical Calculation	80-83
2.9.6. Study of interaction of unnatural C-triazolyl nucleoside 2.81D (^c TBMA ^B Ac) and 2.81E (^c TPy ^B Do) with BSA Protein.	83-88
2.10. Conclusion	89
2.11. Experimental section	89-97
2.12. ¹ H and ¹³ C NMR spectra of selected nucleosides	98-103
2.13. Reference	104-108
CHAPTER 3: DESIGN, SYNTHESIS AND STUDY OF PHOTOPHYSICAL PROPERTIES OF FUSED TRIAZOLYL PHENANTHRENE NUCLEOSIDE	109-152
3.1. Introduction	109
3.2. Hydrophobic base analogues	109-117
3.3. Background	117
3.4. Objective	118
3.5. Results and discussion	118-128
3.5.1. Design and synthesis	118-121
3.5.2. Crystallography	121-122
3.5.3. Space-filling model	122
3.5.4. Photophysical properties	123-124
3.5.5. TDDFT calculation of FTPhen	124
3.5.6. Macromodel calculation	125-126
3.5.7. DFT calculation in the context of DNA	126-127
3.5.8. ONIOM calculation in the context of DNA	127-128
3.6. Conclusion	129
3.7. Experimental section	129-132
3.7.1. General experimental	129
3.7.2. Characterization	129-132
3.8. Studies on Photophysical Properties of the Nucleoside	132

3.9. Representative coordinates	133-140
3.10. ^1H and ^{13}C NMR Spectra	141-148
3.11. Reference	149-152
CHAPTER 4: DESIGN, SYNTHESIS AND PHOTOPHYSICAL PROPERTIES OF FUSED TRIAZOLYL 2-QUINOLINONE NUCLEOSIDE	153-191
4.1. Introduction	153
4.2. Extension of genetic alphabet	153-155
4.3. Size expanded DNA	155-160
4.3.1. Benzo fused size expanded DNA bases: xDNA and yDNA	155-157
4.3.2. Naptho fused size expanded DNA Bases	158
4.3.3. Ethynyl extended DNA bases: Inoyue's base pair	158-159
4.3.4. Unnatural base pairs with four hydrogen bonding	159
4.3.5. 8azaG:C pair	160
4.4. Background	160
4.5. Objective	160-161
4.6. Results and Discussion	161-173
4.6.1. Design and synthesis of fused triazolyl 2-quinolinone (FTQuon)	161-162
4.6.2. Structural Characterization	163
4.6.3. Study of photophysical properties	163-165
4.6.4. Macromodel Study in the context of DNA	165-166
4.6.5. DFT Calculation in the context of DNA	167-168
4.6.6. QM/MM calculation in the context of DNA	169-170
4.6.7. Study of interaction with BSA	170-173
4.7. Conclusion	174
4.8. Experimental section	174-185
4.8.1. General experimental	174

4.8.2. Synthesis and characterization	174-177
4.8.3. Study of Photophysical property of FTQuon	177
4.8.4. Studies On the Interaction of FTQuon with BSA	177-178
4.8.5. Cartesian coordinates of ONIOM optimized trimer	178-185
4.9. ^1H , ^{13}C NMR and NOESY spectra	186-189
4.10. Reference	190-191

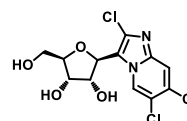
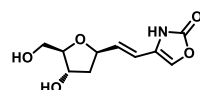
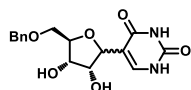
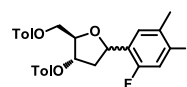
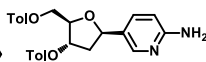
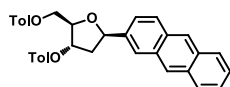
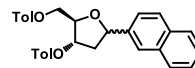
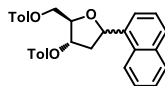
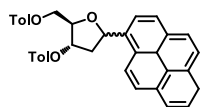
CHAPTER 5: HYBRIDISATION ACCOMPANYING FRET EVENT IN LABELED NATURAL NUCLEOSIDE-UNNATURAL NUCLEOSIDE CONTAINING CHIMERIC DNA DUPLEXES

5.1. Introduction	192-194
5.2. FRET based DNAs and their applications	194-198
5.3. Background	198-199
5.4. Objective	199-200
5.5. Results and discussion	200-218
5.5.1. Study of UV-visible and Fluorescence Photophysical Properties of Nucleoside Monomers.	200-201
5.5.2. Sequence of ODNs Used in This Study	202
5.5.3. Synthesis and Characterization of Modified Oligonucleotides (ODN 1-3)	202-204
5.5.4. Thermal Melting Temperature (T_m) Measurements of the Oligonucleotides.	204-205
5.5.5. UV-visible and Fluorescence Measurements of the Oligonucleotides.	205-208
5.5.6. Förster Resonance Energy Transfer	208-209
5.5.7. Study of Time Resolved Fluorescence of ODNs	209-210
5.5.8. Study of Time Resolved Fluorescence Anisotropy of ODNs	
5.5.9. Calculation of Förster Distance and the FRET Efficiency	211-212
5.5.10. Study of Circular Dichroism Spectroscopy of ODNs	212-213

5.5.11. Macromodel Calculation	214
5.5.11.1. Optimization of B-form DNA structure for the Duplexes	215-218
5.5.11.2. Conformational Search of Optimized B-form DNA structure for the Duplexes	215
5.5.11.3. Molecular Dynamics Simulation of Optimized B-form DNA structure for the Duplexes	216-217
	217-218
5.6. Conclusion	218-219
5.7. Material and methods	219-221
5.7.1. Procedure for oligonucleotide synthesis	219
5.7.2. Studies on photophysical properties of the oligonucleotides	219
5.7.2.1. UV-visible and thermal melting temperature (T_m) measurements of the oligonucleotides	219
5.7.2.2. Steady state fluorescence study of the oligonucleotides	219
5.7.2.3. Time resolved fluorescence and anisotropy decay study of the oligonucleotides	220
5.7.3. Study of circular dichroism spectroscopy of the oligonucleotides	220
5.7.4. Macromodel calculation	220
5.7.4.1. Conformational search of optimized B-form DNA structure for the duplexes	220
5.7.4.2. Molecular dynamics simulation of optimized B-form DNA structures for the duplexes	221
5.8. Reference	222-224
SUMMARY AND OUTLOOK	225-227

Chapter 1

APPLICATIONS OF UNNATURAL C- AND N-NUCLEOSIDES: A REVIEW



1.1. Introduction

DNA is the key genetic macromolecule which is responsible in carrying all the hereditary information into the chromosomes. The discovery of the structure of DNA was pioneered by Watson and Crick.¹ DNA double helix is a nucleotide polymer where two single strand nucleotide chains are holding together by hydrogen bonding and π stacking interaction and thus forming double strand DNA helix (**Figure 1.1**). π stacking and hydrogen bonding (**Figure 1.2**) are the two fundamental factors contributing to the thermodynamic stability of the DNA.^{1,2} The backbone of DNA is a phosphodiester chain and the four natural nucleobases A, T, G and C are connected with each other with this phosphodiester backbone (**Figure 1.3**). Natural nucleobases are flat hydrophobic moiety, stacked over each other and roughly perpendicular to the axis of DNA helix. The intrastrand stacking distance between nucleobases is 3.4 Å.

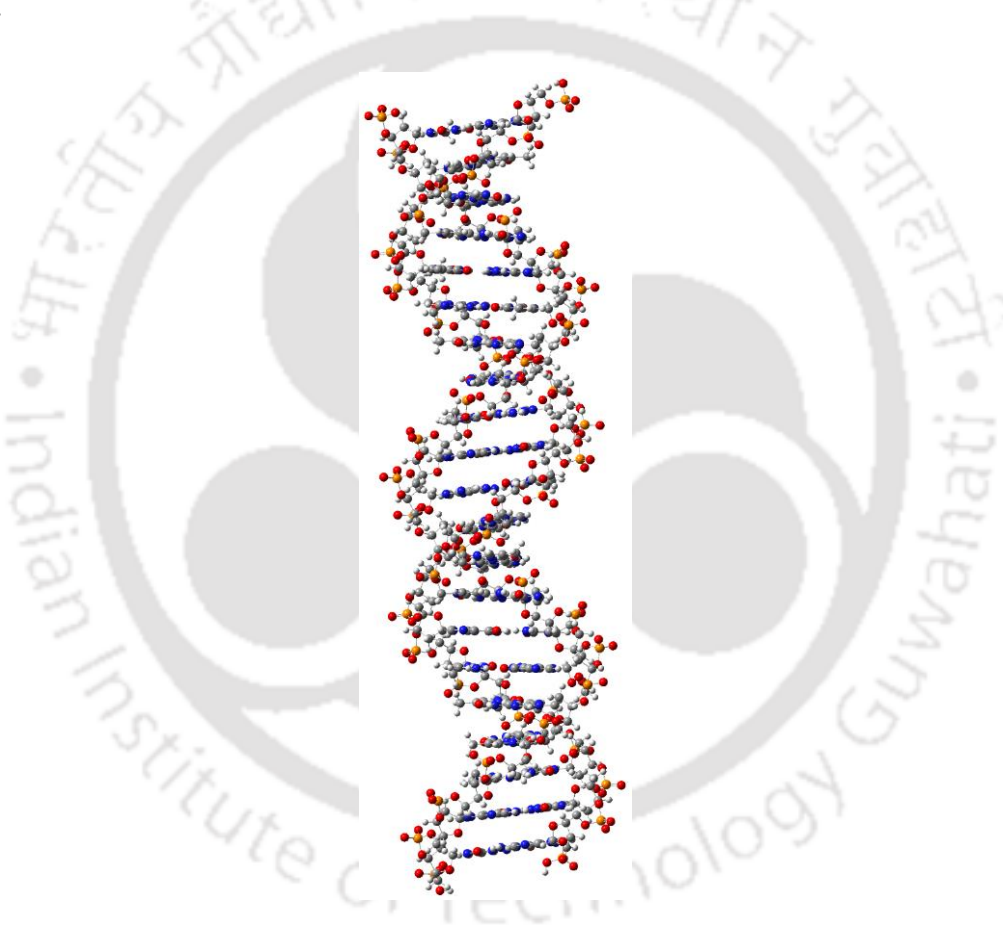


Figure 1.1: B DNA, most common double helical structure found in nature.

Hydrogen bonding and π stacking interactions among the aromatic nucleobases play the major role in determining the structure, function, dynamic behaviour and biological function of DNA. Possible modification of these two forces i.e. π stacking and hydrogen bonding can lead us accessing new information about DNA helix. We chemist are privileged, having the undue advantage of dealing the constitution of DNA in a molecular label. Therefore, efforts have been undertaken by the chemists for modifying the Purine and pyrimidine bases

or full replacement of the bases with a new design. Thus chemically synthesised N-nucleoside with high π -stacking propensity may differ in shape and size, also may provide different pattern of hydrogen bonding inside a DNA helix. In consequence these modifications would ultimately be the part of study of expansion of genetic alphabets.

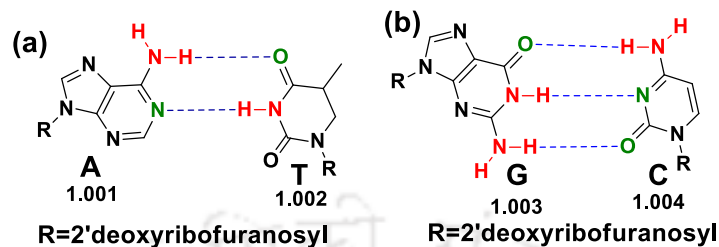


Figure 1.2: (a) A-T hydrogen bonding; (b) G-C hydrogen bonding.

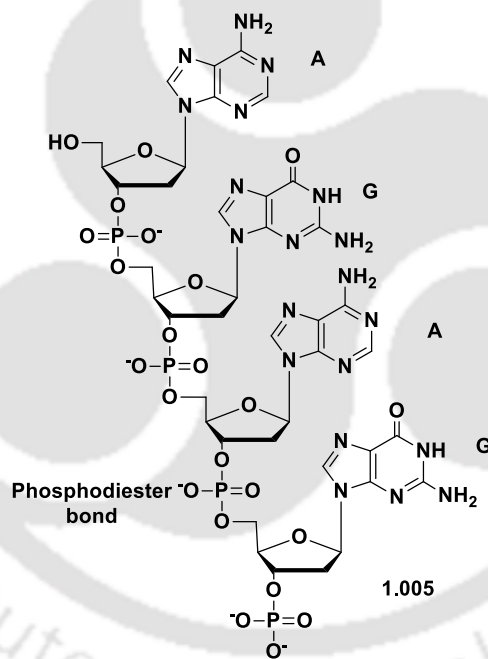


Figure 1.3: A, G, A and G are sequentially connected with phosphodiester backbone.

The incorporation of these unnatural nucleoside into the DNA helix may give rise to distortion or stabilisation depending upon the shape and size of these nucleoside as well the orientation of these nucleoside into the helix. Because of the incorporation of new chemical entities, the modified DNA may differ in photophysical characteristics. A lot of modifications have been done on natural nucleoside and also unnatural nucleosides. Many synthetic methodologies have been developed to modify nucleobases.

1.2. Top down and Bottom up approach: A journey towards extension of genetic alphabet

Altering the hereditary information at the genomic level and study the effects in the living cells is the recent research target. Thus pioneering work by Zamecnik and Stephenson⁴ in the year 1970s about blocking the gene expression of Rous sarcoma virus (RSV) 35S RNA which was responsible for chicken cancer by utilising exogenous antisense oligonucleotide approach (AON) has led to a tremendous development of the field (**Figure 1.4**).⁴ Subsequent development of RNAi (Andrew Fire and Craig C. Mello shared the 2006 Nobel Prize for their work on RNA interference) (**Figure 1.4**) for the modulation of gene expression and an understanding about small interfering RNAs (siRNAs) and miRNA by Fire *et al.* in the year 1998⁵ and Elbashir *et al.*⁶ in the year 2001 which have changed the whole concept of controlling posttranscriptional pathway of gene expression leading to a new era of excitement in the nucleic acid community.

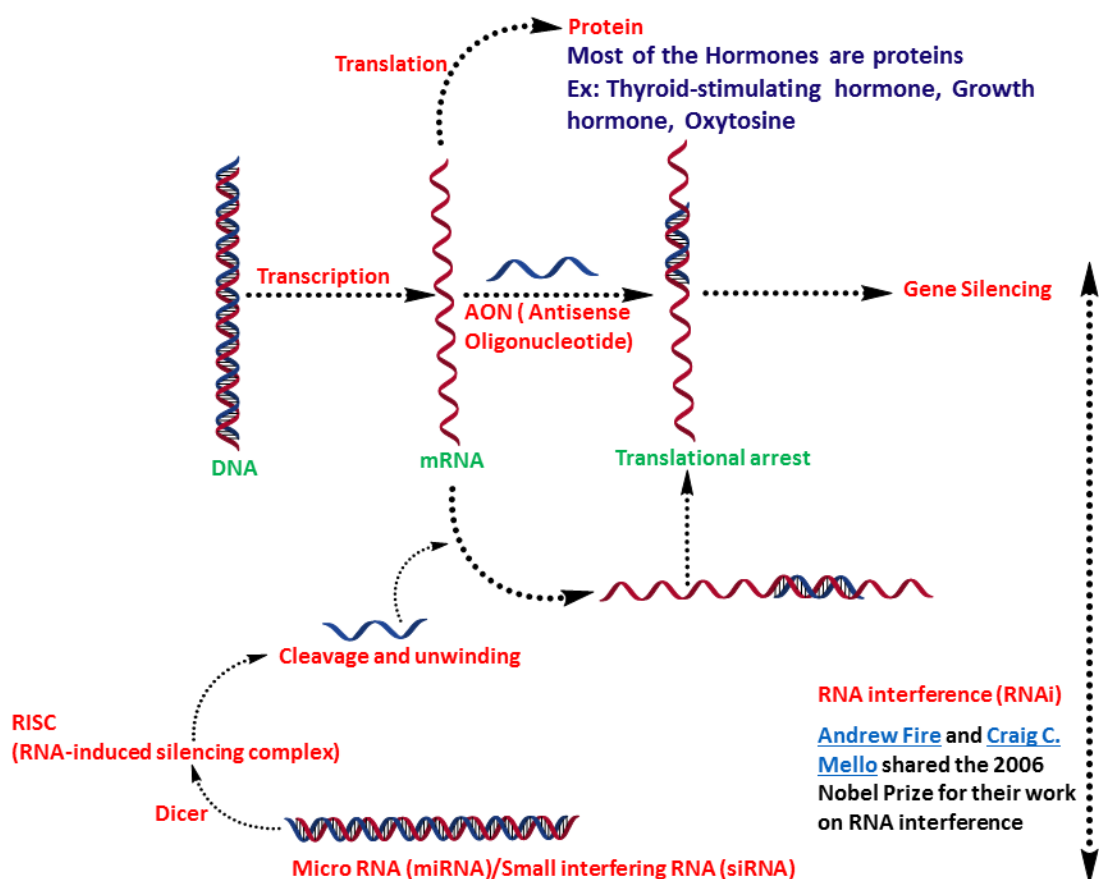


Figure 1.4: AON and RNAi mediated gene silencing.

Chapter 1

Apart from that RNAi has a huge contribution for altering the characteristic encoded to a specific gene of living crops. Like for example the development of blue rose that is hardly exist in the nature was created by suppressing cyanidin genes⁷. Today it is possible to prepare Banana Bract Mosaic Virus (BBrMV) free banana which is a challenge for the production of banana in India and south East Asia⁸. Today it is possible to make genetically engineered tobacco which is nicotine free⁹, coffee which is decaffeinated¹⁰. US is going to give the approval to use Arctic apple which is made by suppressing PPO (polyphenol oxidase) gene. PPO gene suppression saves apple to become brown after being sliced and it is possible because for genetically engineered apple where chlorogenic acid is not converted into quinone product^{11,12}. All these are becoming possible because of the development of technologies which have the access to handle the genetic information present in these crops and not only that today's technology is smart enough to make the variations in genetic level to make all these possible. RNAi (RNA interference) is the recent technology which is operating behind all these successes¹¹.

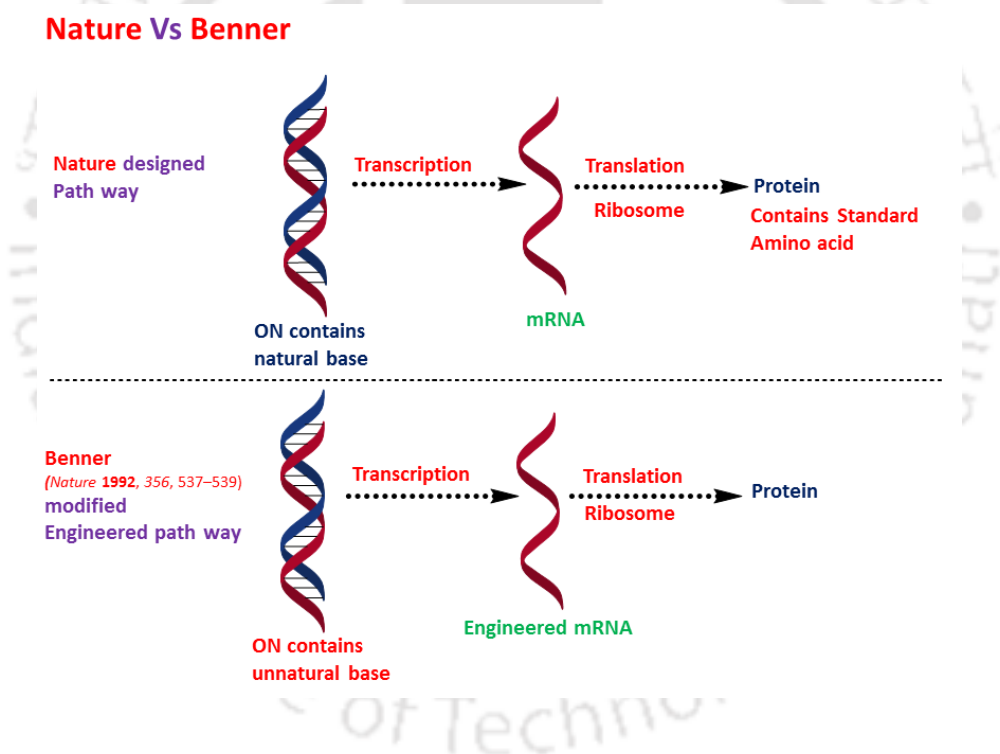


Figure 1.5: Benner designed nonstandard protein formation pathway.

Now all these new genetic expression or gene silencing are nothing but the exogenous supply of a short ODN synthesised from the natural base A, T, G and C. There are no other variations had been made by nature, is said to be known indeed. It's okay to make variation by altering the sequence of the genetic alphabet for new genomic expression leading to new characteristic within living species. But is it possible to come out from the restrained nature giving element and do something new to alter the gene expression for installing new features in a living species where nature will be unable to stream those information? It is the bottom up

approach of a chemist being able to alter the chemical information on a molecular level. There from the modification of the nucleobases have been started.

In the year 1992 the concept of 'protein containing nonstandard amino acid possible to be synthesised' was introduced by Benner¹³⁻¹⁸ where the synthesised unnatural base pairs are shown to be used as a codon-anticodon pair. They were introduced into short ODN. In-vitro replication of the synthesised unnatural base pairs by DNA polymerase make them extended as genetic alphabets and thus genetic alphabet was extended up to 6 letter from 4. This base pairs were able to transcribed correctly to form chemically engineered mRNA and further replication created protein which contains nonstandard amino acid where the versatilities of ribosome are offering new possibilities in protein engineering. This Benner's work was really astounding to think further to put a deep insight into the matter of genetic engineering through nonstandard nucleosides (**Figure 1.5**).

A DNA is a double nucleotide strand where two single strand ribonucleotide are twisting together in the form a regular double strand helix. Here from the structure of DNA it is clear that hydrogen bonding is playing the crucial role to hold the two single strand to form the helix. The incorporation of these pairs and their T_m measurement is revealing that clearly. But if we think further that, is the hydrogen bonding only factor to shape two single strand in the form of a double strand where a uniform helicity is maintaining throughout the DNA. To answer this question, it is essential to remove hydrogen bonding parameter to check whether the DAN is maintaining its helicity or it is perturbed. Now as the nucleobase in a single strand maintained a 3.4 Å uniform distance from each other and as they contain π -cloud there may be some π -interaction which uniformly maintaining helicity throughout the DNA. These curiosities trigger the scientific community to remove hydrogen bonding from nucleobase and introduce hydrophobicity to compensate the removed hydrogen bonding parameter to make the DNA double helix not perturbed from its general form.

Romesberg synthesized some unnatural base pairs. It has been found that they are forming stable DNA double helix. So with the incorporation of hydrophobic nucleobase which are handicapped in providing hydrogen bonding, DNA helicity is still maintaining with high fidelity representing that hydrogen bonding is not the only factor to shape DNA in a regular double helix rather π stacking contributing another major factor in shaping DNA in its inherent form. Moreover, PCR functioning^{19,20,21} of these synthesized unnatural base pairs give another insight into the further preparation of chemically engineered nucleobase hoping to be able to generate further chemically engineered mRNA and protein.

1.3. Introduction of C-nucleoside

Synthesis dealing with anomeric carbon centre present in the natural as well as synthetic N-nucleosides is difficult because of having a major drawback of acid catalysed chemical hydrolysis and enzymatic N-glycosidic bond cleavage leading to degradation of N-nucleosides.²² On the other hand, it is much easier to deal with C-analogues where the carbon-carbon bond provides stability against any enzymatic or acid catalysed hydrolysis.¹ Therefore synthesis of C-nucleoside has got much research interest in recent time. It has been found that an antibiotic, showdomycin^{23,27} and a uridine nucleoside analogue, pseudouridine³, where

glycosidic nitrogen has been replaced by carbon represents the most prevalent C-nucleosides over a couple of other nucleosides. Chemical synthesis makes it easier to strategically design and further develop an ample of C-analogues, having a huge importance in the field of medicinal chemistry as well as chemical biology.^{24-26,28-30} Inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitors are the most important C-nucleoside analogues, which are biologically active.³¹⁻³⁴ A number of C-nucleoside analogues have been designed and synthesised which when paired against natural nucleobases are found to show efficient DNA duplex stabilisation via π - π stacking interaction.

A considerable number of chemical strategies have been developed for synthesising newly modified unnatural synthetic nucleosides, which are widely used in the application of chemical biology.³²⁻³⁵ Among them, the construction of aglycon followed by nucleophilic addition to the carbohydrate moiety at the expense of some leaving group or unsaturation is the most important one.²²⁻⁴¹ Apart from that, addition of organometallic reagents,⁴²⁻⁴⁶ Wittig reaction,^{47-48,51} ozonolysis^{49,50} have been employed to the synthesis of C-nucleosides. For the last 15 years, several modifications have been carried out proficiently for the modification of C-nucleoside analogues, which find abundant application in different areas of chemistry and biotechnology. As for example in the field of medicinal chemistry C-nucleoside, analogues have been used as an antibiotic and cytotoxic material.^{2,6} In the field of chemical biology, C-nucleosides are used toward the extension of genetic alphabet.^{47,51} C-nucleosides having fluorescent nucleobases are also being utilised as a probe for studying structure, function and dynamics of DNA.

1.4. The need of C-Nucleosides in the area of chemical biology

The stability of the duplex DNA is mainly controlled by Watson-Crick hydrogen bonding where adenine makes two hydrogen bonds with Thymine and Guanine makes three hydrogen bonds with cytosine (**Figure 1.2**).¹ Moreover aromatic π - π stacking interactions among the natural DNA bases played a crucial role in stabilising the DNA duplex. Modification of the nucleoside has given us the chance to replace the natural nucleoside by artificially synthesised nucleoside. But instead of investigating the function and structure of the nucleoside that is artificially incorporated into the DNA sculpture, for the last couple of years it is rather drawing more attention towards achieving major goal like stability of the DNA duplex after modification with chemically synthesised nucleoside,⁴⁴ genetic alphabet extension⁴⁵ etc. Nucleoside analogues in the study of the enzyme mechanism, mainly for the enzymes or polymerases of nucleic acid metabolism is another important area of the utilization of synthetic C-nucleosides. Synthetic modification of the nucleosides producing the Carbon-Carbon bond providing extra stability for the nucleosides to be stable enough in comparison with the N-nucleoside analogues have been investigated extensively with promising examples in chemical biology.

1.4.1. Extension of Genetic alphabet

The increase in the number of genetic alphabet is a challenging task and attracted much the current research interest. By the addition of one additional base pair with the existing two base pairs could offer 216 different combination of codons of three letter could be indeed

capable of encoding proteins contain amino acids which are nonbiogenic and therefore cause unnatural properties. The key steps involved in the expansion of the number of genetic alphabet are considered to be the efficient replication with high fidelity. For that reason, the main efforts are involved in the development of a new 3rd base pair which will be replicated efficiently with a great fidelity.⁴⁶ Even now there is a lot of examples where a tremendous amount of effort has been paid to construct a number of designed nucleobases which supposed to provide a considerable amount of stability when incorporated into a DNA duplex.¹⁶⁻¹⁸ On contrary whenever the subject of interest is going to be the enzymatic replication, it is the toughest and challenging area of the science to be explored and that is why till date there are a very few number of artificially synthesised base pairs that have been replicated efficiently and selectively. There are a number of base pairs among the artificially synthesised base pairs which have been designed and synthesised on the basis of hydrogen bonding^{51-61,62-67}. In another way, the shape of the nucleosides have been designed in such a way that, it will be capable of complementing the shape of the natural nucleoside⁴⁸ as well as hydrophobic interactions.^{49,68-93} Metal coordinated base-pairs have also been developed.^{50,94-108}

1.4.2. Additional applications of artificially synthesized C-nucleoside in chemical biology

There are a handful of examples of C-nucleosides in different directions of chemical biology have been reported apart from the investigation in the selectivity and specificity of DNA polymerases. It was reported that the generation of a highly reactive carbene intermediate upon UV irradiation on trifluoromethyldiazirine-benzene-C-nucleoside in a dsDNA forms an interstrand cross-link.¹⁰⁹ For DNA cross linking with proteins a parallel approach was reported by Carell¹¹⁰ where cross linking was done in major groove by the attachment of a diazirine moiety to the 5-position of U of the DNA.

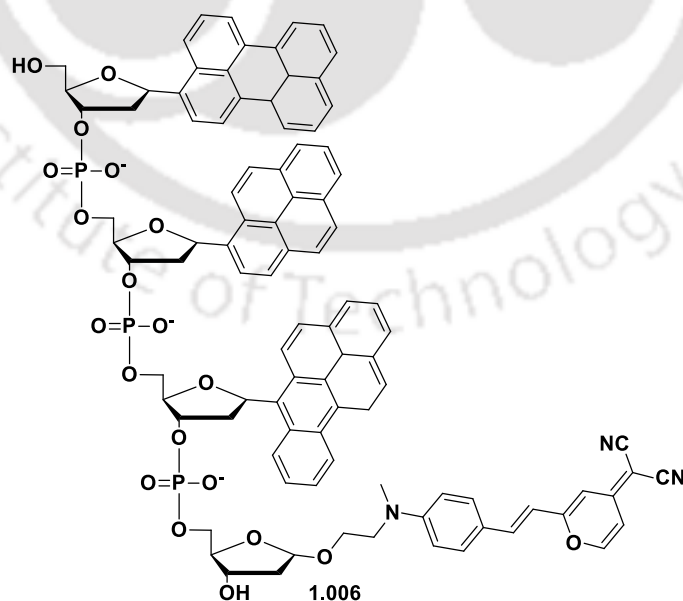


Figure 1.6: Structure of ODF tetramer.¹¹¹

This method might be useful as an essential tool in (1) observation of the interaction of artificial nucleic acids with proteins (2) Protein nucleic acid conjugation (3) Enzyme inhibition. Therefore it is desirable to think about the further growth of this field. A large number of α -aryl C-nucleoside analogues have been reported by Kool⁹⁸ could be termed as C- α -oligodeoxyfluoroside (ODFs). These ODFs could be easily synthesised by an ON-synthesiser with the help of combinatorial approach to develop a library of α -fluoroside which are able to cover the emission maxima within the whole visible region (**Figure 1.6**). Kool's thought was the close proximity of these aromatic fluorophore encouraged by the DNA duplex allow their excited state interaction might be able to produce exciplex, excimer, FRET, H-dimer etc.¹¹² Recently this technique is used in the purpose of staining of cell and zebra fish embryos by C-oligofluoroside to make them fluorescent.¹¹¹

1.5. Role of C-nucleoside in Medicinal Chemistry

1.5.1. Naturally occurring C-nucleoside

In most the rRNA and tRNA¹¹³ abundance of C-nucleoside Pseudouridine (Ψ **1.007**, **Figure 1.7**) is mostly noticeable where it is responsible to make the RNA duplex stable¹¹⁴. Pseudouridine synthases catalysed the formation of Pseudouridine by a unique method through post-translational isomerization within RNA.¹¹⁵ None of the analogues of Ψ **1.007** was reported to be therapeutically active but Showdomycin¹¹⁶ **1.008** to a bit similar with Ψ **1.008** was reported as a natural antibiotic as well as its cytotoxicity are known for decades. In the year 1979 the mechanism of Showdomycin that is how it does act, was fast revealed successfully.¹¹⁷ Here the cytotoxic action of Showdomycin which is an antibiotic C-nucleoside, privileged to be conferred with selectivity explained it to be endowed with alkylating property of its aglycon moiety, maleimide.

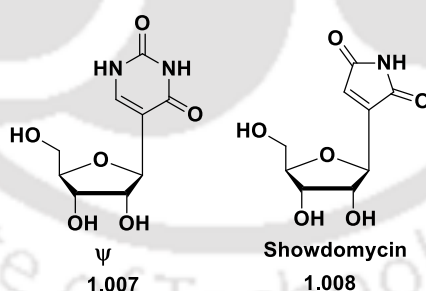


Figure 1.7: Structure of Ψ , a natural C-nucleoside and Showdomycin.

1.5.2. Mechanism and biological activity of C-nucleoside

Nucleic acid metabolism is a process involved in the synthesis and degradation of nucleic acid, DNA and RNA which are the polymers of nucleotide. Any kind of nucleoside might be natural or an artificially synthesised C-/N-nucleoside in principle could be involved as a chemically active target towards any enzymes in the process of nucleic acid metabolism. Both purine, a five-six fused heterocycle, and a pyrimidine, a six membered heterocycle are

connected with sugar moiety through a nitrogen atom. The privilege of C-nucleoside is the replacement of this nitrogen with carbon making the bond highly stable and inert towards hydrolysis and at the same time making this C-C bond resistant towards enzymatic cleavage. This idea of nitrogen heteroatom replacement by carbon is not only making the nucleoside resistant towards any kind of the chemical attack but this replacement dramatically changed the property of the heterocycle. This fact could be simply explained by taking an example where a pyrimidine base is replaced by pyridine or a purine base is replaced by pyrrolopyrimidine changed the heteroatom functionalities (OH, NH₂), acidobasic properties as well as tautomeric population of the responsible functionalities within the heterocycle. A long multistep synthesis of the carba analogue of strong anti-hepatitis C viral (HCV) agent 2'C-methyladenosine¹¹⁸, 9-deazaadenine-2'C-methylribonucleoside is found to be surprisingly completely inactive¹¹⁹ because of just the replacement of one nitrogen atom by carbon instead rest of the rings are intact as it is.

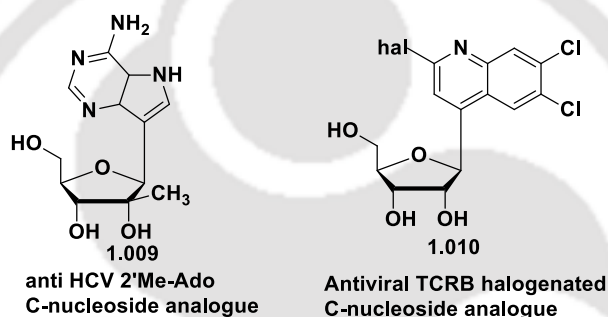


Figure 1.8: Biologically active C-nucleoside analogue.

The changed tautomeric behaviour of the heterocycle as well as the lack of basicity of nitrogen happens to be the reason of the astounding changed behaviour of this anti HCV agent. A range of halogenated C-nucleosides was developed by Townsend as an analogue of the TCRB which is an antiviral agent (**Figure 1.8**). Among these C-analogues a few of them are nonselective yet towards antiviral activity but happened to be cytotoxic in most the instances.

1.5.2.1. Nucleolytic Enzymes inhibitors

It has been known since 1960s that formycins particularly **FA 1.011**, **FB 1.012**, and oxoformycin B (**OFB**) **1.013**, (**Figure 1.9**) are the C-nucleosides were potent to show the properties of cytotoxicity as well as antibiotic activity. But their antiparasitic i.e. antischistosoma, antimalarial etc. types of activity were unravelled latter. As these are the C-nucleoside analogues of purine, made it difficult to comprehend the exact mechanism of action involved through several number of complex and at the same time difficult path ways.

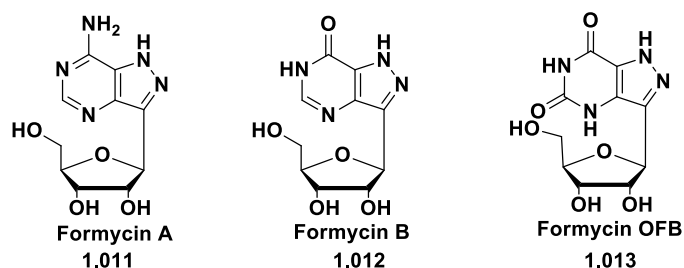


Figure 1.9: Structure of **FA**, **FB** and **FOFB**.

These C-nucleoside analogues are however proved¹²⁰ to be a potent resilient inhibitor of nucleosidases in addition with purine nucleoside phosphorylase (**PNP**) and these are found to be their major part of activity. It was a success in physical organic chemistry of the finding of immucillins which is a completely new PNP inhibitors (**Figure 1.10**).¹²¹ Kinetic isotope effect has been studied systematically by Schramm over PNP inhibitors, a C-nucleoside analogues, in combination with crystal structure,¹²² experimental enzymology,¹²³ molecular modelling accompanied by a number of structural improvements finally made him conclude aza-C-nucleoside analogues of immucillins i.e. ImmA **1.014**, ImmH **1.015**, ImmG **1.016** and their 8-aza-analogues 8-aza-ImmA **1.017** and 8-aza-ImmH **1.018** as the first generation of potent anti-cancer drug (cytostatics) (**Figure 1.10**).

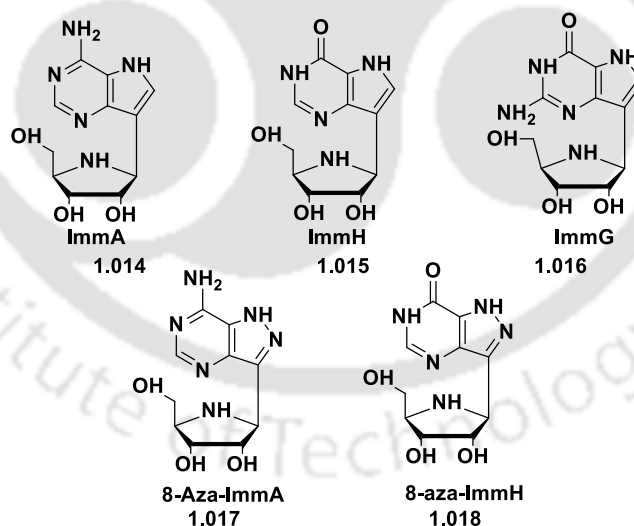
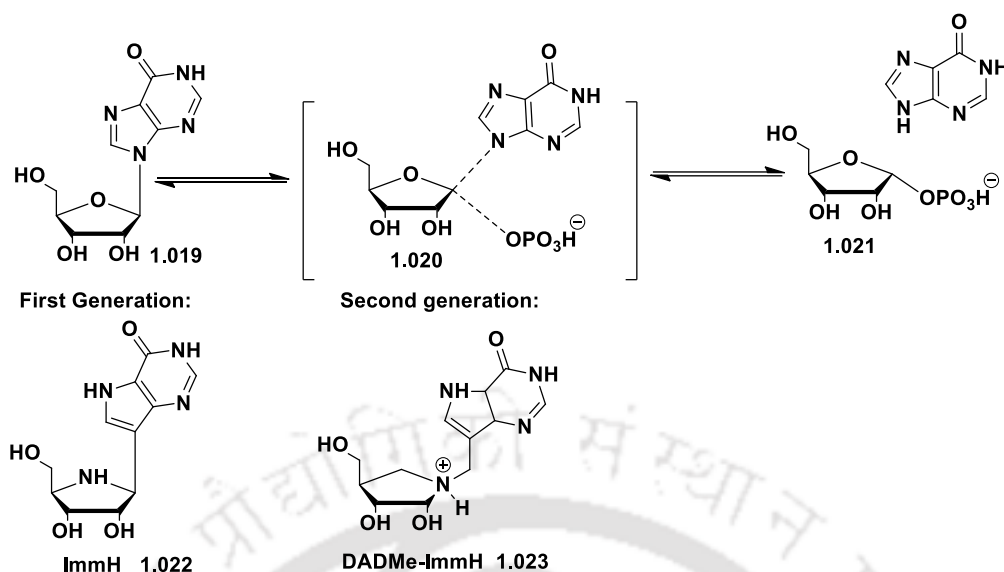


Figure 1.10: Immucillins structure.

A deeper investigation into the mechanism (**Scheme 1.1**) of the reaction and further structural refinement developed a second generation inhibitors although that was not based on C-nucleoside (**Scheme 1.1**).¹²⁴ All these drugs are now involved in clinical trials as an anti-cancer drug (cytostatics).



Scheme 1.1: PNP mechanism and the development of First and Second generation Immucilin.

1.5.2.2. Oxidoreductase inhibitor

The major cofactor of oxidoreductase enzyme is Nicotinamide-adenine dinucleotide (NAD). Oxidoreductase are inhibited by a number of Carboxamide aryl C-nucleoside became capable of acting as a nicotinamide ribonucleoside analogues. In the process of de novo guanylate biosynthesis inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP) conversion is catalysed by inosine 5'-monophosphate dehydrogenase (IMPDH) is the enzyme controls rate limiting step of the process. In tumour cell IMPDH inhibition followed by a further decrease in guanine nucleotides concentration interrupt DNA and RNA synthesis. IMPDH^{125,126} is inhibited by a potent anti-cancer component, Tiazofurin **1.025a** (**Figure 1.11**), found to be able to bio transformed into TAD **1.026**, now is under clinical trial.¹²⁸ It is proved to be the same mechanism in the case of benzamide ribonucleoside (BAR)¹²⁹ **1.024** has been conferred. Even further activity was exhibited by selenazofurin¹³⁰ while the comparable extent of cytostatic activity through the same mechanism was found to be exerted by a number of hetero analogues (e.g. imidazofurin **1.025d**, oxazofurin¹³¹ **1.025c**, selenophenfurin¹³² **1.025f**, furanfurin **1.025g** and thiophenfurin¹³³ **1.025e**) have been investigated further.

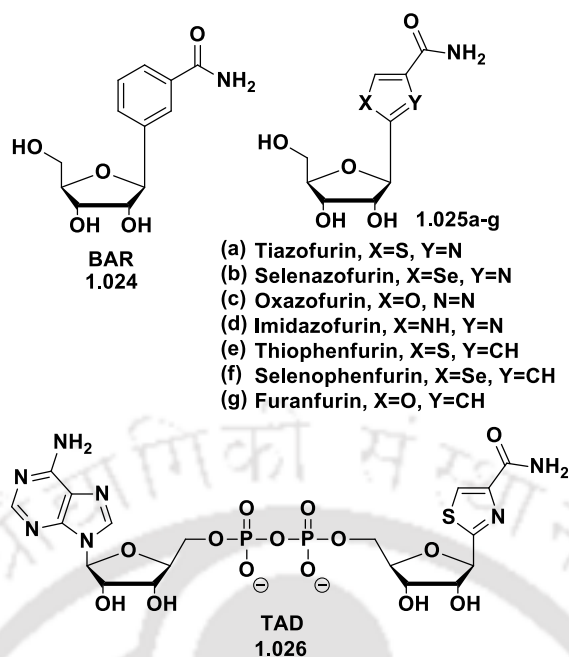


Figure 1.11: Structure of IMPDH C-nucleoside inhibitor.

In supposition through a number of examples, it has been shown that the replacement of the labile C-N bond of an antiviral or anti-cancer or anti-tumour nucleoside by a C-C bond is usually leading to an extent of lack or complete loss of its biological activity. This is probably happened to be the fact because of the change in chemical properties of the heterocyclic base due to, either lacking of basicity because of the absence of nitrogen that are usually taking part in conjugation with the heterocyclic ring, also varying the tautomerisation effect to a great extent, considerably modulates the heterocyclic ring to interact with enzymes. Most of the nucleoside drugs have to go through a very complex bio mechanism to act upon, which might be the reason of the supressing activity of the structurally changed heterocyclic aglycon to be blocked whenever is going through any of these step like phosphorylation, inhibition of polymerase etc. In contrary the synthesis and screening of a large number of C-nucleoside analogues might be able to reveal the uniqueness in the search for a new mechanism of action. To the best of knowledge, no novel attempts have been taken to screen or synthesis C-nucleoside analogues for these purpose. However rational drug designing could be helpful to discover new mechanism of action founded upon C-nucleosides which are biologically active especially in the area of single enzyme inhibition of nucleic acid metabolism. Definitely, improvement and development of new efficient synthetic methodologies further stimulate to unveil new unearth biologically active C-nucleoside which instigate further research to a new realm of science.

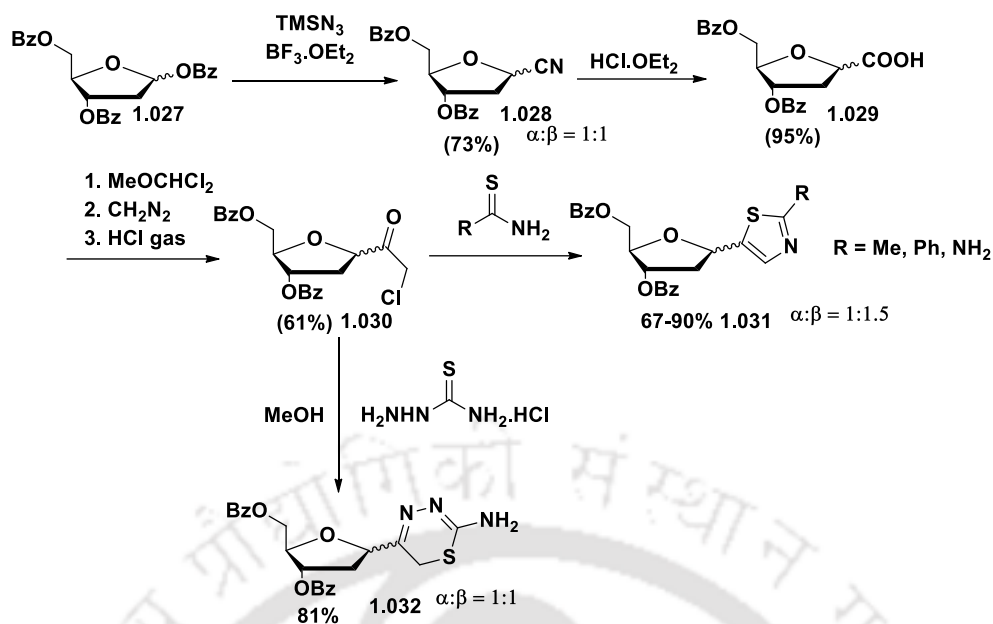
1.6. Synthesis of artificial C-nucleoside

Pseudouridine Ψ **1.007** was first discovered by Cohn which happens to be the first reported biologically active C-nucleoside in the history of science. This natural product has become the part of an active research. Very low abundance of these C-nucleoside analogues in nature in addition with a high degree of expectations to get their artificial analogues are the prompting point to denude things further. There are a number of excellent reviews⁶⁻¹¹ summarised a series of synthetic strategies that have been taken into account. Here the following topics are discussed on the basis of structural variations of C-nucleosides: (a) Construction and modification of aglycon upon carbohydrate (b) Construction of ribose ring upon aglycon. (c) Organometallic coupling with riboside (d) nucleoside modification (e) Cross coupling with preformed aglycon.

1.6.1. Construction and modification of aglycon upon carbohydrate

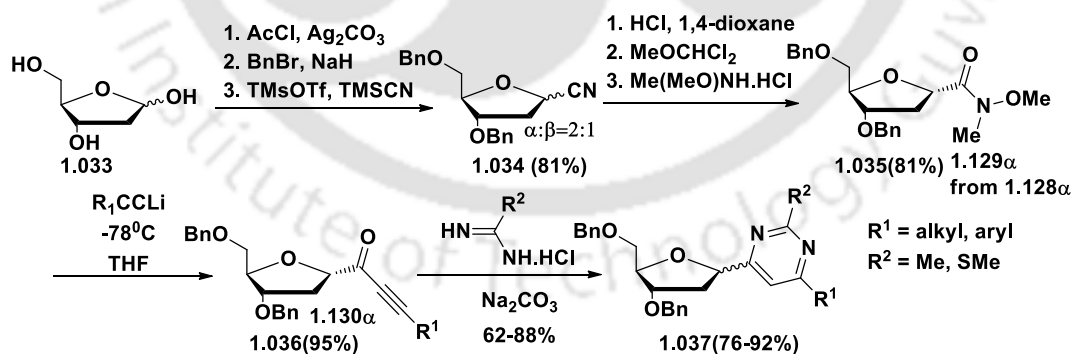
1.6.1.1. Modification through the insertion of nitrile group followed by heterocyclizations

It is needed to introduce the appropriate functional group by following the precise strategy onto a carbohydrate to construct heterocycles.¹²⁷ The introduction of a nitrile functionality is the key classical step to construct a heterocycle underwent through a number of steps of chemical conversion (**Scheme 1.2**). Precursor **1.027** is taken to be fully protected by benzoylated functionality to start with, introduced by Baldwin and co-workers. A nitrile moiety was introduced to the anomeric position by replacing benzoyloxy functionality was achieved to make the benzoyloxy as a good leaving group by the treatment of Lewis acid $\text{BF}_3 \cdot \text{OEt}_2$ followed by the addition of trimethylsilyl cyanide which is the source of nucleophile producing **1.028** as a 1:1 mixture of α and β with a 73% yield. Further acid treatment of this nitrile group hydrolyse it to produce carboxylic acid derivative **1.029** with a high 95% yield. The subsequent addition of α, α -dichloromethyl methyl ether with diazomethane in presence of HCl produce chloroacetyl derivative **1.030** with a moderate 61% yield. The chloroacetyl derivative is further treated with thioamide functionality where the sulphur end is acting as the nucleophilic end to cyclise the ring to harvest a substituted thiazole moiety **1.031** with a good to moderate yield as a 1:1.5 anomeric mixture of α and β . Whereas by using the same concept by keeping the sulphur of semithiocarbazide as the nucleophilic end another aminothiadiazaole **1.032** heterocyclic moiety was synthesised as a equimolar mixture of α and β with a good 81% yield with. The step involved in the introduction of nitrile group although suffers chromatographic separation because of the problem to handle the equimolar anomeric mixture, the next step i.e. involved with the hydrolysis of nitrile to produce carboxylic acid is not associated with the problem of purification, instead it is easy to purify this acid derivative offering the next step to start with a pure diastereomer.



Scheme 1.2: CN group introduction followed by heterocyclic ring formation.

The problem of stereoselectivity towards harvesting the nitrile derivative was solved by starting the synthesis from the commercially available 2-deoxy-D-ribose (**Scheme 1.3**) followed by benzyl protection through the treatment with benzyl bromide and sodium hydride with the subsequent addition of trimethylsilyl triflate and trimethylsilyl cyanide to develop **1.034** as a 2:1 mixture of α and β which is good enough to be separated through the techniques of chromatography with an 81% satisfactory yield.

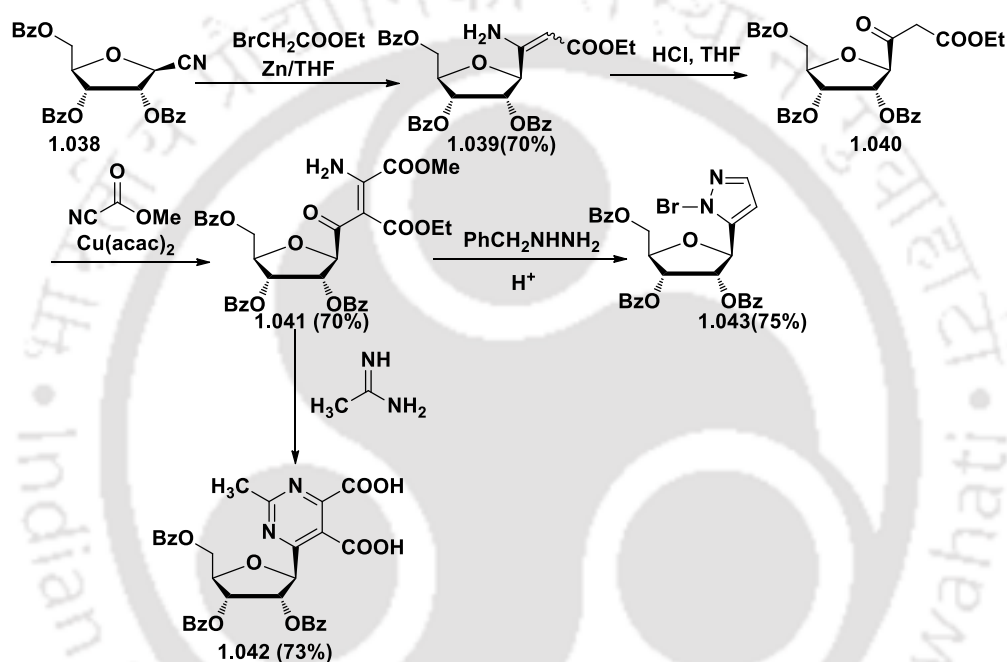


Scheme 1.3: CN group introduction followed by heterocyclic ring formation.

Nitrile hydrolysis with HCl started with the single α -diastereomer followed by α,α -dichloromethyl methyl ether and N,O-dimethylhydroxylamine hydrochloride treatment produce Weinreb amide **1.035 α** with 81% yield which is further treated with lithium acetylide to afford ethynyl ketone **1.036 α** with greater than 95% yield. Conversion of **1.036** to **1.037**

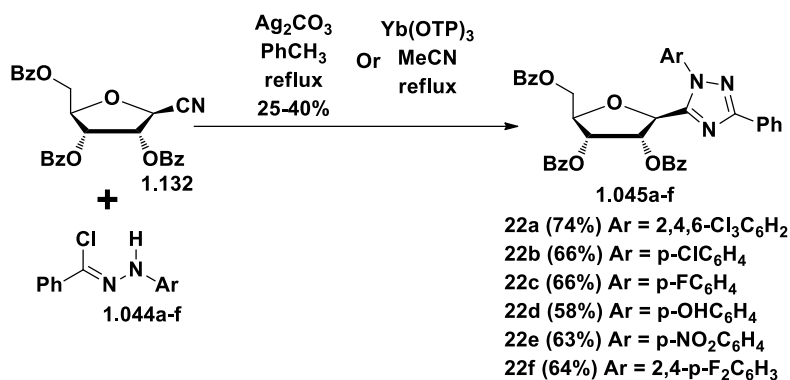
involved cyclisation with the equimolar formation of both anomer revealed the calamity towards controlling stereoselectivity although with a good to moderate yield.¹³⁴

Veronse (Scheme 1.4)¹³⁵ started with 1-cyano- β -D-ribofuranose **1.038** which was converted to **1.039** by Reformatsky reaction and further acid treatment produces unstable β -ketoester **1.040** which was immediately treated with methyl carbonocyanide where Cu(II) has been used as a catalyst to afford **1.041** with a moderate 70% yield. Further treatment of **1.041** with acetimidamide affords **1.042** as cyclised heterocycle with a 73% yield whereas treatment of **1.041** with benzylhydrazine leading to afford **1.043** as a 5 membered nitrogenous heterocycle with a 75% yield.



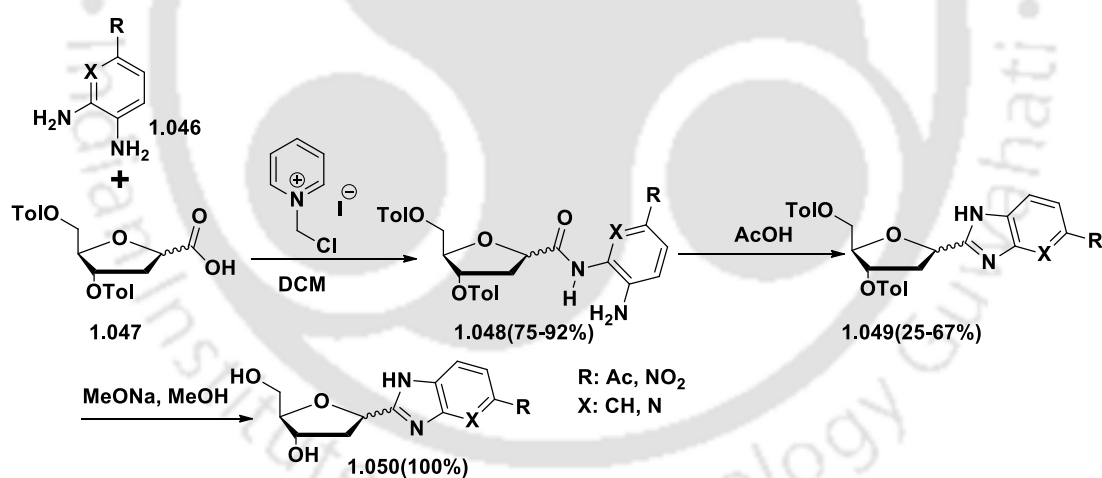
Scheme 1.4: Synthesis of Pyrimidines (**1.137**) and Pyrazoles (**1.136**) C-nucleoside.

Another work with **1.038** was reported by Masoudi *et al.* where the nitrile group is directly involved in one step preparation of five membered heterocycles in the presence of silver or ytterbium metal catalysis. Here **1.038** was treated with a series of hydrozanyl chloride **1.044a-f** in the presence of either AgCO_3 or $\text{Yb}(\text{OTf})_3$ in refluxing condition to afford a series of 5 membered 1,2,4-triazole heterocycle **1.045a-f** in good to moderate yield (Scheme 1.5).¹³⁶



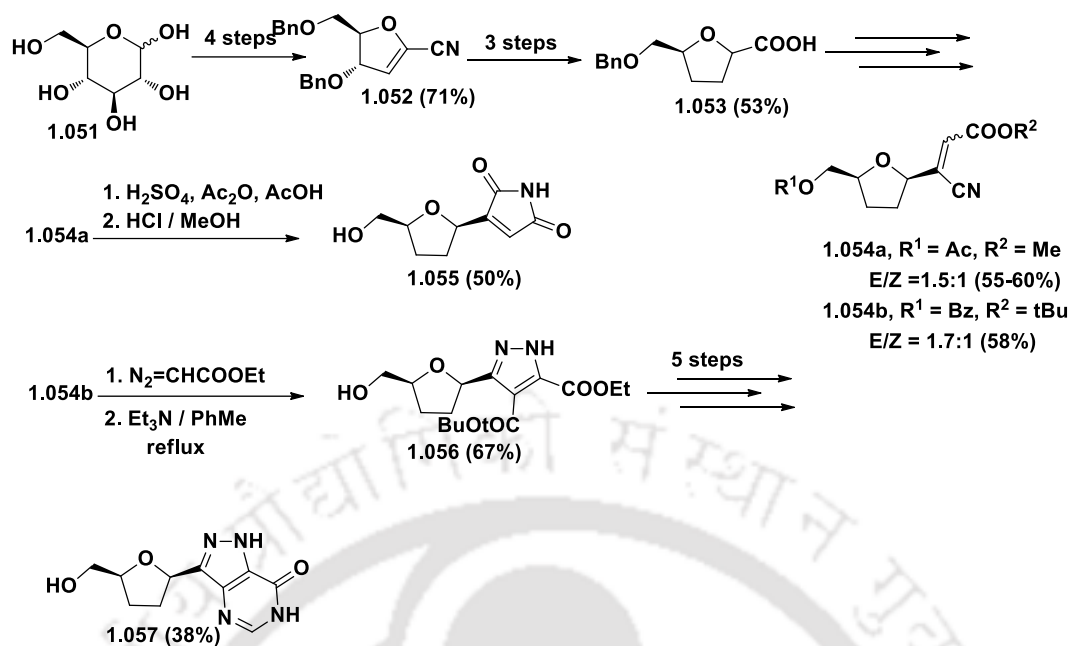
Scheme 1.5: Synthesis of 1,2,4-triazoles through cycloaddition with nitrile.

Benhida¹³⁷ started with a bistoluoyl protected α,β -carboxylic acid of D-ribose **1.047** which is allowed to react with chloromethylpyridiniumiodide, a Mukaiyama reagent in presence of orthobenzodiamine or orthopyridodiamine to produce **1.048** where the carboxylic acid moiety was converted to an amide functionality and then further treated with acetic acid to furnish benzimidazole as well as pyridoimidazole **1.049**. Toluoyl deprotection was done by sodium methoxide in methanol to afford **1.050** in overall moderate yield (**Scheme 1.6**).



Scheme 1.6: Synthesis of Benzimidazole C-nucleoside.

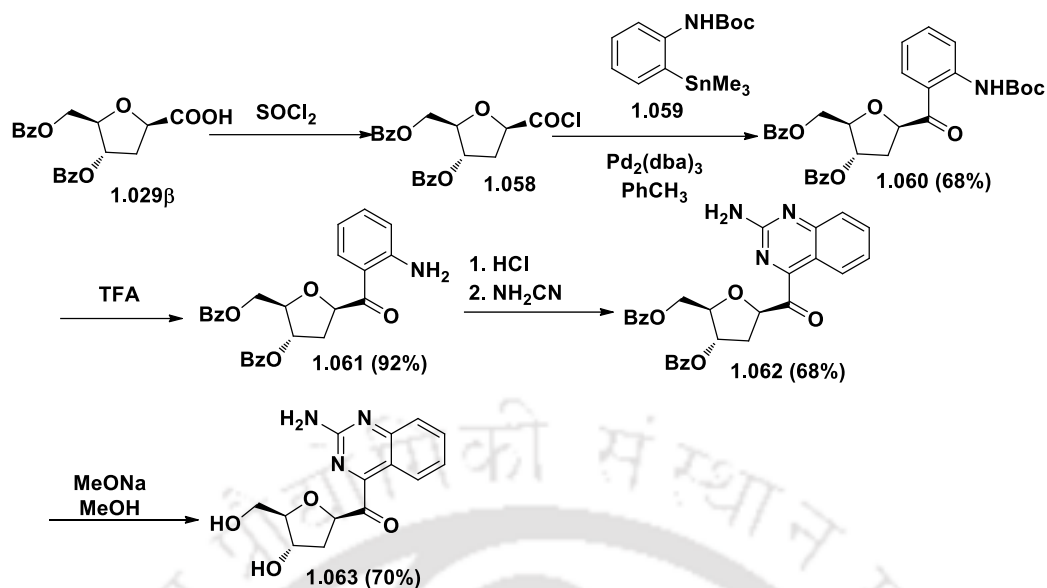
Another important modification started with carboxylic acid was done by Jung (**Scheme 1.7**).¹³⁸ Benzyl protected β -carboxylic acid **1.053** was achieved in pure form by undergoing through a multistep synthesis started from glucosamine **1.051** with an overall 37% yield.



Scheme 1.7: Synthesis of Dideoxysowdomycin and Dideoxyformycin B.

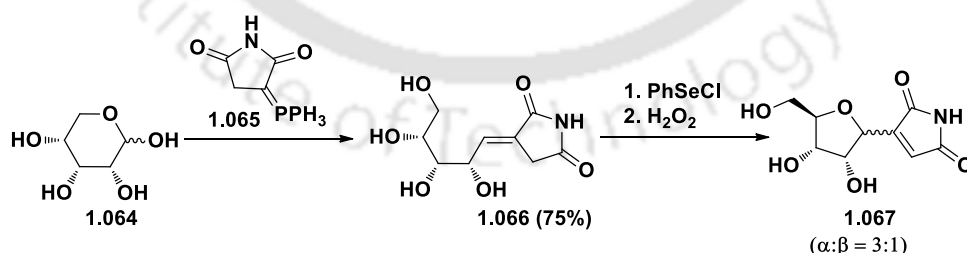
A series of cyanoester **1.054a** and **1.054b** have been synthesised in an overall moderate yield through a multistep synthesis followed by Wittig reaction with a suitable triphenylphosphorane. **1.054a**, was then treated with sulphuric acid, acetic anhydride and acetic acid followed by hydrochloric acid in methanol to afford cyclized dideoxysowdomycin **1.055** in 50% yield. **1.054b** was treated with ethyldiazoacetate in presence of triethyl amine in refluxing condition to afford β -pyrazolediester **1.056**. **1.056** is again further underwent through a 5 step conversation to afford dideoxyformycin B **1.057**.

Gold reported (**Scheme 1.8**)¹³⁹ the synthesis of **1.063** synthesised from bisbenzoyl protected D-ribosidic acid **1.029 β** . Pure **1.029 β** was treated with thionyl chloride to convert carboxylic acid to acid chloride **1.058** which was subsequently converted to **1.060** with the help of Stille coupling where Pd₂(dba)₃ is used as a palladium catalyst and boc protected aniline **1.059** is used as a stannyl precursor. Boc deprotection was achieved by the treatment of TFA to afford **1.061**. Treatment of **1.061** with cyanamide in presence of HCl produce **1.062** in 78% yield. Benzoyl deprotection was done by sodium methoxide in presence of methanol to afford (2R,3S,5R)-5-(2-aminoquinazolin-4-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol **1.063** in 70% yield.

Scheme 1.8: Synthesis of β -benzopyrimidines.

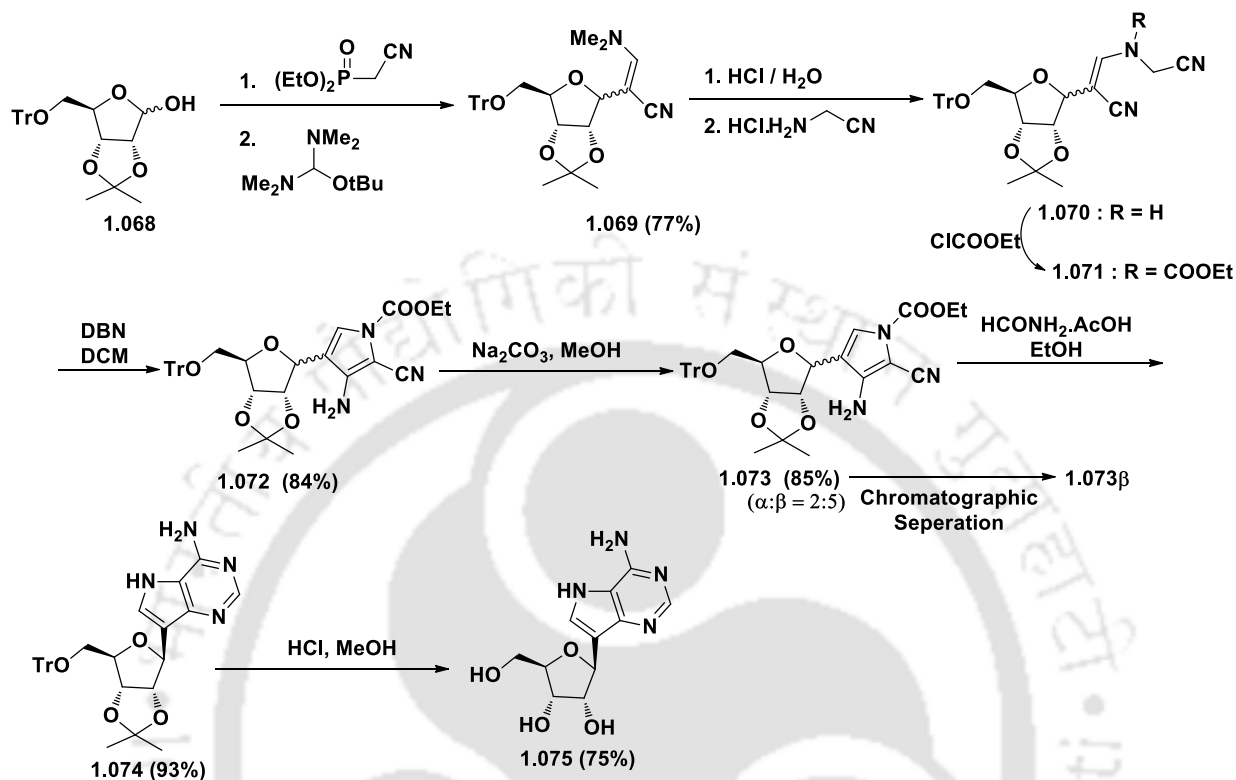
1.6.1.2. Modification through Wittig type reaction

Wittig reaction is another class of reaction which was extensively used in the synthesis of heterocyclic aglycon of riboside.^{140,141} It is a reaction where the two precursors are phosphorus ylide and a carbonyl functionality reacting together to create a double bond unsaturation. Wittig reaction has taken to be the key reaction in the synthesis of **1.067** reported by Barrett (**Scheme 1.9**). Sugar unit **1.064** and ylide **1.065** are the precursor allowed to react in Wittig fashion to produce **1.066** which was further treated with phenylselenenyl chloride and hydrogen peroxide for the purpose of selenoetherification followed by selenoxide elimination to afford a mixture of epi-showdomycine **1.067 α** and showdomycine **1.067 β** in a 3:1 ratio.¹⁴²

Scheme 1.9: Construction of Showdomycin **1.008** by Wittig-Type Chemistry.

Synthesis of 9-deazaadenosine (**Scheme 1.10**)¹⁴³ is a classical one started from protected D-ribose **1.068**. **1.068** was subjected to react through the method of Horner-Wadsworth-Emmons reaction with diethyl methylcyanophosphonate followed by the treatment of tert-butoxy bis(dimethylamino)methane in DMF produce **1.069** which contains an

enamine moiety is further subjected to acidic hydrolysis results an enol which is subsequently reacted in acetate buffer with aminoacetonitrile hydrochloride to afford **1.070** in a good overall yield.



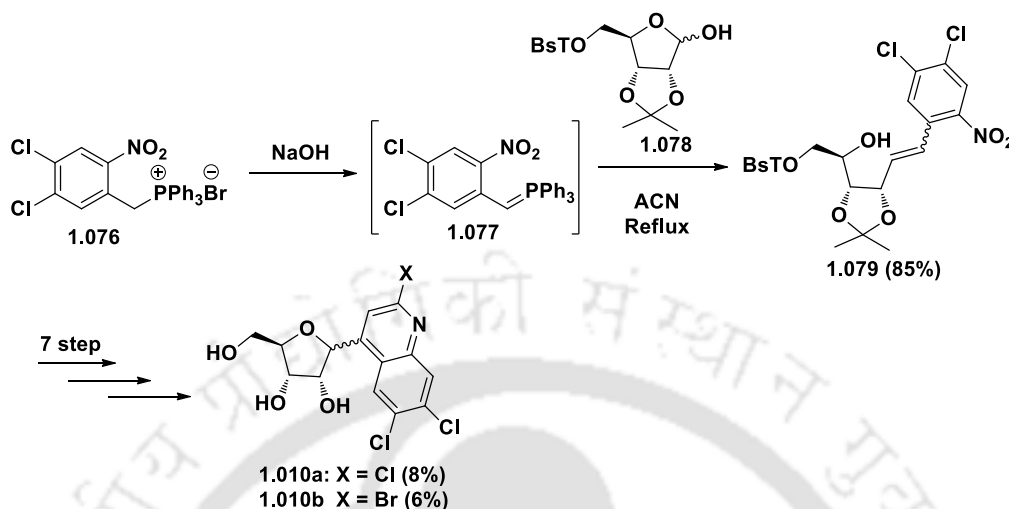
Scheme 1.10: A classical construction of 9-Deazaadenosine.

Unprotected amine nitrogen was protected by the treatment of **1.070** with ethyl chloroformate to produce **1.071**. **1.071** was then treated with a base DBN (1,5-diazabicyclo[4.3.0]non-5-ene) in excess quantity in dichloromethane solvent to afford a functionalized pyrrole heterocycle **1.072** in 84% yield. Deprotection of ester functionality by sodium carbonate in methanol gives **1.073** as a mixture of α and β anomer in 85% yield. The anomeric mixture of **1.073** is then subjected to purify through column chromatography to get cyclised **1.073 β** as a pure anomer which is then treated further in boiling ethanol with formamide acetate to afford 9-deazaadenosine **1.074** in an excellent 93% yield. Acidic deprotection of 2',3' and 5' oxygen of **1.074** produces **1.075** through a number of steps with an overall 22% yield.

Polyhalogenated Quinolines (**1.010a-b**) were synthesised by Townsend *et al.* (**Scheme 1.11**)¹⁴⁴ where the precursor **1.076** was treated with sodium hydroxide to start with phosphorous ylide which was undergone reaction with the another precursor **1.078** in a Wittig fashion to produce **1.079** as a mixture of two isomeric alkene *Z* and *E* with a 12:1 ratio in 85% yield. These mixture of alkenes were then subjected to undergo through a seven multi steps synthesis

Chapter 1

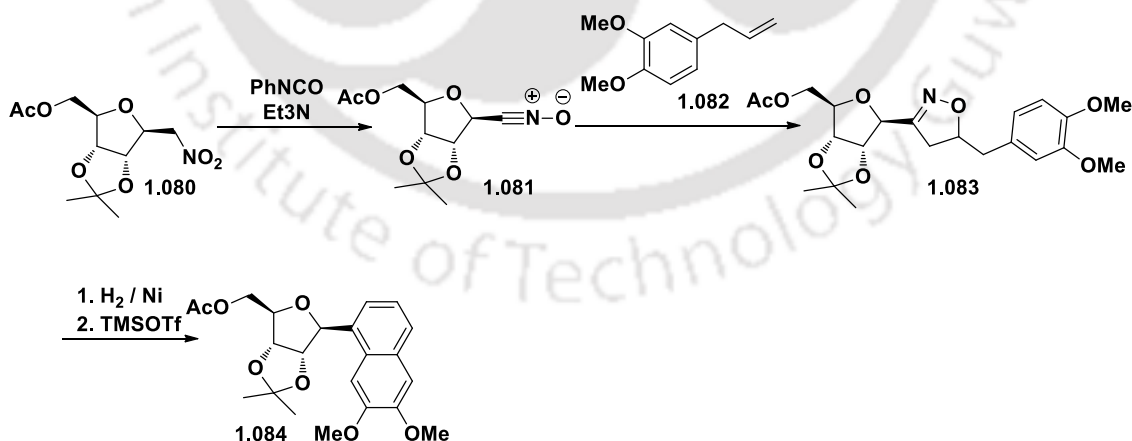
to afford trichloroquinoline aglycon **1.010a** and bromodichloroquinoline aglycon **1.011b** with an undesirably 8% and 6% low overall yield respectively.



Scheme 1.11: Polyhalogenated Quinolines **1.010a** and **1.010b** synthesis.

1.6.1.3. Modification through cycloaddition

The (+)-showdomycin and (-)-6-azapseudouridine are the two examples of C-nucleoside which was synthesised by the first introduction of the concept of cycloaddition into the formation of aglycon.¹⁴⁵

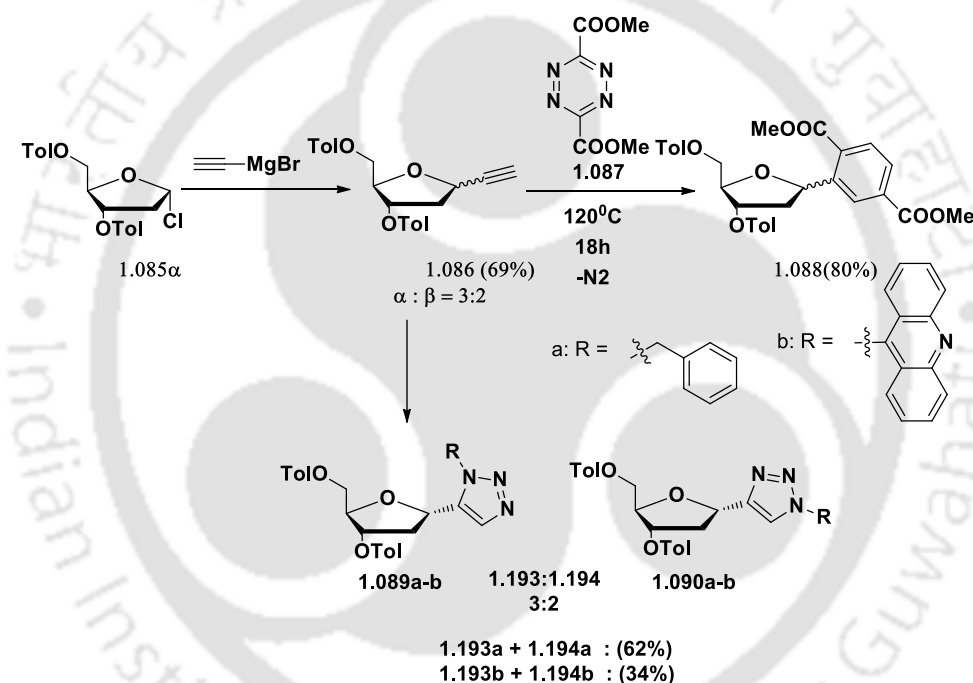


Scheme 1.12: 1,3-dipolar cycloaddition in the synthesis of aglycon moiety.

Latter in the same year 1,3-dipolar cycloaddition concept was again employed by Kozikowski during the synthesis of artificial nucleoside analogues contains isoxazoline ring in

the place of ribose and during the total synthesis of ($\pm$)-blastomycinone.¹⁴⁶ Afterwards Kozikowski reported the synthesis of **1.084** where **1.080** is converted to **1.081** which is bearing an 1,3-dipolar nitrile oxide functional group. **1.082**, which is providing the dienophile part for an 1,3-dipolar cycloaddition, and **1.081** were then subjected to react together to develop isoxazoline **1.083** C-nucleoside analogues which was then converted into **1.084** by Raney nickel reduction followed by trimethylsilyl triflate treatment (Scheme 1.12).

A classic example of cycloaddition reaction in the formation of pyridazine as well as triazole heterocyclic ring was reported by Wamhoff (Scheme 1.13).¹⁴⁷ Where he started with α -ribofuranosyl chloride **1.085 α** which was allowed to react with ethynylmagnesium bromide to convert it into **1.086**.¹⁴⁸ In this reaction the major problem is the formation of undesired α -anomer along with β -anomer.

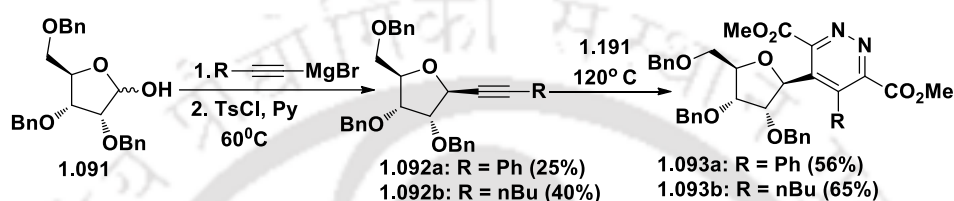


Scheme 1.13: Synthesis of Pyridazines **1.192** and Triazoles **1.193** and **1.194** using cycloaddition as a key step.

Although the formation of α -anomer could be able to reduce by the use of protecting groups (Ac, Bz, Tol) which contains ester functionality but the yield of β -anomer was still not satisfactory. The reaction was further proceeded with toluoyl protection where the anomeric ratio was 3:2 for $\alpha:\beta$. This **1.086** was then subjected to react with dimethyl [1,2,4,5]-tetrazine-3,6-dicarboxylate **1.087** forming an intermediate underwent through Diels Alder reaction was quiet unstable and therefore immediately decomposed by the elimination of one nitrogen unit which was again underwent through retro Diels Alder method finally gives **1.088** with a good 80% yield. In another way the intermediate **1.086** was further involved in the formation of

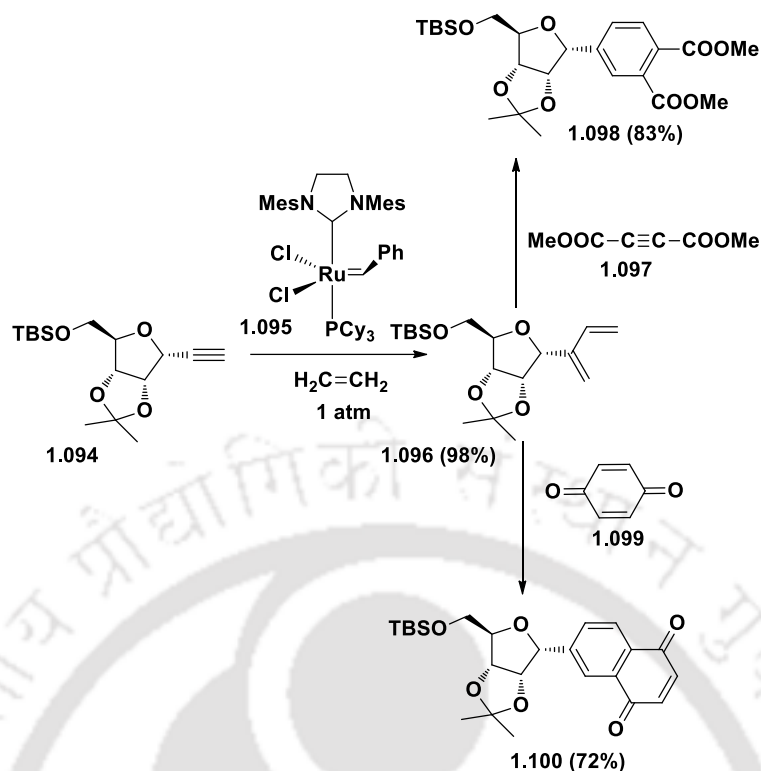
triazole ring through [3+2] cycloaddition to produce regioisomers **1.089a-b** and **1.090a-b** in 3:2 ratio with moderate yield.

Dubreuil reported the synthesis of another substituted pyridazine nucleosides started from **1.091** (Scheme 1.14)¹⁴⁹ which was a benzyl protected ribose sugar unit treated with phenylethynylmagnesium bromide as well as n-butylethynylmagnesium bromide to open up the ring at anomeric centre which was then further closed by the treatment of this open chain sugar with tosyl chloride at refluxing temperature followed by isolation of pure β isomer **1.092a** and **1.092b** were further subjected to Diels Alder cycloaddition reaction with **1.087** produces substituted pyridazine **1.093a** and **1.093b** as a pure β isomer.



Scheme 1.14: Construction of functionally substituted Pyridazine ribofuranose using cycloaddition.

Another interesting work was reported by Kaliappan *et al.* (Scheme 1.15)¹⁵⁰ where they started with protected glycoside **1.094** which was reacted with ethylene in presence of Grub's second generation catalyst **1.095** through an alkyne metathesis produced **1.096** in 98% yield. This intermediate **1.096** was further subjected to Diels Alder reaction with either dimethyl ethynyldicarboxylate **1.097** or benzoquinone **1.099** produced respectively **1.098** and **1.100** in 83% and 72% yield. This is another example where cycloaddition is extensively used in both ways in the formation of C-nucleoside analogues.

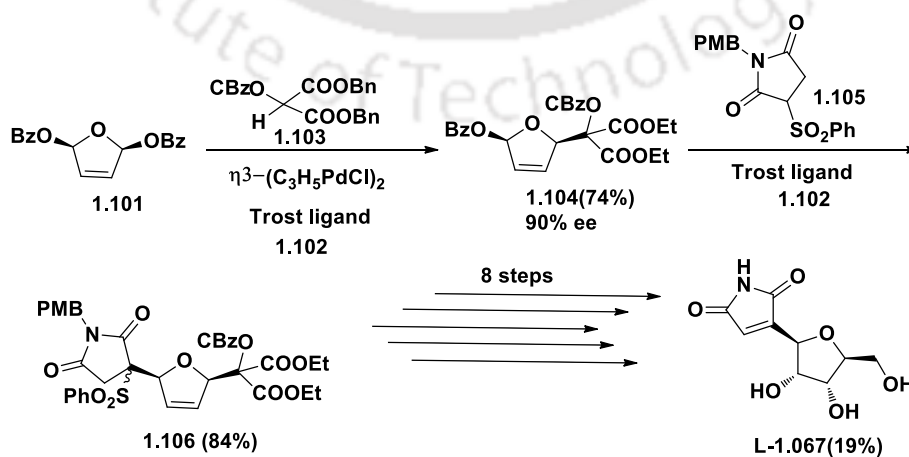


Scheme 1.15: Ring closing metathesis followed by cycloaddition.

As both the steps has been carried out in toluene at 80 °C a one pot two step procedure was further investigated giving approximately same yield with no much significant difference. A similar approach by using Diels Alder reaction was also reported by Kitora *et al.*¹⁵¹ and Yamamoto *et al.*¹⁵² for the synthesis of C-glycosides.

1.6.1.4. Other techniques for the preparation of aglycon upon carbohydrate

A palladium catalyst assisted asymmetric allylic substitution for the synthesis of L-showdomycin **L-1.067** has been reported by Trost (Scheme 1.16).¹⁵³

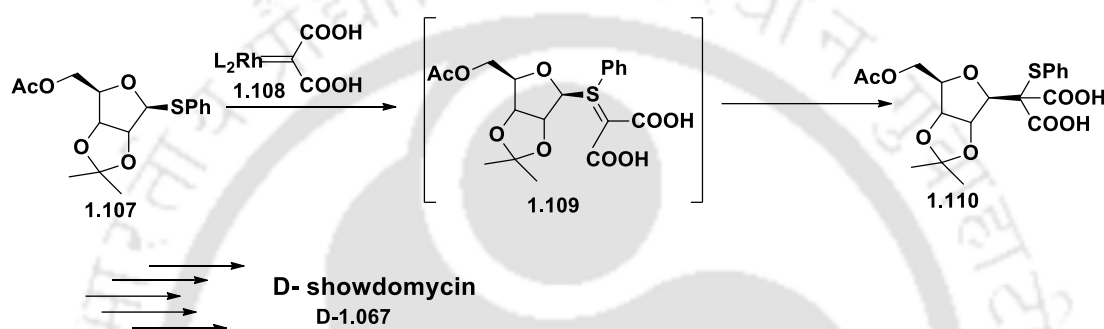


Scheme 1.16: Synthesis of L-Showdomycin involving Trost ligand.

Chapter 1

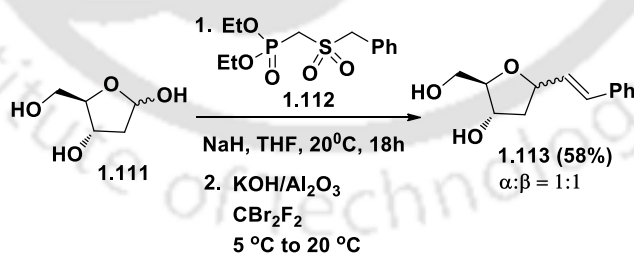
Here $(\eta^3\text{-C}_3\text{H}_5\text{PdCl})_2$ is the Trost ligand **1.102** served as the asymmetric catalysis for the effective allylation at the anomeric centre of **1.101** by maleic diester **1.103** which served as the precursor of the construction of hydroxyl moiety of **L-1.067**. In addition, with this another allylation was carried out by Trost catalyst **1.102** due to the construction of maleimide moiety of **L-1.067**, started from sulfonylsuccinimide **1.105**. The synthesis was carried out through multistep for achieving L- showdomycin **L-1.067** in an overall 10% yield.

The synthesis of D-showdomycin **D-1.067** was achieved started from thiophenynucleoside **1.107**. **1.107** was treated with a Rhodium carbene **1.108** which produced **1.109** underwent rearrangement to afford **1.110** which was then undergone through multistep synthesis to achieve D- showdomycin **D-1.067** (Scheme 1.17).¹⁵⁴



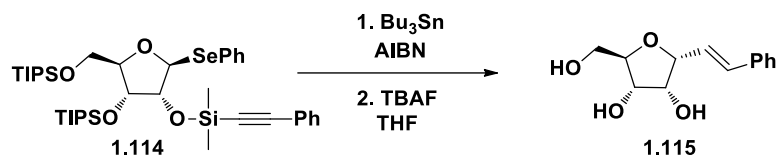
Scheme 1.17: D- showdomycin synthesis from functionalised thioribose.

Synthesis of **1.113** was started from 2-Deoxy-D-ribose **1.111** and underwent through Horner-Wadsworth-Emmons olefination followed by other several steps which produced **1.113** as a mixture of 1:1 ratio of $\alpha:\beta$ (Scheme 1.18).¹⁵⁵



Scheme 1.18: Horner-Wadsworth-Emmons olefination to construct ethenylriboside.

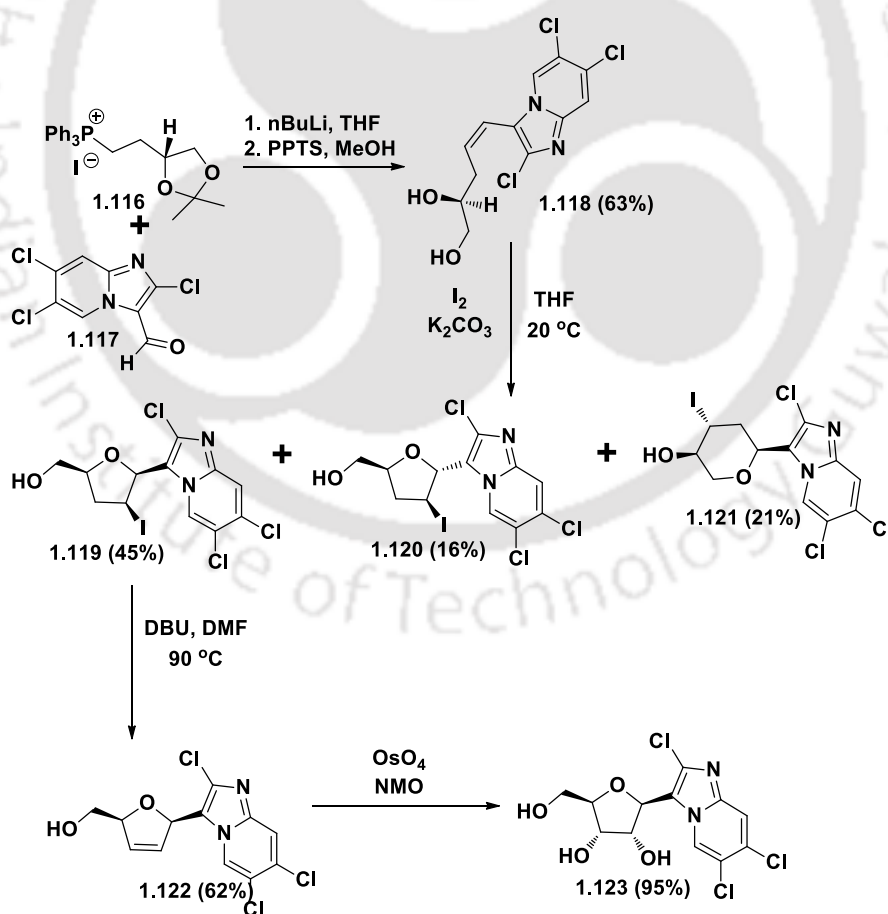
Intramolecular radical cyclisation technic with Bu_3SnH and AIBN is used by Stork (Scheme 1.19)¹⁵⁶ for the synthesis of **1.115** from **1.114** where the diastereoselectivity of this reaction strictly depends on the position of the alkyne. Deprotection of the silylether was done by TBAF to afford **1.115**.



Scheme 1.19: Construction of aglycon through radical cyclisation.

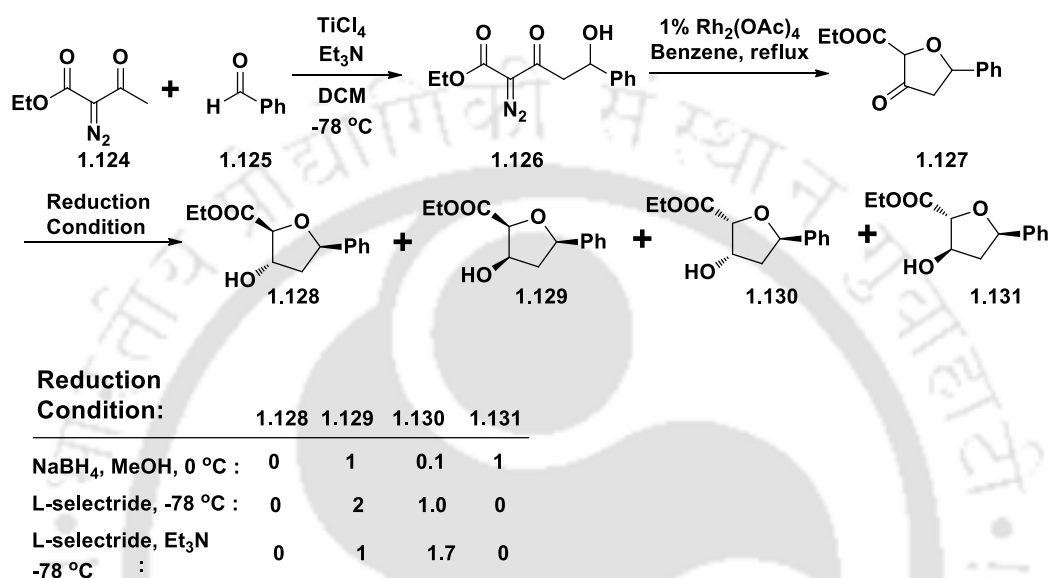
1.6.2. Construction of ribose ring upon aglycon

A classical route for the synthesis of ribose ring was reported by Townsend *et al.* They started with two precursors. One is phosphorous ylide **1.116** and another is aglycon aldehyde **1.117**. They were allowed to react as a Wittig precursor and **1.118** was afforded in a moderate yield which was subjected to ring closing by iodine insertion of the olefinic unsaturation followed by nucleophilic attack produces **1.119**, **1.120** and **1.121**. Iodine elimination by DBU followed by stereoselective OsO_4 mediated olefin hydrolysis afford **1.123** in overall 17% yield (Scheme 1.20).¹⁵⁷



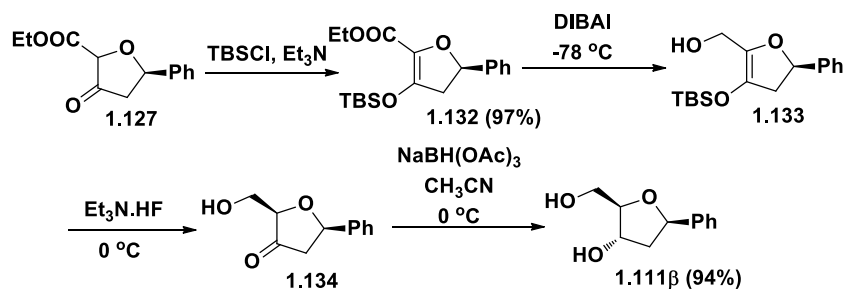
Scheme 1.20: Construction of aglycon upon ribose.

Another classic example is the preparation of 2'-deoxy - β -riboside where any kind of aromatic hetero cycle can be incorporated and the reaction has to be started with aldehyde precursor of that heterocycle. This expedition started with **1.124** and **1.125** by Townsend (Scheme 1.21).¹⁵⁸ Aldol condensation was carried out in DCM at -78 °C in presence of TiCl₄ and Triethylamine to produce **1.126** followed by ring closing by using Rh₂(OAc)₄ as a catalysis afford **1.127** in 95% yield. **1.127** was then allowed to undergo reduction in presence of different reducing agent to produce **1.128-1.131** in different ratio.



Scheme 1.21: Construction of carbohydrate moiety involving carbene.

Now another route was chosen to synthesis **1.111 β** started from **1.127**. **1.132** is produced by TBSCl mediated silylation of **1.127** which was further undergone reduction with DIBAL produced **1.133**. Deprotection of **1.133** followed by NaBH(OAc)₃ mediated carbonyl reduction produced **1.111 β** where all the stereogenic centre in comparison with the natural 2'-Deoxy- β -D-ribose are exactly same with promising β selectivity of the aglycon unit (Scheme 1.22).^{158b}



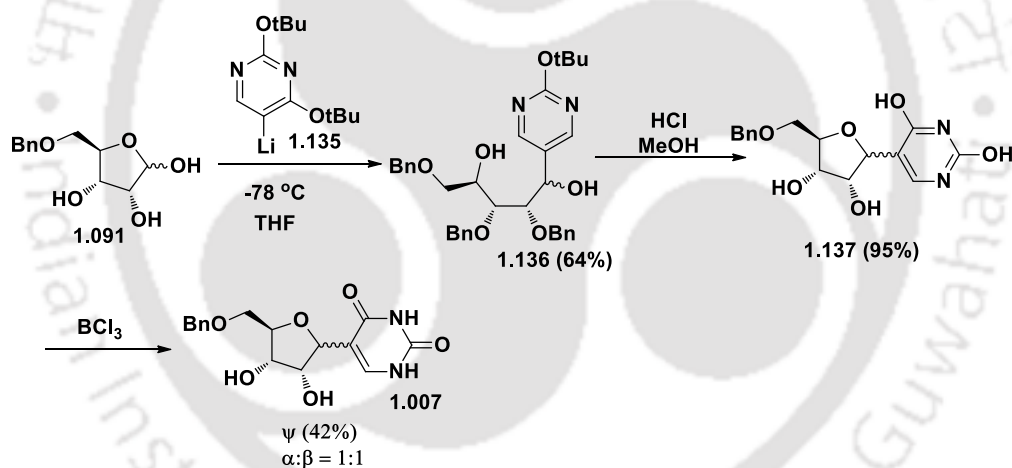
Scheme 1.22: Carbene involving another method of aglycon construction.

1.6.3. Use of organometallic reagents in construction of C-nucleoside analogues

Lithium or magnesium mediated heterocyclic coupling with riboside is a unique concept of forming an aglycon upon ribose ring. Different approaches were conceived and successfully applied to make C-nucleoside analogues.¹⁵⁹ These can be stated as (1) nucleophilic attack to the anomeric centre, (2) started from unsaturation followed by stereoselective addition (3) Nucleophilic attack to halogenose (4) nucleophilic addition to lactone (5) palladium catalysed Heck coupling (6) Lewis acid facilitated aromatic electrophilic substitution.

1.6.3.1. Organolithium and organomagnesium mediated nucleophilic attack to the anomeric centre

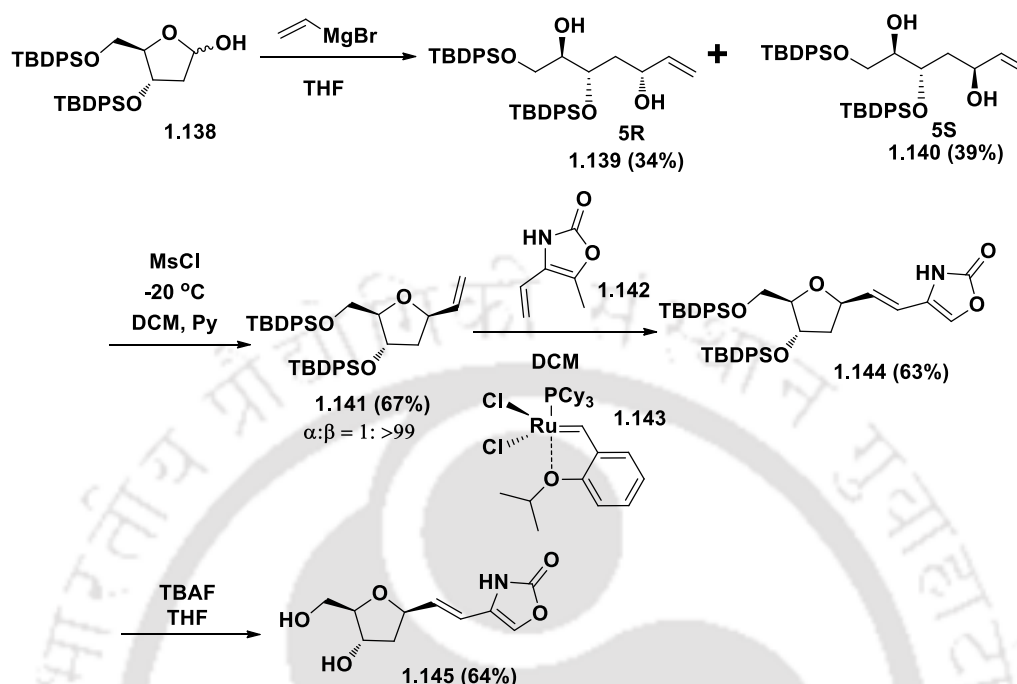
The concept of the nucleophilic attack of organolithium or organomagnesium reagent was started by taking the protected riboside by keeping the anomeric centre intact as it is in the starting material. As the anomeric centre is aldehydic in nature so is easily accessible as a precursor of nucleophilic addition reaction. Addition of organolithium to **1.091** in THF at -78 °C produced **1.136** as an open chain sugar in 64% yield. Further acid catalysed ring closing, which was lacking diastereoselectivity, followed by Lewis acidic deprotection produced pseudouridine (Ψ) **1.007** as a mixture of α : β in 1:1 mixture (Scheme 1.23).^{129a,160}



Scheme 1.23: Coupling of protected ribofuranose **1.091** with organolithium.

The strategy of open chain ring closing suffers because of the lack of diastereoselectivity during ribose ring formation. A novel methodology is further developed by Rothman where even further increased diastereoselectivity was achieved in the synthesis of ribose ring during cyclisation (Scheme 1.24).^{161,162} Silyl protected ribose sugar **1.138** was treated with vinyl magnesium bromide in THF to produce two open chain chromatographically separable sugar **1.139** and **1.140** in 34% and 39% respective yield. Further misylation of **1.139** and **1.140** leading to the cyclisation of the open chain ring via 5(O)*n*-*exo*-tet fashion^{161,162} to afford pure vinylated α and β anomer respectively. Further treatment with Grubs first

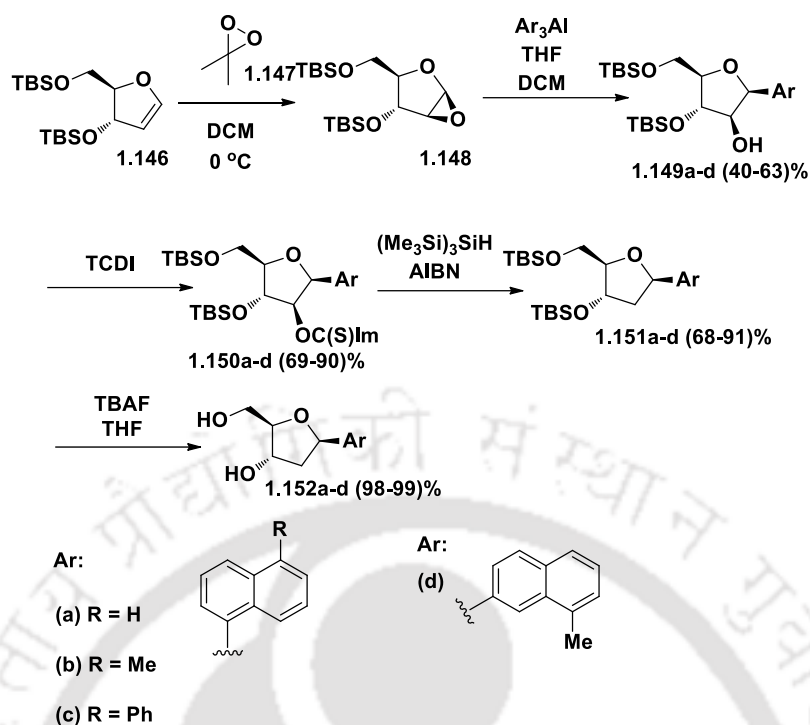
generation catalyst afford external cross metathesis product **1.144** which is undergone TBAF deprotection to give the final C-nucleoside **1.145**.



Scheme 1.24: Vinylmagnesium bromide mediated C-nucleoside synthesis.

1.6.3.2. Triarylluminum mediated nucleophilic coupling with 1,2-dihydrofuran

A completely different concept was considered by Seitz (**Scheme 1.25**).¹⁶³ He started with an olefinic unsaturation. Epoxidation of **1.146** with dimethyldioxirane furnished **1.148** which contains an oxirane ring. The nucleophilic attack of a series of triaryllanes to the oxirane ring finally stereoselectively opened up the ring to afford β -D-arabinofuranoside. Final removal of 2'-hydroxyl group by Barton procedure followed by deprotection afforded β -D-ribofuranoside **1.152a-d** with a series of aryl moiety in moderate to good overall yield with excellent diastereoselectivity. For the case of complex aromatic aglycon there might be some limitation in using 3 equivalent of Ar_3Al .



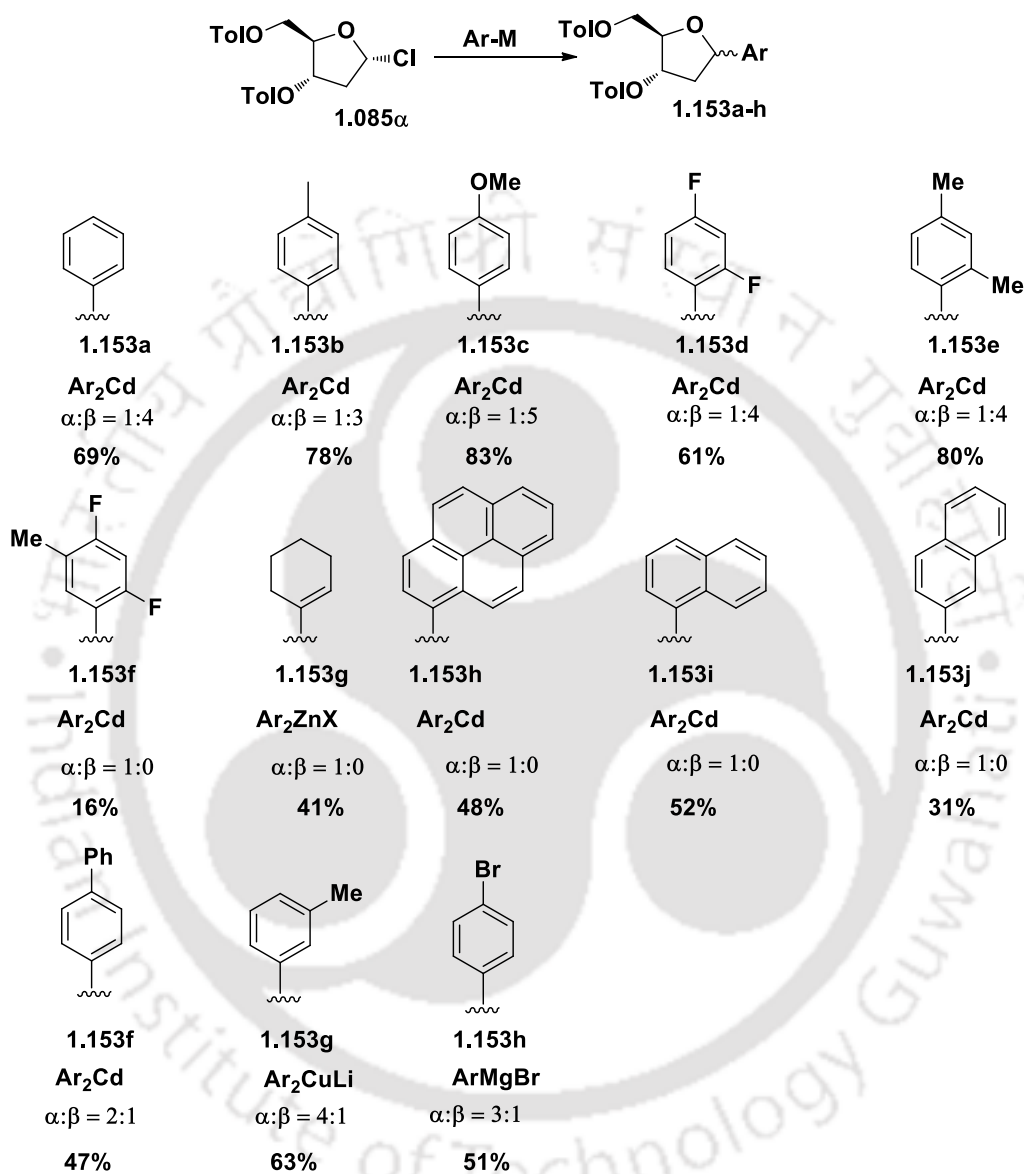
Scheme 1.25: Triarylalluminium mediated aglycon construction.

1.6.3.3. Nucleophilic organometallic addition to sugar Halogenose

Organometallic coupling with sugar halogenose is very common and was given to the utter most importance to construct C-riboside because of its easy direct one step approach to couple the desired aromatic aglycon supplied in the form of organometallic reagent readily replace the halogen from the anomeric C-halogen bond to the protected ribofuranose.¹⁶⁴ Over the last few decades the most extensively used halogenose is Hoffer's Chloro sugar (1-chloro-2-deoxy-3,5-bis[O-(*p*-toluoyl)]-R-D-ribofuranose) **1.085**.¹⁶⁵ Hoffer's Chloro sugar **1.085a** is typically involved with a wide variety of organometallic reagent involving mercury, zinc, cadmium,^{73,166} lithium¹⁶⁹ and magnesium^{167,168} to replace chlorine producing **1.153a-h**. The yield of the reaction was governed by the capability of the organometallic reagent coming into play (Scheme 1.26).^{170,171}

In every cases the major product is α -anomer. The formation of undesirable α -anomer does not depend on the condition of the reaction. That means in each and every cases irrespective of using different reaction condition varied from solvent, metal, temperature the yield of the product still remains at the side of α -anomer probably because the aglycon placed at the β -side experienced 1,3-diaxial interaction with the sterically crowded C5' and forced to preferably form α -anomer. So the problem of getting more desired β -ribonucleoside was basically achieved by the epimerisation of the α -anomer catalysed by acid in turn producing β -anomer as the desired product. Therefore, this method towards the formation of β -anomer comprises with an additional step. But the lack of the formation of the expected yield simply

triggered finding new methodology to produce more yield. This was then done by Seitz using magnesium-cuprate which improved the yield and subsequent acid catalysed epimerisation of the α -anomer produced further desired β -anomer.^{88b,172}

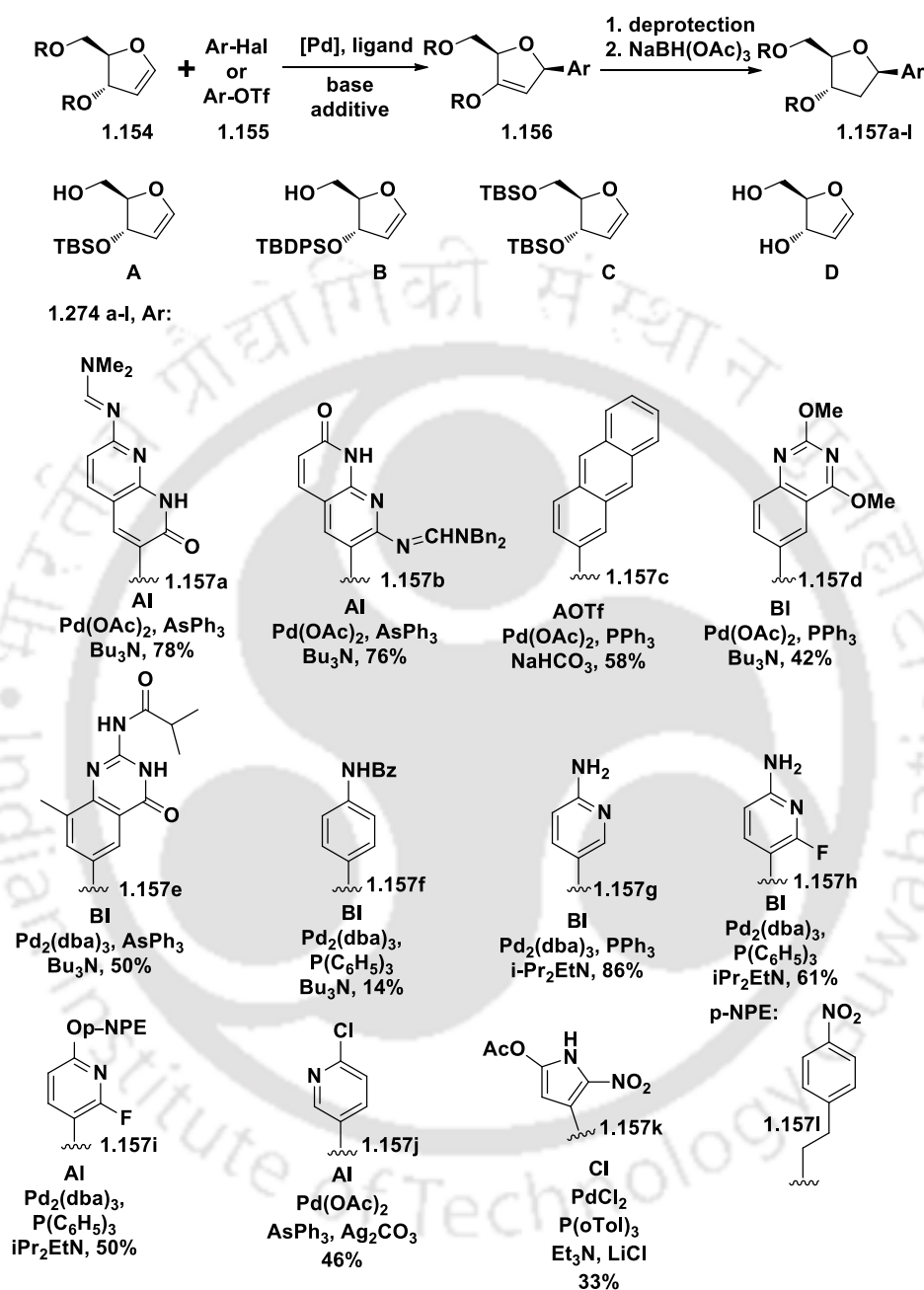


Scheme 1.26: Nucleophilic organometallic addition to sugar Halogenose.

1.6.3.4. Heck mediated C-nucleoside formation

Heck coupling is another method to couple an aromatic aglycon with the sugar moiety. Here it is needed to have a double bond unsaturation in the sugar part with an aromatic halide or triflate. They are allowed to react in presence of palladium catalyst with different ligands in

presence of inclusion of base and additives. The yield and β selectivity depends upon 5' and 3' protecting groups of sugar, palladium catalysis and the ligand that is used in the reaction.

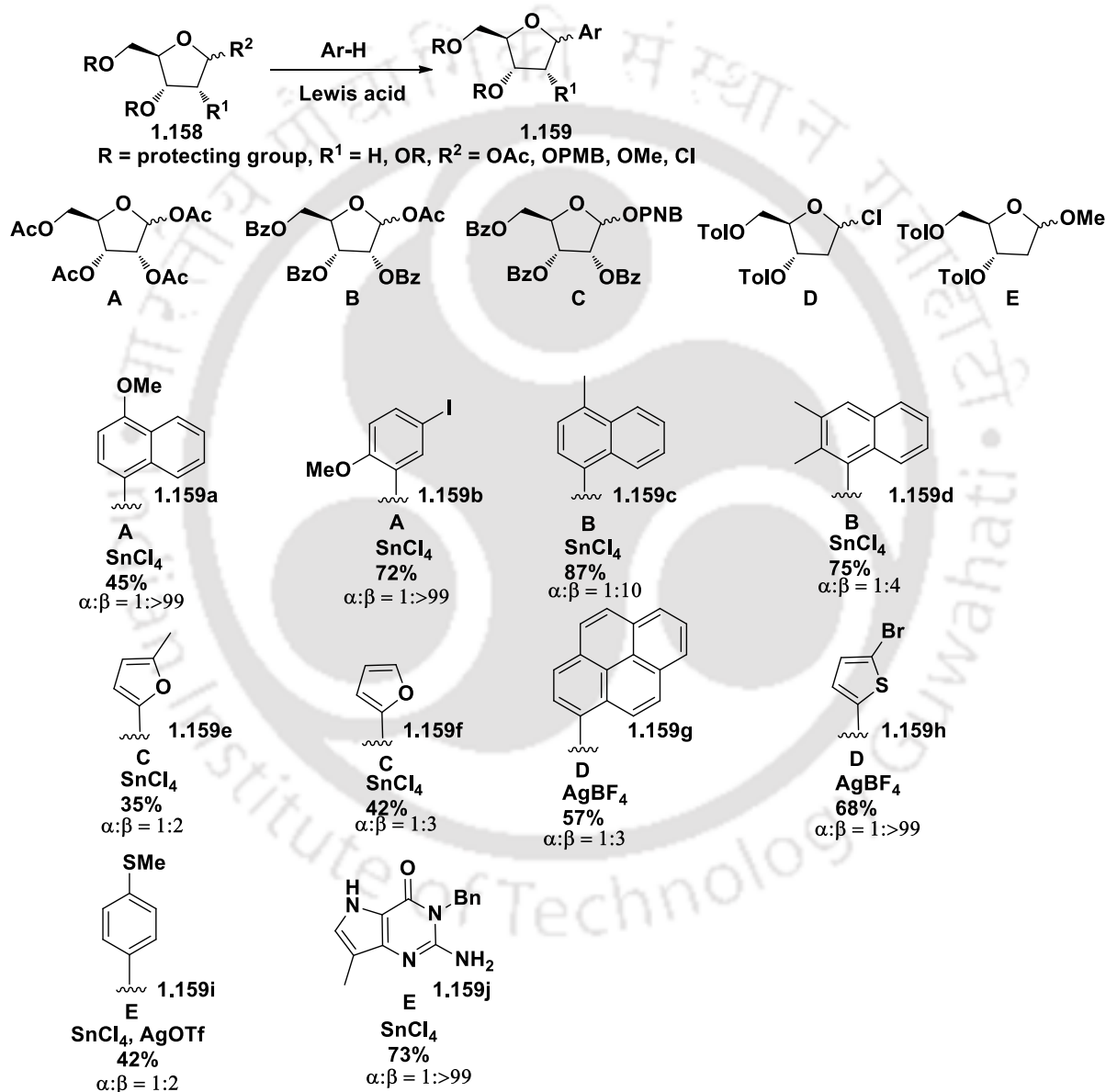


Scheme 1.27: Heck coupling in the construction of aglycon.

Here the aromatic groups are inserted onto the less sterically hindered 1' anomeric position because of the conjugation of oxygen lone pair with the $\text{C}_1'\text{-C}_2'$ double bond which makes C_2' more electron donating and that is why donation to the palladium happened from this end. β selectivity depends on protecting group attached with which oxygen. If it is attached

with 3'-OH group, then because of the 1,3-diaxial interaction it will prevail the aromatic moiety attached from β face. A and B in the **Scheme 1.27**^{47a,173-174} produced good yield because 5'-OH was not protected which led to the formation of α -anomer because of the 1,3 diaxial interaction created on the β face of the molecule. That is why C gives lower yield than A and B. In this way **1.154** and **1.155** are allowed to react in presence of palladium catalyst, base and additives with an aromatic halide/triflate to produce **1.156**. Deprotection followed by NaBH(OAc)₃ reduction provides **1.157a-l** in good to moderate yield (**Scheme 1.27**).

1.6.3.5. Lewis acid mediated C-nucleoside formation



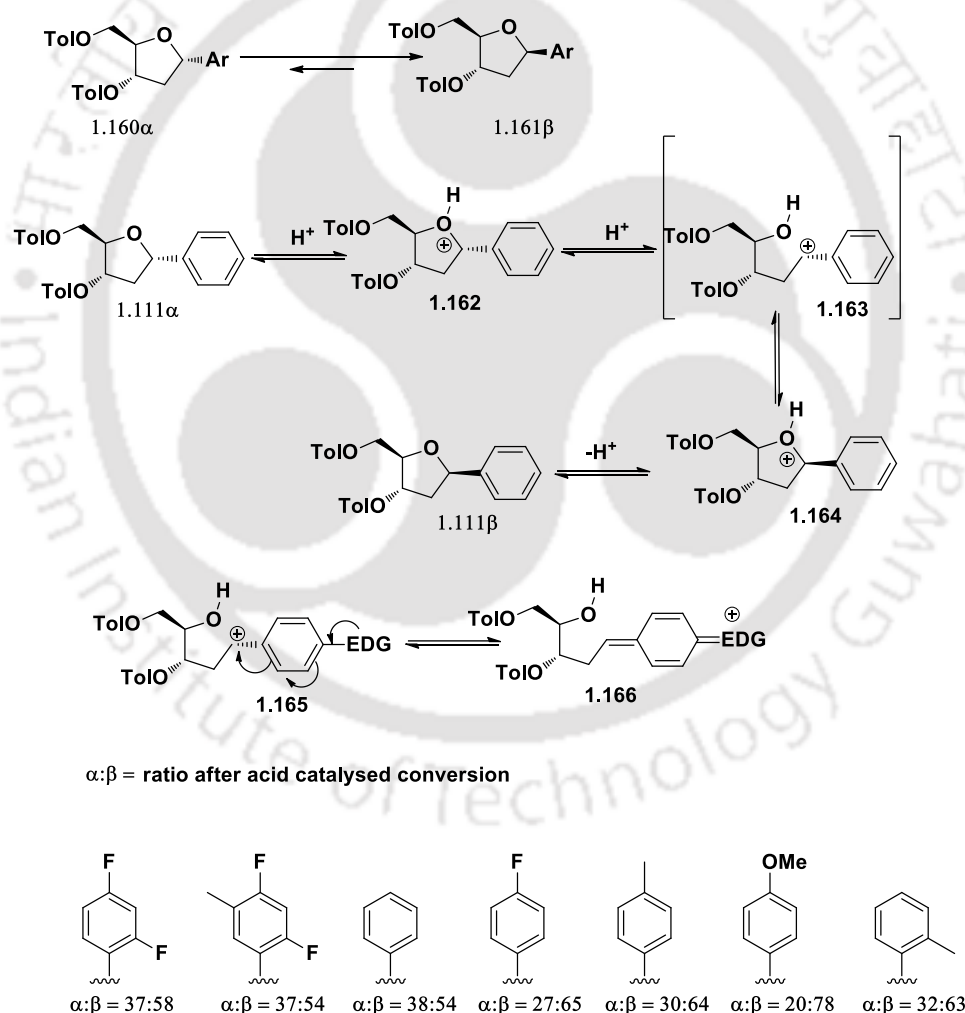
Scheme 1.28: Lewis acid functioning to forming aglycon.

Lewis acids like SnCl_4 , AgOTf , AgBF_4 , $\text{BF}_3\cdot\text{OEt}_3$ are the another class of reagents which helps leaving group, placed at the anomeric centre of the ribose sugar, to come out to make the

centre electrophilic and thus triggering electrophilic substitution reaction. Aromatic moieties those are capable of electron donation willingly takes part in this reaction. Regioselectivity depends on how donating group placed on the aromatic moiety triggering which centre to be more reactive towards electron donation. Whereas diastereoselectivity depends on the nature of the Lewis acid used as well as the nature of the protecting groups which are actually creating diastereoselectivity through providing steric crowding towards anomeric carbon. Formation of **1.159** started from **1.158** which is a Lewis acid mediated pathway towards electrophilic substitution onto the ribose sugar is shown in **Scheme 1.28**.^{175,176-180,133,181}

1.6.4. Nucleoside modification

During the synthesis of C-nucleoside the formation of undesired α -anomer formation is the synthetic drawback which reduced yield of the reaction.



Scheme 1.29: Acid catalysed anomeric conversion.

Most of the time it was observed that it is a major problem towards choosing reagents to synthesis predominantly β -isomer because of the nature of the sugar ring use to choose α -anomer formation. So the methodology has been developed by Stivers where the epimerisation of α to β anomer was done by the addition of a catalytic amount of the mixture of two acid TFA and benzenesulfonic acid (**Scheme 1.29**).^{182,183} Protonation of **1.111 α** makes the anomeric carbon electrophilic which in turn through acid catalysed equilibrium condition is converted into more stable β -anomer. The reaction was more driven towards β -conversion whenever an electron donating group would be present at the ortho or para position of the aromatic moiety in **1.163**. This electron donating group helps the positive charge created at the anomeric carbon to stabilise and thus facilitate the conversion process. Whereas on the other hand like the same way electron withdrawing ortho/para substituted aromatic moiety destabilise the positive charge created at the anomeric position and thus preventing the further progress towards β -conversion.

1.7. Conclusion

Benner's explicit approach towards nonstandard protein formation is a remarkable discovery triggered to explore chemically modified nucleoside with a new hope of installing ideas that are pretty much often interesting and absent in nature. As the nature provided A, T, G and C all are N-nucleoside so primarily the main focus was over N-nucleoside exploration. But step by step modification and a detail investigation over the structural features of the nucleosides gradually started unfolding the secret behind the regular helicity of the double strand DNA. While one group of people started examining how potent the hydrogen bonding parameter to shape the double strand in a regular way with proper helicity the Kool group introduced the world with a new idea called hydrophobicity. This parameter is exceptionally venerable parameter which excludes hydrogen bonding. It was then ascertained that π stacking that is supported by hydrophobicity is potent enough to make the DNA double strand a proper helix without the help of hydrogen bonding. So now the concept of DNA helicity was made clear which helps other to synthesize size expanded base pair which generally either paired through hydrogen bonding or π -stacking. All these base pairs are synthesized and incorporated within short oligonucleotide strand hoping to make them as a replicable base pair. C-nucleoside groups are not in the back side and instead gradually get attention due to the instability of N-nucleoside caused because of the presence of acid sensitive or enzymatically labile anomeric C-N linkage. They are also started designed and their synthesis through various protocol helping us to redesign nucleoside with new chemical features. The synthetic approaches involved Wittig type reaction, organometallic reaction, Heck reaction, Lewis acid mediated reaction, sugar ring formation etc. Although the nucleophilic addition approach in various ways like organometallic addition to the sugar unit followed by leaving group elimination suffers in the formation of desired ratio of β anomer, a new method was developed by which the anomeric conversion from α to β is possible with the treatment of proper acid catalyst. So there are a lot of protocols developed and are to develop to synthesis C-nucleoside and N-nucleoside. All these protocols are now helping us to enrich the nucleoside library with new worth.

1.8. Reference:

1. (a) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 735. (b) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 964.
2. Watson, J. D.; Baker T. A.; Bell S. P.; Gann A.; Levine M.; Richard L.; *Molecular Biology of the Gene* **1987**.
3. Watson, J. D.; *The Double Helix* **1968**.
4. Stephenson, M.L.; Zamecnik, P.C. *Proc. Natl. Acad. Sci. USA* **1998**, *75*, 285.
5. Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. *Nature* **1998**, *391*, 806.
6. Elbashir, S.M.; Harborth, J.; Lendeckel, W.; Yalcin, A.; Weber, K.; Tuschl, T. *Nature* **2001**, *411*, 494.
7. Katsumoto, Y.; Fukuchi-Mizutani, M.; Fukui, Y.; Brugliera, F.; Holton, T. A.; Karan, M.; Nakamura, N.; Yonekura-Sakakibara, K.; Togami, J.; Pigeaire, A.; Tao, G. Q.; Nehra, N. S.; Lu, C. Y.; Dyson, B. K.; Tsuda, S.; Ashikari, T.; Kusumi, T.; Mason, J. G.; Tanaka, Y. *Plant Cell Physiol.* **2007**, *48*, 1589.
8. Rodoni, B. C.; Dale, J. L.; Harding, R. M. *Arch. Virol.* **1999**, *144*, 1725.
9. Lewis, R. S.; Jack, A. M.; Morris, J. W.; Robert, V. J. M.; Gavilano, L. B.; Siminszky, B.; Bush, L. P.; Hayes, A. J.; Dewey, R. E. *Plant Biotechnol. J.* **2008**, *6*, 346.
10. Viswanath, V.; Story, G. M.; Peier, M.; Petrus, M. J.; Lee, V. M.; Hwang, S. W.; Patapoutian, A.; Jegla, T.; Biology, C.; Watmough, S. A.; Dillon, P. J.; Blum, J. D.; Klaue, A.; Carmen, A.; Siccama, T. G.; Eagar, C. *Brief Communication* **2003**, *423*, 19.
11. Abdolhamid Angaji, S.; Hedayati, S. S.; Poor, R. H.; Poor, S. S.; Shiravi, S.; Madani, S. *Plant Omics* **2010**, *3*, 77.
12. Dandekar, A. M.; Teo, G.; Defilippi, B. G.; Uratsu, S. L.; Passey, A. J.; Kader, A. A.; Stow, J. R.; Colgan, R. J.; James, D. J. *Transgenic Res.* **2004**, *13*, 373.
13. Switzer, C.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 8322.
14. Voegel, J. J.; Benner, S. A. *J. Am. Chem. Soc.* **1994**, *116*, 6929.
15. Bain, J. D.; Switzer, C.; Chamberlin, a R.; Benner, S. a. *Nature* **1992**, *356*, 537.
16. Martinot, T. A.; Benner, S. A. *J. Org. Chem.* **2004**, *69*, 3972.
17. Roberts, C.; Bandaru, R.; Switzer, C. *J. Am. Chem. Soc.* **1997**, *119*, 4640.
18. Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 4461.
19. Malyshev, D. A.; Seo, Y. J.; Ordoukhanian, P.; Romesberg, F. E. *J. Am. Chem. Soc.* **2009**, *131*, 14620.

Chapter 1

20. Malyshev, D. A.; Pfaff, D. A.; Ippoliti, S. I.; Hwang, G. T.; Dwyer, T. J.; Romesberg, F. E. *Chemistry* **2010**, 16, 12650.
21. Leconte, A.M.; Hwang, G. T.; Matsuda, S.; Capek, P.; Hari, Y.; Romesberg, F. E. *J. Am. Chem. Soc.* **2008**, 130, 2336.
22. (a) Drohat, A.; Maiti, A. *Org. Biomol. Chem.* **2014**, 12, 8367. (b) Prior, J. J.; Santi, D. V. *J. Biol. Chem.* **1984**, 259 (4), 2429. (c) Stockbridge, R. B.; Schroeder, G. K.; Wolfenden, R. *Bioorg. Chem.* **2010**, 38, 224. (d) Zoltewicz, J. A.; Clark, D. F.; Sharpless, T. W.; Grahe, G. *J. Am. Chem. Soc.* **1969**, 494, 1741.
23. (a) Matsuura, S.; Shiratori, O.; Katagiri, K. *J. Antibiot.* **1964**, 17, 234. (b) Nishimura, H.; Shimaoka, N.; Tanaka, Y.; Komatsu, Y.; Kato, H.; Mayama, M. *J. Antibiot.* **1964**, 17, 148.
24. (a) Cohn, W. E. *J. Biol. Chem.* **1960**, 235, 1488. (b) Cohn, W. E. *Biochim. Biophys. Acta* **1959**, 32, 569.
25. (a) Davis, D. R. *Nucleic Acids Res.* **1995**, 23, 5020. (b) Newby, M. I.; Greenbaum, N. L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, 99, 12697. (c) Desaulniers, J. P.; Chang, Y. C.; Aduri, R.; Abeyirigunawardena, S. C.; Santa Lucia, J.; Chow, C. S. *Org. Biomol. Chem.* **2008**, 6, 3892.
26. Koonin, E. V. *Nucleic Acids Res.* **1996**, 24, 2411.
27. Nakagawa, Y.; Kano, H.; Tsukuda, Y.; Koyama, H. *Tetrahedron Lett.* **1967**, 42, 4105.
28. Harki, D. A.; Graci, J. D.; Korneeva, V. S.; Ghosh, S. K. B.; Hong, Z.; Cameron, C. E.; Peterson, B. R. *Biochemistry* **2002**, 41, 9026. (b) Seela, F.; Jawalekar, A. *Helv. Chim. Acta* **2002**, 85, 1857.
29. Wang, L.; Schultz, P. G. *Chem. Commun.* **2002**, 1.
30. (a) Bain, J. D.; Schwitzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, 356, 537. (b) Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, 115, 4461.
31. Robins, R. K.; Srivastava, P. C.; Narayanan, V. L.; Plowman, J.; Paull, K. D. *J. Med. Chem.* **1982**, 25, 107.
32. Weber, G.; Prajda, N.; Abonyi, M.; Look, K. Y.; Tricot, G. *Anticancer Res.* **1996**, 16, 3313.
33. (a) Fridland, A. *Cancer Res.* **1986**, 46, 532. (b) Zatorski, A.; Watanabe, K. A.; Carr, S. F.; Goldstein, B. M.; Pankiewicz, K. W. *J. Med. Chem.* **1996**, 39, 2422. (c) Pankiewicz, K. W. *Pharmacol. Ther.* **1997**, 76, 89. (d) Szekeres, T.; Sedlak, J.; Novotny, L. *Curr. Med. Chem.* **2002**, 9, 759. (e) Pankiewicz, K. W.; Patterson, S. E. *Chemical Reviews* **2009**, 109, 6761. (f) P. L.; Jayaram, H. N.; Risal, D.; Goldstein, B. M.; Stuyver, L. J.; Schinazi, R. F. *Curr. Med. Chem.* **2004**, 11, 887.

34. (a) Chen, L. Q.; Pankiewicz, K. W. *Curr. Opin. Drug DiscoVery DeV.* **2007**, *10*, 403. (b) Chen, L.; Petrelli, R.; Felczak, K.; Gao, G.; Bonnac, L.; Yu, J. S.; Bennett, E. M.; Pankiewicz, K. W. *Curr. Med. Chem.* **2008**, *15*, 650.
35. Watanabe, K. A. Chemistry of C-nucleosides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York **1994**, **421**.
36. McLaughlin, L. W.; Wilson, M.; Ha, S. B. Use of Nucleoside Analogues to probe Biochemical Processes. In *Comprehensive natural product chemistry, Vol. 7 (DNA and Aspects of Molecular Biology)*; Barton, D. H. R.; Nakanishi, K.; Meth-Cohn, O., Eds. Elsevier: New York **1999**, 252.
37. For general reviews on C-glycosides synthesis, see: (a) Postema, M. H. D. *Tetrahedron* **1992**, *48*, 8545. (b) Jamarillo, C.; Knapp, S. *Synthesis* **1994**, *1*. (c) Shaban, M. A. E.; Nasr, A. Z. *AdV. Heterocycl. Chem.* **1997**, *68*, 223. (d) Du, Y. G.; Linhardt, R. J.; Vlahov, I. R. *Tetrahedron* **1998**, *54*, 9913. (f) Lee, D. Y. W.; He, M. S. *Curr. Top. Med. Chem.* **2005**, *5*, 1333. (e) Adamo, M. F. A.; Pergoli, R. *Curr. Org. Chem.* **2008**, *12*, 1544.
38. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319.
39. Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875.
40. Wu, Q.; Simons, C. *Synthesis* **2004**, *10*, 1533.
41. Kilroy, T. G.; O'Sullivan, T. P.; Guiry, P. J. *Eur. J. Org. Chem.* **2005**, *23*, 4929.
42. Wellington, K. W.; Benner, S. A. *Nucleosides, Nucleotides, Nucleic Acids* **2006**, *25*, 1309.
43. (a) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 735. (b) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 964.
44. Harki, D. A.; Graci, J. D.; Korneeva, V. S.; Ghosh, S. K. B.; Hong, Z.; Cameron, C. E.; Peterson, B. R. *Biochemistry* **2002**, *41*, 9026. (b) Seela, F.; Jawalekar, A. *Helv. Chim. Acta* **2002**, *85*, 1857.
45. (a) Bain, J. D.; Schwitzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537. (b) Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 4461.
46. Henry, A. A.; Romesberg, F. E. *Curr. Opin. Struct. Biol.* **2003**, *7*, 727.
47. (a) Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A. M.; Benner, S. A. *Nucleic Acids Res.* **2006**, *34*, 6095. (b) Sismour, A. M.; Benner, S. A. *Nucleic Acids Res.* **2005**, *33*, 5640.
48. Switzer, C.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 8322.
49. Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863.
50. (a) Clever, G. H.; Kaul, C.; Carell, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 6226. (b) Shionoya, M.; Tanaka, K. *Curr. Opin. Chem. Biol.* **2004**, *8*, 592. (c) Müller, J. *Eur. J. Inorg. Chem.* **2008**, *24*, 3749.

Chapter 1

51. (c) Rao, P.; Benner, S. A. *J. Org. Chem.* **2001**, 66, 5012. (d) Piccirilli, J. A.; Krauch, T.; MacPherson, L. J.; Benner, S. A. *Helv. Chim. Acta* **1991**, 74, 397.
52. Benner, S. A. *Acc. Chem. Res.* **2004**, 37, 784.
53. Leonard, N. J.; Scopes, D. I. C.; Vanderlij, P.; Barno, J. R. *Biochemistry* **1978**, 17, 3677.
54. Liu, H.; Gao, J.; Lynch, S. R.; Saito, D.; Maynard, L.; Kool, E. T. *Science* **2003**, 302, 868.
55. (a) Marx, A.; Summerer, D. *Angew. Chem.* **2004**, 116, 1653. (b) Liu, H.; Gao, J.; Maynard, L.; Saito, D. Y.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, 126, 1102.
56. (a) Liu, H.; Lynch, S. R.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, 126, 6900. (b) Gao, J.; Liu, H.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, 126, 11826. (c) Liu, H.; Gao, J.; Kool, E. T. *J. Org. Chem.* **2005**, 70, 639. (d) Gao, J.; Liu, H.; Kool, E. T. *Angew. Chem., Int. Ed.* **2005**, 44, 3118. (e) Delaney, J. C.; Gao, J.; Liu, H.; Shrivastav, N.; Essigmann, J. M.; Kool, E. T. *Angew. Chem., Int. Ed.* **2009**, 48, 4524.
57. Lynch, S. R.; Liu, H.; Gao, J.; Kool, E. T. *J. Am. Chem. Soc.* **2006**, 128, 14704.
58. (a) Lu, H.; He, K.; Kool, E. T. *Angew. Chem., Int. Ed.* **2005**, 43, 5834. (b) Lee, A. H. F.; Kool, E. T. *J. Am. Chem. Soc.* **2005**, 127, 3331.
59. Doi, Y.; Chiba, J.; Morikawa, T.; Inouye, M. *J. Am. Chem. Soc.* **2008**, 130, 8762.
60. (a) Minakawa, N.; Kojima, N.; Hikishima, S.; Sasaki, T.; Kiyosue, A.; Atsumi, N.; Ueno, Y.; Matsuda, A. *J. Am. Chem. Soc.* **2003**, 125, 9970. (b) Hikishima, S.; Minakawa, N.; Kuramoto, K.; Ogata, S.; Matsuda, A. *ChemBioChem* **2006**, 7, 1970.
61. Minakawa, N.; Ogata, S.; Takahashi, M.; Matsuda, A. *J. Am. Chem. Soc.* **2009**, 131, 1644.
62. (a) Hirao, I. *Curr. Opin. Chem. Biol.* **2006**, 10, 622. (b) Hirao, I.; Kimoto, M.; Mitsui, T.; Fujiwara, T.; Kawai, R.; Sato, A.; Harada, Y.; Yokoyama, S. *Nature Methods* **2006**, 3, 729.
63. Ishikawa, M.; Hirao, I.; Yokoyama, S. *Tetrahedron Lett.* **2000**, 41, 3931.
64. Mitsui, T.; Kimoto, M.; Harada, Y.; Yokoyama, S.; Hirao, I. *J. Am. Chem. Soc.* **2005**, 127, 8652.
65. (a) Hirao, I.; Fujiwara, T.; Kimoto, M.; Mitsui, T.; Okuni, T.; Ohtsuki, T.; Yokoyama, S. *Nucleic Acids Symp. Ser.* **2000**, 44, 261. (b) Fujiwara, T.; Sugiyama, H.; Hirao, I.; Yokoyama, S. *Nucleic Acids Symp. Ser.* **2000**, 44, 43. (c) Mitsui, T.; Kimoto, K.; Sato, A.; Yokoyama, S.; Hirao, I. *Bioorg. Med. Chem. Lett.* **2003**, 13, 4515.

66. Hirao, I.; Ohtsuki, T.; Fujiwara, T.; Mitsui, T.; Yokogawa, T.; Okuni, T.; Nakayama, H.; Takio, K.; Yabuki, T.; Kigawa, T.; Kodama, K.; Yokogawa, T.; Nishikawa, K.; Yokoyama, S. *Nat. Biotechnol.* **2002**, *20*, 177.
67. Tawarada, R.; Seio, K.; Sekine, M. *J. Org. Chem.* **2008**, *73*, 383.
68. Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863.
69. For comprehensive reviews, see: (a) Kool, E. T. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1. (b) Kool, E. T. *Annu. Rev. Biochem.* **2002**, *71*, 191.
70. (a) Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, *59*, 7238. (b) Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 737.
71. (a) Moran, S.; Ren, R. X.-F.; Rumney, S. I. V.; Kool, E. T. *J. Am. Chem. Soc.* **1997**, *119*, 2056. (b) Moran, S.; Ren, R. X.-F.; Kool, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10506.
72. Kool, E. T.; Sintim, H. O. *Chem. Commun.* **2006**, *35*, 3665.
73. Griesang, N.; Richert, C. *Tetrahedron Lett.* **2002**, *43*, 8755.
74. (a) Chiramonte, M.; Moore, C. L.; Kincaid, K.; Kuchta, R. D. *Biochemistry* **2003**, *42*, 10472. (b) Kincaid, K.; Beckman, J.; Zivkovic, A.; Halcomb, R. L.; Engels, J. W.; Kuchta, R. D. *Nucleic Acids Res.* **2005**, *33*, 2620. (c) Beckman, J.; Kincaid, K.; Hocek, M.; Spratt, T.; Engels, J.; Cosstick, R.; Kuchta, R. D. *Biochemistry* **2007**, *46*, 448.
75. Kim, T. W.; Kool, E. T. *J. Org. Chem.* **2005**, *70*, 2048.
76. Lai, J.; Qu, Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 3040. (b) Lai, J. S.; Kool, E. T. *Chem. Eur. J.* **2005**, *11*, 2966.
77. Mathis, G.; Hunziker, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 3203.
78. Ren, R. X.-F.; Narayan, C.; Chaudhuri, P. L.; Rumney, S. I. V.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 7671.
79. (a) Ogawa, A. K.; Wu, Y.; McMinn, D. L.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 3274. (b) Wu, Y.; Ogawa, A. K.; Berger, M.; McMinn, D. L.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 7621. (c) Berger, M.; Ogawa, A. K.; McMinn, D. L.; Wu, Y.; Schultz, P. G.; Romesberg, F. E. *Angew. Chem., Int. Ed.* **2000**, *39*, 2940.
80. (a) Ogawa, A. K.; Wu, Y.; Berger, M.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 8803. (b) Matsuda, S.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 14419. (c) Matsuda, S.; Henry, A. A.; Romesberg, F. E. *J. Am. Chem. Soc.* **2006**, *128*, 6369. (d) Henry, A. A.; Olsen, A. G.; Matsuda, S.; Yu, C.; Geierstanger, B. H.; Romseberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 6923.
81. Kim, Y.; Leconte, A. M.; Hari, Y.; Romesberg, F. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 7809.

Chapter 1

82. Hari, Y.; Hwang, G. T.; Leconte, A. M.; Joubert, N.; Hocek, M.; Romesberg, F. E. *ChemBioChem* **2008**, 9, 2796.
83. Matsuda, S.; Henry, A. A.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2003**, 125, 6134.
84. Hwang, G. T.; Romesberg, F. E. *Nucleic Acids Res.* **2006**, 34, 2037.
85. Berger, M.; Luzzi, S. D.; Henry, A. A.; Romesberg, F. E. *J. Am. Chem. Soc.* **2002**, 124, 1222.
86. (a) Brotschi, C.; Haerberli, A.; Leumann, C. J. *Angew. Chem., Int. Ed.* **2001**, 40, 3012. (b) Brotschi, C.; Mathis, G.; Leumann, C. J. *Chem.sEur. J.* **2005**, 11, 1911. (c) Zahn, A.; Brotschi, C.; Leumann, C. J. *Chem.sEur. J.* **2005**, 11, 2125.
87. Johar, Z.; Zahn, A.; Leumann, C. J.; Jaun, B. *Chem.sEur. J.* **2008**, 14, 1080.
88. (a) Singh, I.; Hecker, W.; Prasad, A. K.; Parmar, V. S.; Seitz, O. *Chem. Commun.* **2002**, 500. (b) Beuck, C.; Singh, I.; Bhattacharya, A.; Hecker, W.; Parmar, V. S.; Seitz, O.; Weinhold, E. *Angew. Chem., Int. Ed.* **2003**, 42, 3958.
89. Kaufmann, M.; Gisler, M.; Leumann, C. J. *Angew. Chem., Int. Ed.* **2009**, 48, 3810.
90. Grigorenko, N. A.; Leumann, C. J. *Chem.sEur. J.* **2009**, 15, 639.
91. (a) Straßler, C.; Davis, N. E.; Kool, E. T. *Helv. Chim. Acta* **1999**, 82, 2160. (b) Gao, J.; Straßler, C.; Tahmassebi, D.; Kool, E. T. *J. Am. Chem. Soc.* **2002**, 124, 11590. (c) Gao, J.; Watanabe, S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, 126, 12748. (d) Wilson, J. N.; Teo, Y. N.; Kool, E. T. *J. Am. Chem. Soc.* **2007**, 129, 15426.
92. Smirnov, S.; Matray, T. J.; Kool, E. T.; de los Santos, C. *Nucleic Acids Res.* **2002**, 30, 5561. (b) Matray, T. J.; Kool, E. T. *Nature* **1999**, 399, 704.
93. Morales, H.; Kool, E. T. *Org. Lett.* **2002**, 12, 4377.
94. Robertson, N.; McGowan, C. A. *Chem. Soc. Rev.* **2003**, 32, 96.
95. Blundell, S. J.; Pratt, F. L. *J. Phys.: Condens. Matter* **2004**, 16, R771.
96. (a) Tanaka, K.; Shionoya, M. *J. Org. Chem.* **1999**, 64, 5002. (b) Shionoya, M.; Tanaka, K. *Bull. Chem. Soc. Jpn.* **2000**, 73, 1945.
97. (a) Tanaka, K.; Clever, G. H.; Takezawa, Y.; Yamada, Y.; Kaul, C.; Shionoya, M.; Carell, T. *Nat. Nanotechnol.* **2006**, 1, 190. (b) Clever, G. H.; Carell, T. *Angew. Chem., Int. Ed.* **2007**, 46, 250.
98. Cao, H.; Tanaka, K.; Shionoya, M. *Chem. Pharm. Bull.* **2000**, 48, 1745.
99. (a) Tanaka, K.; Yamada, Y.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, 124, 8802. (b) Zimmermann, N.; Meggers, E.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, 124, 13684. (c) Polonius, F.-A.; Müller, J. *Angew. Chem., Int. Ed.* **2007**, 46, 5602.

100. (a) Shin, D.; Switzer, C. *Chem. Commun.* **2007**, 4401. (b) Heuberger, B. D.; Shin, D.; Switzer, C. *Org. Lett.* **2008**, *10*, 1091.
101. (a) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shiro, M.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 12494. (b) Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714. (c) Atwell, S.; Meggers, E.; Spraggon, G.; Schultz, P. G. *J. Am. Chem. Soc.* **2001**, *123*, 12364. (d) Zimmermann, N.; Meggers, E.; Schultz, P. G. *Bioorg. Chem.* **2004**, *32*, 13. (e) Weizman, H.; Tor, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3375. (f) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212. (g) Zhang, L.; Meggers, E. *J. Am. Chem. Soc.* **2005**, *127*, 4174.
102. (a) Clever, G. H.; Söhl, Y.; Burks, H.; Spahl, W.; Carell, T. *Chem.sEur. J.* **2006**, *12*, 8708. (b) Clever, G. H.; Polborn, K.; Carell, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 7204.
103. (a) Switzer, C.; Shin, D. *Chem. Commun.* **2005**, 1342. (b) Switzer, C.; Sinha, S.; Kim, P. H.; Heuberger, B. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 1529.
104. Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; Machinami, T.; Ono, A. *J. Am. Chem. Soc.* **2006**, *128*, 2172.
105. (a) Clever, G. H.; Söhl, Y.; Burks, H.; Spahl, W.; Carell, T. *Chem.sEur. J.* **2006**, *12*, 8708. (b) Clever, G. H.; Polborn, K.; Carell, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 7204.
106. (a) Tanaka, K.; Tasaka, M.; Cao, H.; Shionoya, M. *Eur. J. Pharm. Sci.* **2001**, *13*, 77. (b) Tasaka, M.; Tanaka, K.; Shiro, M.; Shionoya, M. *Supramol. Chem.* **2001**, *13*, 671. (c) Tanaka, K.; Tasaka, M.; Cao, H.; Shionoya, M. *Supramol. Chem.* **2002**, *14*, 255.
107. (a) Seo, Y. J.; Hwang, G. T.; Ordoukhanian, P.; Romesberg, F. E. *J. Am. Chem. Soc.* **2009**, *131*, 3246. (b) Seo, Y. J.; Matsuda, S.; Romesberg, F. E. *J. Am. Chem. Soc.* **2009**, *131*, 5046.
108. Leconte, A. M.; Hwang, G. T.; Matsuda, S.; Capek, P.; Hari, Y.; Romesberg, F. E. *J. Am. Chem. Soc.* **2008**, *130*, 2336.
109. Qui, Z.; Lu, L.; Jian, X.; He, C. *J. Am. Chem. Soc.* **2008**, *130*, 14398.
110. Warncke, S.; Gégout, A.; Carell, T. *ChemBioChem* **2009**, *10*, 728.
111. Teo, N. Y.; Wilson, J. N.; Kool, E. T. *J. Am. Chem. Soc.* **2009**, *131*, 3923.
112. (a) Cuppoletti, A.; Cho, Y.; Park, J. S.; Strassler, S.; Kool, E. T. *Bioconjugate Chem.* **2005**, *16*, 528. (b) Wilson, J. N.; Cho, Y.; Tan, S.; Cuppoletti, A.; Kool, E. T. *ChemBioChem* **2008**, *9*, 279. (c) Wilson, J. N.; Gao, J.; Kool, E. T. *Tetrahedron* **2007**, *63*, 3427.
113. (a) Cohn, W. E. *J. Biol. Chem.* **1960**, *235*, 1488. (b) Cohn, W. E. *Biochim. Biophys. Acta* **1959**, *32*, 569.

Chapter 1

114. (a) Davis, D. R. *Nucleic Acids Res.* **1995**, 23, 5020. (b) Newby, M. I.; Greenbaum, N. L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, 99, 12697. (c) Desaulniers, J. P.; Chang, Y. C.; Aduri, R.; Abeyasirigunawardena, S. C.; Santa Lucia, J.; Chow, C. S. *Org. Biomol. Chem.* **2008**, 6, 3892.
115. Koonin, E. V. *Nucleic Acids Res.* **1996**, 24, 2411.
116. (a) Matsuura, S.; Shiratori, O.; Katagiri, K. *J. Antibiot.* **1964**, 17, 234. (b) Nishimura, H.; Shimaoka, N.; Tanaka, Y.; Komatsu, Y.; Kato, H.; Mayama, M. *J. Antibiot.* **1964**, 17, 148. (c) Nakagawa, Y.; Kano, H.; Tsukuda, Y.; Koyama, H. *Tetrahedron Lett.* **1967**, 42, 4105.
117. Uehara, Y. I.; Fisher, J. M.; Rabinowicz, M. *Biochem. Pharmacol.* **1980**, 29, 2199.
118. Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q. L.; Tomassini, J. E.; Xia, J. *J. Med. Chem.* **2004**, 47, 2283.
119. Butora, G.; Olsen, D. B.; Carroll, S. S.; McMaster, D. R.; Schmitt, C.; Leone, J. F.; Stahlhut, M.; Burlein, C.; MacCoss, M. *Bioorg. Med. Chem.* **2007**, 15, 5219.
120. Comprehensive review: Bzowska, A. In *Modified Nucleosides: Biochemistry, Biotechnology and Medicine*; Herdewijn, P., Ed.; Wiley-VCH: Weinheim, Germany, 2008, 473.
121. (a) Miles, R. W.; Tyler, P. C.; Furneaux, R. H.; Bagdassarian, C. K.; Schramm, V. L. *Biochemistry* **1998**, 37, 8615. (b) Kicska, G. A.; Long, L.; Hořrig, H.; Fairchild, C.; Tyler, P. C.; Furneaux, R. H.; Schramm, V. L.; Kaufman, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, 98, 4593. (c) Evans, G. B.; Furneaux, R. H.; Gainsford, G. J.; Hanson, J. C.; Kicska, G. A.; Sauve, A. A.; Schramm, V. L.; Tyler, P. C. *J. Med. Chem.* **2003**, 46, 155.
122. (a) Shi, W.; Basso, L. A.; Santos, D. S.; Tyler, P. C.; Furneaux, R. H.; Blanchard, J. S.; Almo, S. C.; Schramm, V. L. *Biochemistry* **2001**, 40, 8204. (b) Federov, A.; Shi, W.; Kicska, G.; Federov, E.; Tyler, P. C.; Furneaux, R. H.; Hanson, J. C.; Gainsford, G. J.; Larese, J. Z.; Schramm, V. L.; Almo, S. C. *Biochemistry* **2001**, 40, 853. (c) Shi, W.; Li, C. M.; Tyler, P. C.; Furneaux, R. H.; Grubmeyer, C.; Schramm, V. L.; Almo, S. C. *Nat. Struct. Biol.* **1999**, 6, 588.
123. (a) Basso, L. A.; Santos, D. S.; Shi, W.; Furneaux, R. H.; Tyler, P. C.; Schramm, V. L.; Blanchard, J. S. *Biochemistry* **2001**, 40, 8196. (b) Kicska, G. A.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H.; Kim, K.; Schramm, V. L. *J. Biol. Chem.* **2002**, 277, 3219. (c) Kicska, G. A.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H.; Schramm, V. L.; Kim, K. *J. Biol. Chem.* **2002**, 277, 3226.
124. (a) Evans, G. B.; Furneaux, R. H.; Lewandowicz, A.; Schramm, V. L.; Tyler, P. C. *J. Med. Chem.* **2003**, 46, 5271. (b) Murkin, A. S.; Tyler, P. C.; Schramm, V. L. *J. Am. Chem. Soc.* **2008**, 130, 2166.

125. (a) Fridland, A. *Cancer Res.* **1986**, 46, 532. (b) Zatorski, A.; Watanabe, K. A.; Carr, S. F.; Goldstein, B. M.; Pankiewicz, K. W. *J. Med. Chem.* **1996**, 39, 2422. (c) Pankiewicz, K. W. *Pharmacol. Ther.* **1997**, 76, 89. (d) Szekeres, T.; Sedlak, J.; Novotny, L. *Curr. Med. Chem.* **2002**, 9, 759. (e) Pankiewicz, K. W.; Patterson, S. E.; Black, P. L.; Jayaram, H. N.; Risal, D.; Goldstein, B. M.; Stuyver, L. J.; Schinazi, R. F. *Curr. Med. Chem.* **2004**, 11, 887.
126. (a) Chen, L. Q.; Pankiewicz, K. W. *Curr. Opin. Drug DiscoVery DeV.* **2007**, 10, 403. (b) Chen, L.; Petrelli, R.; Felczak, K.; Gao, G.; Bonnac, L.; Yu, J. S.; Bennett, E. M.; Pankiewicz, K. W. *Curr. Med. Chem.* **2008**, 15, 650.
127. (a) Sarma, M. S. P.; Wilson, P.; Otter, B. A.; Klein, R. S. *Nucleosides, Nucleotides Nucleic Acids* **1995**, 14, 397 Synthesis of thiazofurines: (b) Ramasamy, K. S.; Lau, J. Y. N. *Nucleosides, Nucleotides Nucleic Acids* **2001**, 20, 1329. (d) Rothman, J. H. *J. Org.Chem.* **2007**, 72, 3945.
128. Weber, G.; Prajda, N.; Abonyi, M.; Look, K. Y.; Tricot, G. *Anticancer Res.* **1996**, 16, 3313.
129. (a) Krohn, K.; Heins, H.; Wielckens, K. *J. Med. Chem.* **1992**, 35, 511. (b) Jayaram, H. N.; Gharehbaghi, K.; Jayaram, N. H.; Rieser, J.; Krohn, K.; Paull, K. D. *Biochem. Biophys. Res. Commun.* **1992**, 186, 1600. (c) Gharehbaghi, K.; Paull, K. D.; Kelley, J. A.; Barchi, J. J.; Marquez, V. E.; Cooney, D. A.; Monks, A.; Scudiero, D.; Krohn, K.; Jayaram, H. N. *Int. J. Cancer* **1994**, 56, 892. (d) Saunders, P. P.; Arimilli, S.; Krohn, K.; Muhs, M. A.; Alvarez, E.; Survelyer, R. *Anti- Cancer Drugs* **1996**, 7, 93. (e) Grush, M.; Rosenberger, G.; Fuhrman, G.; Braun, K.; Titscher, B.; Szekeres, T.; Fritzer-Szekeres, M.; Oberhuber, G.; Krohn, K.; Hengstschlaeger, M.; Krupitza, G.; Jayaram, H. N. *Cell Death Differ.* **1999**, 8, 736. (f) Krohn, K.; Dorner, H.; Zukowski, M. *Curr. Med. Chem.* **2002**, 9, 727. (g) Gharehbaghi, K.; Grunberger, W.; Jayaram, H. N. *Curr. Med. Chem.* **2002**, 9, 743.
130. (a) Srivastava, P. C.; Robins, R. K. *J. Med. Chem.* **1983**, 26, 445. (b) Kirsi, J. J.; North, J.; McKernan, P. A.; Murray, B. K.; Canonico, P. G.; Huggins, J. W.; Srivastava, P. C.; Robins, R. K. *Antimicrob. Agents Chemother.* **1983**, 24, 353.
131. Franchetti, P.; Marchetti, S.; Cappellacci, L.; Yalowitz, J. A.; Jarayam, H. M.; Goldstein, B. M.; Grifantini, M. *Bioorg. Med. Chem. Lett.* **2001**, 11, 67.
132. Franchetti, P.; Cappellacci, L.; Abu Sheikha, G.; Jayaram, H. N.; Gurudutt, V. V.; Sint, T.; Schneider, B. P.; Jones, W. D.; Goldstein, B. M.; Perra, G.; De Montis, A.; Loi, A. G.; La Colla, P.; Grifantini, M. *J. Med. Chem.* **1997**, 40, 1731.
133. Franchetti, P.; Cappellacci, L.; Grifantini, M.; Barzi, A.; Nocentini, G.; Yang, H.; O'Connor, A.; Jayaram, H. N.; Carrell, C.; Goldstein, B. M. *J. Med. Chem.* **1995**, 38, 3829.
134. Adlington, R. M.; Baldwin, J. E.; Pritchard, G. J.; Spencer, K. C. *Tetrahedron Lett.* **2000**, 41, 575.

135. Veronse, A. C.; Morelli, C. F. *Tetrahedron Lett.* **1998**, 39, 3853.
136. Al-Masoudi, N. A.; Al-Soud, Y. A.; Ali, I. A. I. *Nucleosides, Nucleotides Nucleic Acids* **2007**, 26, 37.
137. Adelfinskaya, O.; Wu, W.; Davisson, V. J.; Bergstrom, D. E. *Nucleosides, Nucleotides Nucleic Acids* **2005**, 24, 1919.
138. Jung, M. E.; Trifunovich, I. D. *Tetrahedron Lett.* **1992**, 33, 2921.
139. Li, J.-S.; Gold, B. *J. Org. Chem.* **2005**, 70, 8764.
140. Butora, G.; Olsen, D. B.; Carroll, S. S.; McMaster, D. R.; Schmitt, C.; Leone, J. F.; Stahlhut, M.; Burlein, C.; MacCoss, M. *Bioorg. Med. Chem.* **2007**, 15, 5219.
141. (a) Liang, C.; Ma, T.; Cooperwood, J. S.; Du, J.; Chu, C. K. *Carbohydr. Res.* **1997**, 303, 33. For an example on the first Wittig-type strategy ever, see: (c) Ohru, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, 97, 4602. (d) De Bernardo, S.; Weigele, M. *J. Org. Chem.* **1977**, 42, 109. Utilization of exocyclic CHO group in Wittig reaction: (e) Krishna, P. R.; Reddy, V. V. R.; Srinivas, R. *J. Org. Chem.* **2007**, 63, 9871.
142. Barrett, A. G. M.; Broughton, H. B.; Attwood, S. V.; Gunatilaka, A. A. L. *J. Org. Chem.* **1986**, 51, 495.
143. Lim, M.-I.; Klein, R. S. *Tetrahedron Lett.* **1981**, 22, 25.
144. Chen, J. J.; Drach, J. C.; Townsend, L. B. *J. Org. Chem.* **2003**, 68, 4170.
145. Ohno, M.; Ito, Y.; Arita, M.; Shibata, T.; Adachi, K.; Sawai, H. *Tetrahedron* **1984**, 40, 145.
146. Kozikowski, A. P.; Ghosh, A. K. *J. Org. Chem.* **1984**, 49, 2762.
147. Wamhoff, H.; Warnecke, H. *ARKIVOC* **2001**, 95.
148. (a) Adamo, M. F. A.; Pergoli, R. *Org. Lett.* **2007**, 9, 4443. (b) Heinrich, D.; Wagner, T.; Diederichsen, U. *Org. Lett.* **2007**, 9, 5311. (c) Chiba, J.; Takeshima, S.; Mishima, K.; Maeda, H.; Nanai, Y.; Mizuno, K.; Inouye, M. *Chem. Eur. J.* **2007**, 13, 8124. (d) Lubin-Garmain, N.; Baltaze, J.-P.; Coste, A.; Hallonet, A.; Laure'ano, H.; Legrave, G.; Uziel, J.; Auge', J. *Org. Lett.* **2008**, 10, 725.
149. Joshi, U.; Josse, S.; Pipelier, M.; Chevallier, F.; Prade're, J.-P.; Hazard, R.; Legoupy, S.; Huet, F.; Dubreuil, D. *Tetrahedron Lett.* **2004**, 45, 1031.
150. Kaliappan, K. P.; Subrahmanyam, A. V. *Org. Lett.* **2007**, 9, 1121.
151. Nova'k, P.; Pohl, R.; Katora, M.; Hocek, M. *Org. Lett.* **2006**, 8, 2051.
152. (a) Yamamoto, Y.; Saigoku, T.; Ohgai, T.; Nishiyama, H.; Itoh, K. *Chem. Commun.* **2004**, 2702. (b) Yamamoto, Y.; Saigoku, T.; Nishiyama, H.; Ohgai, T.; Itoh, K. *Org. Biomol. Chem.* **2005**, 3, 1768. (c) Yamamoto, Y.; Hashimoto, T.; Hattori, K.; Kikuchi,

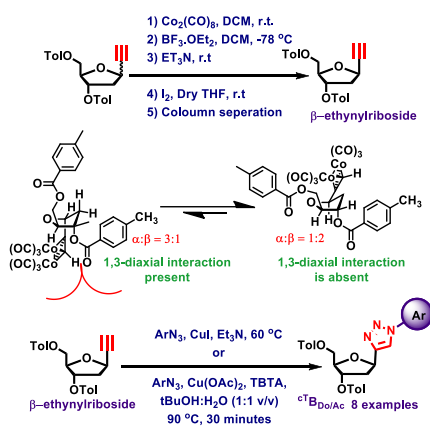
- M.; Nishiyama, H. *Org. Lett.* **2006**, *8*, 3565. (d) Yamamoto, Y.; Yamashita, K.; Hotta, T.; Hashimoto, T.; Kuikuchi, M.; Nishiyama, H. *Chem. Asian J.* **2007**, *2*, 1388.
153. Trost, B. M.; Kallander, L. S. *J. Org. Chem.* **1999**, *64*, 5427.
154. Kametani, T.; Kawamura, K.; Honda, T. *J. Am. Chem. Soc.* **1987**, *109*, 3010.
155. Jeanmart, S.; Taylor, R. J. K. *Tetrahedron Lett.* **2005**, *46*, 9043.
156. Stork, G.; Suh, H. S.; Kim, G. *J. Am. Chem. Soc.* **1991**, *113*, 7054.
157. Gudmundsson, K. S.; Drach, J. C.; Townsend, L. B. *J. Org. Chem.* **1998**, *63*, 984.
158. (a) Calter, M. A.; Sugathapala, P. M.; Zhu, C. *Tetrahedron Lett.* **1997**, *38*, 3837. (b) Calter, M. A.; Zhu, C. *J. Org. Chem.* **1999**, *64*, 1415. (c) Angle, S. R.; Nietzel, M. L. *J. Org. Chem.* **1999**, *64*, 8754. (d) Jones, K.; Toutounji, T. *Tetrahedron* **2001**, *57*, 2427.
159. Shaban, M. A. E. *Adv. Heterocycl. Chem.* **1997**, *70*, 163.
160. Brown, D. M.; Ogden, R. C. *J. Chem. Soc., Perkin. Trans.* **1981**, *4*, 723.
161. Koc̣ovsky', P.; Pour, M. *J. Org. Chem.* **1990**, *55*, 5580.
162. Rothman, J. H. *J. Org. Chem.* **2009**, *74*, 925.
163. Singh, I.; Seitz, O. *Org. Lett.* **2006**, *8*, 4319.
164. Hurd, C. D.; Bonner, W. A. *J. Am. Chem. Soc.* **1945**, *67*, 1972.
165. Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777.
166. (a) Klein, R. S.; Kotick, M. P.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1971**, *36*, 4113. (b) Sharma, R. A.; Bobek, M.; Bloch, A. *J. Med. Chem.* **1975**, *18*, 473. (c) Pirrung, M. C.; Zhao, X.; Harris, S. V. *J. Org. Chem.* **2001**, *66*, 2067. (d) Singh, I.; Hecker, W.; Prasad, A. K.; Parmar, V. S.; Seitz, O. *Chem. Commun.* **2002**, 500.
167. Chen, D. W.; Beuscher, A. E.; Stevens, R. C.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *J. Org. Chem.* **2001**, *66*, 1725.
168. Butora, G.; Olsen, D. B.; Carroll, S. S.; McMaster, D. R.; Schmitt, C.; Leone, J. F.; Stahlhut, M.; Burlein, C.; MacCoss, M. *Bioorg. Med. Chem.* **2007**, *15*, 5219.
169. Shapiro, R.; Chambers, R. W. *J. Am. Chem. Soc.* **1961**, *83*, 3920.
170. Chaudhuri, N. C.; Kool, E. T. *Tetrahedron Lett.* **1995**, *36*, 1795.
171. Hocek, M.; Pohl, R.; Klepeta' r'ova', B. *Eur. J. Org. Chem.* **2005**, 4525.
172. Hainka, S.; Singh, I.; Hemmings, J.; Seitz, O. *J. Org. Chem.* **2007**, *72*, 8811.
173. Coleman, R. S.; Berg, M. A.; Murphy, C. J. *Tetrahedron* **2007**, *63*, 3450.
174. Joubert, N.; Pohl, R.; Klepeta' r'ova', B.; Hocek, M. *J. Org. Chem.* **2007**, *72*, 6797.

Chapter 1

175. Peyron, C.; Navarre, J. M.; Dubreuil, D.; Vierling, P.; Benhida, R. *Tetrahedron Lett.* **2008**, 49, 6171.
176. Ba'rtá, J.; Pohl, R.; Klepeta'ř'ova', B.; Ernsting, N. P.; Hocek, M. *J. Org. Chem.* **2008**, 73, 3798.
177. (a) Cermola, F.; Iesce, M. R.; Buonerba, G. *J. Org. Chem.* **2005**, 70, 6503. Pyrazoline buildup, see: (b) Cermola, F.; Iesce, M. R. *Tetrahedron* **2006**, 62, 10694.
178. Liu, M.-C.; Luo, M.-Z.; Mozdziesz, D. E.; Sartorelli, A. C. *Nucleosides, Nucleotides Nucleic Acids* **2005**, 24, 45.
179. Hainke, S.; Arndt, S.; Seitz, O. *Org. Biomol. Chem.* **2005**, 3, 4233.
180. Hamamichi, N.; Miyasaka, T. *J. Org. Chem.* **1991**, 56, 3731.
181. (a) Inoue, T.; Kuwajima, I. *J. Chem. Soc., Chem. Commun.* **1980**, 251. (b) Girgis, N. S.; Michael, M. A.; Smee, D. F.; Alaghamandan, H. A.; Robins, R. K.; Cottam, H. B. *J. Med. Chem.* **1990**, 33, 2750. (c) Farr, R. N.; Kwok, D. I.; Daves, G. D. *J. Org. Chem.* **1992**, 57, 2093. (d) Patil, S. A.; Otter, B. A.; Klein, R. S. *J. Heterocycl. Chem.* **1993**, 30, 509. (e) Mumper, M. W.; Aurenge, C.; Hosmane, R. S. *Bioorg. Med. Chem. Lett.* **1993**, 3, 2847. (f) Hatano, A.; Makita, S.; Kirihara, M. *Tetrahedron* **2005**, 61, 1723. For an example utilizing protected ribofuransyl fluorides, see: (g) Chen, J. J.; Wei, Y.; Williams, J. D.; Drach, J. C.; Townsend, L. B. *Nucleosides, Nucleotides Nucleic Acids* **2005**, 24, 1417.
182. Chaudhuri, N. C.; Ren, R. X. F.; Kool, E. T. *Synlett* **1997**, 341.
183. (a) Jiang, Y. L.; Stivers, J. T. *Tetrahedron Lett.* **2003**, 44, 4051. (b) Jiang, Y. L.; Stivers, J. T. *Tetrahedron Lett.* **2003**, 44, 85.

Chapter 2

SYNTHESIS, PHOTOPHYSICAL PROPERTY OF TRIAZOLYL C- NUCLEOSIDE AND STUDY OF INTERACTION WITH PROTEIN BSA



2.1. Introduction

Acid catalysed hydrolysis and enzymatic C-N glycosidic bond disruption leading us to think about C-nucleoside synthesis.¹ As a result of tremendous research effort a lot of methodologies are available now a day for the synthesis of C-nucleosides.²⁻¹⁷ Organometallic reagents,¹⁸⁻²² Wittig reaction,²³⁻²⁴ ozonolysis^{25,26} are the techniques enormously employed in developing unnatural C-nucleosides. Last couple of years C-nucleosides find enormous application in different areas of chemistry, biochemistry, bio medical science and biotechnology. Besides they can be used as antibiotic and cytotoxic material.^{2,6}

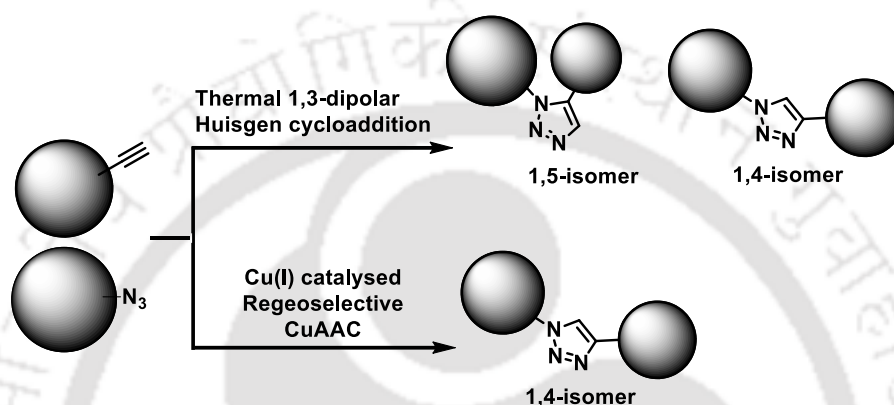


Figure 2.1: Huisgen 1,3-dipolar azide-alkyne cycloaddition vs Sharpless Copper catalysed 1,3-dipolar azide-alkyne cycloaddition.

In recent times click reaction has got attention in various ways in almost all branches of chemistry and biology. In the year of 1963, Rolf Huisgen,²⁷ a German chemist has discovered 1,3-dipolar cycloaddition reaction²⁷ between an organic azide and an alkyne to generate a five membered heterocyclic triazole moiety.²⁸ But the major drawback of this reaction includes the robust reaction condition, slow reaction rate and more over the electronic limitations on the azide and alkyne, where only the LUMO of an electron poor dipolarophile i.e. an alkyne can interact with the HOMO of an electron rich dipole which is an azide. But, after that in the year of 2001, such limitations were overcome by an American chemist, K. Barry Sharpless. He discovered Copper catalysed azide-alkyne cycloaddition (CuAAC)²⁹ reaction (**Figure 2.1**) which is a Cu(I) catalysed 1,3-dipolar azide-alkyne cycloaddition.³⁰ It is an environment friendly reaction. Here water can be used as a solvent. It does not demand any drastic condition and the rate of the reaction is also comparatively faster than Huisgen 1,3 dipolar cycloaddition. It doesn't produce any undesirable biproducts (**Figure 2.1-2.2**). Most of the protocols involve either Cu(I) or Cu(II) with a reducing agent like sodium ascorbate as the catalyst. In addition, this copper catalysed click reaction offers an array of electron donating and withdrawing azide and alkyne to form 5 membered 1,2,3-triazole ring with an excellent regioselectivity.

So in comparison with Huisgen 1,3-dipolar cycloaddition, Sharpless 1,3-dipolar cycloaddition is believed to be accepted more wide in scope, flexible, fully regiospecific and more over this is an environment friendly reaction. **Figure 2.2** explains the proposed mechanism of click reaction where the role of Cu(I) in forming triazole heterocycle followed by subsequent electrophilic substitution at the cuprated nucleophilic carbon is explained.

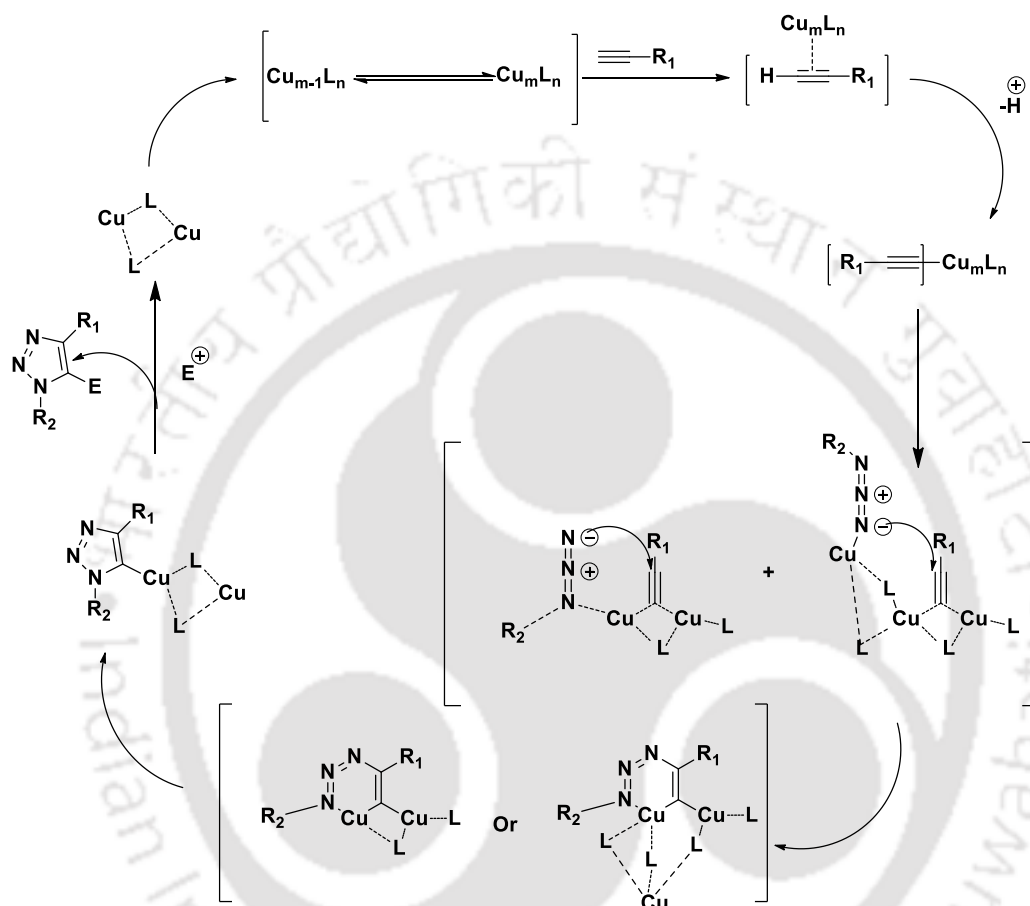
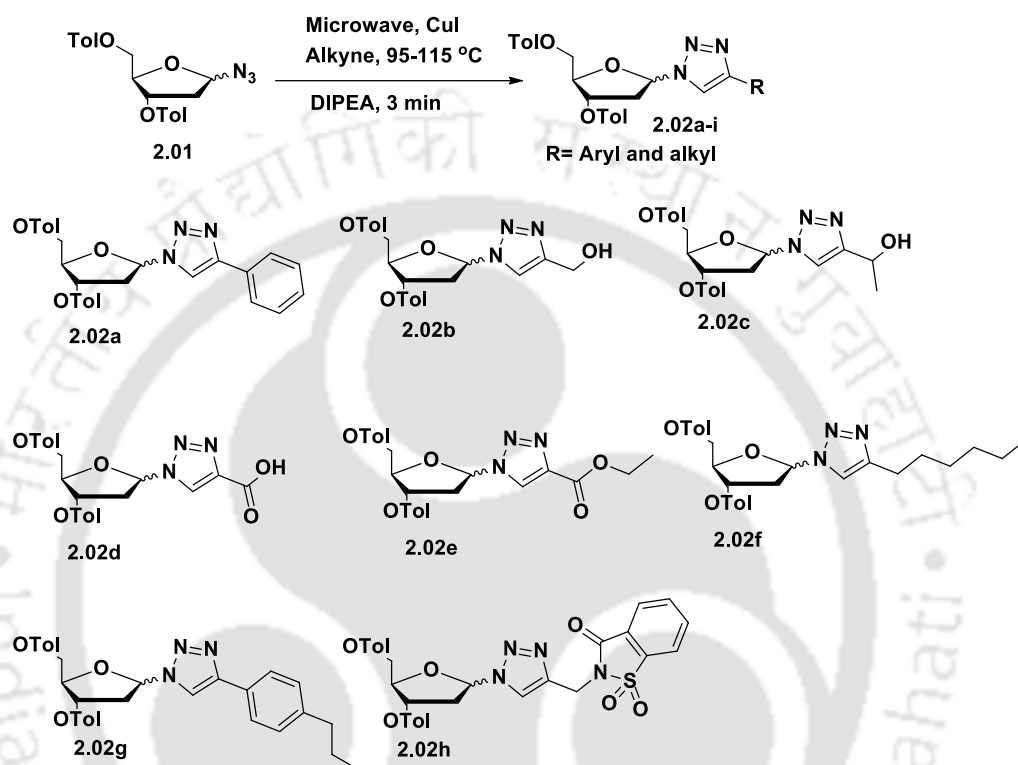


Figure 2.2: Mechanism of Click chemistry

2.2. Click chemistry in the modification of nucleosides

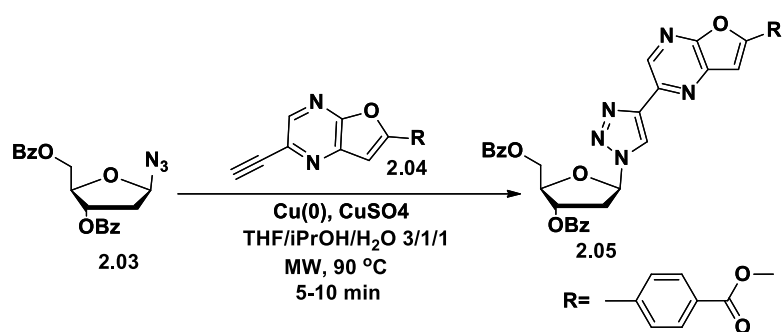
A handful of examples exist here, where click chemistry is the key step to synthesize synthetic nucleosides. Generally, click chemistry can be performed in either room temperature or a moderate heat is needed to form the triazole moiety with the use of Cu(I) as a catalyst in presence of amine base. Sometimes it has been found that this reaction condition is not enough for complete and efficient conversion to product with a required time frame. To enhance the yield of the reaction and to minimise the duration of reaction time, microwave irradiation has turned attention to the click chemistry.

Guezguez *et al.*³² treated β -1'-azido isomer of the sugar **2.01** with a series of alkyne and observed that it is impossible to achieve the complete conversion of this type click reaction before 24 h. Here they have used aromatic and aliphatic alkyne. But the yield of the reaction is dissatisfied in both the cases. So to accelerate the speed of the reaction they used microwave irradiation with 95-115 °C heat and observed that the reaction time was dramatically changed from 24 h to 3 min with CuI as a catalyst and DIPEA as a base (**Scheme 2.1**).



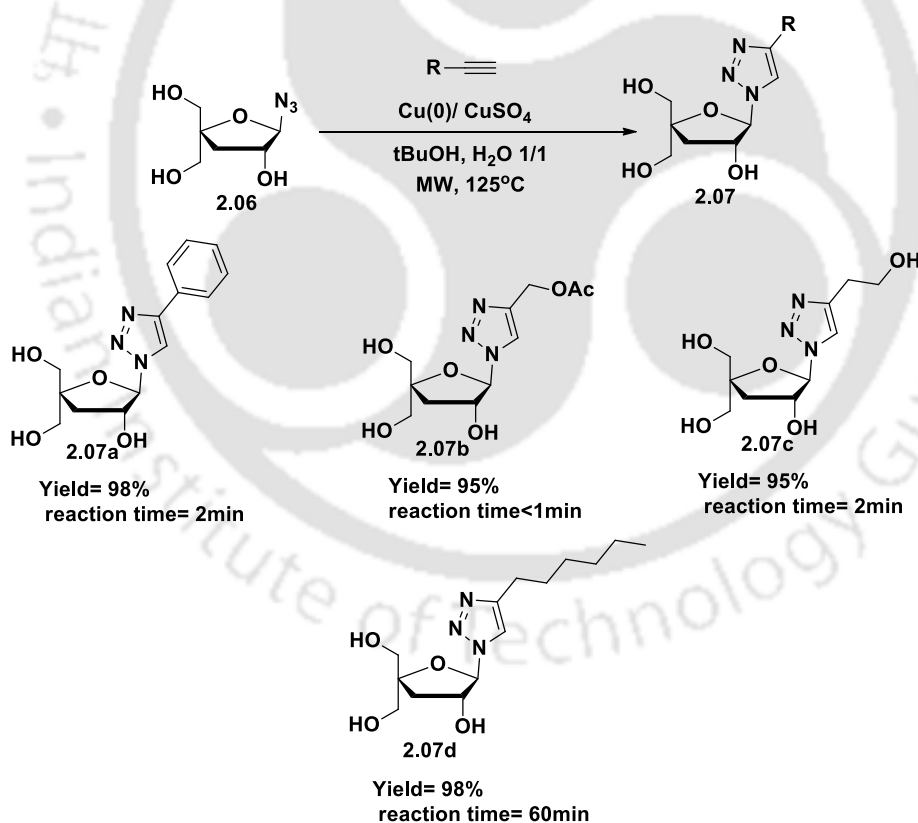
Scheme 2.1: Microwave irradiated click reaction by Guezguez *et al.*

Eycken *et al.*³³ and Broggi *et al.*³⁴ have also shown that microwave irradiation has a marked impact on click chemistry. This will help to complete the reaction within a few minutes. Benzyl protected β -1'-azidodeoxyribose **2.03** reacted with an alkyne **2.04** remain in conjugation with an heterocyclic ring producing the 1,4-substituted 1,2,3-triazole ring **2.05** catalysed by Cu(0) and Cu(II) within 5-10 minutes (**Scheme 2.2**).



Scheme 2.2: Microwave irradiated click reaction by Eycken *et al.*

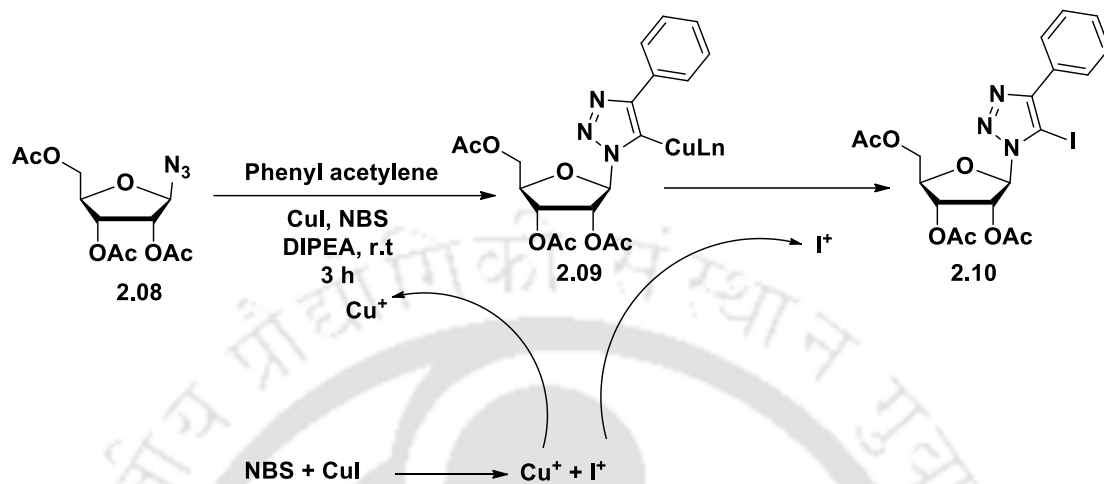
Whereas Broggi *et al.*³⁴ has shown that in the the β -1'-azido sugar **2.06** catalysed by the same Cu(0) – Cu(II) catalyst surprisingly produced the 1,2,3-triazole ring **2.07** within 2 minutes only (**Scheme 2.3**).



Scheme 2.3: Microwave irradiated click reaction by Broggi *et al.*

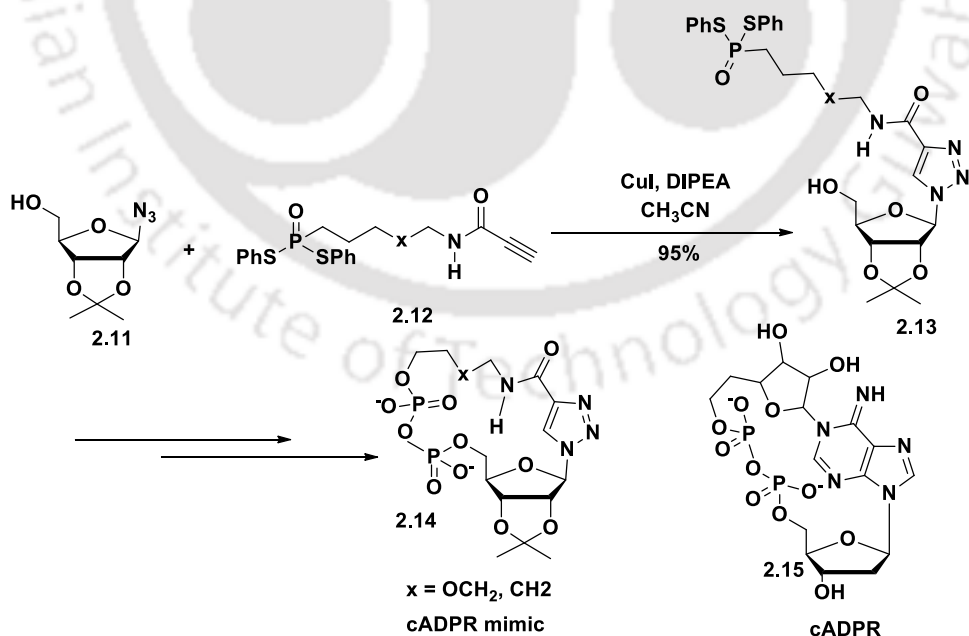
Further modification of the heterocyclic 1,2,3-triazole ring was done by Li *et al.*³⁵ They introduced one iodine in 5 position of the triazole ring which would open a new way to modify the 5 position of the triazole ring. The azido sugar moiety **2.08** and phenyl alkyne reacted in

presence of 1 equivalent of CuI and 1 equivalent of NBS to produce 1,4 disubstituted 5-iodo-1,2,3-triazole **2.10**. According to the author the CuI and NBS catalytic system provides both of Cu^+ and I^+ for the efficient formation of 5-iodo triazole ring in presence of restricted amount DIPEA (**Scheme 2.4**).



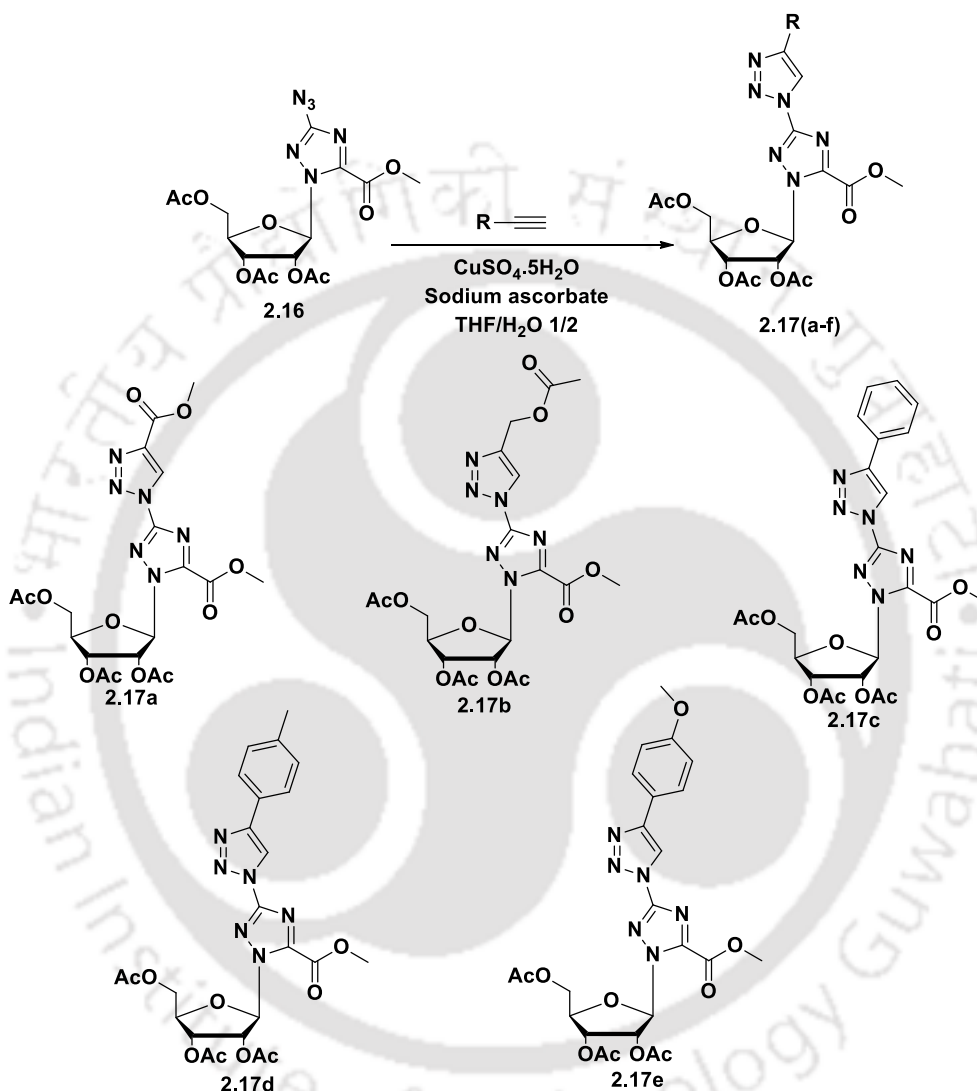
Scheme 2.4: 5-iodo triazole formation by Li *et al.*

Li *et al.* has also applied click chemistry to modify the cADPR **2.15**,³⁶ which is an endogenous cellular messenger nucleotide, controls calcium signalling (**Scheme 2.5**).



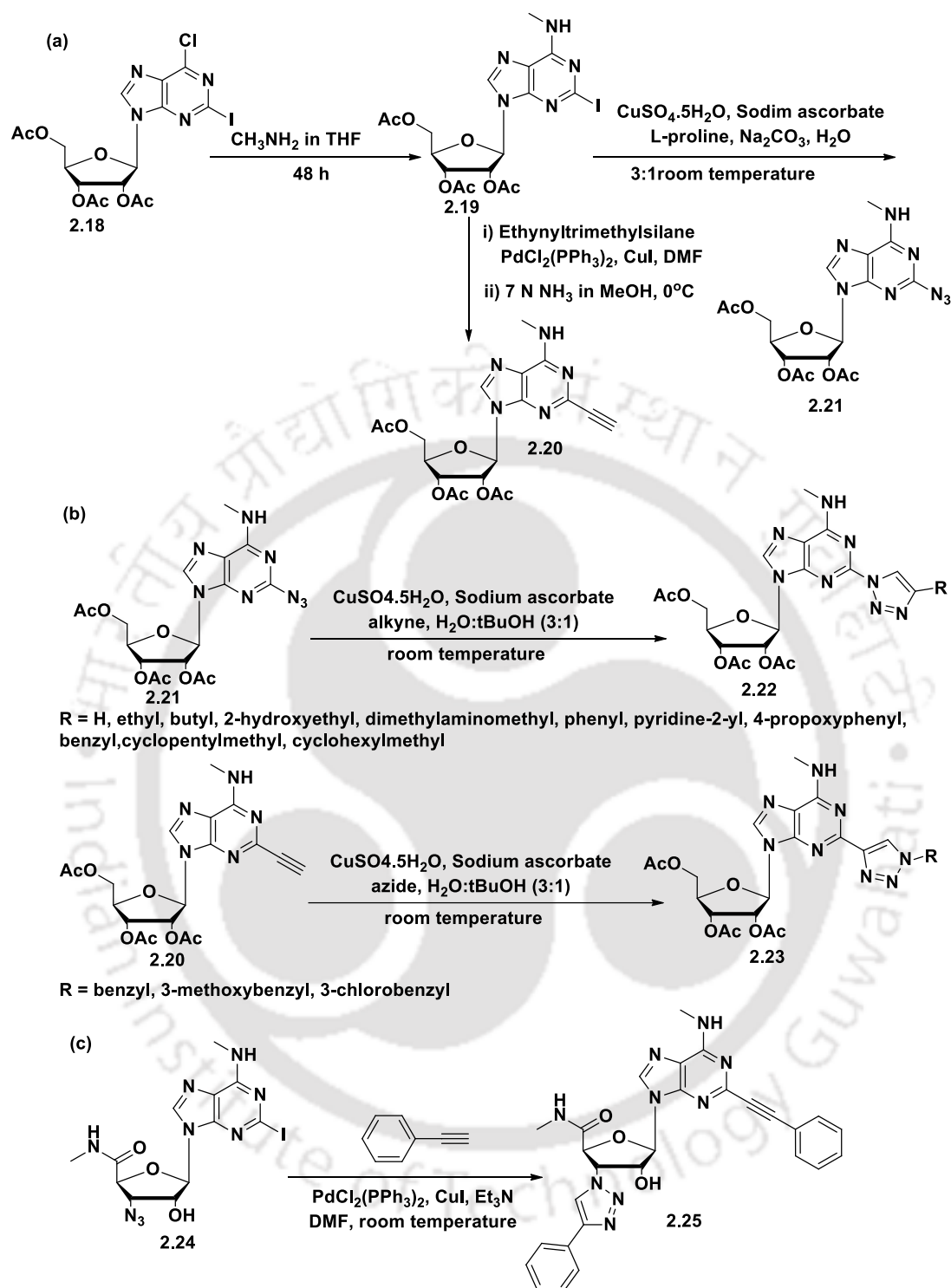
Scheme 2.5: Synthesis of cADPR mimic

Nucleosides with bis-triazole as modified bases have also been reported in which one triazole part is 1,2,3-triazole is synthesised via copper catalysed click reaction and the another triazole part is 1,2,4-triazole. These new nucleosides which find application as antiviral agent against tobacco mosaic virus (TMV) was reported by Xia *et al.*³⁷ They have shown that compound **2.17c** and **2.17d** showed comparable antiviral activity with ribavirin (**Scheme 2.6**).



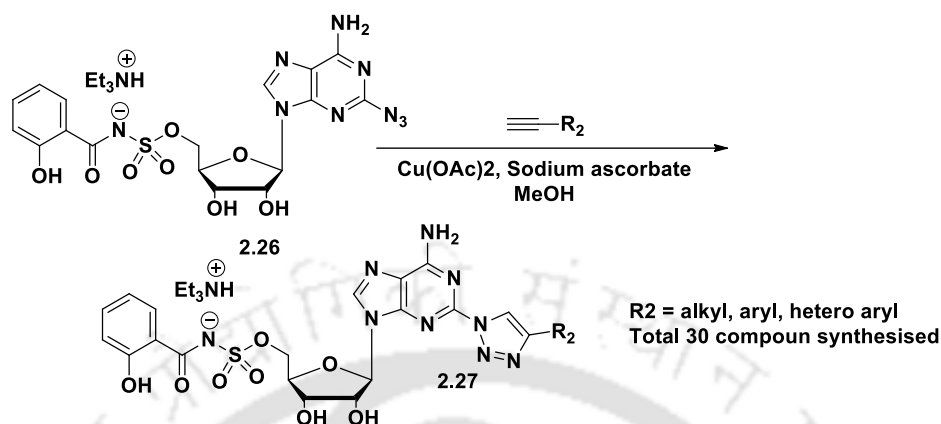
Scheme 2.6: Anti TMV nucleoside analogues.

A range of Adenosine analogues have been prepared by Cosyn *et al.*³⁸ starting from the common intermediate **2.19** where two types of reaction were involved to prepare the alkyne **2.20** and azide **2.21** derivative of the 6-chloro-2-iodo-adenosine **2.18** (**Scheme 2.7a**). After that **2.21** and **2.20** separately reacted with different types of azide and alkyne respectively to prepare the 1,2,3-triazolo derivative of adenosine (**Scheme 2.7b**). Cosyn *et al.*³⁹ (**Scheme 2.7c**) reported that if the precursor contains both azide and a halo substituent **2.24** then under sonogashira condition the azide part also reacted with the alkyne.



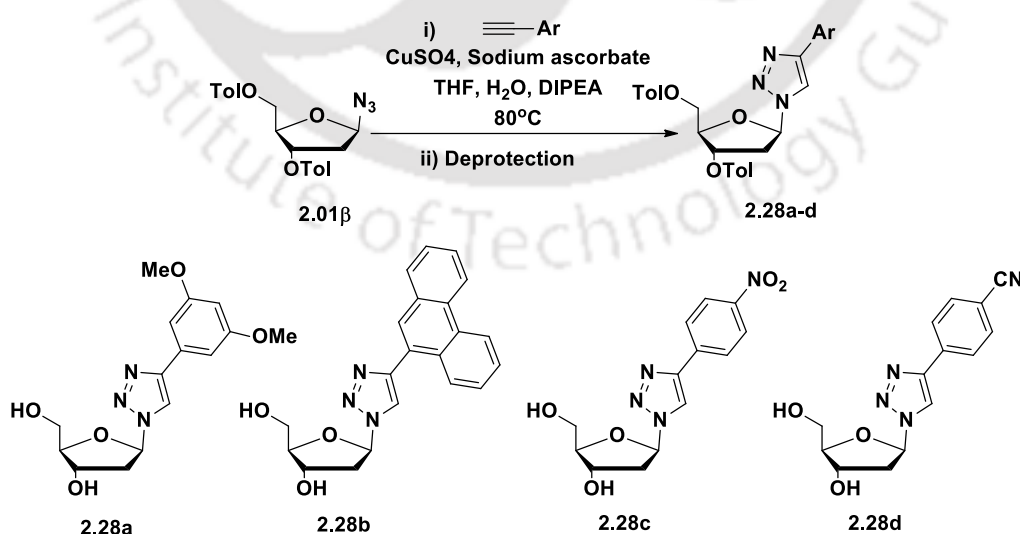
Scheme 2.7: (a) Modification of N⁶methyl-2-iodo-adenosine. (b) Triazole modified Adenosine analogues. (c) Sonogashira condition assisted one pot click Sonogashira.

Inspired by the effort of Cosyn *et al.*, Gupte *et al.*⁴⁰ synthesised some novel adenosine analogues **2.27** via click chemistry which showed anti-tuberculosis activity in submicromolar to micromolar range (**Scheme 2.8**).



Scheme 2.8: Click assisted synthesis of anti-tuberculosis adenosine analogues.

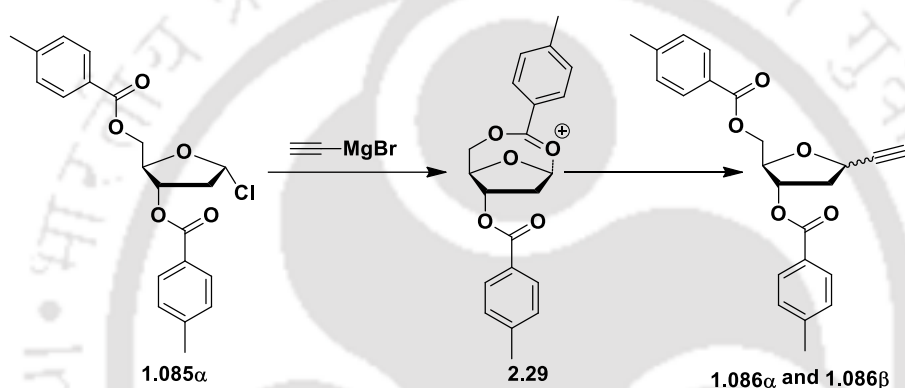
In recent years the synthesis and modification of nucleoside analogues, incorporation into short DNA and study of duplex stability has drawn a special attention. On the basis of that concept our research group previously reported the synthesized donor acceptor unnatural nucleosides **2.28a-d** via click chemistry approach and incorporated these nucleosides into the DNA to measure duplex stability, photophysical properties etc. (**Scheme 2.9**).^{41h} A pair of such donor-acceptor bases was found to stabilize a duplex via ground state charge transfer interaction to the extent comparable to that offered by a natural A-T pair.



Scheme 2.9: Donor-acceptor triazolyl nucleoside synthesis through click chemistry.

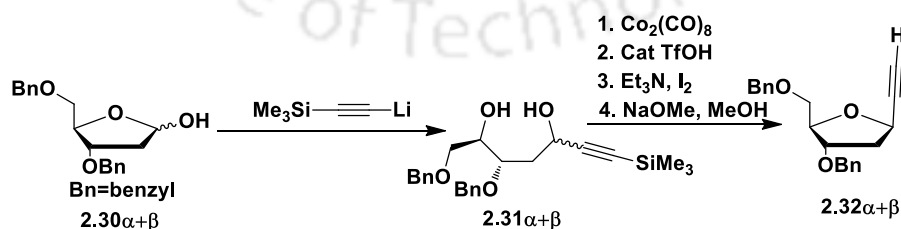
2.3. Synthesis of 1-ethynylriboside

In this section we will discuss about different protocol to synthesize **1.086**. In most of the cases it has been found that the α -anomer is the major product. In the year 2001 Heinrich *et al.*⁴² (Scheme 2.10) synthesised 2-deoxy-3,5-bis[O-(p-toluoyl)]- α -D-ethynylribofuranoside **1.086 α** as a major product where the minor product is β isomer. They have started it with Hoffer's chloro sugar **1.085 α** . Then ethynyl magnesium bromide was allowed to react with **1.085 α** to form this 1-ethynylriboside **1.086 α** and **1.086 β** . They have postulated intermediate **2.29** and according to them through this intermediate the reaction was proceeded. They explained that here neighbouring group i.e. 5'-toluoyl group participation assisted the elimination of the chlorine which is the key to generate preferably α -isomer, **1.086 α** where the α : β ratio of this 1-ethynylriboside is found to be 3:1.



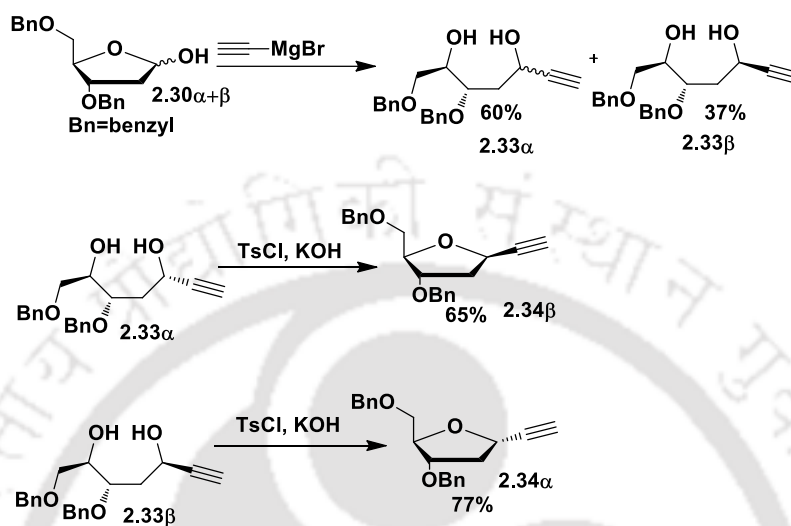
Scheme 2.10: Ethynylmagnesium bromide mediated synthesis of 1-ethynylriboside.

In the year 2002, Inoyue⁴³ has synthesised trimethylsilylated β -ethynylriboside (Scheme 2.11) started from bisbenzylprotected riboside **2.30**. Treatment of alkynyl lithium gives **2.31 α** and **2.31 β** . Treatment of **2.31 α** and **2.31 β** with cobalt carbonyl and subsequent treatment of TfOH, Et₃N and I₂ produces **2.36 β** in a very good 99% yield.



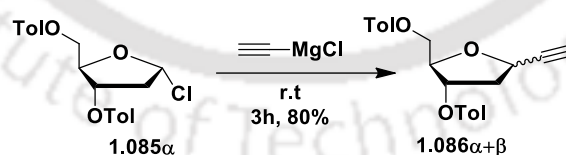
Scheme 2.11: Inoyue method of synthesising trimethylsilylated β -ethynylriboside.

Adamo *et al.*⁴⁴ in the year 2007 synthesised also 1-ethynyl riboside **2.34 α** and **2.34 β** via different procedure. They first used ethynyl magnesium bromide to **2.30** which was also used by Inoyue as a substrate. Then **2.33 α** and **2.33 β** were differently treated with TsOH and KOH which gives **2.34 α** and **2.34 β** in 65% and 77% yield respectively (**Scheme 2.12**).



Scheme 2.12: Adamo method of synthesising diastereo pure α - and β -ethynylriboside.

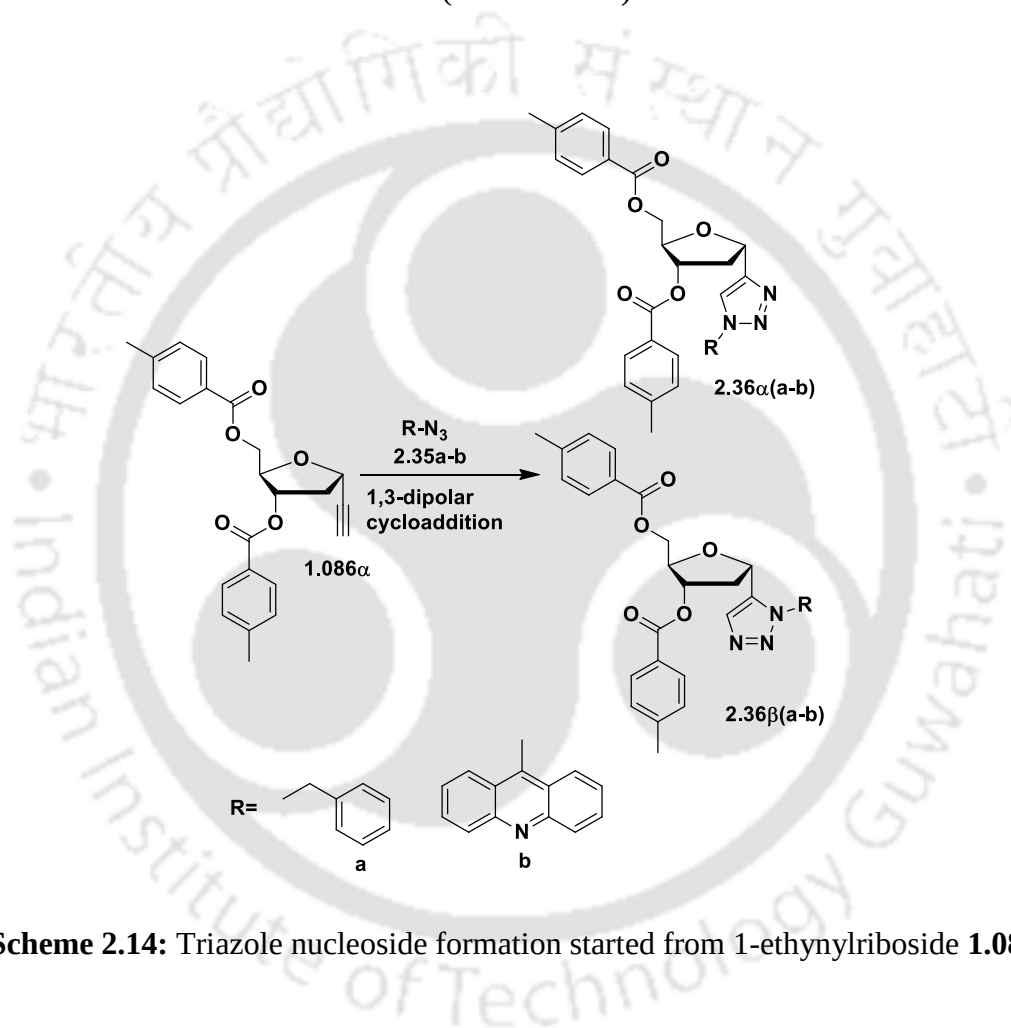
In the year 2008 Kotora⁴⁵ synthesised 1-ethynyl riboside **1.086** started from Hoffer's chloro sugar **1.085 α** . They treated Hoffer's chloro sugar **1.085 α** with ethynyl magnesium chloride in dry THF and got 80% yield of the 1-ethynylriboside **1.086** which was a mixture of α and β anomer. Here the α : β ratio is stated as 2:1 (**Scheme 2.13**).



Scheme 2.13: Kotora method of synthesising 1-ethynylriboside, predominantly α -anomer.

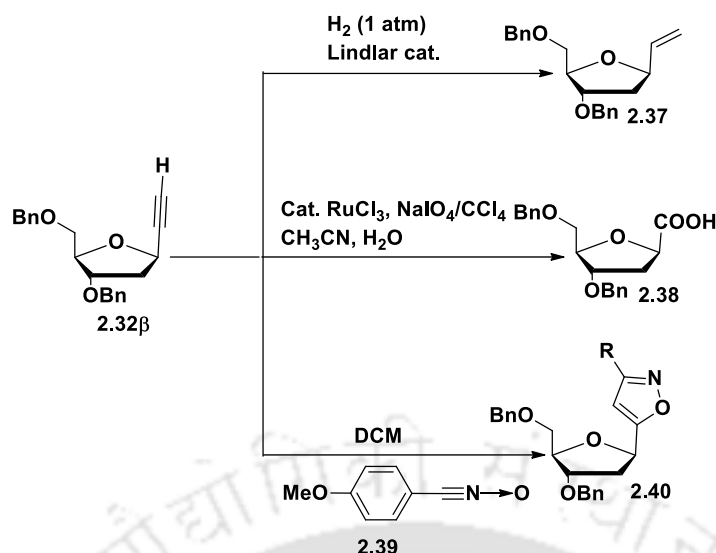
2.4. Synthesis of different C-nucleoside analogues starting from 1-ethynyl riboside

Wamhoff⁴² *et al.* used Huisgen 1,3 dipolar cycloaddition where azide **2.35a-b** were allowed to react with one alkyne and as the stereoselectivity of the reaction is low it produced 2 products. So bistoluoylprotected ethynylriboside **1.086a** was allowed to react with azide **2.35a-b** and produced **2.36a** (**a-b**) and **2.36b** (**a-b**) in 62% and 34% yield respectively. The ratio of these two product is roughly around 3:2. Although the separation of these isomer through column chromatography was failed but NOE and COSY gave some conclusive signal to determine the structure of these isomer (**Scheme 2.14**).



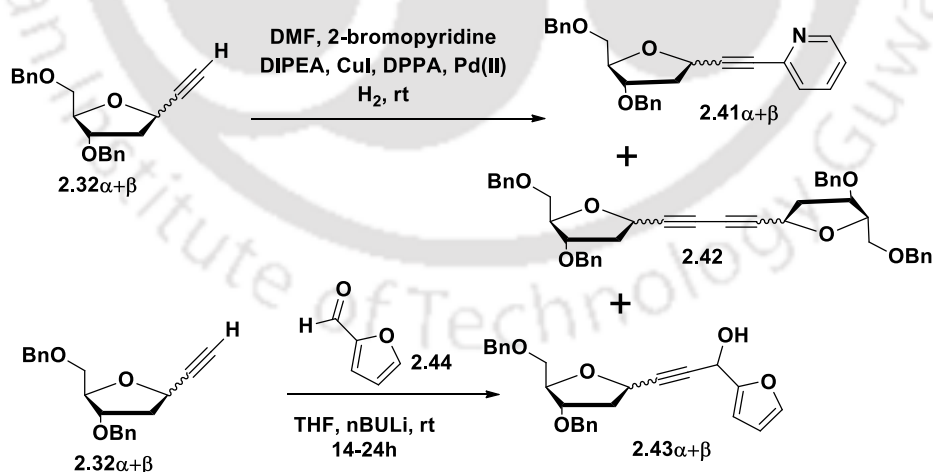
Scheme 2.14: Triazole nucleoside formation started from 1-ethynylriboside **1.086a**

Inoye⁴³ *et al.* have used sugar 1-ethynylriboside **2.32b** in different purposes. The reduction of 1-ethynylriboside **2.32b** with lindlar catalyst in presence of hydrogen give **2.37**. Treatment of catalytic amount of RuCl₃ with NaIO₄ in a mixture of carbon tetrachloride, acetonitrile and water afforded **2.38**. Treatment of 1-ethynylriboside **2.32b** with nitrile N-oxide **2.39** produced **2.40** (**Scheme 2.15**).



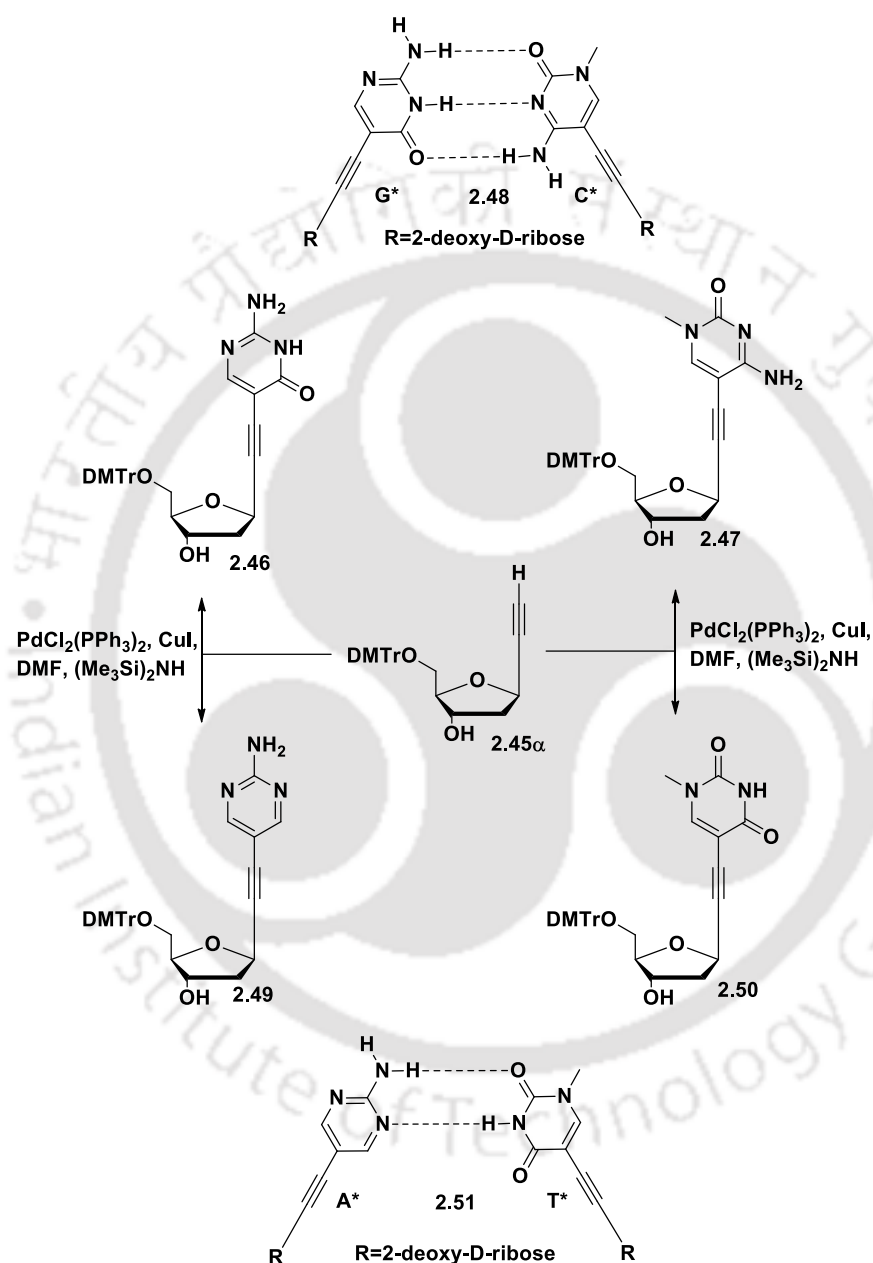
Scheme 2.15: Inoye synthesised heterocyclic.

Adamo *et al.*⁴⁴ has started with **2.32α** and **2.32β** 1-ethynylribose separately and then applied it to Sonogashira reaction to form a mixture of **2.41α** and **2.41β** and double substituted ethynyl nucleoside **2.42**. In the further application they have shown the formation of alcohol where the ethynyl hydrogen was abstracted by *n*BuLi and then attacked to aldehyde **2.44** to form a mixture of alcohol **2.43α** and **2.43β** along with **2.42** (Scheme 2.16).



Scheme 2.16: Adamo's 1-ethynylribose mediated synthetic route to form C-triazolyl nucleoside

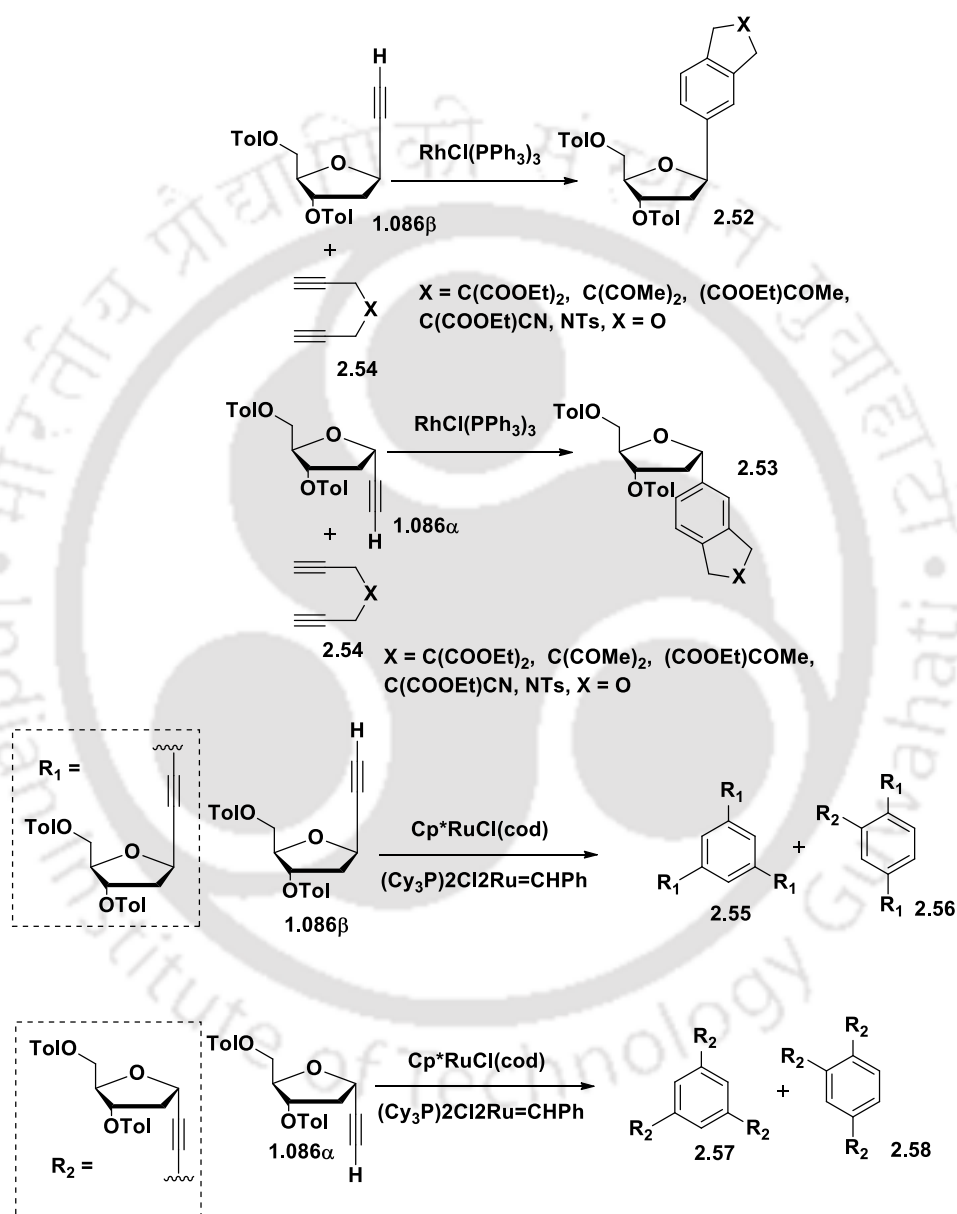
A new class of hydrogen bonded base pair in DNA was presented by Inoyue⁴⁶ in the year 2008. He used Sonogashira coupling in shaping the nucleosides in a way so that a pair could be capable to be paired either with three or two hydrogen bonds. They prepared iG* **2.46** and iC* **2.47**. These two are making 3 hydrogen bonds whereas A* **2.49** and T* **2.50** paired with two hydrogen bonds. (Scheme 2.17).



Scheme 2.17: Designing and construction of 3 point and 2-point hydrogen bonding.

In the year 2008 Kotora *et al.*⁴⁷ reported that a Rh catalysed [2+2+2] cyclotrimerization reaction can proceed smoothly with bistoluoyl protected **1.086α** and **1.086β** 1-

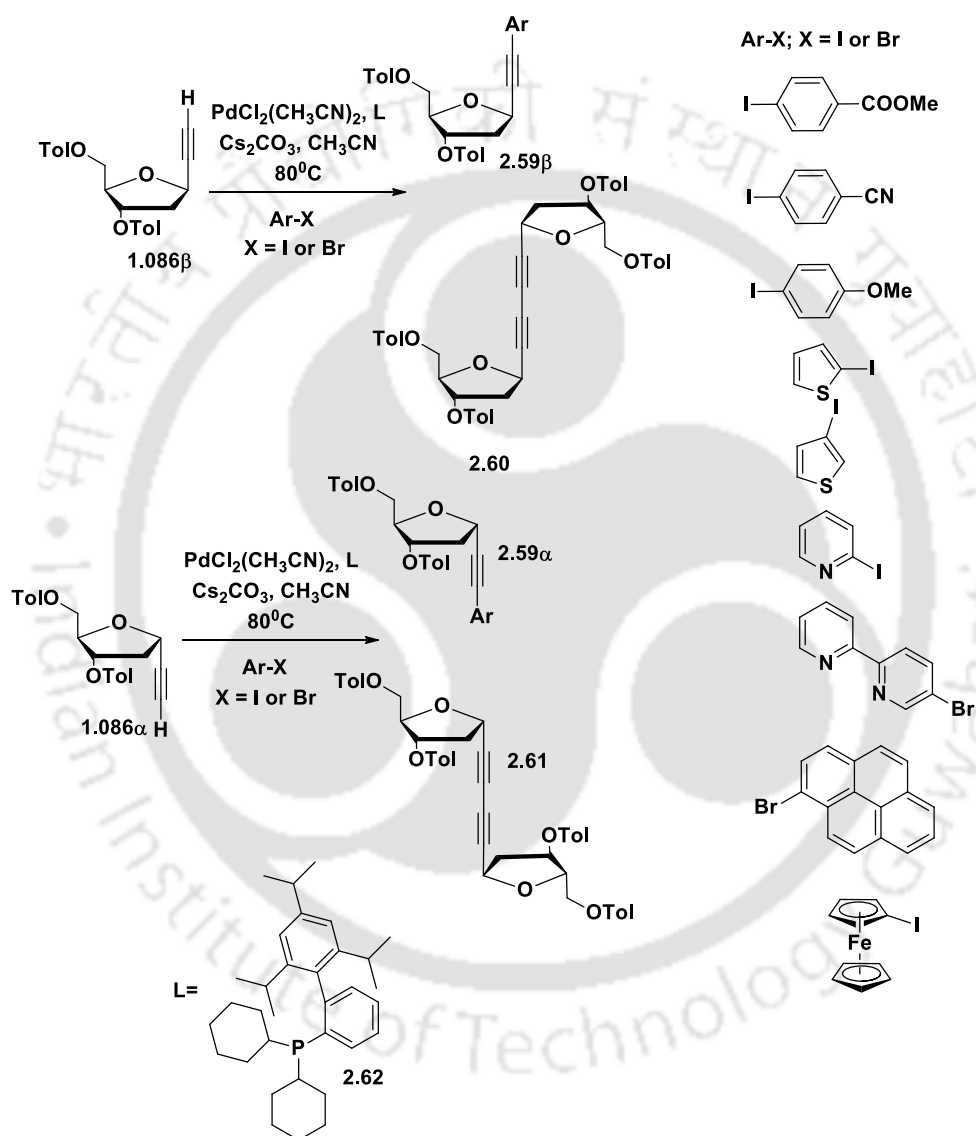
ethynyldeoxyribose to synthesize an array of C-nucleosides. However analogous alkyne like α - or β -phenylethynyl or propynyl ribosides only underwent reaction in presence of Rh catalyst under microwave condition. Homologous cyclotrimerization was also tried under Rh catalysed condition but failed to produce such desired trimerization product whereas Ru catalyst was able to produce such desired homocyclotrimerised product **2.55**, **2.56**, **2.57**, **2.58** from both **1.086 α** and **1.086 β** anomers (Scheme 2.18).



Scheme 2.18: Heterocyclic triazolyl nucleoside formation started from 1-ethynylriboside

Here Kotora *et al.*⁴⁸ developed a protocol where 1-arylethynylriboside **2.59 β** and **2.59 α** could be made easily from α and β anomer of the 1-ethynylriboside **1.086 α** and **1.086 β** . The problem to prepare arylethynylriboside through Sonogashira coupling is ending up with the

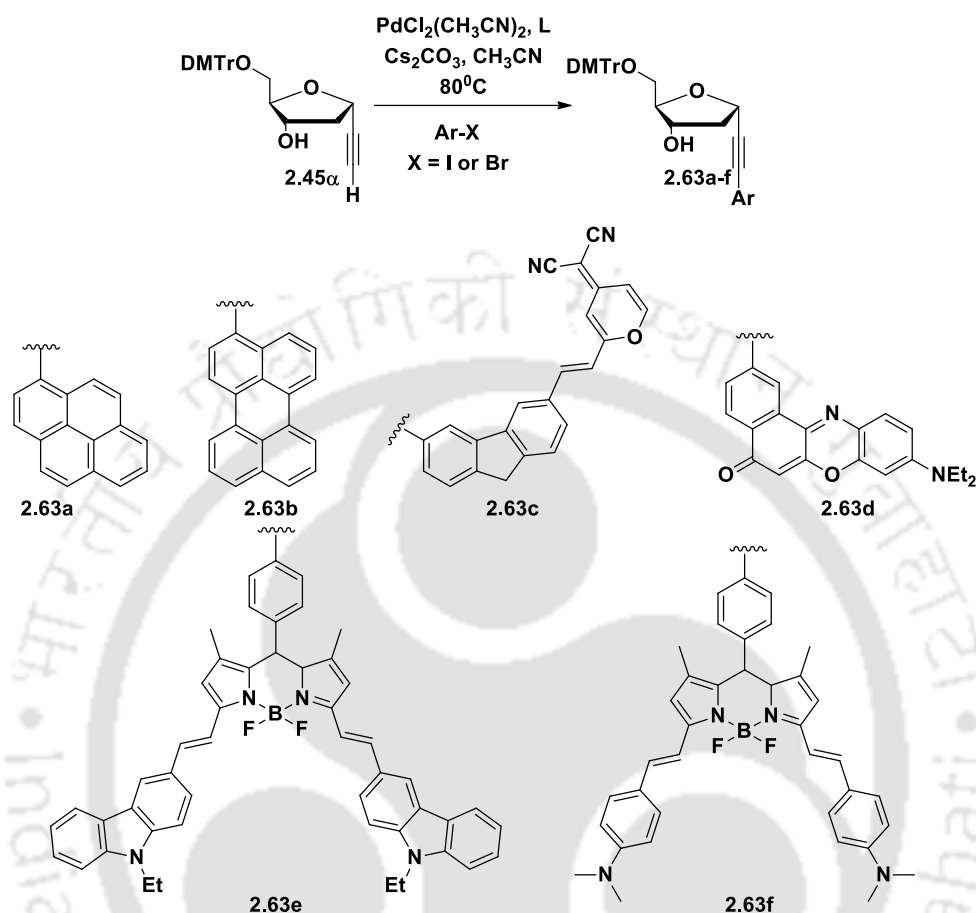
dimerization of 1-ethynylriboside **2.60** and **2.61** so that the desired arylethynylriboside **2.59 β** and **2.59 α** was expected to be failed to produce in a substantial yield. The problem was solved by not using the copper catalyst which generally promoted dimerization of alkyne. To avoid the problem, they have used modified Sonogashira condition and got a substantial yield of the arylated product. They applied it on various aryl substituents (**ArX**) and successfully eliminated the dimerization problem (**Scheme 2.19**).



Scheme 2.19: 1-ethynylriboside in Sonogashira coupling with different aromatic halide.

Recently another work was done by E. T. Kool in 2016. They started with α -ethynylriboside **2.45 α** and developed some fluorescently labelled alkynylriboside **2.63a-f**. Sonogashira coupling protocol was used as the key step to develop these dyes tagged

alkynylriboside. They have used these fluorophoric nucleosides in the preparation of DNA based polyfluorophores (**Scheme 2.20**).⁴⁹



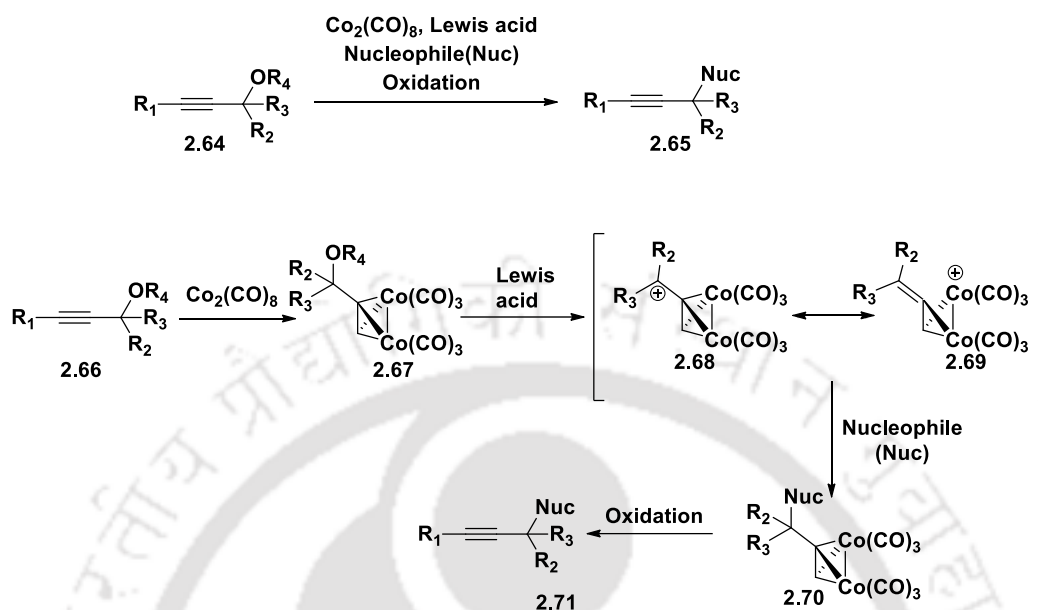
Scheme 2.20: Synthesis of fluorophore tagged alkynyl riboside.

2.5. Nicholas reaction

Nicholas reaction⁵⁰ is involved in generating a carbocation which is stabilised by the support of formed complex within alkyne and dicobalt octacarbonyl. Further we will explain here how Nicholas reaction can be used as a tool in increasing the β -selectivity of an acid catalysed anomeric conversion of 1'-ethynylpyranose. We have extended this concept into our work where we have used 1'-ethynylfuranose and achieved higher β -selectivity.

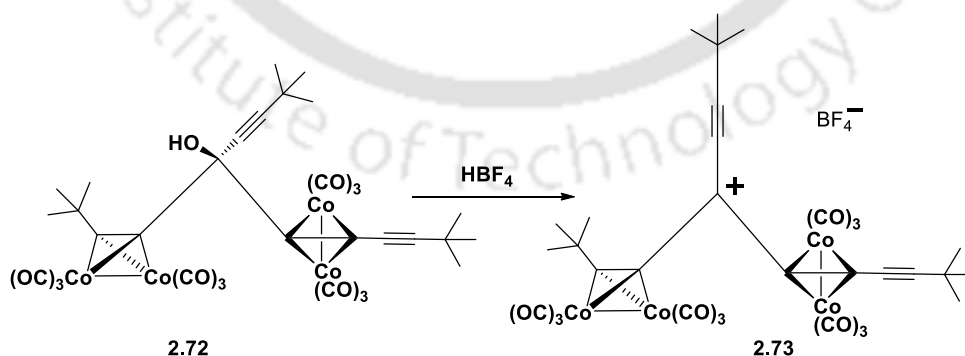
In Nicholas reaction an alkyne **2.66** was employed to react with cobalt carbonyl to afford a brown colour complex **2.67**. With the treatment of Lewis acid like TfOH , $\text{BF}_3\cdot\text{OEt}_2$, the $-\text{OR}_4$ functionality would remove as a leaving group. The positive charge is delocalised into the complex and form carbocation **2.69**. Next the intermolecular or intramolecular

nucleophilic attack to this cationic centre gave rise to **2.70** which was then oxidised with iodine to decomplex the cobalt, generating **2.71** as a product (**Scheme 2.21**).



Scheme 2.21: Synthetic Scheme of Nicholas reaction.

As a proof of formation of carbocation, **2.73** was isolated as a X ray crystal structure. The reason behind the stability of this carbocation was remaining in the delocalisation of this cationic charge within the $Co_2(CO)_6$ moiety. In support of the experimental evidence it was observed that the IR stretching frequency of the CO moiety in the cation **2.73** was significantly increased by 40-60 cm^{-1} compare to the parent molecule **2.72**.

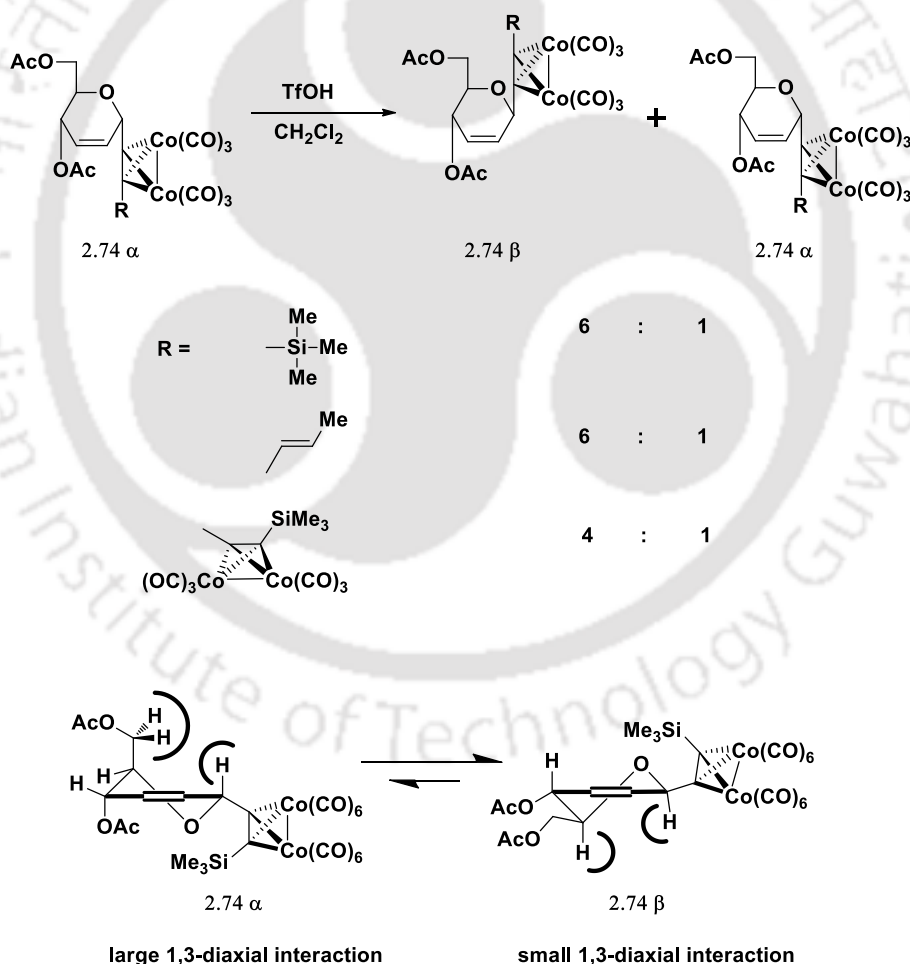


Scheme 2.22: Isolated stable carbocation **2.73**

This is due to the delocalisation of cationic charge into the $\text{Co}_2(\text{CO})_6$ moiety decreasing the $d(\text{Co}) \rightarrow \pi^*(\text{CO})$ donation which automatically increased bond distance increasing stretching frequency. ^1H NMR shows an enhanced deshielding effect on the α carbon with respect to the alkyne in the **2.73** compare to the parent molecule **2.72**. The molecule **2.73** chosen by Melikyan *et al.*⁵¹ consisted of two $\text{Co}_2(\text{CO})_6$ moiety was imparting greater crystallinity and thus greater thermal stabilisation (**Scheme 2.22**) was the clear evidence of the existence of carbocation formation.

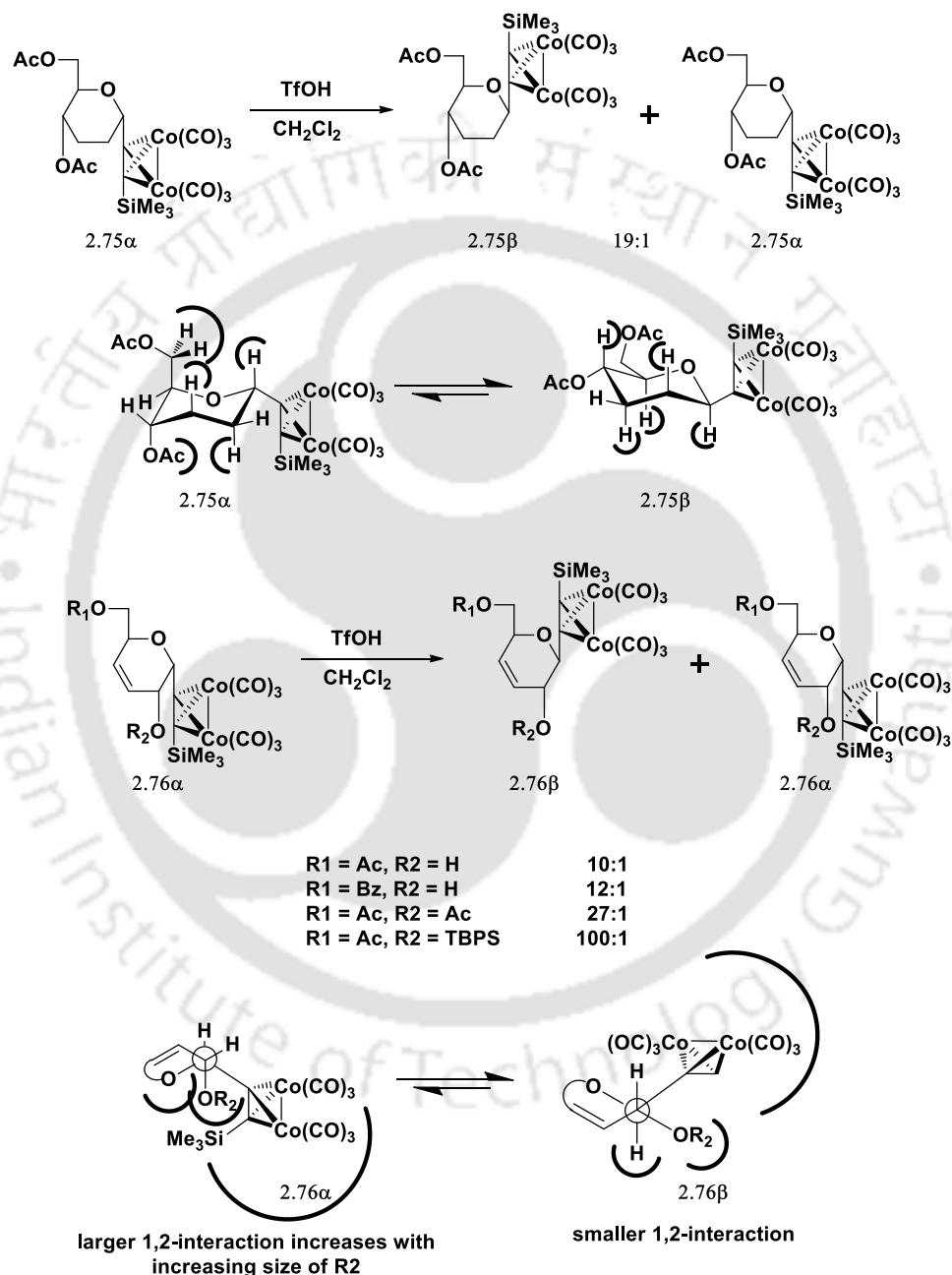
2.6. Intramolecular Nicholas reaction and β -selectivity

Intramolecular Nicholas reaction can lead to facile a higher extent of epimerisation of α to β . The concept was first introduced by Isobe and applied over a range of pyranose ring. The placement of a α -alkynyl substituent at the anomeric position of a pyranose ring led to be epimerised by forming cobalt complexation. **2.74 α** epimerised to **2.74 β** (**Scheme 2.23**) and **2.74 α** in a 4:1 to 6:1 β : α ratio depending on the R substituent.⁵²



Scheme 2.23: Isomerisation of **2.74 α** to **2.74 α** and **2.74 β**

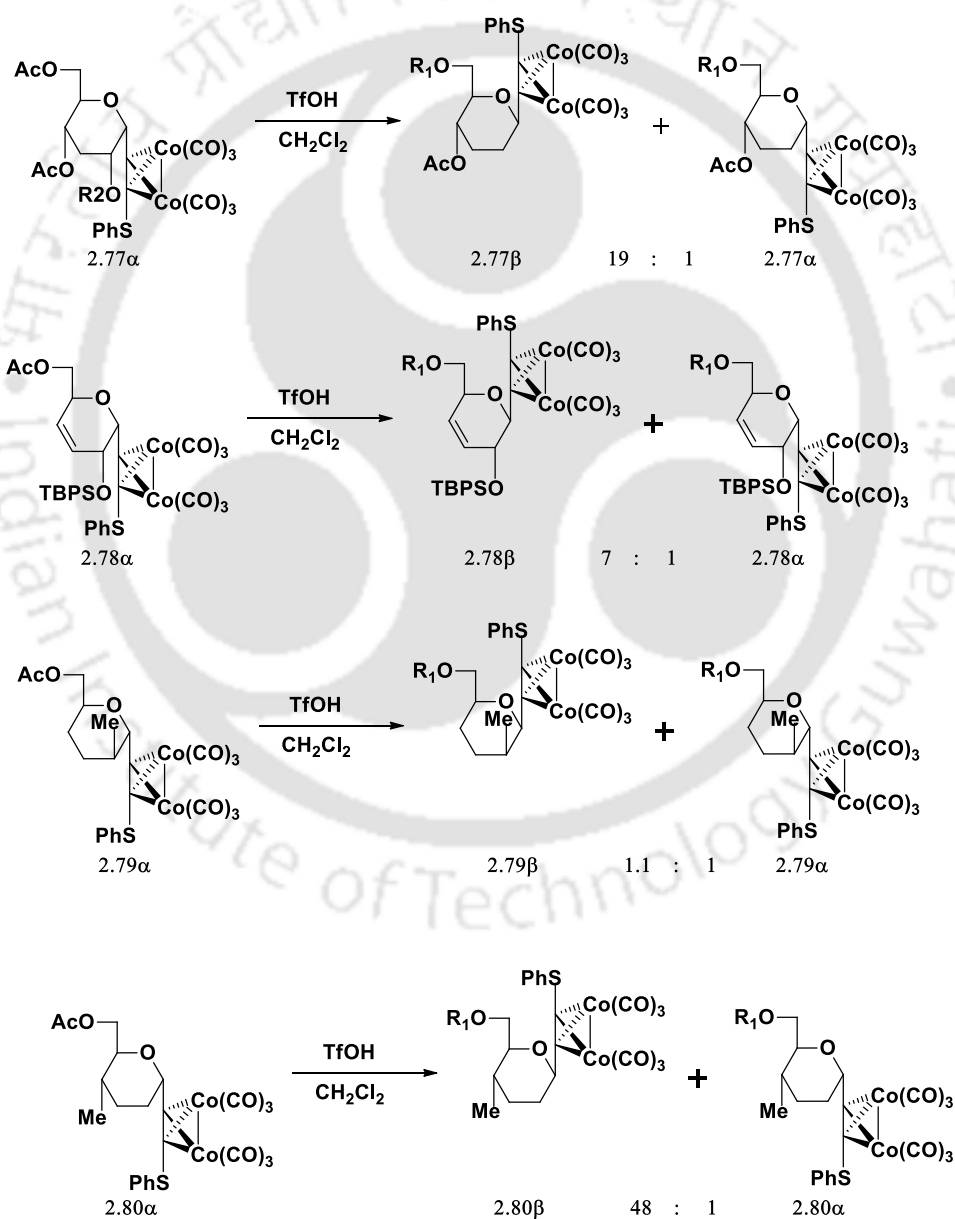
Compound **2.75 α** epimerised to **2.75 β** and **2.75 α** in 19:1 β : α ratio, whereas **2.76 α** epimerised to 10:1 to 100:1 β : α ratio depending on the size of the R₁ and R₂ (**Scheme 2.24**). The driving force behind the epimerisation depends on the enhanced 1,3-diaxial interaction depending on the substituent attached with alkyne or the other end of the pyranose ring.



Scheme 2.24: Steric dependent cobalt carbonyl catalysed α to β conversion.

Higher the 1,3-diaxial interaction between the interacting partner, higher will be the steric crowding leading to higher extent of formation of the β -anomer. **2.76** experienced large amount of steric crowding rather than **2.75** and that is why the ration of β : α for **2.76** is higher than **2.75** (Scheme 2.24). On the other hand, the steric crowding imposed by C₂ substituent was explored. It has been found that with the increase of the steric crowding on the C₂ substituent of **2.76 α** the ratio β : α increases from 10:1 to 100:1.⁵²

The same concept was extended to apply on phenylthioalkynylpyranose **2.77-2.80** by Isobe group and the same argument was made to conclude that cobalt catalysed isomerisation from α to β at a substantial ratio is said to be highly feasible in the case of α -alkynyl substituents in a pyranose ring through Lewis acid treatment (Scheme 2.25).⁵³⁻⁵⁵



Scheme 2.25: α to β steric dependent cobalt carbonyl catalysed anomeric conversion.

2.7. Background

Unnatural *N*-nucleosides are found to be most accessible nucleoside analogues, which often are, designed to mimic the shape, size and H-bonding pattern of natural nucleosides. Apart from that, the significance in synthesizing *N*-nucleoside lies on their huge impact in the field of chemistry and biology. They can be used as a potent therapeutic drug as well as capable of fulfilling criteria to make stable DNA double helix via π - π stacking and H-bonding interaction. However, the *N*-glycosidic linkage is generally labile and suffers from enzymatic chemical hydrolysis. The stability and importance of *C*-glycosidic bond as evident from naturally occurring *C*-nucleoside, showdomycin and pseudouridine have attracted attention to the scientist for the development of *C*-nucleoside analogues. As a result of tremendous research efforts, several methodologies have been developed for the synthesis of various types of *C*-nucleosides. These *C*-nucleosides are found to have application in chemical biology as well as medicinal chemistry. They are also capable of providing π stacking as well as hydrogen bonding interaction in stabilizing DNA duplexes. Moreover, the fluorescent analogues have been utilized as fluorescent probe within a DNA helix or within a biological system. In the field of expansion of genetic alphabet, *C*-nucleosides have also attracted recent research interest. In addition, it is also interesting and changing research effort to compare the structure, stability and property of DNA containing *C*- and *N*-nucleoside. They could also be capable of generating a differential photophysical outcome in comparison to the *N*-nucleoside having a lone pair sharing with the rest of the π -system. Therefore, design of *C*-nucleoside is currently an attractive research area.

2.8. Objective

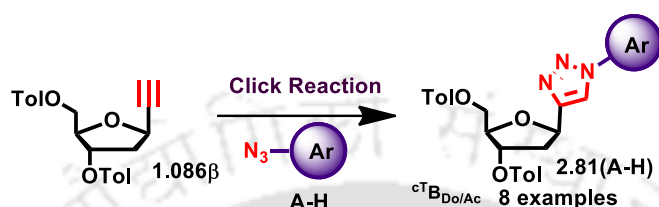
Over the year lots of methodologies have been developed for the synthesis of *C*-nucleoside analogues. Among them, the umpolung synthesis is an effective technique. In this method, a neutral carbon atom is converted to a nucleophile, which is participating in a nucleophilic substitution reaction at the anomeric carbon of the furanose ring. Versatile organometallic reagents thus could allow for the development of newer *C*-nucleosides. The other way is to use the metal catalyzed reaction i.e. palladium catalyzed Heck coupling to attach aglycon unit to the sugar. Apart from that, cross coupling, Wittig type reaction, ozonolysis, are recently being employed for the generation of new *C*-nucleosides. Keeping in mind the accessibility of the starting material as well the expense of the reagent, we have chosen Click reaction as a part of our continuous research effort to synthesis triazolyl donor-acceptor *C*-nucleoside.

Thus with the above literature and background, we framed our objective as follows:

- (a) The synthesis of key intermediate, the β -ethynyl-2-deoxyribose. For that purpose, we employed a modified literature protocol utilizing intramolecular Nicholash reaction. We also tried to rectify the shortcomings of the published protocol.
- (b) Synthesis of triazolyl *C*-nucleosides containing various donor/acceptor aromatics via alkyne-azide cycloaddition reaction.
- (c) Study of photophysical properties.

(d) Study of interaction with BSA.

The nucleosides were designed in such a way that they could provide π -stacking interaction in stabilizing the DNA duplex if incorporated and the large π -system might also be capable of stabilizing abasic site as well. Apart from that, the triazole aromatic π -conjugate is expected to tune the photophysical properties of the chromophore in particular and leading to the generation of fluorescent triazolyl C-nucleoside **2.81A-H** with interesting photophysical properties (**Scheme 2.26**).



Scheme 2.26: Schematic presentation of unnatural triazolyl donor-acceptor aromatic C-nucleosides ($cTBD_{Do}/Ac$).

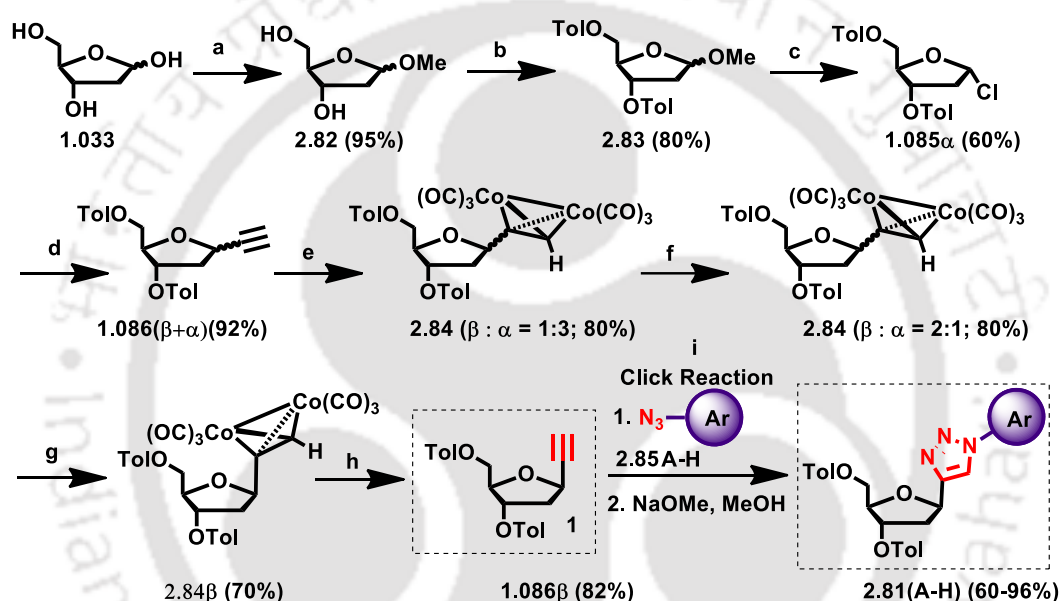
2.9. Results and discussion

2.9.1. Synthesis of β -1-ethynylribose via intramolecular Nicholas reaction and the synthesis of triazolyl nucleoside derivatives.

Several methodologies exist here for the generation of novel C-nucleosides and interesting nucleobase pairs usable for various DNA based applications. The Cu(I)-catalyzed azide-alkyne cycloaddition reaction got attention only in recent years in the field of nucleic acid chemistry for the design of artificial nucleobases with interesting properties, functional nucleic acids of potential value in biology, medicine, material sciences, and in nanotechnology. As a part of our continuous effort we also adopted the click chemistry protocol utilizing alkynyl sugar and chromophoric aromatic azides for the synthesis of C-triazolyl nucleoside with interesting photophysical property.

The synthesis of the key intermediate, alkynyl sugar **1.086 β** was started from commercially available 2-Deoxy-D-ribose **1.033**. The conversion of **1.033** to 1-O-methyl-2-deoxy- α/β -D-ribofuranose **2.82** was carried out in methanolic HCl solution with 95% yield. Next toluoyl protection of the hydroxyl group of **2.82** was performed in dry acetone in presence of p-toluoyl chloride and dry trimethylamine to afford **2.83** as a yellowish oil in an overall 80% yield. Then the conversion of **2.83** to Hoffer's chloro sugar **1.085 α** was achieved by dissolving **2.83** into dry ether and passing HCl vapour through the ethereal solution of **2.83** for about 20 minutes to get a thick white precipitate of diastereo pure α -chloro sugar **1.085 α** which was then washed with dry ether to get a diastereo pure α -chloro sugar in 60% yield. Thus, ethynyl magnesium chloride was allowed to react with Hoffer's chlorosugar **1.085 α** to afford bistoluoylated 1'-ethynyl-2'-deoxy C-nucleoside **1.086** as an anomeric mixture ($\beta/\alpha = 1/3$) in 92 % overall isolated yield. As the yield of the β anomer is exceptionally low and the anomeric separation by open column chromatography is a tedious job, we followed intramolecular

Nicholas reaction to achieve the separation as well as the β -anomer in high yield. Thus, the treatment of α and β anomeric mixture of ethynyl C-nucleoside **1.086** with dicobalt octacarbonyl $\text{Co}_2(\text{CO})_8$ afforded the cobalt complex as a 3:1 mixture of α : β anomers **2.84** in 80 % isolated yield. Next, the mixture **2.84** was treated with $\text{BF}_3 \cdot \text{OEt}_2$ at -78°C to get β -anomer as a major product with a 1:2 mixture of α : β anomers **2.84** in overall 80% yield (**Scheme 2.27**). At this stage, we separated the anomeric mixture via silica-gel column chromatography to afford the desired **2.84 β** anomer. Second step acid treatment of separated **2.84 α** -anomer would produce again **2.84 β** . Therefore, the overall yield of **2.84 β** started from **2.84(α + β)** mixture is 70%. Finally, the decomplexation of **2.84 β** was done by the treatment of iodine in THF at 0°C and the crude material was subjected to silica-gel column chromatography to get bis toluoylated 1'-ethynyl-2'- β -deoxy C-nucleoside **1.086 β** in 82 % yield.



Scheme 2.27: Synthesis of unnatural triazolyl donor-acceptor C-nucleosides.

After getting the key intermediate **1.086 β** , various donor-acceptor aromatic azides **2.85(A-H)** were allowed to undergo copper (I) catalyzed Huisgen 1,3-dipolar azide alkyne cycloaddition reaction followed by deprotection with sodium methoxide in methanol afford triazolyl donor-acceptor aromatic C-nucleosides **2.81A-H** in 60-96% yields (**Scheme 2.27**, **Figure 2.3**). All the compounds were characterized by NMR, IR and mass spectrometry.

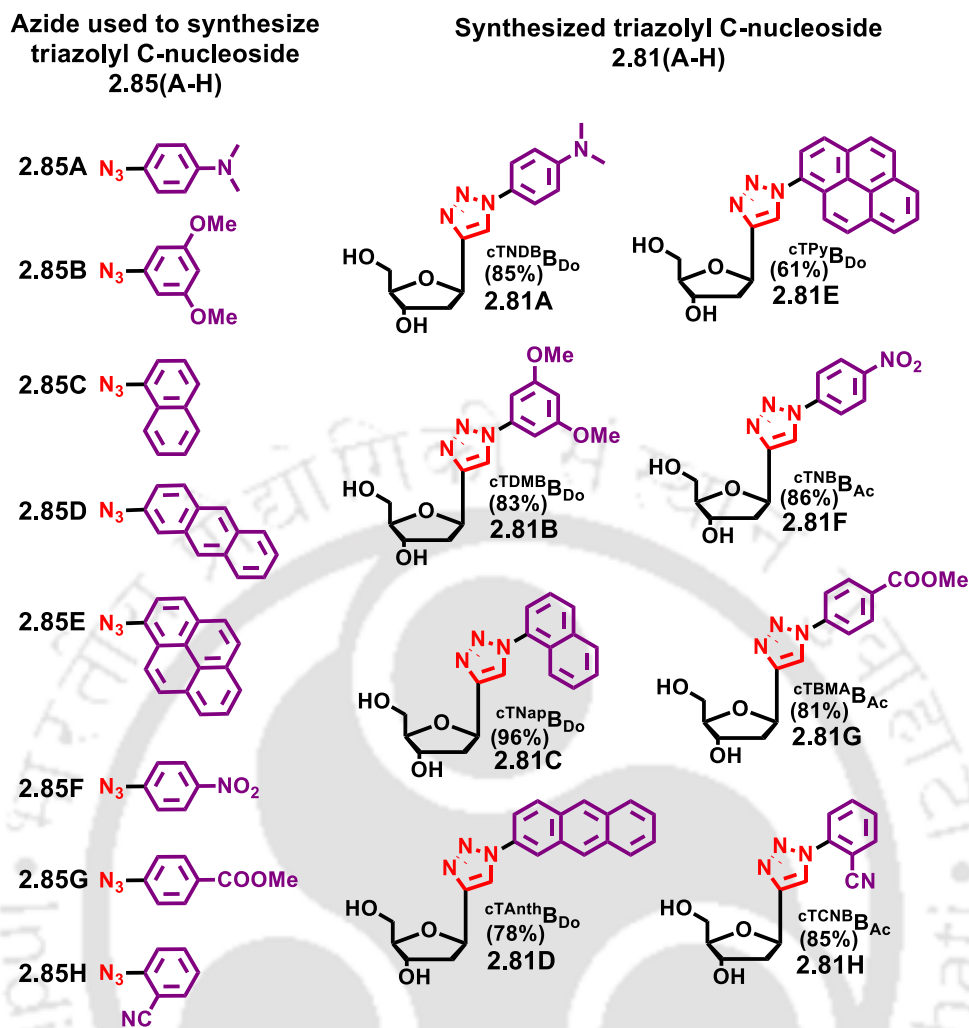


Figure 2.3: Structure of the synthesized unnatural triazole nucleoside. Yield in parentheses.

2.9.2. Structural elucidation by Single crystal analysis

β -conformation of the C-triazolyl nucleoside was confirmed by single crystal X-Ray structure of ${}^{\text{cTNMB}}\text{B}_{\text{D}_0}$ (**2.81B**) (Figure 2.4).

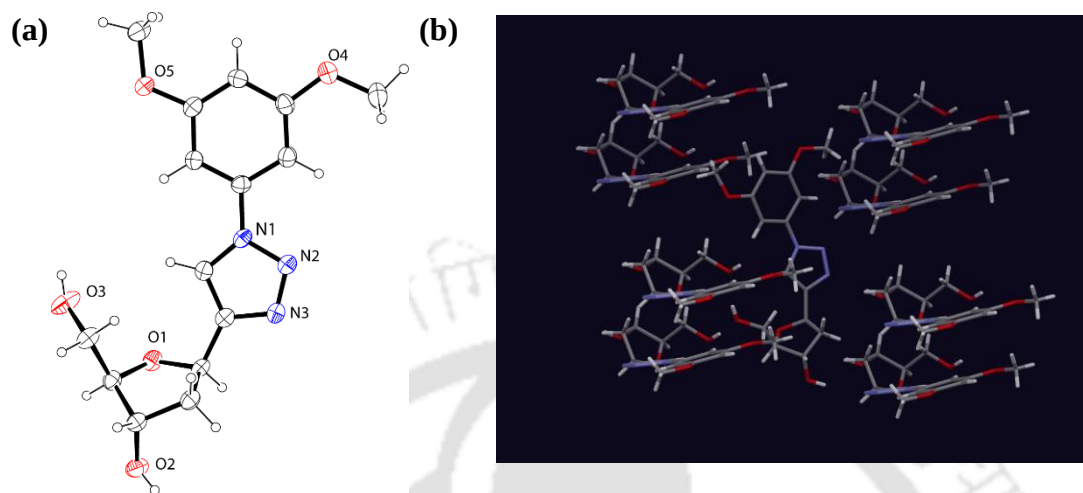


Figure 2.4: (a) ORTEP diagram of ${}^{\text{cTNMB}}\text{B}_{\text{D}_0}$ (**2.81B**) with thermal ellipsoid at 50% probability. (b) Crystal packing arrangement of ${}^{\text{cTNMB}}\text{B}_{\text{D}_0}$ (**2.81B**).

Table 2.1: Crystal parameters of C-triazolyl nucleoside ${}^{\text{cTNMB}}\text{B}_{\text{D}_0}$ (**2.81B**).

Parameters	Descriptions
Z	2
Molecular Formula	C ₁₅ H ₁₉ N ₃ O ₅
Temperature (K)	296
Space Group	P 21 Monoclinic
a (Å)	12.1915(17)
b (Å)	4.9618(7)
c (Å)	12.4620(17)
α (degree)	90
β degree)	93.593(7)
γ (degree)	90
M_r	321.33
V (Å ³)	752.37(18)
μ (mm ⁻¹)	0.108
F(000)	340.0
R(reflections)	0.0509(1790)
wR2(reflections)	0.1149(2691)
Correction method	Multi Scan

2.9.3. 1,3-diaxial interaction triggering 2.84 α to 2.84 β conversion

It has been found that after treatment with Lewis acid ($\text{BF}_3 \cdot \text{OEt}_2$), $\alpha:\beta$ ratio of **2.84** changed from 3:1 to 1:2. This is due to the fact that **2.84 α** experienced 1,3-diaxial interaction between the cobalt complex held at C_1' position with the toluoyl group situated at C_3' position. After treatment with Lewis acid, the formation of carbo cation removed the steric strain by moving the cobalt complex at C_1' from axial to equatorial position. Thus the formation of **2.84 β** was favoured (**Figure 2.5**).

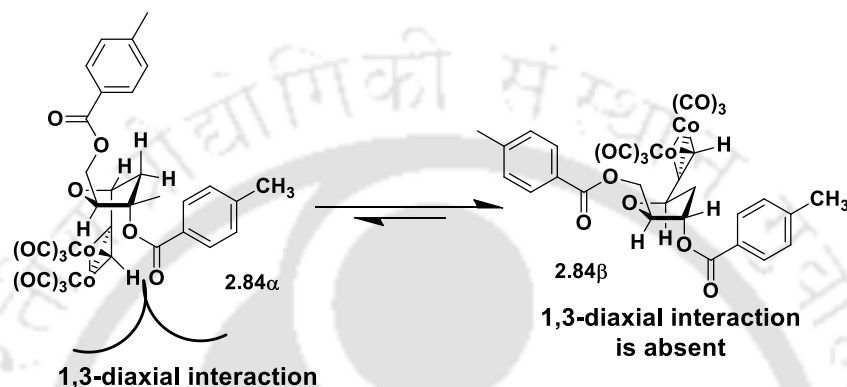


Figure 2.5: 1,3-diaxial interaction enables **2.84 α** to **2.84 β** conversion.

2.9.4. Study of photophysical properties

After having all the nucleosides in hand we measured the photophysical properties of few nucleosides in various organic solvents and in buffer. All the UV-visible spectra were recorded with a 10 μM sample concentration at 25 $^\circ\text{C}$ with a cell of 1cm path length and 1nm slit width. The same 10 μM sample was used to measure its fluorescence emission property with a cell of 1 cm path length and 3nm emission/excitation slit width at 25 $^\circ\text{C}$.

The UV visible spectra of triazolyl anthracene nucleoside **2.81D** ($^{\text{cTAnth}}\text{B}_{\text{D}_0}$) exhibited structured absorption bands at around 346 nm, 364 nm, 385 nm in various organic solvent. With increasing polarity of the solvent, the band blue shifted by about 5 nm from dioxane to methanol with slight hyperchromicity. Upon excitation at absorption maxima of 365 nm the nucleoside **2.81D** showed structured emission at 392, 417 and 442 nm in different organic solvent. The emission spectra experienced a gradual decrease in emission intensity as the solvent polarity increased indicating a solvent-solute or hydrogen bonding interaction mediated fluorescence quenching (**Figure 2.6 a-b**, **Table 2.2**).

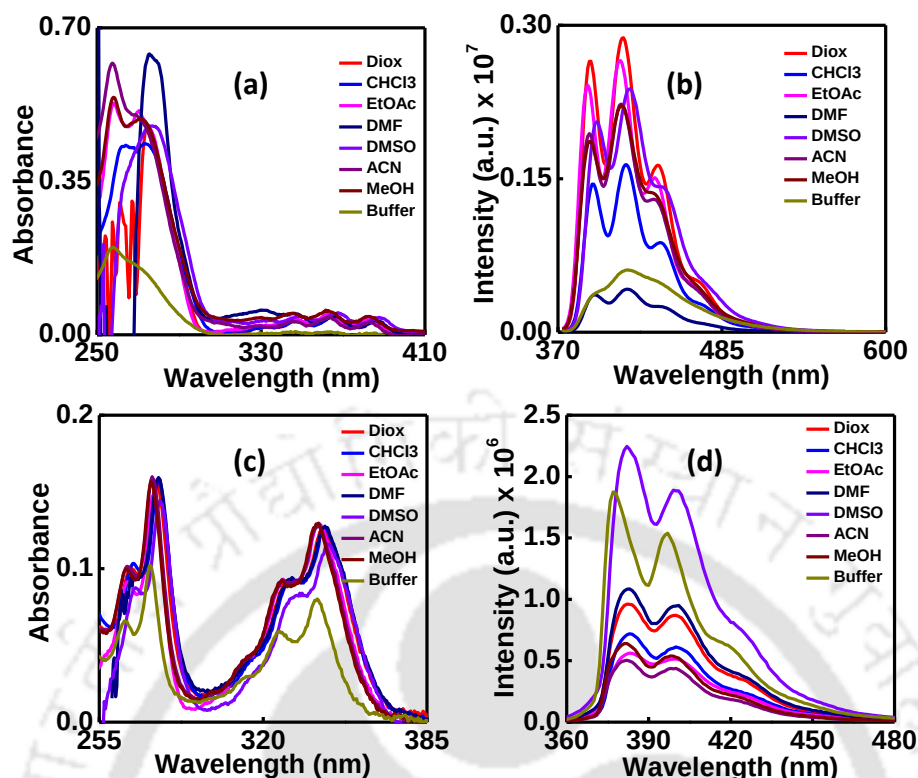


Figure 2.6: UV-visible absorption and fluorescence emission spectra of C-triazolyl nucleoside **2.81D** and **2.81E** in various organic solvents; (a-b) for **2.81D** ($cTAnthBDo$) and (c-d) for **2.81E** ($cTPyBDo$).

In case of triazolyl pyrene nucleoside, **2.81E** ($cTPyBDo$) the UV visible spectra displayed structured absorption bands at around 275, 330 and 345 nm in various polar and nonpolar organic solvents. The UV-visible spectra experienced 4-5 nm blue shift with increasing solvent polarity like triazolyl anthracene nucleoside. On an average, excitation at absorption maxima displayed structured emission characteristic of pyrene emission band at around 380 and 400 nm. The emission spectra revealed that except in buffer and DMSO with increasing polarity of the solvent it experienced a hypochromic shift with no promising shift in wavelength of the emission maxima. Interestingly in buffer it experienced about 4-5 nm blue shift with increasing fluorescence intensity due to microenvironmental effect (**Figure 2.6 c-d**, **Table 2.2**).

Table 2.2: Summary of UV-Visible and Fluorescence spectra of C-nucleoside **2.81D** and **2.81E**.

Entry →	(2.81D)							
Solvents →	Diox	CHCl ₃	EtOAc	DMF	DMSO	ACN	MeOH	Buffer
λ_{max}^{abs} (nm)	346, 365, 385	347, 365, 386	345, 363, 384	346, 366, 386	348, 368, 388	345, 362, 383	344, 362, 382	345, 363, 384
$\epsilon_{max} \times 10^5$	0.032 (346 nm)	0.031 (347 nm)	0.037 (345 nm)	0.047 (346 nm)	0.042 (348 nm)	0.031 (345 nm)	0.049 (345 nm)	0.006 (345 nm)
λ_{max}^{fl} (nm)	393, 415, 440	393, 417, 441	391, 414, 438	394, 419, 445	398, 420, 443	392, 414, 436	390, 413, 436	395, 415, 438
(2.81E)								
λ_{max}^{abs} (nm)	278, 328, 343	278, 330, 344	275, 326, 341	278, 330, 344	279, 332, 346	275, 327, 341	275, 327, 341	275, 325, 341
$\epsilon_{max} \times 10^5$	0.125 (343 nm)	0.125 (344 nm)	0.129 (341 nm)	0.128 (344 nm)	0.115 (345 nm)	0.129 (341 nm)	0.129 (341 nm)	0.079 (341 nm)
λ_{max}^{fl} (nm)	382, 400	383, 400	383, 400	382, 400	381, 400	381, 399	380, 399	376, 396

The UV visible spectra of C-triazolyl naphthalene nucleoside **2.81C** (^cTN^{ap}B_{Do}) in various organic solvents showed structureless broad absorption bands centred at 280-293 nm. On an average with increasing polarity of the solvent it experienced an hyperchromic effect. Excitation at absorption maxima, the emission spectra in various solvents showed emission bands at around 345 nm whereas in buffer it experienced a 25 nm red shift. With increasing polarity of the solvent the intensity of the emission spectra decreased (**Figure 2.7a-b, Table 2.3**).

On the other hand, the acceptor nucleoside C-triazolyl *o*-cyanobenzene nucleoside **2.81H** (^cTN^BB_{Ac}) exhibited structureless broad absorption band at around 279-286 nm. The band position shifted towards lower wavelength region by 5-6 nm with increasing polarity of the solvent. It was also found that, the absorbance decreases with increasing polarity of the solvent with a hypochromic effect. When the excitation spectra were recorded it was found that the band at 366 nm was much higher in intensity than the band appeared at 276 nm. Upon excitation at maxima of each solvent (340-373 nm) showed strong emission band at higher wavelength at 366 nm in buffer and 427-439 nm for other organic solvent. The emission spectra experienced 12 nm red shift with increasing polarity of the organic solvent due to internal charge transfer from donor triazole to acceptor cyano benzene moiety (**Figure 2.7c-e, Table 2.3**).

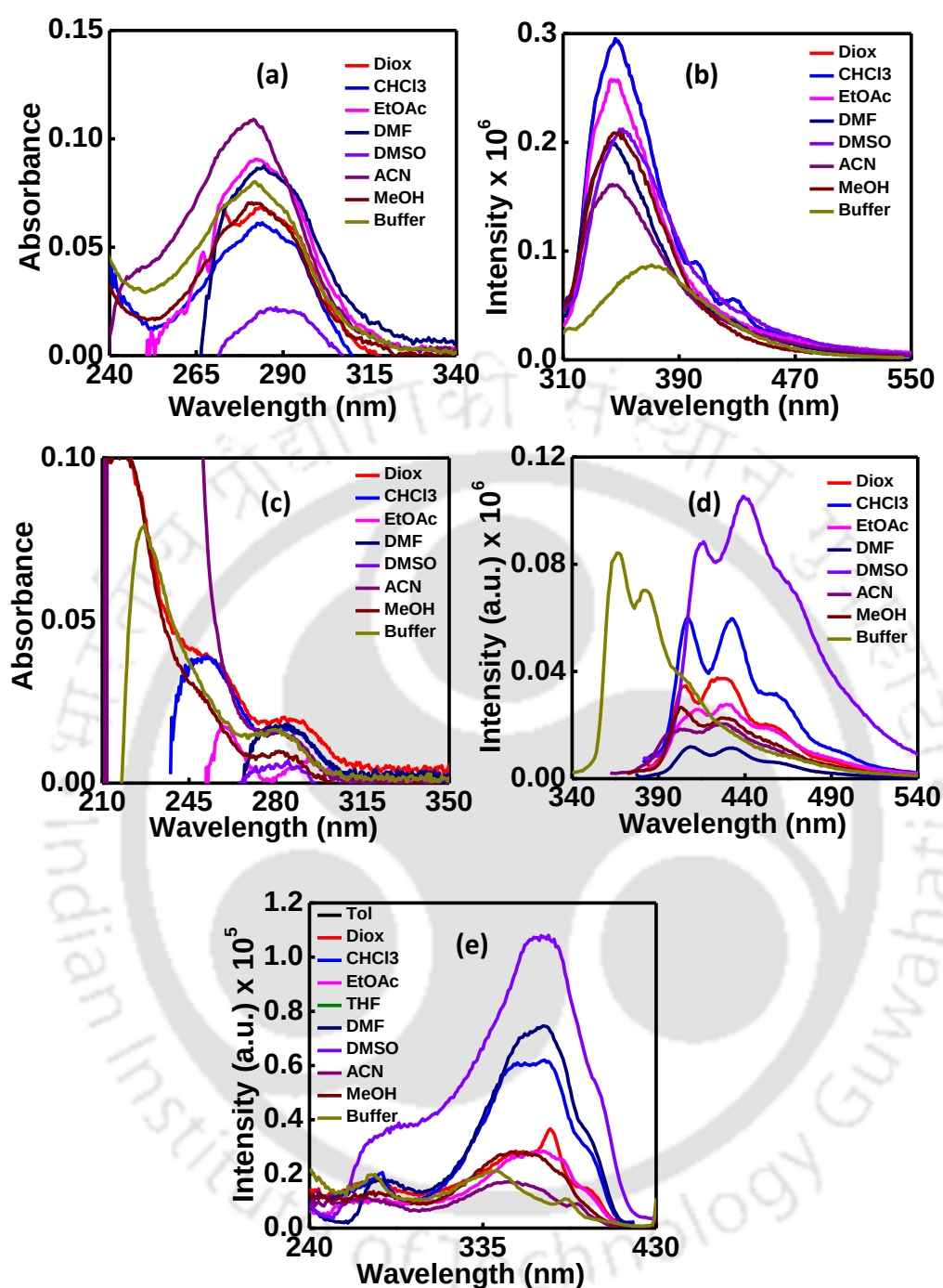


Figure 2.7: UV-visible absorption and fluorescence emission spectra of C-triazolyl nucleoside **2.81C** ($c^{TNap}B_{D0}$) and **2.81H** ($c^{TCNB}B_{Ac}$) in various organic solvents; (a-b) for **2.81C** ($c^{TNap}B_{D0}$) and (c-e) for **2.81H** ($c^{TCNB}B_{Ac}$), (d) fluorescence excitation spectra of **2.81H** ($c^{TCNB}B_{Ac}$).

Table 2.3: Summary of UV-Visible and Fluorescence spectra of C-nucleoside **2.81C** and **2.81H**.

Entry →	(2.81C)							
Solvents →	Diox	CHCl ₃	EtOAc	DMF	DMSO	ACN	MeOH	Buffer
λ_{max}^{abs} (nm)	283	283	283	284	290	281	281	281
$\epsilon_{max} \times 10^5$	0.068	0.061	0.090	0.086	0.021	0.1086	0.070	0.079
λ_{max}^{fl} (nm)	346	347	344	342	350	344	347	370
(2.81H)								
λ_{max}^{abs} (nm)	287	285	293	281	282	281	280	280
$\epsilon_{max} \times 10^5$	0.019	0.0168	0.0059	0.0177	0.0053	0.016	0.0096	0.016
λ_{max}^{fl} (nm)	405, 426, 458	406, 432, 459	413, 430, 457	408, 432, 462	415, 440, 468	400, 429, 459	402, 429, 456	363, 381, 405

The UV visible spectra of triazolyl 3,5-dimethoxy benzene **2.81B** (^cTDMB^BD₀) nucleoside exhibited structureless broad absorption band. With increasing polarity of the organic solvent the absorption band shifted towards higher wavelength except in buffer where no shift in wavelength was observed. Absorption maxima of different solvents also experienced a hyperchromic shift with an increase in the polarity of the solvent. The emission spectra recorded by excitation at UV maxima ranging from 255-258 nm exhibited structureless emission band. In dioxane the emission band appeared at 284 nm and in buffer it appeared at 410 nm. So overall the emission band experienced a huge red shift of 124 nm with increasing polarity of the solvent (**Figure 2.8 a-b, Table 2.4**).

In dioxane **2.81B** (^cTDMB^BD₀) showed a structureless absorption band at 261 nm which indicates resonance conjugation in between triazole ring and dimethoxy benzene ring. With increasing the volume of water the absorption at 261 nm has been started shifted toward lower wavelength region and at 100% water the absorption maxima shifted to 253 nm. Thus hypochromicity with 8 nm blue shift with the increase of the volume of water was observed in absorption spectra (**Figure 2.8c**).

Upon excitation at 260 nm again a structureless broad emission band was observed at 358 nm indicating conjugation of the triazole ring with the phenyl methyl ester ring. With increasing volume of water, the emission maxima were shifted towards higher wavelength region and in 100% water the emission maxima were appeared at 414 nm. About 56 nm red shift of the emission maxima with the increase of the volume of water was observed probably due to strong electronic coupling between the triazole and trimethoxy benzene ring. This long

conjugation decreased the HOMO-LUMO energy gap which allow the emission maxima shifted to the higher wavelength region (**Figure 2.8d**).

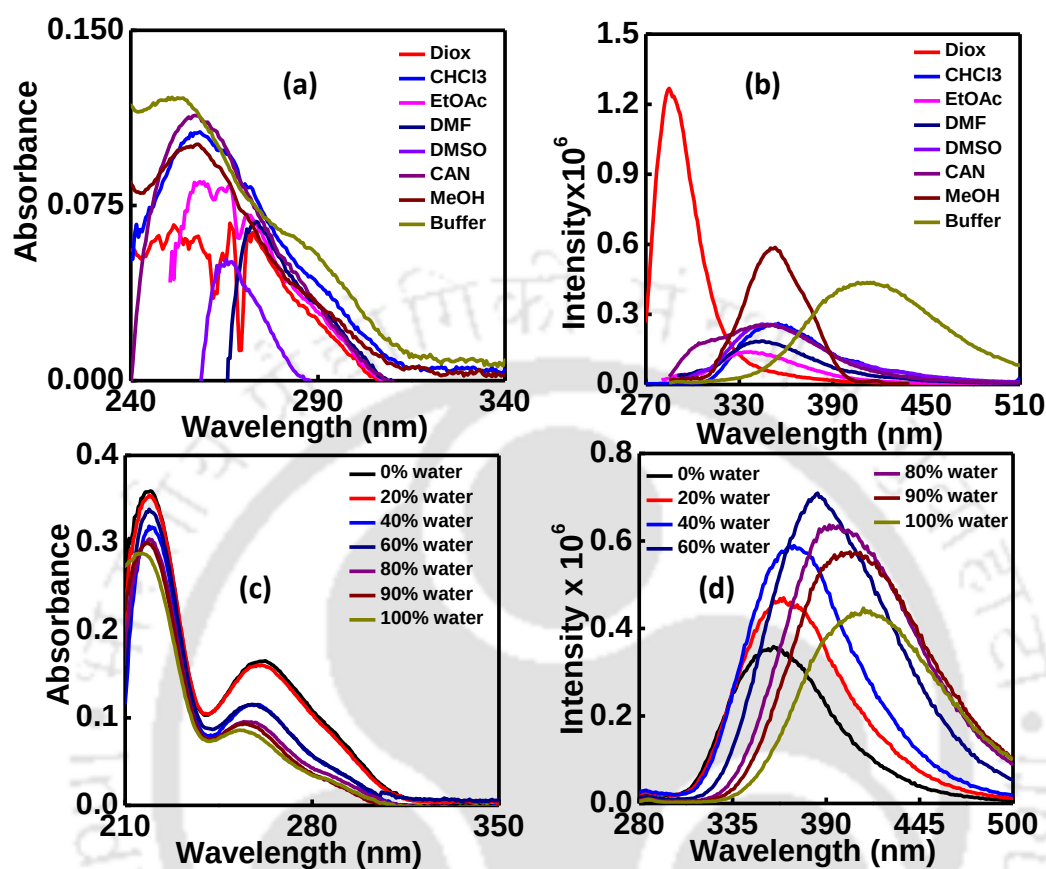


Figure 2.8: UV-visible absorption and fluorescence emission spectra of C-triazolyl nucleoside **2.81B** (${}^{\text{cTDMB}}\text{B}_{\text{D0}}$) (a-b) in various organic solvents; (c-d) in different concentration of water and dioxance.

Table 2.4: Summery of UV-Visible and Fluorescence spectra of C-nucleoside **2.81B**

Entry →	(2.81B)							
Solvents →	Diox	CHCl ₃	EtOAC	DMF	DMSO	ACN	MeOH	Buffer
$\lambda_{\text{max}}^{\text{abs}}$ (nm)	255	258	258	273	265	257	257	252
$\epsilon_{\text{max}} \times 10^5$	0.058	0.106	0.084	0.067	0.05	0.113	0.100	0.121
$\lambda_{\text{max}}^{\text{fl}}$ (nm)	284	355	334	344	347	349	351	409

The UV visible spectra of triazolyl N,N-dimethylamino phenyl nucleoside **2.81A** ($^{\text{cTND}}\text{B}_{\text{D}_0}$), showed structureless absorption band ranging from 285-300 nm in various organic solvent. With the increase in the polarity of the solvent the absorption band shifted towards higher wavelength whereas in the case of ethyl acetate, acetonitrile and buffer the absorption band experienced a 4-5 nm blue shift. Upon excitation at absorption maxima structureless emission band appeared in each solvent. With increasing polarity of the solvent the emission maxima experienced a large red shift started from 380 to 432 nm. In buffer the emission intensity was almost appeared to be negligible in comparison to other solvents (**Figure 2.9 a-b, Table 2.5**).

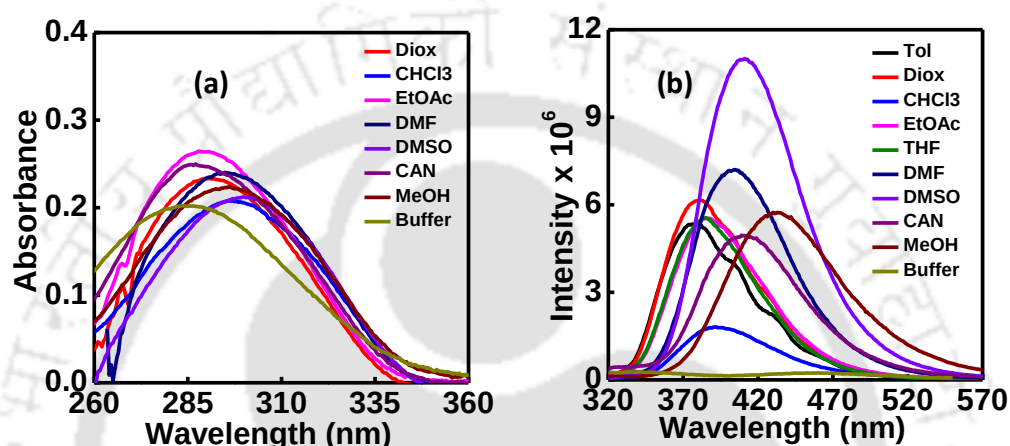


Figure 2.9: (a) UV-visible absorption and (b) fluorescence emission spectra of C-triazolyl nucleoside **2.81A** ($^{\text{cTND}}\text{B}_{\text{D}_0}$) in various organic solvents.

Table 2.5: Summary of UV-Visible and Fluorescence spectra of C-nucleoside **2.81A**.

Entry →	(2.81A)							
Solvents →	Diox	CHCl ₃	EtOAc	DMF	DMSO	ACN	MeOH	Buffer
$\lambda_{\text{max}}^{\text{abs}}$ (nm)	290	297	288	295	300	286	296	285
$\epsilon_{\text{max}} \times 10^5$	0.234	0.20	0.265	0.24	0.213	0.249	0.221	0.199
$\lambda_{\text{max}}^{\text{fl}}$ (nm)	380	391	386	403	410	410	432	461

The UV visible spectra for triazolyl phenyl-p-methylester nucleoside **2.81G** ($^{\text{cTBMA}}\text{B}_{\text{Ac}}$) showed structureless absorption band at 262- 274 nm. With increasing polarity of the solvent the absorption band shifted towards lower wavelength region by 12 nm and showed hyperchromic effect. Excitation at absorption maxima ($\lambda_{\text{ex}} = 257\text{-}274$ nm) generated

structureless emission band. As the polarity of the solvent increased the emission band experienced a 36 nm red shift with increasing emission intensity (Figure 2.10 a-b, Table 2.6).

In dioxane $c^{TBMA}B_{Ac}$ showed a structureless absorption band at 268 nm which indicated π -electron cloud conjugation of the triazole ring with the phenylmethylester ring. With increase in water concentration the absorption maxima were shifted to the lower wavelength region and in 100% water a 9 nm blue shift was observed with hypsochromism (Figure 2.10c).

The analysis of emission spectra revealed that $c^{TBMA}B_{Ac}$ nucleoside exhibited a structureless emission band at 342 nm. With gradual addition of water, the fluorescence intensity increased and shifted to the higher wavelength region. The shift of emission maxima towards higher wavelength region was 21 nm which may be due to solvent solute interaction and hydrogen bonding. (Figure 2.10d).

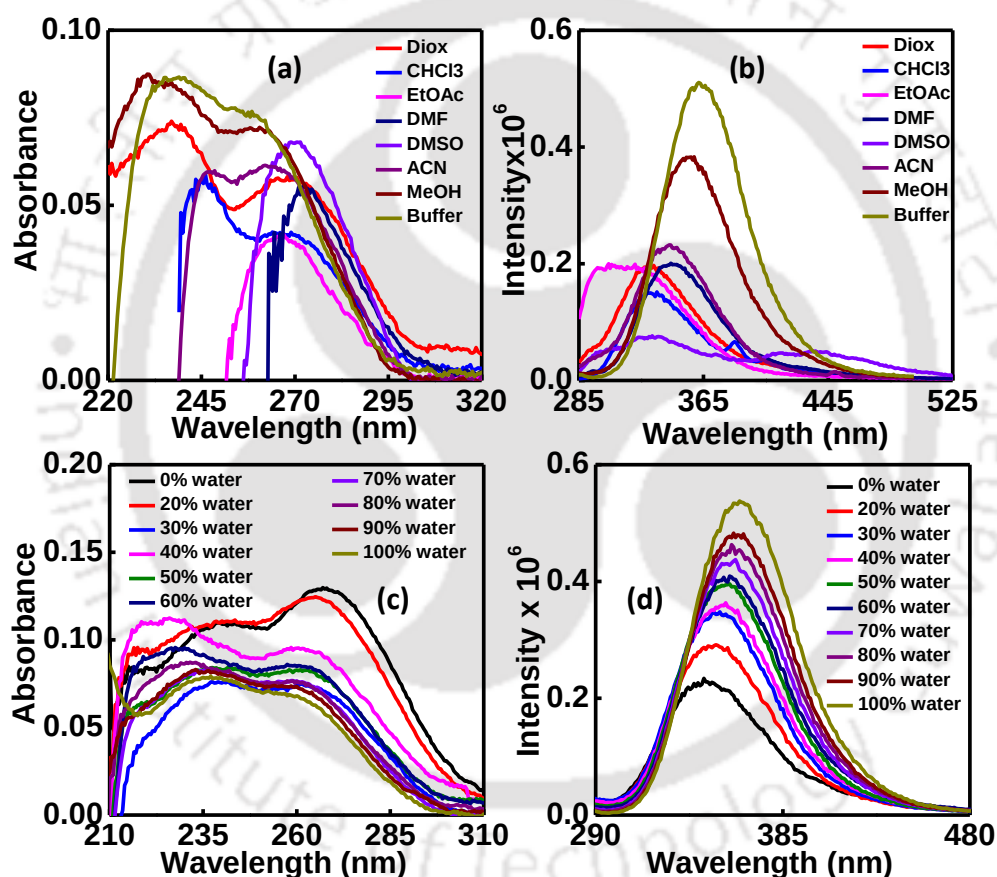


Figure 2.10: UV-visible absorption and fluorescence emission spectra of C-triazolyl nucleoside 2.81G ($c^{TBMA}B_{Ac}$) (a-b) in various organic solvent; (c-d) in different concentration of water and dioxane.

Table 2.6: Summary of UV-Visible and Fluorescence spectra of C-nucleoside **2.81G**.

Entry →	(2.85G)							
Solvents →	Diox	CHCl ₃	EtOAc	DMF	DMSO	ACN	MeOH	Buffer
λ_{max}^{abs} (nm)	268	269	265	274	269	262	260	257
$\epsilon_{max} \times 10^5$	0.041	0.042	0.040	0.054	0.067	0.061	0.072	0.0755
λ_{max}^{fl} (nm)	328	332	320	346	333	342	355	362

2.9.5. Theoretical Calculation

To get an overview over how the variation of solvent polarity is effecting the absorption and emission property of our triazolyl nucleosides, we carried out TDDFT calculation using G09 program package.⁵⁶ It is clear from the HOMO-LUMO orbital overlap and transition oscillator strength (f) of the donor/acceptor triazolyl nucleosides that the S_0 and S_1 electronic transition or the other possible electronic transition are quite feasible. So the feasibility of the reverse transition S_1 to S_0 transition is revealing the fluorophoric nature of our unnatural triazolyl nucleosides. The redistribution of the electronic charge density among the possible energy states certainly support the solvatochromic nature and the intramolecular charge transfer characteristic of our synthesized unnatural C-triazolyl donor/acceptor nucleosides (**Figure 2.11-2.14**).^{57,58}

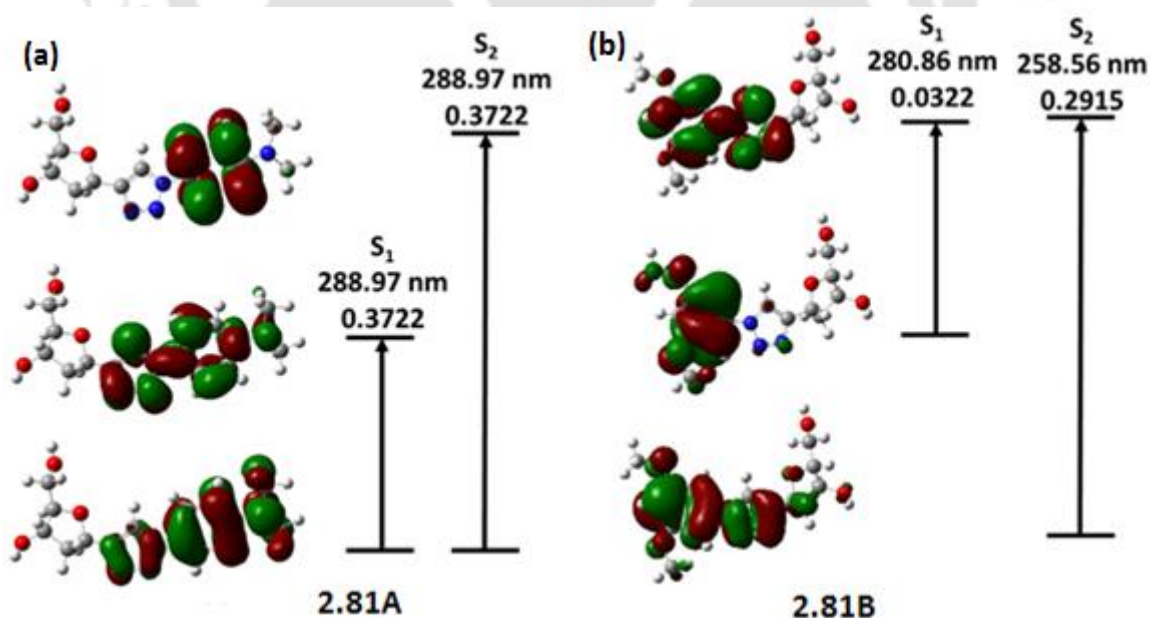


Figure 2.11: (a) HOMO-LUMO energy diagram for nucleoside **2.81A** ($c^{TND\mathbf{B}}\mathbf{D_0}$). (b) HOMO-LUMO energy diagram for nucleoside $c^{TDM\mathbf{B}}\mathbf{D_0}$ (**2.81B**).

The experimental UV-absorption values for **2.81A** in dioxane was found to be 290 nm which are closed to the theoretical value 289 nm with a prominent HOMO→LUMO ($S_0 \rightarrow S_1$) and HOMO→LUMO+1 ($S_0 \rightarrow S_2$) transitions with highest CI value of 0.55 and 0.42 respectively (**Figure 2.11 2.81A**).

The experimental UV-absorption values for **2.81B** in dioxane was found to be 255 nm which is closed to the theoretical value 258 nm with a prominent HOMO-1→LUMO ($S_0 \rightarrow S_2$) transition with highest CI value of 0.68. The another transition for 281 nm which is a HOMO→LUMO ($S_0 \rightarrow S_1$) transition with CI value 0.66 was also prominent (**Figure 2.11 2.81B**).

The experimental UV-absorption values for **2.81C** in dioxane was found to be 283 nm which is closed to the theoretical value 300 nm with a prominent HOMO→LUMO ($S_0 \rightarrow S_1$) transition with highest CI value of 0.69 (**Figure 2.12 2.81C**).

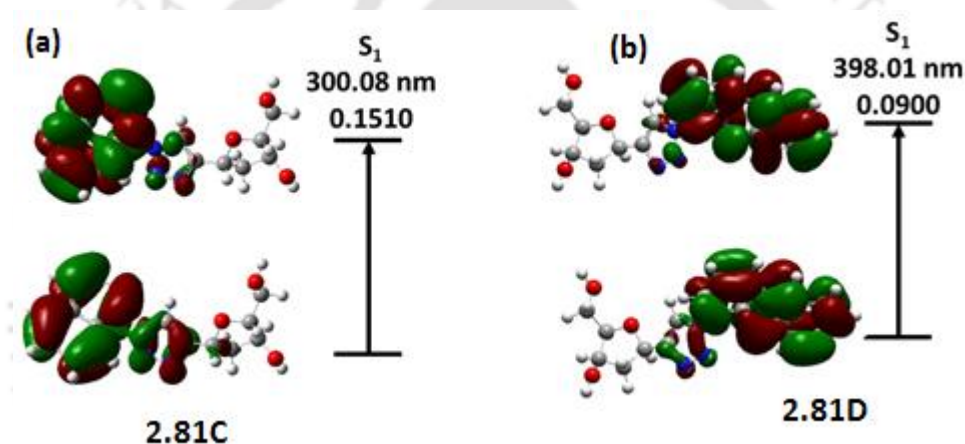


Figure 2.12: (a) HOMO-LUMO energy diagram for nucleoside **2.81C** (${}^{\text{cT Nap}}\text{B}_{\text{D}_0}$). (b) HOMO-LUMO energy diagram for nucleoside **2.81D** (${}^{\text{cT Anth}}\text{B}_{\text{D}_0}$).

The experimental UV-absorption values for **2.81E** dioxane was found to be 278 nm which is closed to the theoretical value 274 nm with a prominent HOMO→LUMO+1 ($S_0 \rightarrow S_2$) transition with highest CI value of 0.33. The another transition for 352 nm is also a prominent HOMO→LUMO ($S_0 \rightarrow S_1$) transition with CI value 0.68. (**Figure 2.13 2.81E**).

The experimental UV-absorption values for **2.81G** in dioxane was found to be 268 nm which is closed to the theoretical value 281 nm with a prominent HOMO→LUMO ($S_0 \rightarrow S_1$) transition with highest CI value of 0.69 (**Figure 2.13 2.81G**).

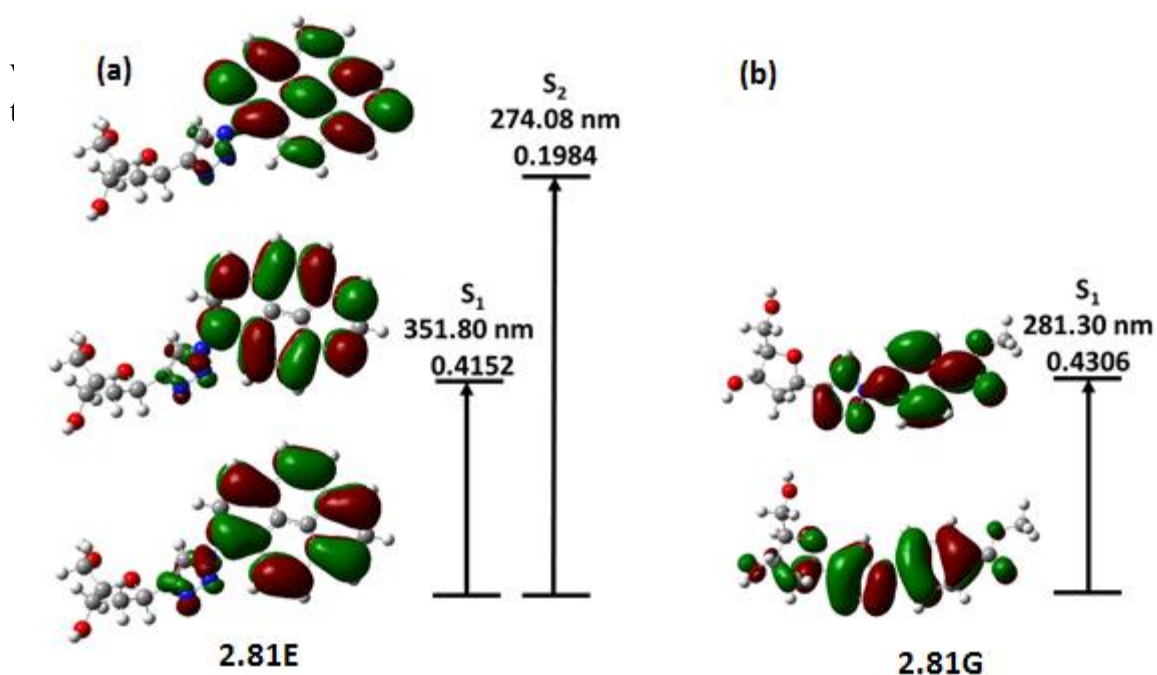


Figure 2.13: **2.81E** HOMO-LUMO energy diagram for nucleoside **2.81E** ($^{cTPy}B_{D0}$). **2.81G** HOMO-LUMO energy diagram for nucleoside **2.81D** ($^{cTBAM}B_{Ac}$).

The experimental UV-absorption values for **2.81H** in dioxane was found to be 287 nm which is near about similar to the theoretical value 284 nm with a prominent HOMO→LUMO ($S_0 \rightarrow S_1$) transition with highest CI value of 0.69 (**Figure 2.14 2.81H**).

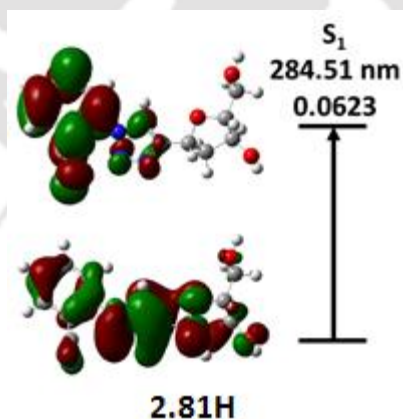


Figure 2.14: HOMO-LUMO energy diagram for nucleoside **2.81H** ($^{cTCNB}B_{Ac}$).

Table 2.7: Summary of HOMO-LUMO transition

Entry	Optimized Energy (a.u.)	Electronic transition	Energy (eV) (Wavelength)	<i>f</i>	Composition	CI
2.81A	-1028.15698626	S ₀ -> S ₁	4.2905 eV 288.97 nm	0.3722	HOMO -> LUMO (81 -> 82)	0.54873
		S ₀ -> S ₂	4.2910 eV 288.94 nm	0.3722	HOMO -> LUMO+1 (81 -> 83)	0.41803
2.811B	-1123.23300280	S ₀ -> S ₁	4.4145 eV 280.86 nm	0.0322	HOMO -> LUMO (85 -> 86)	0.66252
		S ₀ -> S ₂	4.7951 eV 258.56 nm	0.2915	HOMO-1 -> LUMO (84 -> 86)	0.68117
2.81C	-1047.83136174	S ₀ -> S ₁	4.1317 eV 300.08 nm	0.1510	HOMO -> LUMO (82 -> 83)	0.68916
2.81D	-1201.51375679	S ₀ -> S ₁	3.1151 eV 398.01 nm	0.0900	HOMO -> LUMO (95 -> 96)	0.70003
2.81E	-1277.74218599	S ₀ -> S ₁	3.5243 eV 351.80 nm	0.4152	HOMO -> LUMO (101 ->102)	0.67825
		S ₀ -> S ₂	4.5237 eV 274.08 nm	0.1984	HOMO -> LUMO+1 (101 ->103)	0.32812
2.81G	-1122.06438112	S ₀ -> S ₁	4.4076 eV 281.30 nm	0.4306	HOMO -> LUMO (84 -> 85)	0.68995
2.81H	-986.411119530	S ₀ -> S ₁	4.3577 eV 284.51 nm	0.0623	HOMO -> LUMO (75 -> 76)	0.68496

2.9.6. Study of interaction of unnatural C-triazolyl nucleoside 2.81D (^{cTBAM}B_{Ac}) and 2.81E (^{cTPy}B_{D0}) with BSA Protein.

From the interesting photophysical properties of 2.81D (^{cTAnth}B_{D0}) and 2.81E (^{cTPy}B_{D0}) we next studied their interaction behaviour with BSA protein.

Triazolyl C-nucleoside ^{cTAnth}B_{D0} showed structured band in sodium phosphate buffer at 345 nm, 364 nm, 385 nm and 414 nm in the UV spectra. With increasing concentration of BSA hyperchromic absorption at 345 nm, 364 nm and 385 nm were prominent. With hypochromicity of absorption at 414 nm a clear isobestic point was also observed at 395 nm (**Figure 2.15a-b**). All these observations clearly suggested a strong interaction with BSA.

Excitation at 280 nm a gradual decrease in emission intensity of the probe and on the other hand the emission band appeared at 418 nm due to ^{cTAnth}B_{D0} was observed with increasing concentration of BSA (**Figure 2.15c**).

The emission spectra at λ_{ex} = 363 nm was also recorded. With increasing concentration of BSA a gradual increase in the fluorescence intensity at characteristic bands of ^{cTAnth}B_{D0} at 414 nm was observed. The enhancement of fluorescent intensity at 415 nm was occurred due to hydrophobic interaction of the ^{cTAnth}B_{D0} with hydrophobic pocket of BSA (**Figure 2.15d**).

A gradual enhancement of anisotropy with increasing concentration of BSA finally revealed that ^{cTAnth}B_{D0} nucleoside resided in the hydrophobic pocket of BSA (**Figure 2.15e**).

Stern-Volmer quenching constant was found to be $1.026 \times 10^3 \text{ Lmol}^{-1}$ suggested static quenching (Figure 2.15f).

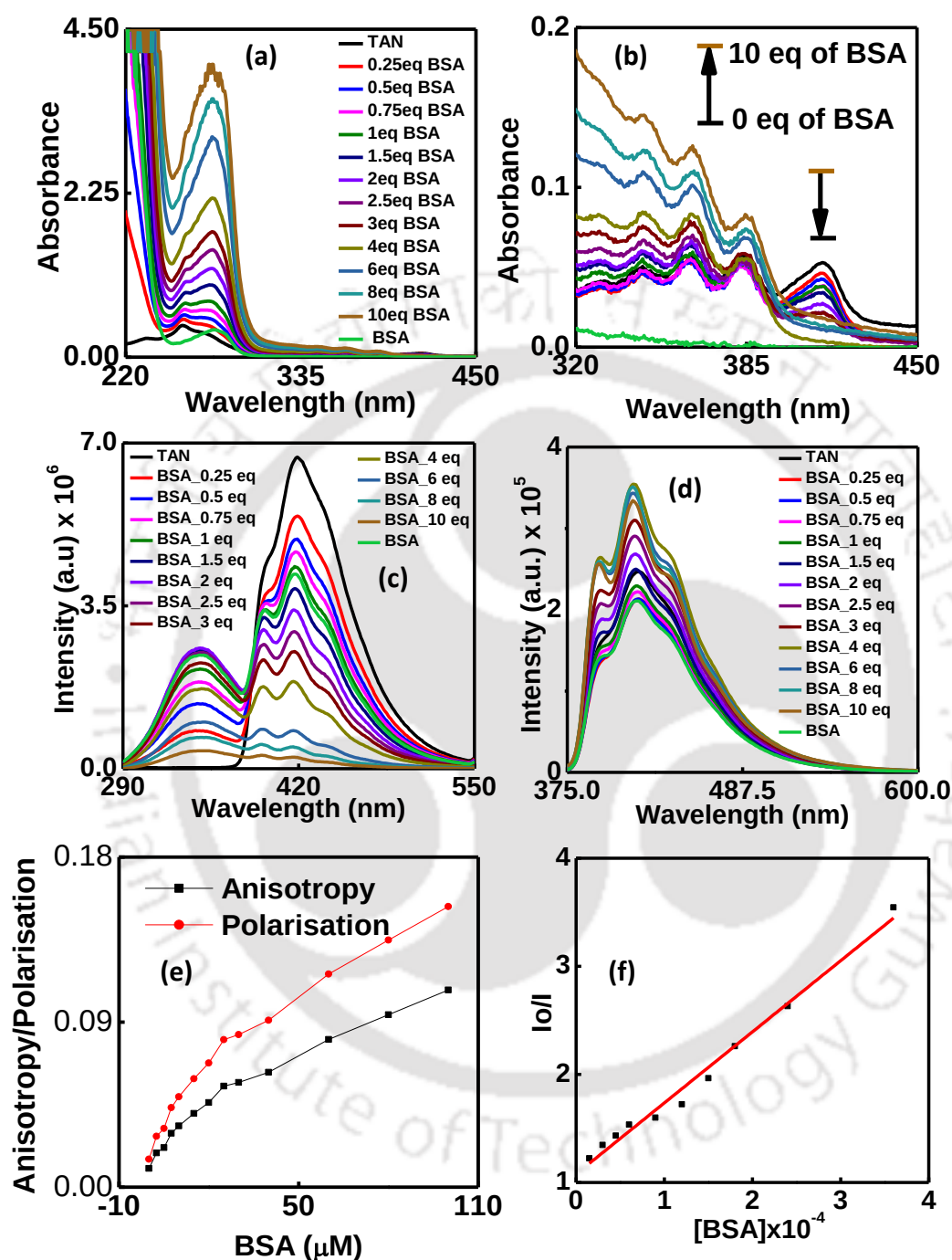


Figure 2.15: (a) UV-visible spectra of **2.81D**. (b) Extended UV-Visible spectra. (c) and (d) fluorescence titration of **2.81D** in absence or in presence of BSA at $\lambda_{\text{ex}} = 280 \text{ nm}$ and 363 nm respectively. (e) Steady-state anisotropy and polarisation plot ($\lambda_{\text{em}} = 420 \text{ nm}$) of **2.81D** in presence of increasing BSA concentration. (f) Stern-Volmer plot. $[\text{cTBAM BAc}] = 10 \mu\text{M}$ and BSA added from 0.25eq to 6eq.

In absence of BSA ${}^c\text{TPyB}_{\text{D}0}$ showed structured absorption band at 325 nm and 341 nm in phosphate buffer, which are the characteristic bands of pyrene. Upon gradual addition of BSA both the bands experienced hypochromic shift and a 2 nm. This observation indicated a strong interaction of the ${}^c\text{TPyB}_{\text{D}0}$ nucleoside with the hydrophobic pocket of BSA (**Figure 2.16a-b**).

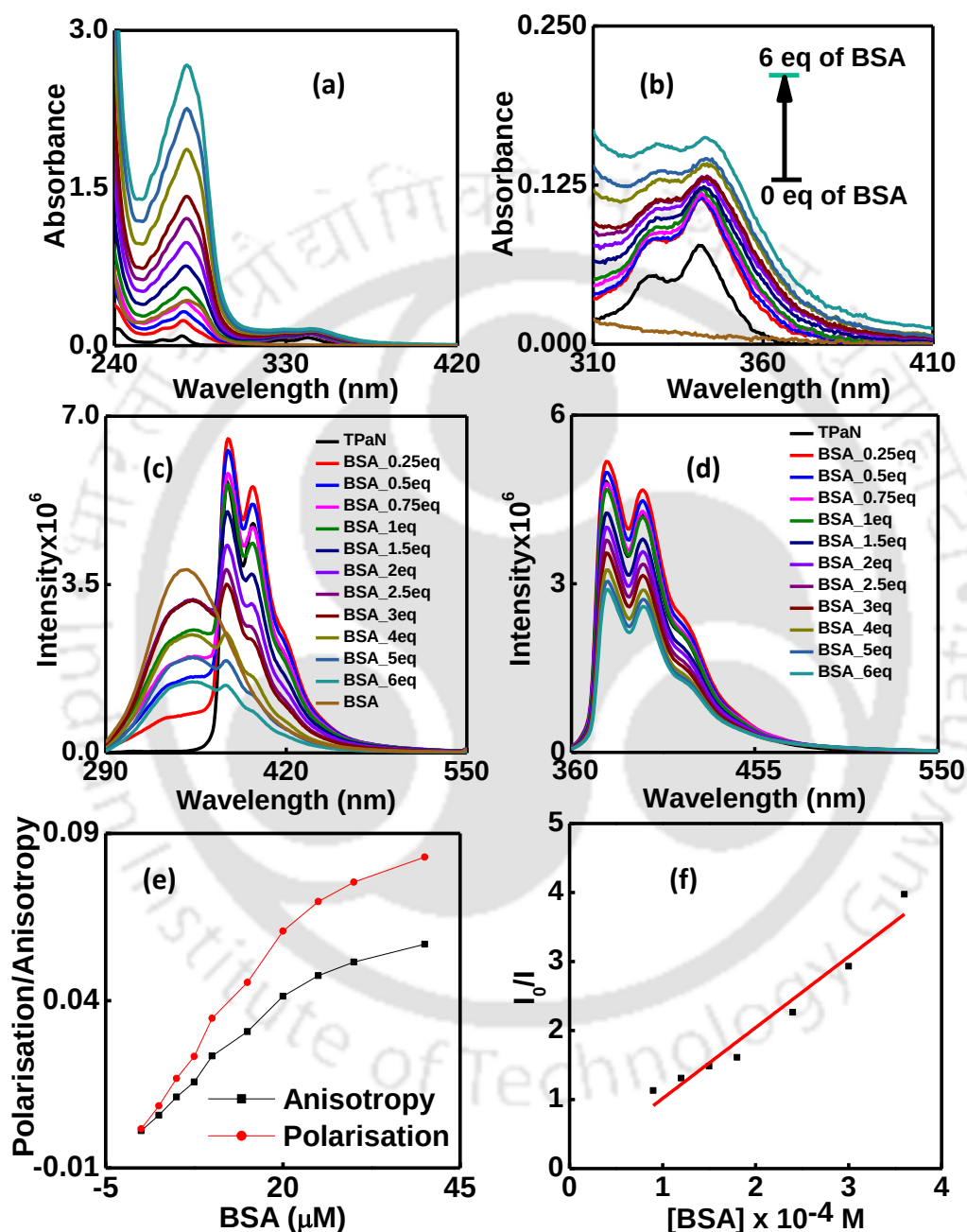


Figure 2.16: (a) UV-visible spectra of **2.81E**. (b) Extended UV-Visible spectra. (c) and (d) fluorescence titration of **2.81E** in absence or in presence of BSA at $\lambda_{\text{ex}} = 280$ nm and 345 nm respectively. (e) Steady-state anisotropy and polarisation plot ($\lambda_{\text{em}} = 380$ nm) of **2.81D** in presence of increasing BSA concentration. (f) Stern-Volmer plot. $[\text{}^c\text{TPyB}_{\text{D}0}] = 10 \mu\text{M}$ and BSA added from 0.25eq to 6eq.

This was also clarified from the emission spectra. Thus upon excitation at 345 nm the $cTPyB_{D0}$ nucleoside showed structured absorption band 378 nm, 397 nm and 417 nm. With gradual addition of BSA the bands at 417 nm shifted by 2 nm towards higher wavelength region and quenching of fluorescence was the result (**Figure 2.16d**). The quenching of fluorescence intensity was also observed upon excitation at 280 nm which is the absorption maxima of BSA (**Figure 2.16c**). This phenomenon was also supported by the restricted motion of $cTPyB_{D0}$ in the hydrophobic pocket of BSA as was resulted from the enhanced fluorescence anisotropy with gradual increase of the concentration of BSA (**Figure 2.16e**). Stern-Volmer quenching constant was found to be $6.578 \times 10^3 \text{ Lmol}^{-1}$ (**Figure 2.16f**).

The binding of nucleosides **2.81D** ($c^{TAnth}B_{D0}$) and **2.81E** ($c^{TPy}B_{D0}$) with BSA did not perturb the conformation of BSA as was revealed from CD spectra (**Figure 2.17a-b**).

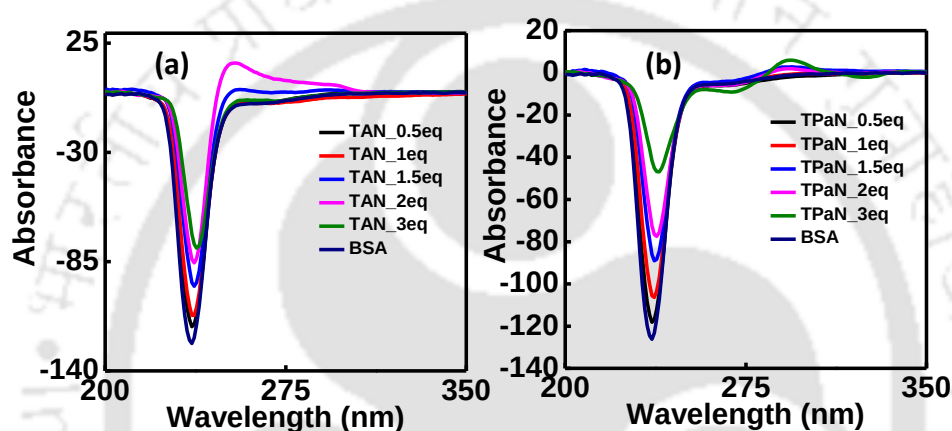


Figure 2.17: (a) CD-spectra of BSA with **2.81D** and (b) CD-spectra of BSA with **2.81E** in methanol solvent (60 μM concentration).

Molecular Docking:

Docking calculations were carried out using Autodock4 (Bikadi, Hazai, 2009). The amino acid sequence of BSA protein was observed from the NCBI website, <http://www.ncbi.nlm.nih.gov/protein/CAA76847.1>. The 3D model of the BSA protein was built using the 3D structure 1AO6 chain 'A' as template using the ESyPred3D11 web server. This template shares 72.4% identities with the BSA sequence. Following is the BSA sequence which was used to generate the 3D model. >gi|3336842|emb|CAA76847.1| bovine serum albumin [Bos taurus]

```

MKWVTFISLLLLFSSAYSRGVFRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQ
YLQQCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYG
DMADCCQKQEPERNECFLSHKDDSPDLPLKLPDPNTLCDEFKADEKKFWGKYLVEI
ARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLTSARQRL
RCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDLLECA
DDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAED
KDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATLEECCA KDDPHA
CYSTVFDKCLKHLVDEPQNLIKQNCQFEKLGEYGFQNALIVRYTRKVPQVSTPTLVE

```


VSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTESLVN
RRPCFSALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKATE
EQLKTVMENFVAFVDKCCAADDKEACFAVEGPKLVVSTQTALA

Binding energy plot suggest that **-9.11 kcal/mol** is the maximum binding energy (**Figure 2.18**) for complexation of **2.81D** ($c^{T\text{Anth}}\text{B}_{\text{D}_0}$). Docking study supported the binding of the fluorophoric moiety of the probe nucleoside $c^{T\text{Anth}}\text{B}_{\text{D}_0}$ in the hydrophobic pocket of BSA (**Figure 2.19a-b**).

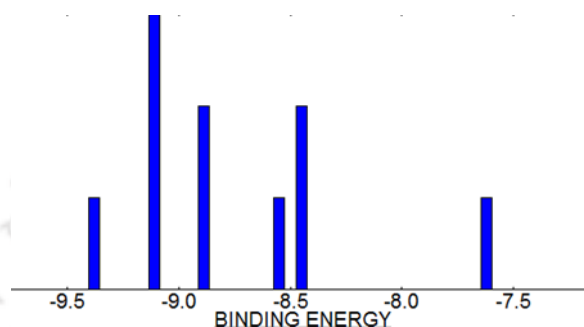


Figure 2.18: Binding Energy plot of the complexation of $c^{T\text{Anth}}\text{B}_{\text{D}_0}$ with BSA.

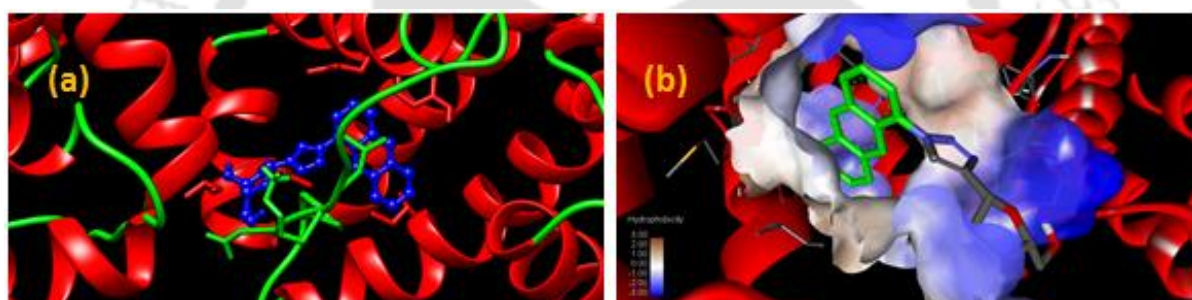


Figure 2.19: (a) Complex structure of $c^{T\text{Anth}}\text{B}_{\text{D}_0}$ with BSA. (b) Interaction of Anthranyl triazolyl moiety of $c^{T\text{Anth}}\text{B}_{\text{D}_0}$ with the hydrophobic pocket of BSA.

Estimated Free Energy of Binding	= -9.11 kcal/mol [(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, K_i	= 210.72 nM (nanomolar) [Temperature = 298.15 K]
(1) Final Intermolecular Energy	= -10.60 kcal/mol
vdW + Hbond + desolv Energy	= -10.59 kcal/mol
Electrostatic Energy	= -0.01 kcal/mol
(2) Final Total Internal Energy	= -1.53 kcal/mol
(3) Torsional Free Energy	= +1.49 kcal/mol
(4) Unbound System's Energy [(2)]	= -1.53 kcal/mol

From the binding energy graph -9.43 kcal/mol (**Figure 2.20**) is the highest binding energy for the complexation of ${}^{\text{cTPy}}\text{BD}_{\text{o}}$ with **BSA**. Docking study supported the binding of the fluorophoric moiety of the probe nucleoside ${}^{\text{cTPy}}\text{BD}_{\text{o}}$ in the hydrophobic pocket of BSA (**Figure 2.21a-b**).

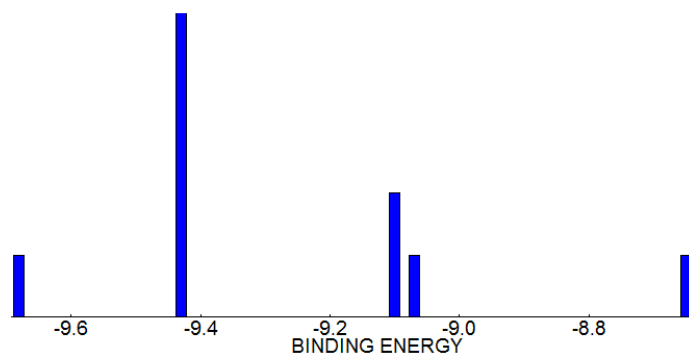


Figure 2.20: Binding energy plot of the complexation of ${}^{\text{cTPy}}\text{BD}_{\text{o}}$ with **BSA**.

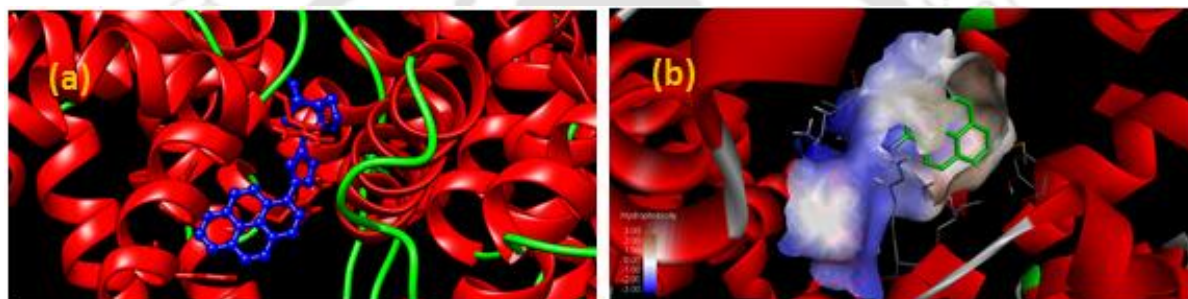


Figure 2.21: (a) Complex structure of ${}^{\text{cTPy}}\text{BD}_{\text{o}}$ with **BSA**. (b) Interaction of pyrenayl triazolyl moiety of ${}^{\text{cTPy}}\text{BD}_{\text{o}}$ with the hydrophobic pocket of **BSA**.

$$\text{Estimated Free Energy of Binding} = -9.43 \text{ kcal/mol } [= (1) + (2) + (3) - (4)]$$

$$\text{Estimated Inhibition Constant, } K_i = 123.19 \text{ nM (nanomolar) [Temperature} = 298.15 \text{ K]}$$

$$(1) \text{ Final Intermolecular Energy} = -10.92 \text{ kcal/mol}$$

$$\text{vdW} + \text{Hbond} + \text{desolv Energy} = -10.65 \text{ kcal/mol}$$

$$\text{Electrostatic Energy} = -0.26 \text{ kcal/mol}$$

$$(2) \text{ Final Total Internal Energy} = -1.45 \text{ kcal/mol}$$

$$(3) \text{ Torsional Free Energy} = +1.49 \text{ kcal/mol}$$

$$(4) \text{ Unbound System's Energy } [= (2)] = -1.45 \text{ kcal/mol}$$

2.10. Conclusion

In conclusion we have successfully synthesised few triazolyl donor-acceptor C-nucleoside applying Nicholash reaction as the key to prepare the intermediate β -sugar alkyne which undergone 1,3-dipolar azide –alkyne cycloaddition reaction with various donor-acceptor aromatics. Few of the synthesised nucleoside showed interesting photophysical property. Two of the fluorescent nucleoside were used to study interaction with BSA and found a strong interaction with a quenching incidence of fluorescence emission.

2.11. Experimental Section

2.11.1. General Experimental

All reactions were carried out under a nitrogen atmosphere. Organic extracts were dried over anhydrous sodium sulphate. Solvents were removed in a rotary evaporator under reduced pressure. Silica gel (60-120 mesh size) was used for the column chromatography. Reactions were monitored by TLC on silica gel 60 F254 (0.25mm). ^1H NMR spectra were measured with Bruker 600 (600 MHz) and ^{13}C NMR spectra were measured with Bruker 600 (150 MHz) spectrometer. Coupling constant (J value) was reported in hertz. The chemical shifts were shown in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in ^1H NMR, $\delta = 77.23$ in ^{13}C NMR), methanol ($\delta = 3.34$ in ^1H NMR, $\delta = 49.2$ in ^{13}C NMR), dimethyl sulfoxide ($\delta = 2.48$ in ^1H NMR, $\delta = 39.5$ in ^{13}C NMR), as an internal standard. Mass spectra were recorded using WATERS MS system, Q-tof premier and data analyzed using Mass Lynx 4.1. IR spectra were recorded in KBr or neat on a Perkin Elmer Spectrum one FT-IR spectrometer.

2.11.2. Crystallographic Description and ORTEP Diagram

Crystal data were collected with Bruker Smart Apex-II CCD diffractometer using graphite monochromated $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 298 K. Cell parameters were retrieved using SMART [a] software and refined with SAINT[a] on all observed reflections. Data reduction was performed with the SAINT software and corrected for Lorentz and polarization effects. Absorption corrections were applied with the program SADABS[b]. The structure was solved by direct methods implemented in SHELX-97[c] program and refined by full-matrix least-squares methods on F^2 . All non-hydrogen atomic positions were located in difference Fourier maps and refined anisotropically. The hydrogen atoms were placed in their geometrically generated positions. Crystals were isolated in from acetonitrile/chloroform mixture at room temperature.

a. SMART V 4.043 Software for the CCD Detector System; Siemens Analytical Instruments Division: Madison, WI, 1995.

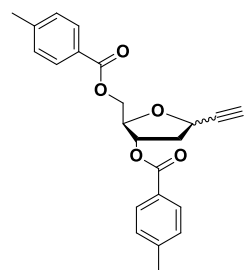
b. SAINT V 4.035 Software for the CCD Detector System; Siemens Analytical Instruments Division: Madison, WI, 1995.

c. Sheldrick, G. M. SHELXL-97, Program for the Refinement of Crystal Structures; University of Göttingen: Göttingen (Germany), 1997.

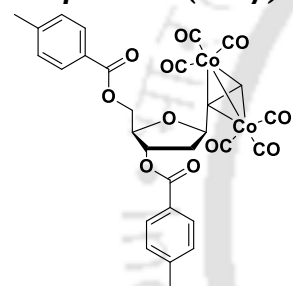
2.11.3. Synthesis and characterization

Synthesis of 1-Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -/ β -D-ribofuranose (1.086):

In a nitrogen flushed 2 neck round bottom flask fitted with magnetic stirring bar and septum, charged with (2R, 3S, 5R)-5- α -chloro-2-(((4-methylbenzoyl) oxy) methyl) tetrahydrofuran-3-yl 4-methylbenzoate (1.8 g, 4.64 mmol), THF was added to it to make it dissolve. A 0.5 M solution of ethynyl magnesium chloride (13 ml, 6.5 mmol) was added to the reaction mixture and allowed to stir for 3h to achieve complete conversion of the starting material which was checked by TLC. After the completion of the reaction the reaction mixture was diluted with ethyl acetate and the combined organic fraction was washed with aqueous solution of ammonium chloride and brine. The organic fraction was then evaporated and concentrated in vacuo and the residue was purified by Column chromatography by hexane: ethyl acetate (25:1) to get a mixture of α and β anomer. Overall yield of the reaction is 1.4 g (92%). R_f = 0.3 in 25:1 (Hexane: Ethyl acetate).

**Synthesis of cobalt complexed 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose (2.84 β):**

A 2 neck round bottom flask fitted with magnetic stirring bar and septum was degassed by high vacuum pump and filled with nitrogen. The degassing procedure was repeated for 3 times and each time the reaction vessel was back filled by nitrogen. Now 1-Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -/ β -D-ribofuranose (1.4 g, 3.7 mmol) was added to the reaction vessel by keeping it properly in nitrogen atmosphere. A volume of 15 ml dry Dichloro methane was added to the reaction vessel followed by $\text{Co}_2(\text{CO})_8$ (1.45 g, 4.25 mmol) by keeping it again in proper nitrogen atmosphere. The reaction mixture was then allowed to stir for 1 h. The completion of the cobalt complexation was then achieved which was checked by TLC. Now the reaction mixture was allowed to come to -78°C and at this temperature $\text{BF}_3\cdot\text{OEt}_2$ (2 ml, 14.8 mmol) was added to the reaction mixture. Maximum conversion of the α anomer to the β anomer was achieved after 1 h which was checked by TLC. Now the reaction mixture was allowed to strictly come to the room temperature and an excess aqueous solution of NaHCO_3 was added to the reaction mixture to quench excess $\text{BF}_3\cdot\text{OEt}_2$. The organic fraction was washed with brine and concentrated in vacuum. The crude material was purified by column chromatography by eluting through Hexane: Ethyl acetate (35:1). The remaining mixture of the α and β complex was again treated with $\text{BF}_3\cdot\text{OEt}_2$ and following the above procedure overall yield of the cobalt complexed 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose was 1.73 g (70%) which was a brown coloured gummy material.

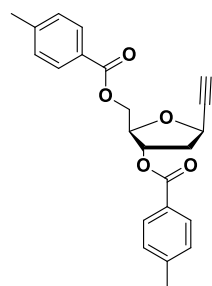


^1H NMR (600 MHz, CDCl_3) δ 7.94 (dd, J = 15.5, 8.2 Hz, 4H), 7.28 – 7.25 (m, 4H), 7.23 (d, J = 8.0 Hz, 2H), 6.07 (s, 1H), 5.61 (d, J = 6.0 Hz, 1H), 5.33 (dd, J = 10.6, 4.7 Hz, 1H), 4.57 – 4.45 (m, 3H), 2.57 (dd, J = 13.5, 4.8 Hz, 1H), 2.42 (d, J = 8.3 Hz, 6H), 2.18 (ddd, J = 13.6, 10.7, 6.1 Hz, 1H). **^{13}C NMR** (150 MHz, CDCl_3) δ 199.4, 166.2, 144.4, 144.0, 130.0, 129.8, 129.4, 129.3, 127.2, 127.0, 93.6, 82.9, 79.1, 77.2, 72.2, 68.1, 66.0, 64.9, 41.7, 21.8, 15.4. **IR**(cm^{-1}): 2924.62, 2096.49, 2056.60, 2028.90, 1721.38, 1612.44, 1453.97, 1270.34, 1177.02,

1105.05, 1020.59, 804.33, 753.06; HRMS calculated for $C_{29}H_{29}Co_2O_{11}([M+H]^+)$ 664.9899, found 664.990.

Synthesis of 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose (1.086 β):

Cobalt complexed 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose (1.73 g, 2.6 mmol) was taken in round bottom flask. 10 ml THF was added to it to dissolve the starting material. The reaction mixture was then allowed to come to 0 °C. I_2 (9.9 g, 39 mmol) was added to the reaction mixture. After 1h there was complete conversion of the starting material which was checked by TLC. The reaction mixture was diluted with DCM and washed with sodium thiosulfate to quench the excess iodine. The organic fraction was collected and concentrated in vacuo. Crude was purified by eluting 10:1 (hexane: ethyl acetate) through the column chromatography to afford 800 mg white solid. Yield of the product is 82 %.



1H NMR (600 MHz, $CDCl_3$) δ 7.96 (dd, J = 32.5, 8.1 Hz, 4H), 7.27 (dd, J = 15.3, 6.9 Hz, 4H), 5.56 (dt, J = 4.8, 2.3 Hz, 1H), 4.95 – 4.84 (m, 1H), 4.56 (qd, J = 11.8, 4.5 Hz, 2H), 4.45 (td, J = 4.4, 2.3 Hz, 1H), 2.55 (t, J = 3.9 Hz, 1H), 2.53 – 2.49 (m, 2H), 2.43 (d, J = 7.8 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.5, 166.1, 144.4, 143.6, 129.9, 129.8, 129.3, 129.3, 127.2, 126.9, 82.9, 81.9, 77.2, 76.5, 74.4, 68.2, 64.6, 40.0, 21.9, 21.8. ^{13}C NMR (150 MHz, $CDCl_3$, DEPT-135) δ 130.0, 129.9, 129.4, 129.3, 82.9, 76.6, 68.3, 64.6, 40.0, 21.9, 21.9. IR(cm^{-1}): 2989.75, 2964.95, 2908.19, 1722.93, 1611.82, 1471.01, 1393.78, 1318.95, 1286.07, 1262.95, 1247.59, 1212.65, 1128.57, 1102.14, 931.65752.07. HRMS Calculated for $C_{23}H_{23}O_5([M+H]^+)$ 379.154, found 379.1547.

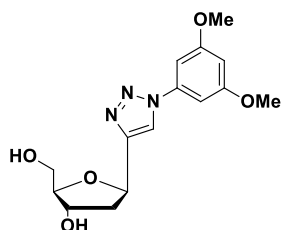
General procedure for the synthesis of triazole nucleoside (2.81A-H)

Procedure A: 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose (50 mg, 0.132 mmol) was taken in a 2 neck round bottom flask. THF was added to it followed by CuI (3 mg, 0.0132 mmol) and triethyl amine (0.0132 mmol). Aromatic azide (0.145 mmol) was added to the reaction mixture. The reaction mixture was stirred for 30 minutes at 80 °C. Completion of the reaction was checked by TLC. The reaction mixture was diluted with ethyl acetate and washed with ammonium chloride. The organic fraction was concentrated by evaporation and the crude material was purified by column chromatography by using 5:1 (Hexane: ethyl acetate). This material was then dissolved in 10 ml MeOH and NaOMe (29 mg, 0.53 mmol) was added to it. The reaction mixture was then allowed to stir for 10 h at room temperature. Completion of the reaction was checked by TLC. The reaction mixture was then concentrated and purified by column chromatography.

Prosedure B: 1- β -Ethynyl-2-deoxy-3, 5-bis-(O-p-toluoyl)- α -D-ribofuranose (50 mg, 0.132 mmol) was taken in a 2 neck round bottom flask charged with magnetic stirring bar and septum. The reaction vessel was flushed with nitrogen. t BuOH (5 ml) and H_2O (5 ml) were added to it followed by anhydrous $Cu(OAc)_2$ (3 mg, 0.0132 mmol) and TBTA (7 mg, 0.0132 mmol). The reaction mixture was then heated at 90 °C for 30 min. After completion of the reaction t BuOH was evaporated and the product was collected by ethyl acetate. The organic fraction was washed with ammonium chloride and brine and purified by column

chromatography by 5:1 (Hexane: ethylacetate). This material was then treated with sodium methoxide in 10 ml dry methanol for 10 h to afford the product. This crude material was then purified by column chromatography.

Synthesis of (2R,3S,5R)-5-(1-(3,5-dimethoxyphenyl)-1H-1,2,3-triazol-4-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (2.81B): Product was synthesised by Procedure A.

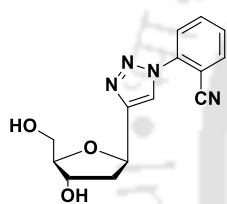


Purified by 10:1 (CHCl₃:MeOH). White solid. Yield 28.3 mg (83%).

¹H NMR (600 MHz, MeOD) δ 8.52 (s, 1H), 6.98 (d, *J* = 2.1 Hz, 2H), 6.57 (t, *J* = 2.1 Hz, 1H), 5.32 (t, *J* = 7.9 Hz, 1H), 4.59 (s, 10H), 4.39 – 4.35 (m, 1H), 3.93 (td, *J* = 4.7, 2.7 Hz, 1H), 3.64 (ddd, *J* = 31.8, 11.9, 4.7 Hz, 2H), 3.28 (s, 5H), 2.27 (dd, *J* = 7.9, 3.9 Hz, 2H). ¹³C NMR (150 MHz, MeOD) δ 163.2, 150.6, 139.8, 122.2, 101.6, 99.8, 89.3,

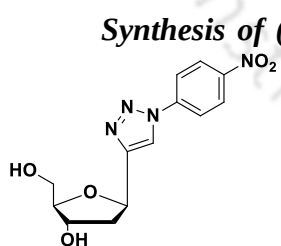
74.1, 73.9, 63.9, 56.3, 49.0, 42.7. ¹³C NMR (150 MHz, MeOD, DEPT-135) δ 122.2, 101.6, 99.8, 89.3, 74.1, 73.9, 63.9, 56.3, 42.7. IR(cm⁻¹): 2956.86, 2924.66, 1620.14, 1598.66, 1497.82, 1478.43, 1379.38, 1342.83, 1267.86, 1209.74, 1155.94, 1053.81, 1012.30, 859.85, 760.28. HRMS Calculated for C₁₅H₂₀N₃O₅ ([M+H]⁺) 322.1397, found 322.1397.

Synthesis of 2-(4-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1H-1,2,3-triazol-1-yl)benzonitrile (2.81H): Product was synthesised by Procedure A. Purified



by 10:1 (CHCl₃:MeOH). White solid. Yield 32 mg (85%). ¹H NMR (600 MHz, MeOD) δ 8.53 (s, 2H), 8.01 (dd, *J* = 7.8, 1.1 Hz, 2H), 7.94 – 7.88 (m, 2H), 7.82 (d, *J* = 7.9 Hz, 2H), 7.74 (t, *J* = 7.7 Hz, 2H), 5.39 (t, *J* = 7.9 Hz, 2H), 4.60 (s, 1H), 4.40 (dd, *J* = 6.6, 3.9 Hz, 2H), 3.96 (td, *J* = 4.8, 2.7 Hz, 2H), 3.65 (qd, *J* = 11.9, 4.9 Hz, 4H), 2.38 – 2.29 (m, 4H). ¹³C NMR (150 MHz, MeOD) δ 150.8, 139.7, 135.8, 135.7, 131.4, 126.8, 124.7, 116.5, 109.1, 89.4, 74.1,

73.8, 63.9, 42.6. ¹³C NMR (150 MHz, MeOD, DEPT-135) δ 135.8, 135.7, 131.4, 126.8, 124.7, 89.4, 74.1, 73.8, 63.9, 42.6. IR(cm⁻¹): 2927.02, 2232.79, 1642.08, 1508.74, 1449.41, 1345.67, 1229.42, 1085.28, 1052.33, 956.54, 848.84, 773.00. HRMS Calculated for C₁₄H₁₅N₄O₃ ([M+H]⁺) 287.1139, found 287.1139.



Synthesis of (2R,3S,5R)-2-(hydroxymethyl)-5-(1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)tetrahydrofuran-3-ol (2.81F): Product was synthesised by Procedure A. Purified by 10:1 (CHCl₃:MeOH). Yellowish white solid.

Yield 34 mg (86%). ¹H NMR (600 MHz, MeOD) δ 8.72 (s, 1H), 8.46 (d, *J* = 9.1 Hz, 2H), 8.16 (d, *J* = 9.1 Hz, 2H), 5.45 – 5.24 (m, 1H), 4.64 (s, 5H), 4.40 (dt, *J* = 5.3, 2.7 Hz, 1H), 3.97 (td, *J* = 4.8, 2.7 Hz, 1H),

3.66 (ddd, *J* = 27.7, 11.9, 4.8 Hz, 2H), 2.34 – 2.29 (m, 2H). ¹³C NMR (150 MHz, MeOD, DEPT-135) δ 125.1, 120.8, 120.4, 87.9, 72.6, 72.4, 62.4, 41.2. IR(cm⁻¹): 1626.81, 1596.28, 1538.0, 1504.7, 1404.79, 1354.84, 1229.95, 1204.98, 1080.09, 1049.56, 994.06, 858.07. HRMS Calculated for C₁₃H₁₅N₄O₅ ([M+H]⁺) 307.1037, found 307.1035.

Synthesis of (2R,3S,5R)-5-(1-(4-(dimethylamino)phenyl)-1H-1,2,3-triazol-4-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (2.81A): Product was synthesised by Procedure A.

Purified by 12:1 (CHCl₃:MeOH). Yellowish white solid. Yield 34 mg (85%). ¹H NMR (600 MHz, MeOD) δ 8.32 (s, 1H), 7.57 (d, *J* = 9.0 Hz, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 5.31 (t, *J* = 7.9 Hz, 1H), 4.60 (s, 2H), 4.37 (d, *J* = 2.4 Hz, 1H), 3.93 (dd, *J* = 7.1, 4.6 Hz, 1H), 3.64 (ddd, *J* = 30.8, 11.9, 4.7 Hz, 2H), 2.99 (s, 6H), 2.26 (dd, *J* = 8.7, 4.6 Hz, 2H). ¹³C NMR (150 MHz, MeOD) δ 151.2, 149.0, 126.7, 121.6, 120.7, 112.3, 88.1, 72.9, 72.8, 62.7, 41.5, 39.4. ¹³C NMR (150 MHz, MeOD, DEPT-135) δ 121.6, 120.7, 112.3, 88.1, 72.8, 72.77, 62.7, 41.5, 39.4. IR(cm⁻¹): 2906.72, 1634.46, 1531.29, 1448.38, 1356.29, 1227.61, 1089.27, 1049.50, 990.34, 966.06, 819.99. HRMS Calculated for C₁₅H₂₁N₄O₃ ([M+H]⁺) 305.1602, found 305.1602.

Synthesis of (2R,3S,5R)-2-(hydroxymethyl)-5-(1-(naphthalen-1-yl)-1H-1,2,3-triazol-4-yl)tetrahydrofuran-3-ol (2.81C): Product was synthesised by Procedure A. Purified by 13:1 (CHCl₃:MeOH). Yield 39 mg (96%). ¹H NMR (600 MHz, MeOD) δ 8.38 (s, 1H), 8.14 – 8.10 (m, 1H), 8.04 (d, *J* = 8.1 Hz, 1H), 7.64 (dt, *J* = 9.4, 3.0 Hz, 2H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 5.43 (dd, *J* = 9.7, 6.1 Hz, 1H), 4.51 – 4.34 (m, 1H), 3.98 (td, *J* = 4.7, 2.8 Hz, 1H), 3.67 (ddd, *J* = 30.7, 11.9, 4.8 Hz, 2H), 2.98 (s, 1H), 2.85 (s, 1H), 2.42 – 2.32 (m, 2H). ¹³C NMR (150 MHz, MeOD) δ 150.0, 135.7, 134.9, 131.8, 129.9, 129.5, 129.0, 128.3, 126.7, 126.2, 124.9, 123.1, 89.4, 74.1, 73.6, 63.9, 49.0, 42.7. ¹³C NMR (150 MHz, MeOD, DEPT-135) δ 131.8, 129.5, 129.1, 128.3, 126.7, 126.3, 124.9, 123.1, 89.4, 74.1, 73.9, 63.9, 42.7. IR(cm⁻¹): 2922.97, 2852.56, 1736.80, 1646.08, 1598.17, 1540.70, 1467.55, 1430.40, 1389.60, 1224.22, 1089.57, 1049.98, 803.05, 772.85. HRMS Calculated for C₁₅H₂₁N₄O₃ ([M+H]⁺) 312.1343, found 312.1346.

Synthesis of (2R,3S,5R)-5-(1-(anthracen-2-yl)-1H-1,2,3-triazol-4-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (2.81D): Product was synthesised by Procedure A. Purified by 15:1 (CHCl₃:MeOH). White solid. Yield 37 mg (78%). ¹H NMR (600 MHz, DMSO) δ 8.50 – 8.40 (m, 3H), 8.33 (s, 1H), 8.11 (d, *J* = 9.1 Hz, 1H), 7.97 (d, *J* = 2.6 Hz, 2H), 7.90 (dd, *J* = 9.0, 1.8 Hz, 1H), 7.49 – 7.41 (m, 2H), 5.48 – 5.28 (m, 1H), 4.83 (d, *J* = 3.9 Hz, 1H), 4.33 (dd, *J* = 17.3, 10.9 Hz, 2H), 3.94 (s, 1H), 3.71 – 3.51 (m, 2H), 2.37 – 2.17 (m, 2H). ¹³C NMR (150 MHz, CDCl₃) δ 149.9, 133.4, 131.8, 131.5, 130.2, 129.9, 126.4, 126.2, 125.8, 125.6, 119.7, 117.2, 87.5, 72.3, 72.3, 62.5, 41.6, 39.5. IR(cm⁻¹): 2925.64, 2876.6, 1629.28, 1468.05, 1434.92, 1384.16, 1180.67, 1095.28, 1049.83, 895.38, 745.22, 526.34; HRMS Calculated for C₂₁H₂₀N₃O₃ ([M+H]⁺) 362.1481, found 362.1499.

Synthesis of (2R,3S,5R)-5-(1-(5a,10-dihydropyren-1-yl)-1H-1,2,3-triazol-4-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (2.81E): Product was synthesised by Procedure B. Purified by 20:1 (CHCl₃:MeOH). Yield 31 mg (61%). ¹H NMR (600 MHz, MeOD) δ 8.47 – 8.43 (m, 1H), 8.25 – 8.12 (m, 3H), 8.10 – 7.89 (m, 5H), 7.64 (dd, *J* = 10.9, 9.3 Hz, 1H), 5.48 (dd, *J* = 9.8, 5.9 Hz, 1H), 4.60 (s, 2H), 4.45 (dd, *J* = 5.2, 2.5 Hz, 1H), 4.04 – 3.98 (m, 1H), 3.70 (ddd, *J* = 30.6, 11.9, 4.8 Hz, 2H), 2.48 – 2.35 (m, 2H). ¹³C NMR

(150 MHz, MeOD) δ 150.2, 133.7, 132.4, 131.9, 131.4, 130.8, 130.0, 127.9, 127.6, 128.0, 127.0, 125.9, 124.5, 121.7, 89.4, 74.2, 74.0, 63.9, 49.0, 42.8. **^{13}C NMR** (150 MHz, MeOD) δ 130.8, 130.0, 128.0, 127.6, 127.2, 127.0, 125.9, 124.5, 121.7, 89.5, 74.2, 74.0, 64.0, 42.8. IR(cm^{-1}): 2923.12, 2850.7, 1681.30, 1636.0, 1615.6, 1456.1, 1419.3, 1382.5, 1284.3, 1263.9, 1047.1, 752.65. **HRMS** Calculated for $\text{C}_{23}\text{H}_{20}\text{N}_3\text{O}_3$ ($[\text{M}+\text{H}]^+$) 386.1499, found 386.1497.

2.11.4. Photophysical Studies of the Nucleosides

UV-visible measurements: All the UV-visible spectra of the nucleoside monomers (10 μM) were measured in different organic solvents using a UV-Visible spectrophotometer with a cell of 1 cm path length at 25 $^{\circ}\text{C}$. Equal volume of each sample solution in different solvents were used. The measurements were taken in absorbance mode. The absorbance values of the sample solutions were measured in the wavelength regime of 200–550 nm. All the sample solutions were prepared just before doing the experiment.

Fluorescence experiments: All the sample solutions of the nucleoside monomers (10 μM) were prepared as described in UV measurement experiments. Fluorescence spectra were obtained using a fluorescence spectrophotometer at 25 $^{\circ}\text{C}$ using 1 cm path length cell. The excitation wavelengths for monomers were set at $\lambda_{\text{abs}}^{\text{max}}$, and emission spectra were measured in the wavelength regime of 315–600 nm with an integration time of 0.1 sec.

2.11.5. Studies On the Interaction of 2.81D ($^{\text{cTAnthBDo}}$) and 2.81E ($^{\text{cTPyBDo}}$) with BSA

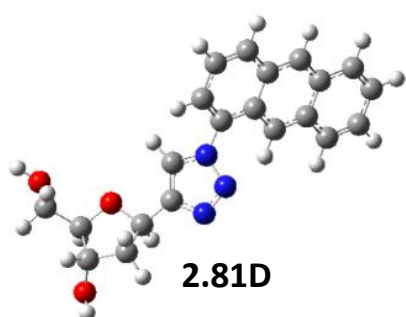
Preparation of BSA solution: Phosphate buffer of pH 7.02 was used to prepare the solution of BSA (Merck). A 1200 μM of stock BSA solution was prepared by dissolving 0.08 gm of BSA in 1 mL phosphate buffer (20 mM) of pH 7.0. From that stock solution sub stock of 1000 μM BSA was prepared. The compound stock solution was prepared in DMF because of the poor solubility in water. 1.0 mg of nucleoside **2.81D** ($^{\text{cTAnthBDo}}$) and **2.81E** ($^{\text{cTPyBDo}}$) was dissolved in 1.5 mL DMF to make a stock probe solution of concentration 910.3 μM .

General experimental on interaction study of BSA by photophysical study: All the spectral measurements were carried out at room temperature. To study the interaction of compound with BSA, an aqueous solution of nucleoside **2.81D** ($^{\text{cTAnthBDo}}$) and **2.81E** ($^{\text{cTPyBDo}}$) (10 μM) was titrated with different concentrations of BSA (ranging from 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 2.5, 3.0, 4, 5, 6, 10, 14, 18, 20 equivalent). The total volume of the final solution for each sample was 3 mL. The % of DMF content was 2%. The presence of 2% DMF does not induce structural changes to biomolecules. Each sample solution was mixed well before spectral measurements.

UV-Visible study with BSA: The UV-Visible absorbance measurements were performed using UV-visible spectrophotometer with a cell of 1 cm path length at 298 K. All the UV-Visible studies were carried out in 20 mM phosphate buffer of pH 7.02 containing solution at 298 K. 2 % DMF was used to solubilize the probe. The measurements were taken in absorbance mode and the absorbance values of the sample solutions were measured in the wavelength regime of 200–700 nm. All the experiments were carried out with freshly prepared sample solutions.

Fluorescence study with BSA: All fluorescence and steady state anisotropy experiments were performed using a steady state fluorescence spectrophotometer with a cell of 1 cm path length at 298 K. The excitation wavelength for probe nucleoside **2.81D** (${}^{\text{cTAnth}}\text{B}_{\text{D}_0}$) and **2.81E** (${}^{\text{cTPy}}\text{B}_{\text{D}_0}$) was set at 220 nm, 420 nm and emission spectra were measured in the wavelength regime of 290–650 nm. Steady state anisotropy of the solutions was measured using steady state fluorescence spectrophotometer. The fluorescence lifetime experiment was carried out as described above.

2.11.6. b3lyp/6-31+g(d,p) Optimized Structure, Energy and Cartesian Coordinates of the Synthesized C-nucleosides 2.81D and 2.81E.

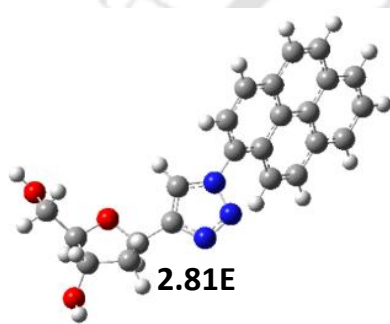


E(RB3LYP) = -1201.62823455 a.u.

1 O1	3.8334	-0.0834	0.7483 O
2 N2	-0.0161	0.4707	-0.7304 N
3 N3	1.1860	-1.0854	-1.5916 N
4 N4	0.0170	-0.5122	-1.6707 N
5 O5	6.3959	-1.9441	-0.5312 O
6 H6	6.7442	-2.1794	-1.4010 H
7 O7	5.6111	1.0518	2.7290 O
8 H8	6.0279	1.8520	3.0713 H
9 C9	1.1654	0.5183	-0.0511 C
10 H10	1.3566	1.2086	0.7537 H
11 C11	5.2429	-0.3232	0.8059 C
12 H12	5.4462	-1.1839	1.4592 H
13 C13	1.9245	-0.4835	-0.6130 C
14 C14	5.6801	-0.7100	-0.6328 C
15 H15	6.3332	0.0570	-1.0723 H
16 C16	3.3101	-0.9316	-0.2774 C
17 H17	3.2802	-1.9726	0.0835 H
18 C18	5.9553	0.8969	1.3556 C
19 H19	7.0382	0.7391	1.2380 H
20 H20	5.6615	1.7807	0.7696 H
21 C21	4.3499	-0.8156	-1.4062 C
22 H22	4.3322	-1.6648	-2.0943 H
23 H23	4.1634	0.0988	-1.9788 H
24 C24	-1.1486	1.3290	-0.5754 C
25 C25	-2.4355	0.7706	-0.2563 C
26 C26	-0.9646	2.6836	-0.7051 C
27 C27	-2.6665	-0.6014	-0.0916 C
28 C28	-3.5350	1.6959	-0.0809 C
29 C29	-2.0534	3.5810	-0.5216 C
30 H30	0.0183	3.0662	-0.9609 H

Chapter 2

31	C31	-3.9375	-1.0963	0.2333	C
32	H32	-1.8529	-1.3026	-0.2368	H
33	C33	-4.8044	1.1943	0.2385	C
34	C34	-3.3012	3.0995	-0.2232	C
35	H35	-1.8849	4.6478	-0.6301	H
36	C36	-4.1808	-2.4966	0.4015	C
37	C37	-5.0384	-0.1770	0.4010	C
38	H38	-5.6283	1.8925	0.3673	H
39	H39	-4.1370	3.7805	-0.0872	H
40	C40	-5.4324	-2.9601	0.7174	C
41	H41	-3.3518	-3.1868	0.2709	H
42	C42	-6.3287	-0.7014	0.7300	C
43	C43	-6.5201	-2.0507	0.8841	C
44	H44	-5.6038	-4.0254	0.8413	H
45	H45	-7.1563	-0.0080	0.8556	H
46	H46	-7.5043	-2.4363	1.1338	H

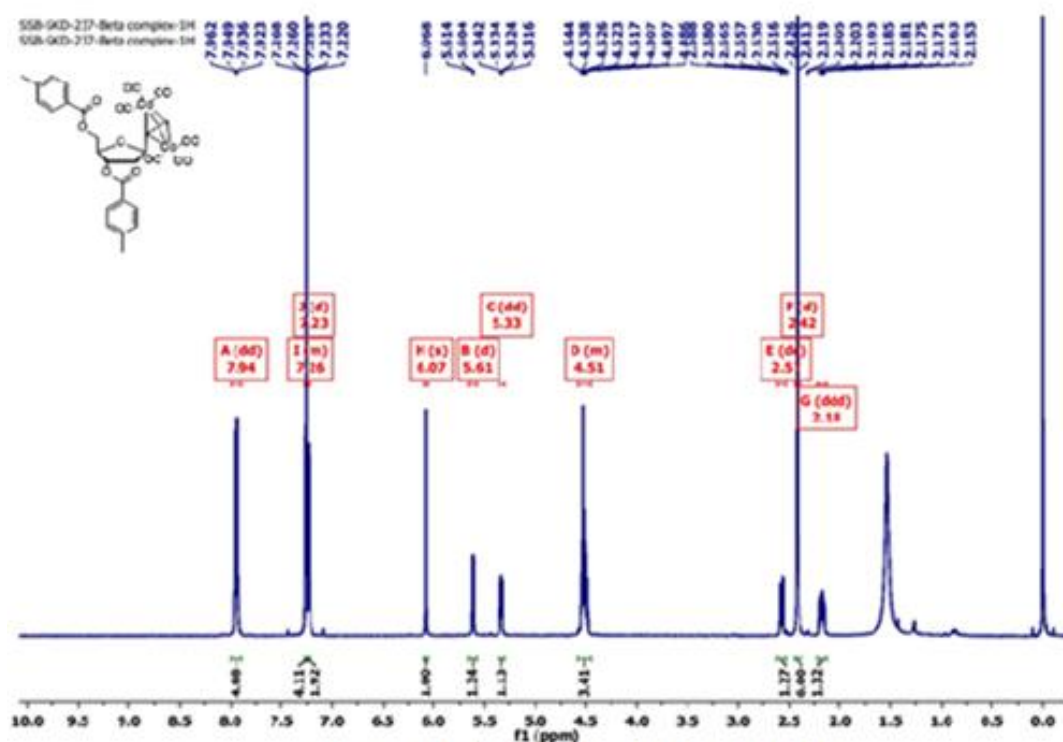
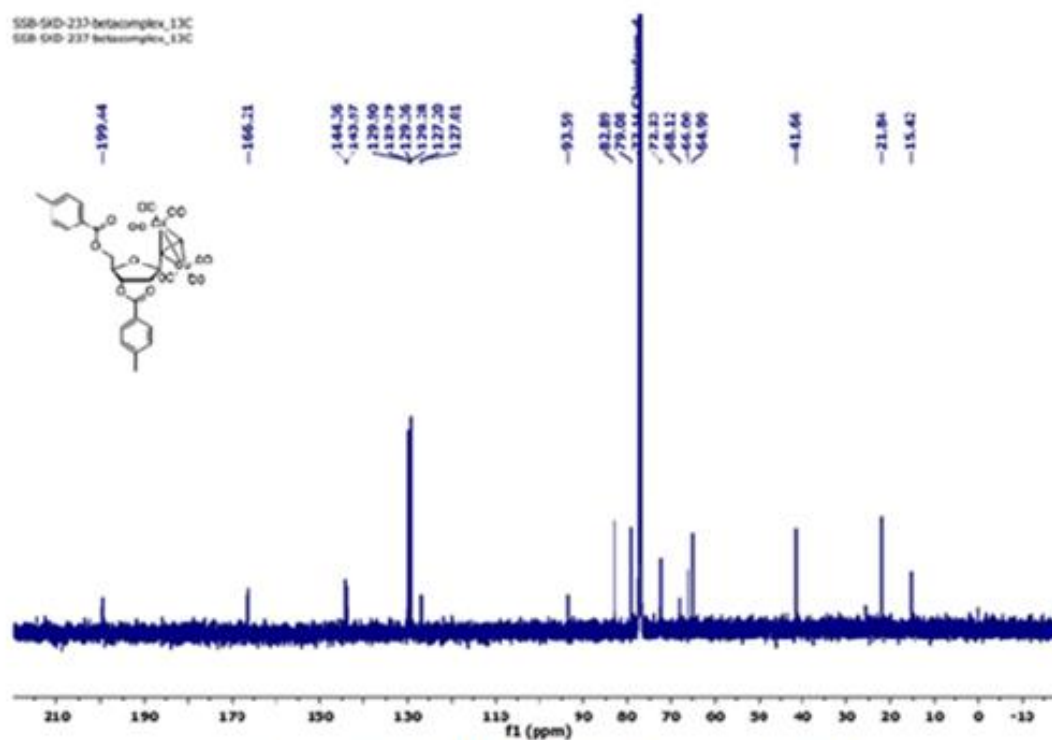


E(RB3LYP) = -1277.87169999 a.u.

1	O1	4.3361	0.2033	0.7478	O
2	N2	0.4507	-0.2011	-0.6885	N
3	N3	1.9142	-1.6481	-1.2995	N
4	N4	0.6636	-1.3094	-1.4492	N
5	O5	7.2030	-1.3876	-0.2461	O
6	H6	7.5922	-1.7090	-1.0698	H
7	O7	5.9000	1.9466	2.4548	O
8	H8	6.1790	2.8517	2.6395	H
9	C9	1.6007	0.1684	-0.0556	C
10	H10	1.6622	1.0101	0.6143	H
11	C11	5.7651	0.2117	0.8123	C
12	H12	6.1051	-0.4737	1.6023	H
13	C13	2.5282	-0.7675	-0.4553	C
14	C14	6.2658	-0.3498	-0.5455	C
15	H15	6.7611	0.4302	-1.1407	H
16	C16	3.9682	-0.9039	-0.0794	C
17	H17	4.1130	-1.8438	0.4779	H
18	C18	6.2636	1.6098	1.1194	C
19	H19	7.3575	1.6141	0.9978	H
20	H20	5.8250	2.3151	0.3976	H
21	C21	4.9781	-0.8410	-1.2395	C
22	H22	5.1148	-1.8034	-1.7395	H
23	H23	4.6362	-0.1111	-1.9805	H

24 C24	-0.8163	0.4590	-0.6566 C
25 C25	-1.9736	-0.2254	-0.2264 C
26 C26	-0.8654	1.7994	-1.0466 C
27 C27	-3.2093	0.4983	-0.2076 C
28 C28	-1.9681	-1.5966	0.2092 C
29 C29	-2.0667	2.4993	-1.0187 C
30 H30	0.0449	2.2837	-1.3856 H
31 C31	-4.4075	-0.1500	0.2246 C
32 C32	-3.2534	1.8702	-0.6078 C
33 C33	-3.1143	-2.2058	0.6241 C
34 H34	-1.0370	-2.1490	0.1862 H
35 H35	-2.0931	3.5398	-1.3293 H
36 C36	-4.3732	-1.5147	0.6437 C
37 C37	-5.6449	0.5655	0.2435 C
38 C38	-4.5123	2.5642	-0.5787 C
39 H39	-3.0892	-3.2427	0.9484 H
40 C40	-5.5635	-2.1330	1.0636 C
41 C41	-6.8101	-0.0940	0.6711 C
42 C42	-5.6545	1.9411	-0.1743 C
43 H43	-4.5338	3.6054	-0.8893 H
44 C44	-6.7674	-1.4286	1.0753 C
45 H45	-5.5365	-3.1718	1.3817 H
46 H46	-7.7510	0.4497	0.6849 H
47 H47	-6.5982	2.4800	-0.1592 H
48 H48	-7.6778	-1.9221	1.4026 H



2.12. ^1H and ^{13}C NMR spectra of selected nucleosidesFigure 2.22: ^1H NMR Spectra of 2.84βFigure 2.23: ^{13}C NMR Spectra of 2.84β

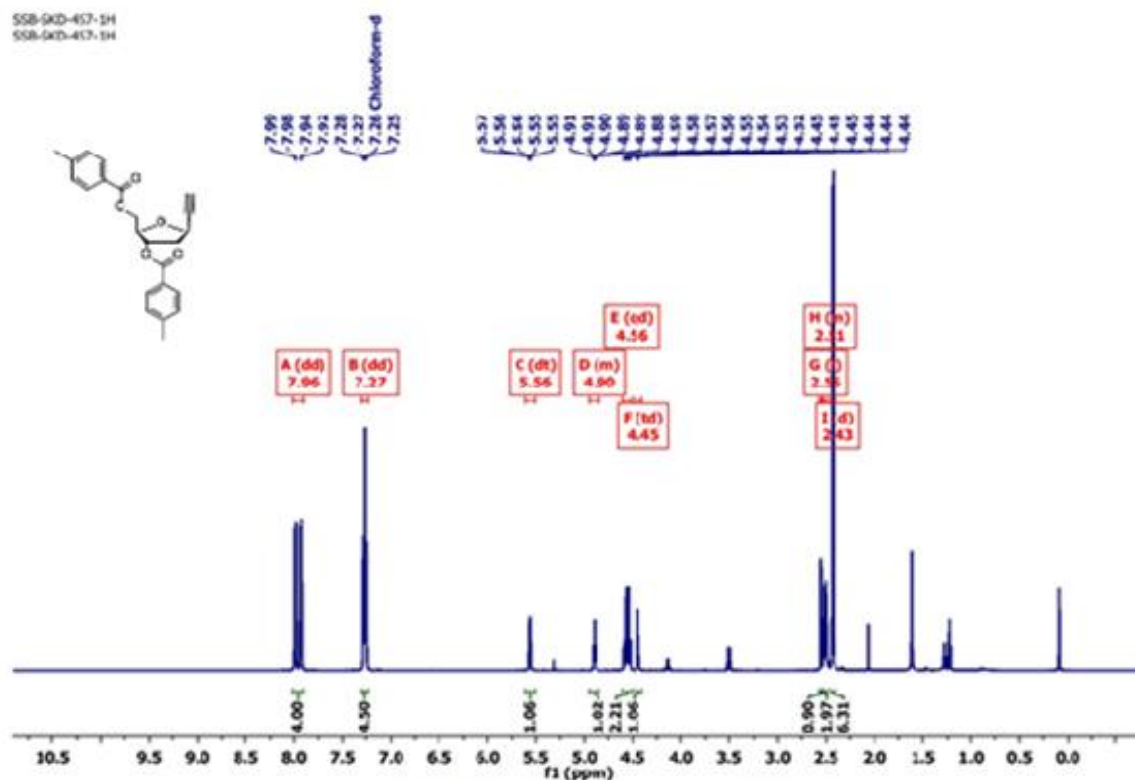


Figure 2.24: ^1H NMR Spectra of 1.086β

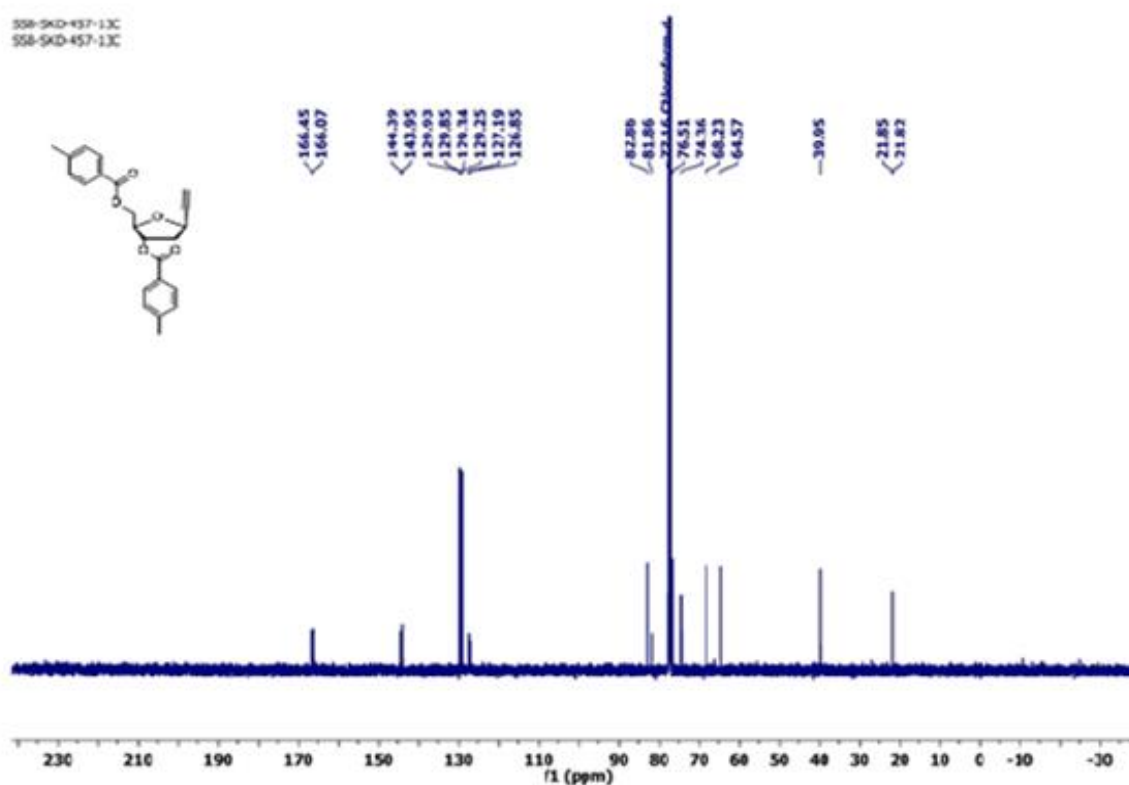
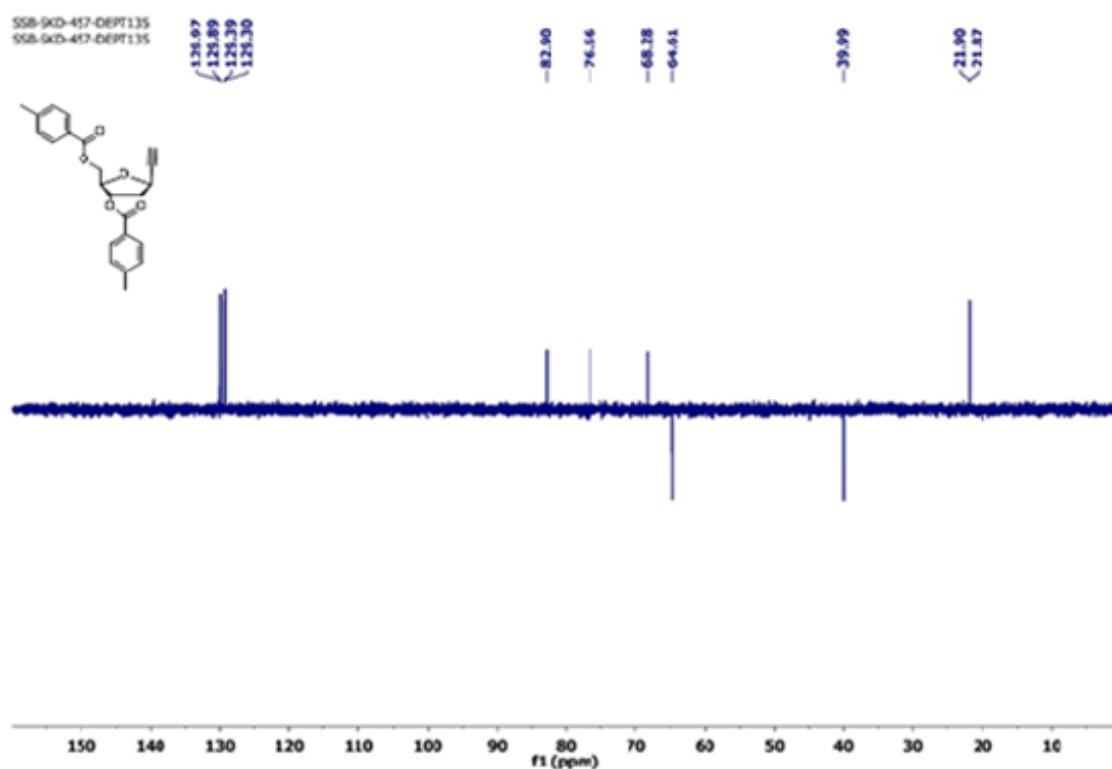
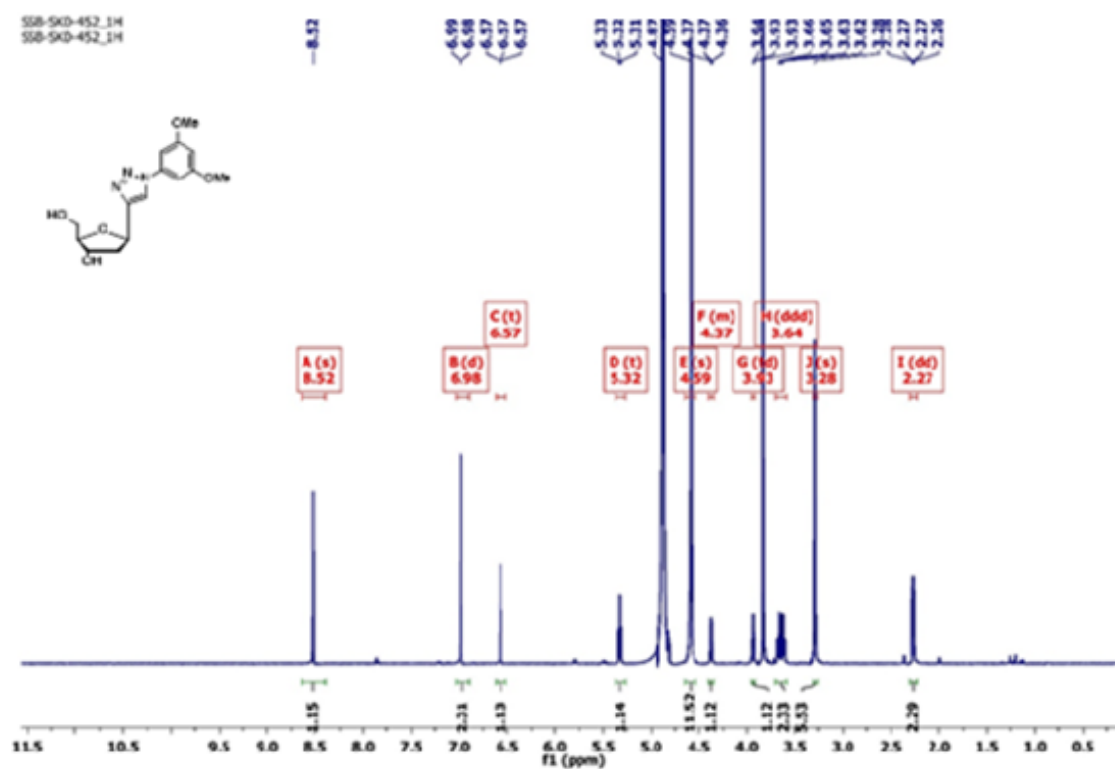


Figure 2.25: ^{13}C NMR Spectra of 1.086β

Figure 2.26: DEPT 135 of 1.086 β Figure 2.27: ^1H NMR Spectra of 2.81B

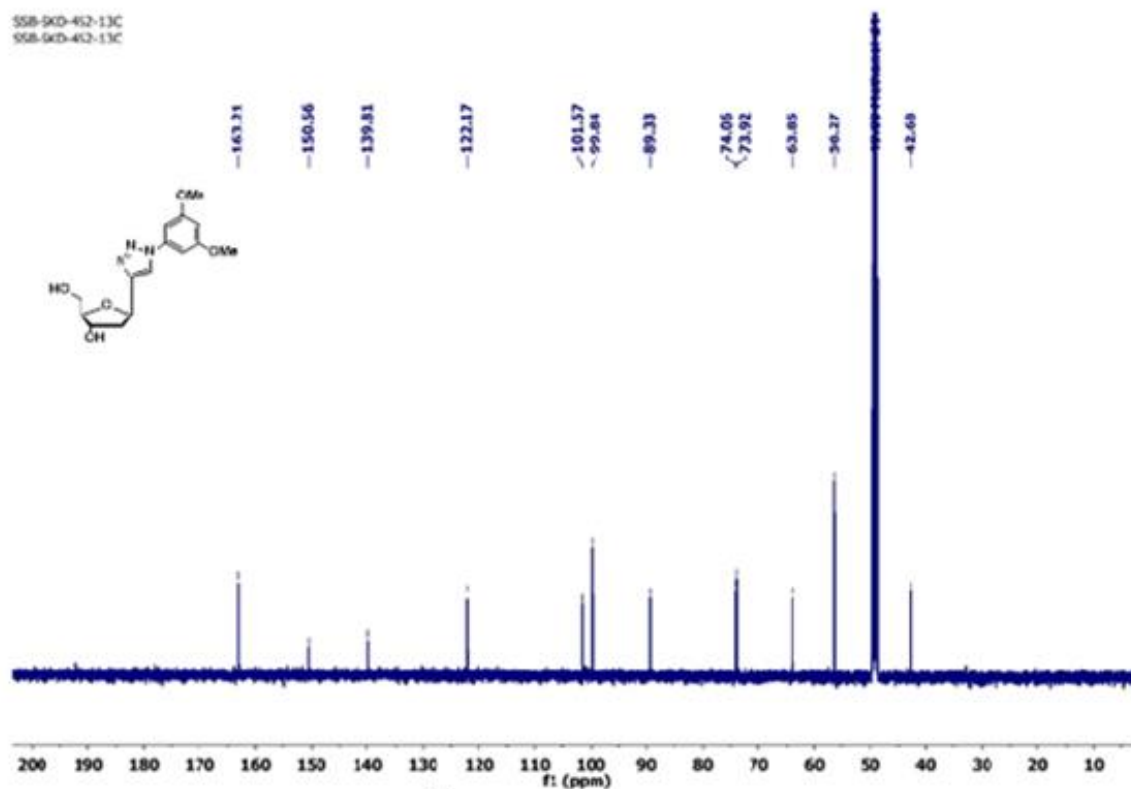


Figure 2.28: ^{13}C NMR Spectra of 2.81B

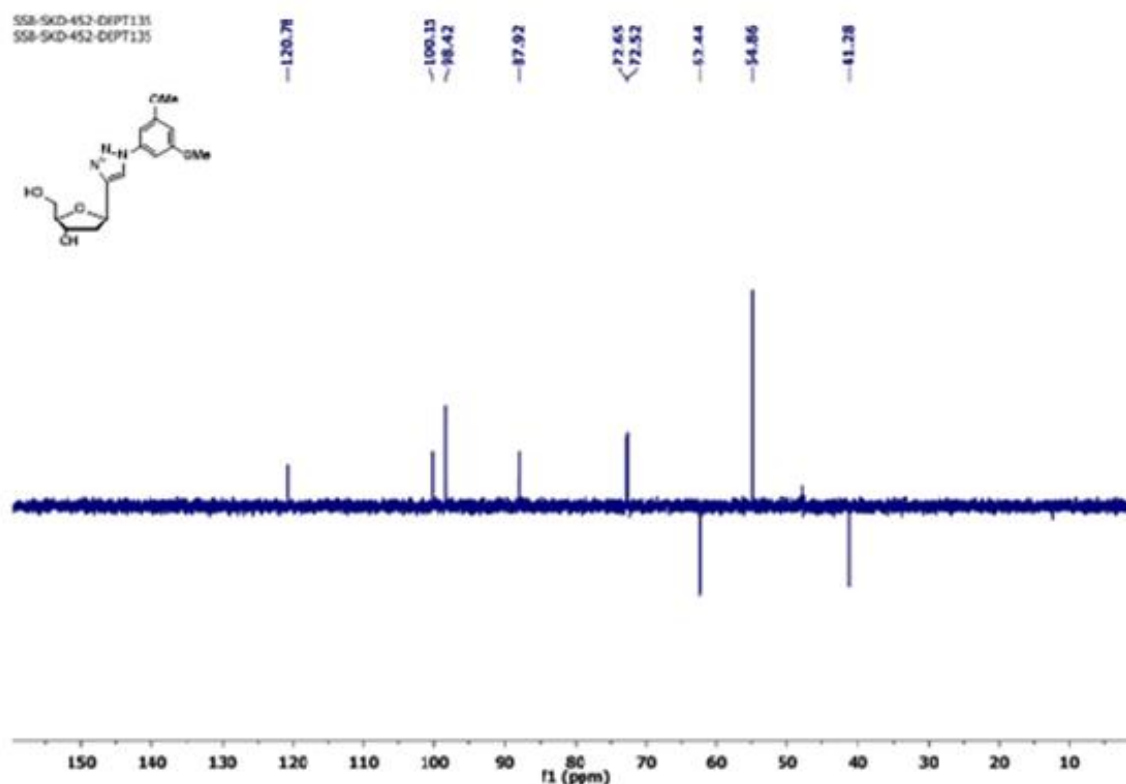
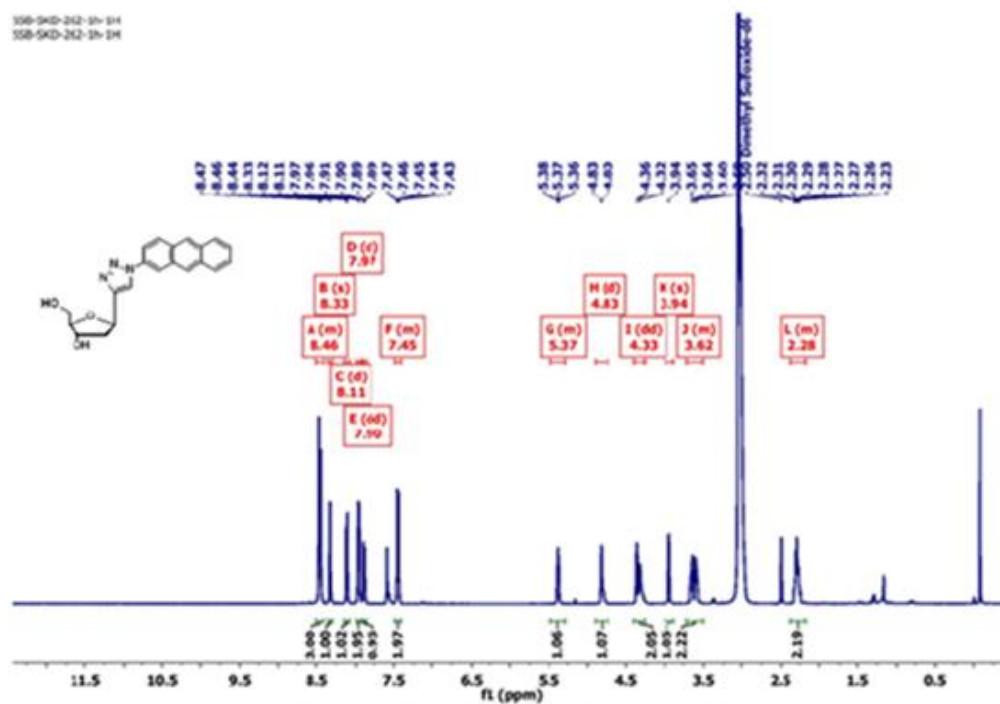
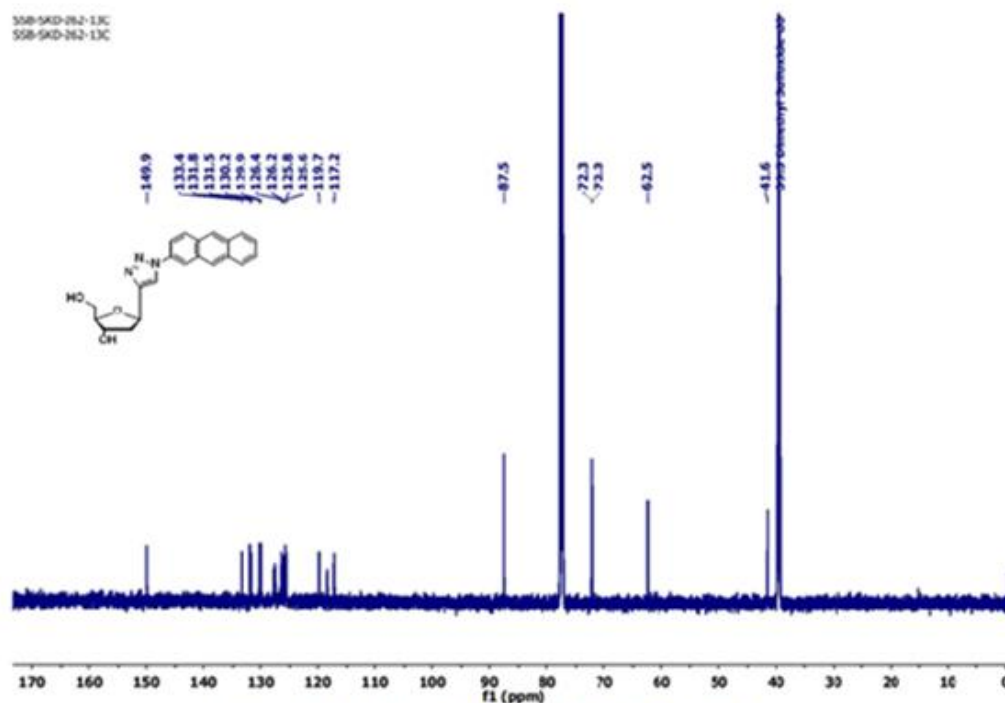


Figure 2.29: DEPT 135 of 2.81B

Figure 2.30: ^1H NMR Spectra of 2.81DFigure 2.31: ^{13}C NMR Spectra of 2.81D

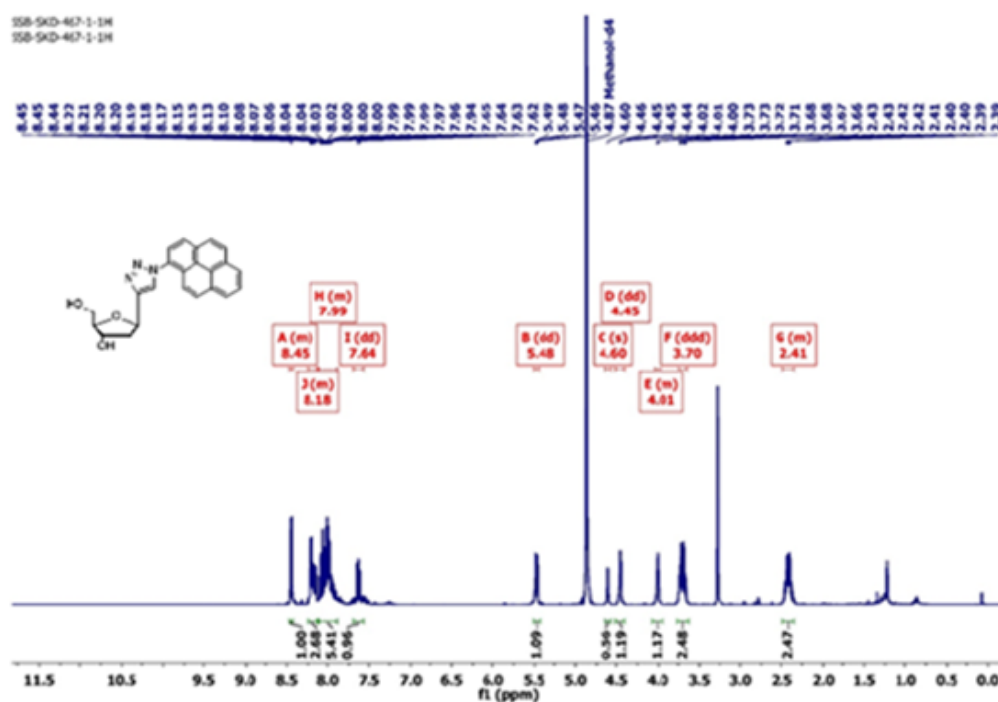


Figure 2.32: ^1H NMR Spectra of 2.81E

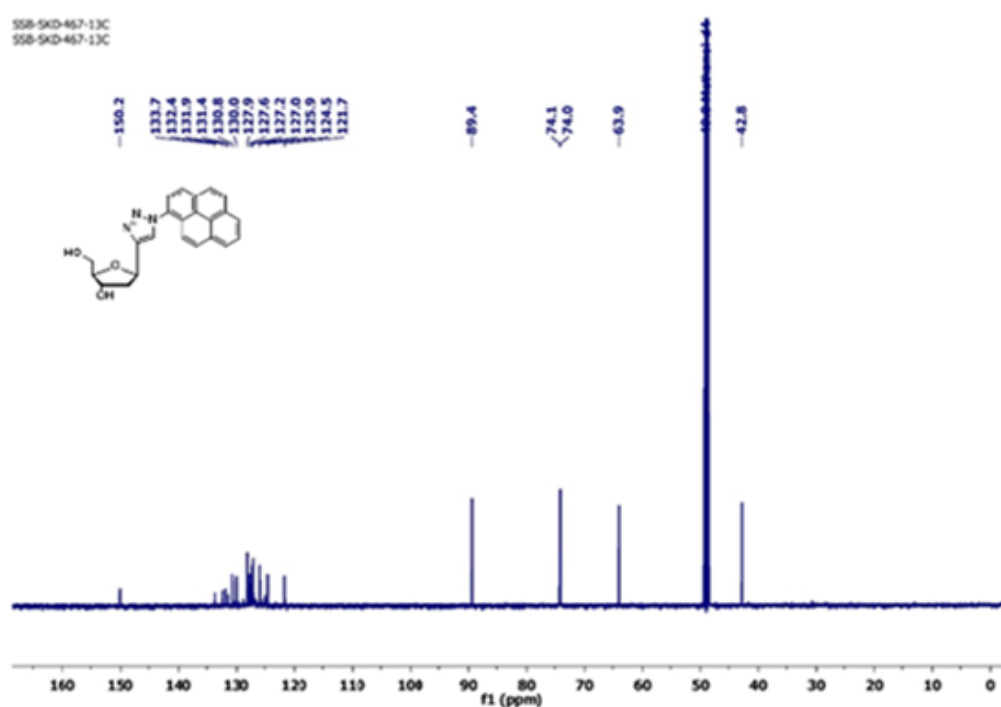


Figure 2.33: ^{13}C NMR Spectra of 2.81E

2.13. Reference:

1. (a) Drohat, A.; Maiti, A. *Org. Biomol. Chem.* **2014**, *12*, 8367. (b) Prior, J. J.; Santi, D. V. *J. Biol. Chem.* **1984**, *259*, 2429. (c) Stockbridge, R. B.; Schroeder, G. K.; Wolfenden, R. *Bioorg. Chem.* **2010**, *38*, 224. (d) Zoltewicz, J. A.; Clark, D. F.; Sharpless, T. W.; Grahe, G. *J. Am. Chem. Soc.* **1969**, *494*, 1741.
2. (a) Matsuura, S.; Shiratori, O.; Katagiri, K. *J. Antibiot.* **1964**, *17*, 234. (b) Nishimura, H.; Shimaoka, N.; Tanaka, Y.; Komatsu, Y.; Kato, H.; Mayama, M. *J. Antibiot.* **1964**, *17*, 148.
3. (a) Cohn, W. E. *J. Biol. Chem.* **1960**, *235*, 1488. (b) Cohn, W. E. *Biochim. Biophys. Acta* **1959**, *32*, 569.
4. (a) Davis, D. R. *Nucleic Acids Res.* **1995**, *23*, 5020. (b) Newby, M. I.; Greenbaum, N. L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 12697. (c) Desaulniers, J. P.; Chang, Y. C.; Aduri, R.; Abeyisirigunawardena, S. C.; Santa Lucia, J.; Chow, C. S. *Org. Biomol. Chem.* **2008**, *6*, 3892.
5. Koonin, E. V. *Nucleic Acids Res.* **1996**, *24*, 2411.
6. Nakagawa, Y.; Kano, H.; Tsukuda, Y.; Koyama, H. *Tetrahedron Lett.* **1967**, *42*, 4105.
7. Harki, D. A.; Graci, J. D.; Korneeva, V. S.; Ghosh, S. K. B.; Hong, Z.; Cameron, C. E.; Peterson, B. R. *Biochemistry* **2002**, *41*, 9026. (b) Seela, F.; Jawalekar, A. *Helv. Chim. Acta* **2002**, *85*, 1857.
8. Wang, L.; Schultz, P. G. *Chem. Commun.* **2002**, *1*.
9. (a) Bain, J. D.; Schwitzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537. (b) Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 4461.
10. Watanabe, K. A. Chemistry of C-nucleosides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1994; 421.
11. McLaughlin, L. W.; Wilson, M.; Ha, S. B. Use of Nucleoside Analogues to probe Biochemical Processes. In *Comprehensive natural product chemistry, Vol. 7 (DNA and Aspects of Molecular Biology)*; Barton, D. H. R., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier: New York, 1999; 252.
12. (a) Postema, M. H. D. *Tetrahedron* **1992**, *48*, 8545. (b) Jamarillo, C.; Knapp, S. *Synthesis* **1994**, *1*. (c) Shaban, M. A. E.; Nasr, A. Z. *AdV. Heterocycl. Chem.* **1997**, *68*, 223. (d) Du, Y. G.; Linhardt, R. J.; Vlahov, I. R. *Tetrahedron* **1998**, *54*, 9913. (f) Lee, D. Y. W.; He, M. S. *Curr. Top. Med. Chem.* **2005**, *5*, 1333. (e) Adamo, M. F. A.; Pergoli, R. *Curr. Org. Chem.* **2008**, *12*, 1544.
13. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319.
14. L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875.
15. Wu, Q.; Simons, C. *Synthesis* **2004**, *10*, 1533.

16. T. G.; O'Sullivan, T. P.; Guiry, P. J. *Eur. J. Org. Chem.* **2005**, 23, 4929.
17. Wellington, K. W.; Benner, S. A. *Nucleosides, Nucleotides, Nucleic Acids* **2006**, 25, 1309.
18. Griesang, N.; Richert, C. *Tetrahedron Lett.* **2002**, 43, 8755.
19. (a) Klein, R. S.; Kotick, M. P.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1971**, 36, 4113. (b) Sharma, R. A.; Bobek, M.; Bloch, A. *J. Med. Chem.* **1975**, 18, 473. (c) Pirrung, M. C.; Zhao, X.; Harris, S. V. *J. Org. Chem.* **2001**, 66, 2067. (d) Singh, I.; Hecker, W.; Prasad, A. K.; Parmar, V. S.; Seitz, O. *Chem. Commun.* **2002**, 500.
20. Chen, D. W.; Beuscher, A. E.; Stevens, R. C.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *J. Org. Chem.* **2001**, 66, 1725.
21. (a) Adamo, M. F. A.; Pergoli, R. *Org. Lett.* **2007**, 9, 4443. (b) Heinrich, D.; Wagner, T.; Diederichsen, U. *Org. Lett.* **2007**, 9, 5311. (c) Chiba, J.; Takeshima, S.; Mishima, K.; Maeda, H.; Nanai, Y.; Mizuno, K.; Inouye, M. *Chem. Eur. J.* **2007**, 13, 8124. (d) Lubin-Garman, N.; Baltaze, J.-P.; Coste, A.; Hallonet, A.; Laure'ano, H.; Legrave, G.; Uziel, J.; Auge', J. *Org. Lett.* **2008**, 10, 725.
22. Shapiro, R.; Chambers, R. W. *J. Am. Chem. Soc.* **1961**, 83, 3920.
23. Butora, G.; Olsen, D. B.; Carroll, S. S.; McMaster, D. R.; Schmitt, C.; Leone, J. F.; Stahlhut, M.; Burlein, C.; MacCoss, M. *Bioorg. Med. Chem.* **2007**, 15, 5219.
24. (a) Liang, C.; Ma, T.; Cooperwood, J. S.; Du, J.; Chu, C. K. *Carbohydr. Res.* **1997**, 303, 33. For an example on the first Wittig-type strategy ever, see: (c) Ohrui, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, 97, 4602. For the first report on a HWE modification, see: (d) De Bernardo, S.; Weigle, M. *J. Org. Chem.* **1977**, 42, 109. Utilization of exocyclic CHO group in Wittig reaction: (e) Krishna, P. R.; Reddy, V. V. R.; Srinivas, R. *J. Org. Chem.* **2007**, 63, 9871.
25. Kalvoda, L.; Farkas', J.; S'orm, F. *Tetrahedron Lett.* **1970**, 26, 2297.
26. Bobek, M. *Collect. Czech. Chem. Commun.* **1969**, 34, 1690.
27. (a) Huisgen, R. *Angew. Chem.* **1963**, 75, 604. (b) Huisgen, R. *Angew. Chem.* **1963**, 11, 633.
28. Huisgen, R.; Szeimies, G.; Mobius, L. *Chem. Ber.* **1967**, 100, 2494.
29. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem.* **2001**, 40, 2004.
30. (a) Tornøe, C. W.; Meldal, M. *Proceedings of the Second International and the Seventeenth American Peptide Symposium* **2001**, 263. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, 67, 3057. (c) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, 41, 2565.
31. Tornøe, C. W.; Meldal, M. *Chem. Rev.* **2008**, 108, 2952.
32. Guezguez, R.; Bougrin K.; Akri, K. E.; Benhida, R. *Tetrahedron Lett.* **2006**, 47, 4807.

33. Ermolat'ev, D. S.; Metha, V. P.; Van der Eycken, E. V. *QSAR Comb. Sci.* **2007**, 26, 1266.
34. (a) Broggi, J.; Joubert, N.; Aucagne, V.; Berteina-Raboin, S.; DiezGonzalez, S.; Nolan, S. P.; Topalis, D.; Deville-Bonne, D.; Balzarini, J.; Neyts, J.; Andrei, G.; Snoeck, R.; Agrofoglio, L. *Nucleosides Nucleotides*, **2007**, 26, 1391. (b) Broggi, J.; Joubert, N.; Diez-Gonzalez, S.; Berteina-Raboin, S.; Zevaco, T.; Nolan, S. P.; Agrofoglio, L. *Tetrahedron*, **2009**, 65, 1162.
35. Li, L.; Zhang, G.; Zhu, A.; Zhang, L. *J. Org. Chem.* **2008**, 73, 3630.
36. Li, L.; Lin, B.; Yang, Z.; Zhang, L.; Zhang, L. *Tetrahedron Lett.* **2008**, 49, 4491.
37. Xia, Y.; Li, W.; Qu, F.; Fan, Z.; Liu, X.; Berro, C.; Rauzy, E.; Peng, L. *Org. Biomol. Chem.* **2007**, 5, 1695.
38. Cosyn, L.; Palaniappan, K. K.; Kim, S.-K.; Duong, H. T.; Gao, Z.-G.; Jacobson, K. A.; Van Calenbergh, S. *J. Med. Chem.* **2006**, 49, 7373.
39. Cosyn, L.; Gao, Z.-G.; Van Rompaey, P.; Lu, C.; Jacobson, K. A.; Van Calenbergh, S. *Bioorg. Med. Chem.* **2006**, 14, 1403.
40. Gupte, A.; Boshoff, H. I.; Wilson, D. J.; Neres, J.; Labello, N.; Somu, R. V.; Xing, C.; Barry, C. E.; Aldrich, C. C. *J. Med. Chem.* **2008**, 51, 7495.
41. (a) McLaughlin, L. W.; Wilson, M.; Ha, S. B. Use of Nucleoside Analogues To Probe Biochemical Processes. In *Comprehensive Natural Product Chemistry, Vol. 7, DNA and Aspects of Molecular Biology*; Barton, D. H. R., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier: New York **1999**, 252. (b) Kool, E. T.; Morales, J. C.; Guckian, K. M. *Angew. Chem., Int. Ed.* **2000**, 39, 990. (c) Kool, E. T. *Acc. Chem. Res.* **2002**, 35, 936. (d) Geyer, C. R.; Battersby, T. R.; Benner, S. A. *Structure* **2003**, 11, 1485. (e) Benner, S. A. *Nature* **2003**, 421, 118. (f) Benner, S. A.; Sismour, A. M. *Nat. Rev. Genet.* **2005**, 6, 533. (g) Kimoto, M.; Cox, R. S., 3rd.; Hirao, I.; *Expert Rev. Mol. Diagn.* **2011**, 3, 321. (h) Bag, S. S.; Talukdar, S.; Matsumoto, K.; Kundu, R. *J. Org. Chem.* **2013**, 78, 278.
42. Wamhoff, H.; Warnecke, H. *ARKIVOC* **2001**, 2, 1085-1090.
43. Takase, M.; Morikawa, T.; Abe, H.; Inouye, M. *Org. Lett.* **2003**, 5, 625.
44. Adamo, M. F. A.; Pergoli, R. *Org. Lett.* **2007**, 9, 4443.
45. Novák, P.; Číhalová, S.; Otmar, M.; Hocek, M.; Kotora, M. *Tetrahedron* **2008**, 64, 5200.
46. Doi, Y.; Chiba, J.; Morikawa, T.; Inouye, M. *J. Am. Chem. Soc.* **2008**, 130, 8762.
47. Novák, P.; Pohl, R.; Kotora, M.; Hocek, M. *Org. Lett.* **2006**, 8, 2051.
48. Bobula, T.; Hocek, M.; Kotora, M. *Tetrahedron* **2010**, 66, 530.

49. Kölmel, D. K.; Barandun, L. J.; Kool, E. T. *Org. Biomol. Chem.* **2016**, *14*, 6407.
50. Teobald, B. J. *Tetrahedron* **2002**, *58*, 4133.
51. Melikyan G. G.; Bright, S.; Monroe, T.; Hardcastle, K. I.; Ciurash, J. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 161-164.
52. Tanaka, S.; Tsukiyama, T.; Isobe, M. *Tetrahedron Lett.* **1993**, *34*, 5757.
53. Isobe, M.; Jiang, Y. *Tetrahedron Lett.* **1995**, *36*, 567.
54. Jiang, Y.; Isobe, M. *Tetrahedron* **1996**, *52*, 2877.
55. Tsuboi, K.; Ichikawa, Y.; Naganawa, A.; Isobe, M.; Ubukata, M.; Isono, K. *Tetrahedron* **1997**, *53*, 5083.
56. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A. Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc. Wallingford CT, **2009**.
57. (a) Ji, L.; Lorbach, A.; Edkins, R. M.; Marder, T. B. *J. Org. Chem.* **2015**, *80*, 5658. (b) Bucevicius, J.; Skardziute, L.; Dodonova, J.; Kazlauskas, K.; Bagdziunas, G.; Jursenas, S.; Tumkevicius, S. *RSC Adv.* **2015**, *5*, 38610. (c) Nagarajan, N.; Velmurugan, G.; Venuvanalingam, P.; R. Renganathan *J. Photochem. Photobiol. A: Chemistry* **2014**, *284*, 36. (d) Denneval, C.; Moldovan, O.; Baudequin, C.; Achelle, S.; Baldeck, P.; Plé, N.; Darabantu, M.; Ramondenc, Y. *Eur. J. Org. Chem.* **2013**, *2013*, 5591. (e) Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH Verlag: GmbH: Weinheim, Germany, **2001**.
58. (a) Fromherz, P. *J. Phys. Chem.* **1995**, *99*, 7188. (b) Albinsson, B. *J. Am. Chem. Soc.* **1997**, *119*, 6369. (c) Shim, T.; Lee, M. H.; Kim, D.; Ouchi, Y. *J. Phys. Chem. B* **2008**, *112*, 1906. (d) Panigrahi, M.; Dash, S.; Patel, S.; Behera, P. K.; Mishra, B. K. *Spectrochim. Acta, Part A* **2007**, *68*, 757. (e) Kim, H. J.; Hong, J.; Hong, A.; Ham, S.; Lee, J. H.; Kim, J. S. *Org. Lett.* **2008**, *10*, 1963. (f) Benniston, A. C.; Harriman, A.; Llarena, I.; Sams, C. A. *Chem. Mater.* **2007**, *19*, 1931. (g) Maeda, H.; Maeda, T.; Mizuno, K.; Fujimoto, K.; Shimizu, H.; Inouye, M. *Chem.—Eur. J.* **2006**, *12*, 824. (h) Perez -Inestrosa, E.; Montenegro, J. -M.; Collado, D.; Suau, R. *Chem. Commun.* **2008**,

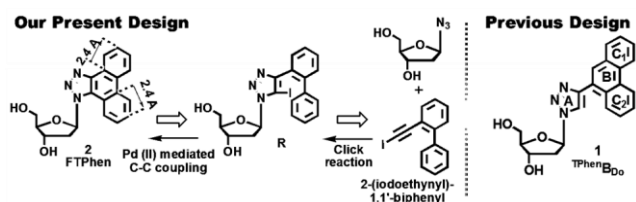
Chapter 2

1085. (i) Yang, S. -W.; Elangovan, A.; Hwang, K. -C.; Ho, T. -I. *J. Phys. Chem. B* **2005**, 109, 16628. (m) Senthilkumar, S.; Nath, S.; Pal, H. *Photochem. Photobiol.* **2004**, 80, 104.



Chapter 3

DESIGN, SYNTHESIS AND PHOTOPHYSICAL PROPERTY OF A DOUBLY WIDENED FUSED TRIAZOLYL PHENANTHRENE UNNATURAL NUCLEOSIDE



3.1. Introduction

DNA is nothing but the sequential arraying of nucleobases A, T, G and C that are provided by nature. DNA is regular double helix that attracts the attention towards searching for its route cause. The only parameter that is responsible for shaping and maintaining its helicity is nonetheless the hydrogen bonding. In contrast although the hydrogen bonding parameter is apparently making its regular helicity possible to happen but certainly not the one and only criteria out of all the respective parameters like hydrogen bonding, π -stacking and Van der Waals force. The extent of contribution of all these parameters to shaping the regular helicity should be one of the primary focus to explore the behavior of DNA. As the gradual progress happened it was progressively unfolded that hydrogen bonding is not the only parameter in forming the regular helicity but π -stacking is the another route cause of its happening.¹⁹ Apart from that it has been found that without the hydrogen bonding the DNA helix shamelessly existing in the form of a regular helix.^{14-17,19} The pioneering experimentation by E. T. Kool over shape complementarity^{14,15} is the crucial point indeed strongly empowering the role of hydrophobicity more clear in the existence of hybridized duplex nucleotide polymer. Further Romesberg's²⁻¹³ and Hirao's^{18,20-22} synthetic strategies in complete removing hydrogen bonding parameter to construct unnatural base pairs based on hydrophobicity and subsequent incorporation of these base pairs are clearly revealing the essence of hydrophobicity in constructing the regular hybridized DNA double strand. After revealing the crux of hydrophobicity it was further drawn by Kool's groups towards size expansion by introducing large heterocycle or hydrocarbon to enhance the steric bulk¹⁶ and it has been found that the size expansion is not indeed perturbing the whole of its helicity, instead because of its large size it is providing more π -stacking which in turn made the DNA helix stable enough in comparison to the natural DNA. Not only that some of the base pairs show high fidelity towards replication and transcription. So we will see how the design and synthesis of hydrophobic base pairs and size expanded hydrophobic base pairs are really potent enough to stabilize the DNA double strand.

3.2. Hydrophobic base analogues

E. T. Kool *et al.* established that “hydrogen bonds in a base pair are not absolutely required for efficient nucleotide insertion”.¹⁹ Generally during replication DNA polymerase hardly does any mistake and if happened that also once per 10^4 - 10^5 nucleotide insertion. So to speak primarily it was thought that this high critical fidelity factor depends on the hydrogen bonding between purine and pyrimidine nucleobases. Replacing the polar hydrogen bonded base pair **dA-dT** by nonpolar hydrophobic isostere **dZ-dF**^{14,15} (**Figure 3.1**) could reveal the extent of the essence of this purine-pyrimidine hydrogen bonding in replication.

To maintain the shape complementarity, the shape analogues or the isostere of **dA 1.001** i.e. **dZ 3.01** was placed against the isostere of **dT 1.002** i.e. **dF 3.02** and they found to be doing a good job in mimicking the natural nucleoside **dA 1.001** and **dT 1.002** whenever incorporated in

DNAs. This result established that hydrogen bonding does not need absolutely in efficient nucleotide insertion and replication (**Figure 3.1**).¹

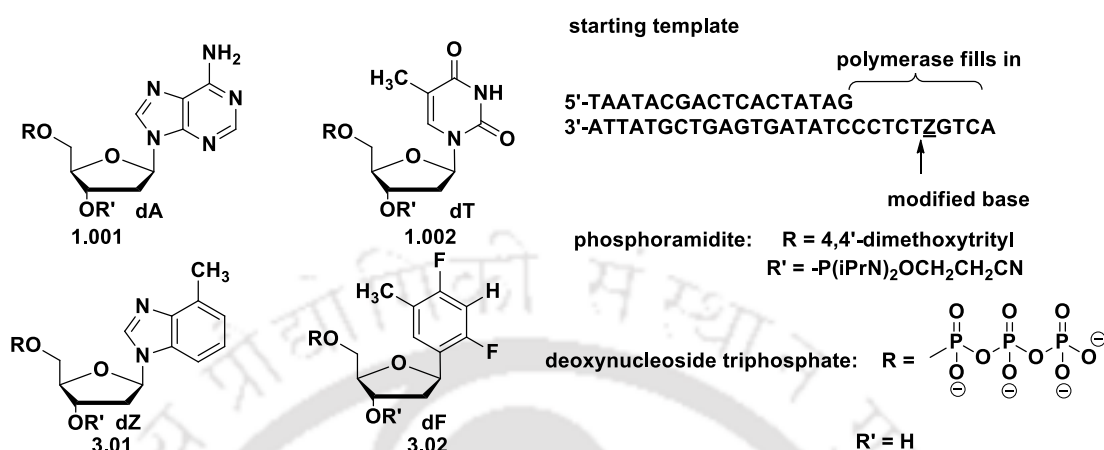


Figure 3.1: E. T. Kool's shape analogue hydrophobic base pair of A-T

A number of non-natural nucleoside was synthesized by Kool *et al.*¹⁶ replaced the natural nucleobases with unnatural fluorophores and thus these can be aided as a biophysical probe. The aromatic moiety used for probing the nucleosides are 1-naphthyl **3.05α** and **3.05β**, 2-naphthyl **3.06α** and **3.06β**, 9-phenanthrenyl **3.04α** and **3.04β**, and 1-pyrenyl **3.03α** and **3.03β**.

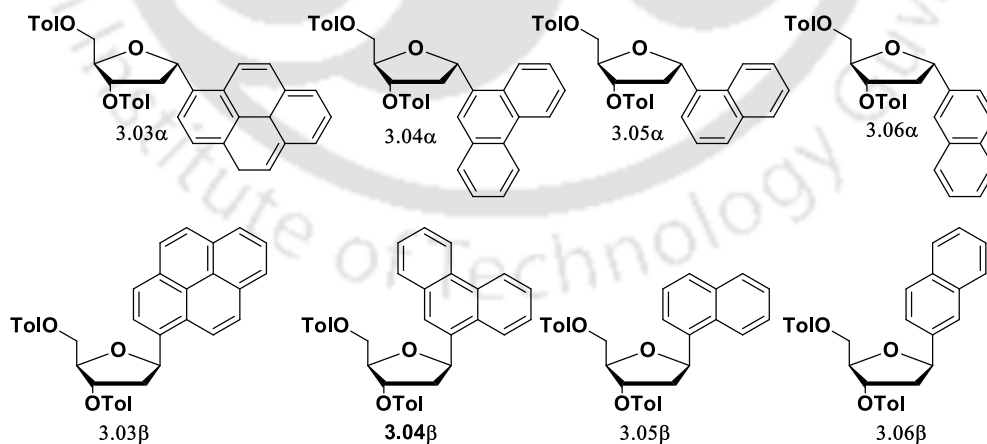


Figure 3.2: Fluorescent unnatural hydrophobic nonpolar nucleoside reported by Kool *et al.*

The nucleosides were synthesized by the coupling of organocadmium derivative of these aromatic moieties with Hoffer's Chloro sugar **1.189a**. The product of this coupling reaction is α -nucleoside which is then effectively converted into its β -derivative by acid catalyzed epimerization. These all fluorescent aromatic moieties do not possess any site to take part in hydrogen bonding. Moreover, they are non-polar hydrophobic moieties and forming stable DNA duplex only by providing nonpolar hydrophobic interaction (**Figure 3.2**).

Strässler *et al.*^{17a} synthesized a number of fluorescent nucleosides where they have used known hydrocarbon or thiophene fluorophore to prepare fluorescent nucleoside. These nucleosides can be used as a fluorescent probe in studying the structure and dynamics of these nucleosides and their interaction with proteins. They may also be used as a fluorescent probe in nucleic acid based biomedical research. The fluorophore attached with deoxyribose at anomeric carbon in α -D form includes terphenyl **3.10**, stilbene **3.09**, terthiophene **3.08**, benzoterthiophene **3.07**. Synthesis of these nucleosides (**Figure 3.3**) were achieved by the addition of aromatic Grignard of these corresponding fluorophore to Hoffer's chloro sugar **1.189a**.

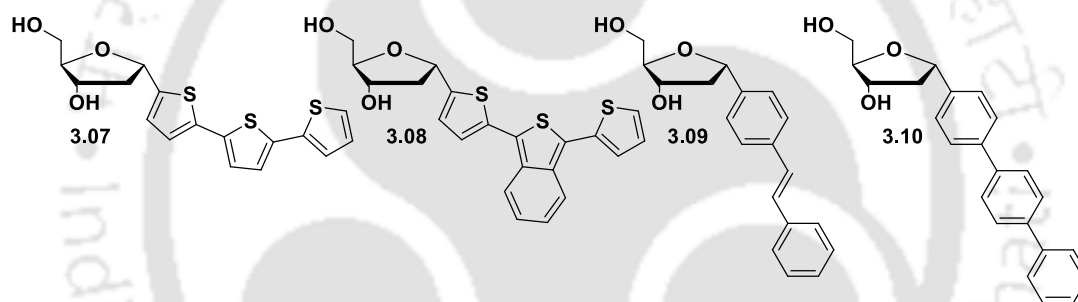


Figure 3.3: Fluorescent unnatural thiophene and hydrocarbon based **C-nucleosides** by Strässler *et al.*

Naptho substituted pyrimidine have also been synthesized by different groups. Godde *et al.*^{17b} synthesized two pyrimidine fused unnatural nucleosides **3.11 β** and **3.12 β** , and then they have incorporated it into DNA (**Figure 3.4**).

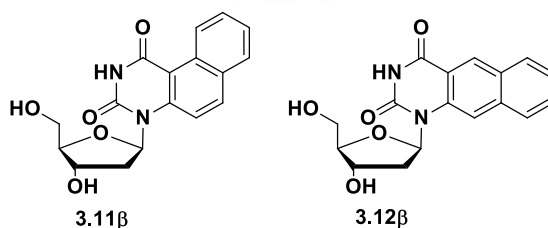


Figure 3.4: Naptho substituted pyrimidine unnatural nucleoside reported by Godde *et al.*

In the year 1999 Romesberg's group introduced propynyl isocarbostyryl (**PICS**) **3.13** (**Figure 3.5**) where the synthesis was started from Hoffre's chloro sugar **1.189a**. Although **PICS 3.13** is not targeted to be designed to have shape complementarity but it has been found that **PICS 3.13** was efficiently incorporated opposite to itself with sound efficiency by the Klenow fragment of *Escherichia coli* DNA polymerase I. High thermal stability helping **PICS 3.13** to be paired of and appearing as a selective hydrophobic base pair. This study suggests that hydrophobicity itself without the need of hydrogen bonding is sufficient enough for the enzymatic incorporation as well as selective self-pairing of **PICS 3.13**.²

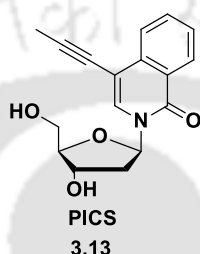


Figure 3.5: Romesberg **PICS** unnatural nucleoside

To examine the potential of hydrophobicity within nonstandard base pairing, Ogawa *et al.*³ synthesized **TM 3.14**, **DM 3.15**, **2MN 3.17**, **DMN 3.16**, **ICS 3.18**, **7AI 3.19** nucleosides. Here they have found that initially self and hetero pairing between **TM 3.14** and **DM 3.15** (**Figure 3.6**) are less stable by natural **dA:dT** pair by 4.0-6.4 °C.

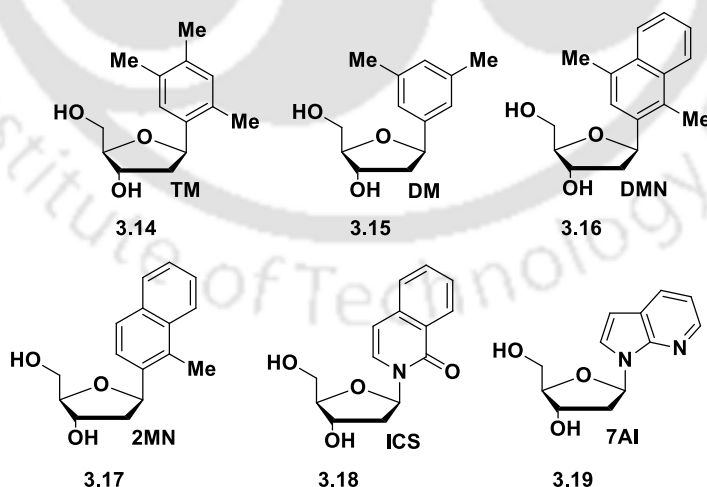


Figure 3.6: Structure of unnatural hydrophobic nucleobases reported by Ogawa *et al.*

Whereas an increasing effort to enhance the aromatic surface area by synthesizing **2MN 3.17** and **DMN 3.16** is suggesting enhanced thermodynamic stability compare to the previous results, further improvement to **ICS 3.18** results a self-pairing which exhibited a comparable thermal stability with natural **dA:dT** pair ($T_m = 59.2\text{ }^\circ\text{C}$ for both ICS self-pair and dA:dT pair).

Wu *et al.*⁴ has synthesized six novel unnatural nucleosides (**Figure 3.7**). These nucleosides form stable base pair whenever incorporated into duplex DNA. The thermal stability of the formed base pair within DNA duplex does not depend on the hydrogen bonding interaction. As the surface area of the nucleobases increased it caused enhanced thermal stability due to increased hydrophobicity of the nucleobases.

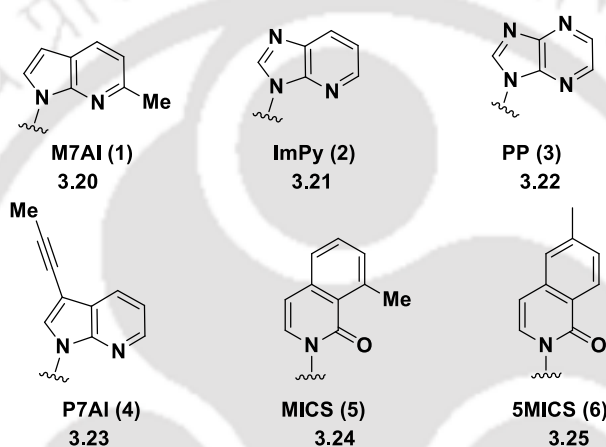


Figure 3.7: Six hydrophobic nucleosides reported by Wu *et al.*

All these nucleobases are derivative of **7AI 3.19** and **ICS 3.18**. These nucleobases are proved to be efficiently incorporated through Klenow fragment of Escherichia coli DNA polymerase I.

Henry *et al.* started investigation with relatively smaller base pairs compare to the previous one.⁵⁻⁸ They started with fluorine substituted aromatic moiety for their study. Six unnatural nucleoside **3.26-3.31** (**Figure 3.8**) they synthesized. Insertion of these nucleoside by Klenow fragment of Escherichia coli DNA polymerase I efficiently synthesized modified ODN. Thermal temperature between self-paired and hetero paired unnatural fluorine substituted nucleobases suggested a nearly identical stability with the natural base pairs. CD spectra for each base pairs were appeared to be almost identical with the natural base pairs and suggested that with the incorporation of hydrophobic base pairs although completely lacks the hydrogen bonding features but indeed showed no distortion and rather showed B form DNA with significant thermal stability.

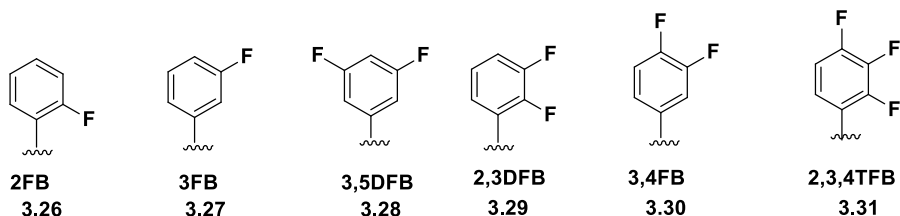


Figure 3.8: Fluorine substituted unnatural nucleobases

For the case of **3FB** it was possible to incorporate it with high efficiency and then also extended by Kf exo^- .⁸ The structural studies of **3FB** self-pair after incorporation suggest that they form planner paired structure which is suitable for further extension by Kf exo^- .⁹

Thus Romesberg's group synthesized an array of more than 60 analogues of unnatural nucleosides. They have applied a combinatorial approach and investigated 3600 (60 x 60) combinations to ascertain candidate that are efficiently replicable.¹⁰ All the possible combinations of base pairs are evaluated by insertion through Kf exo^- and then further extension also accomplished by Kf exo^- . Among all the possible combinations were investigated, **SICS-MMO2** pair was proved to be the best as far as the selectivity and efficient extension by Kf exo^- was concerned. Afterwards they have investigated the structural activity of the synthesized nucleoside in base pairing and then optimized hydrophobicity of these nucleosides to get effective pairing to maximize the efficiency and selectivity in extension.^{10,11}

After all the investigation they finally reached to the conclusion that **5SICS:MMO2** (Figure 3.9d), **5SICS:NaM** (Figure 3.9h) and **5SICS:DMO** (Figure 3.9l) pairs are the 3 combinations who were functioning in PCR.^{12,13} **5SICS-5SICS** mispairing was reduced by the methyl group present in **5SICS**. A slight aromatic expansion and rearrangement of methoxy group over **MMO2 3.33** results **NaM 3.36**.¹¹ **DMO 3.44** was just a modification of **MMO2 3.33** was done to fine tune shape complementarity. Para methyl substituent of **MMO2 3.33** was replaced by methoxy functionality results **DMO 3.44**.¹³ The incorporation efficiency of **dDMOTP** opposite to **5SICS 3.32** was then increased compare to **MMO2 3.33**.¹³ The structure of the **5SICS:MMO2** within a DNA duplex was also determined which revealed that this unnatural base pair were lacking of hydrogen bonding and somewhat stacked over each other.¹² They proposed that this slight stacking of the nucleobase opposite to each other is the unique features of **5SICS:MMO2** which is making them differentiated from other base pairs who were not potentially able to stack over each other in efficient replication. **5SICS:MMO2**, **5SICS:NaM**, and **5SICS:DMO**, these base pairs are functioning well in PCR using Deep Vent DNA polymerase. For **5SICS:MMO2** the fidelity is about 92.9-99.4%. For **5SICS:NaM** it is about 98.0-99.8% and for **5SICS:DMO** it is about 90.7-99.8%. **5SICS:MMO2**, **5SICS:NaM**, these base pairs are also functioning in transcription by the use of T7 RNA polymerase.¹¹

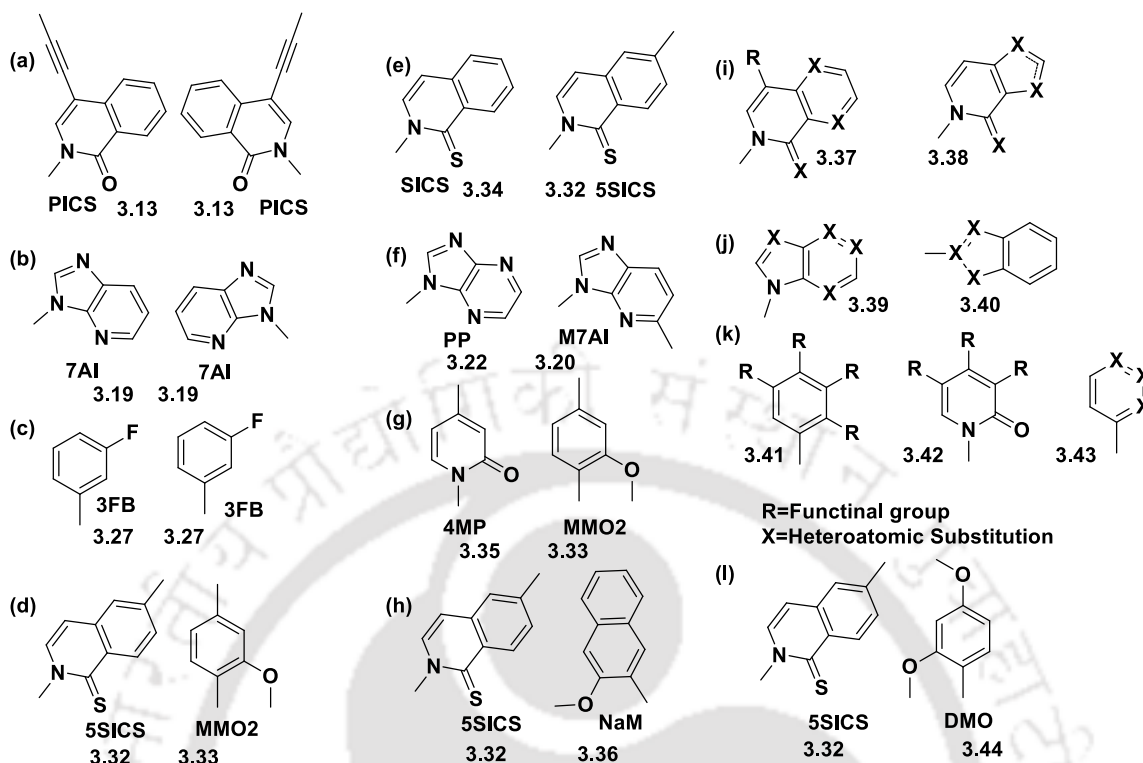


Figure 3.9: Unnatural base pairs synthesized by Romesberg *et al.* 5SICS:MMO2, 5SICS:NaM and 5SICS:DMO are three base pairs function efficiently in PCR.

Mitsui *et al.* developed an unnatural hydrophobic base pyrrole-2-carbaldehyde denoted as **Pa 3.47**. **Pa** was recognized as a specific pairing partner of 9-methylimidazo[(4,5)-b]pyridine, **Q 3.45**. **Q** was known to be effectively paired with **F 3.46** as an isostere of natural **A-T** pair and **F** also be capable to pair with **A** efficiently in replication. So in contrast the **Q-Pa 3.10c** pair was found to be specifically selective in replication. Apart from that **Pa 3.47** was found to have superior interaction with DNA polymerases compare to **F 3.46**. The aldehyde part of the **Pa 3.47** was well recognized by the Klenow fragment of *Escherichia coli* DNA polymerase I and reverse transcriptase (**Figure 3.10**).¹⁸⁻²⁰

Hirao *et al.* reported that **Ds-Pa (Figure 3.11a)** pair functions effectively in transcription and replication.²⁰ The PCR amplification of the oligonucleotide containing **Ds-Pa** pair (**Figure 3.11**) using Vent DNA polymerase (exo⁺) has given unnatural base pair selectivity of 99% per replication and after 20 cycles of PCR approximately 96-97% of the **Ds-Pa** pair was remained in the amplified DNA fragments. This revealed the fidelity of the **Ds-Pa** pair in replication and transcription.²⁰

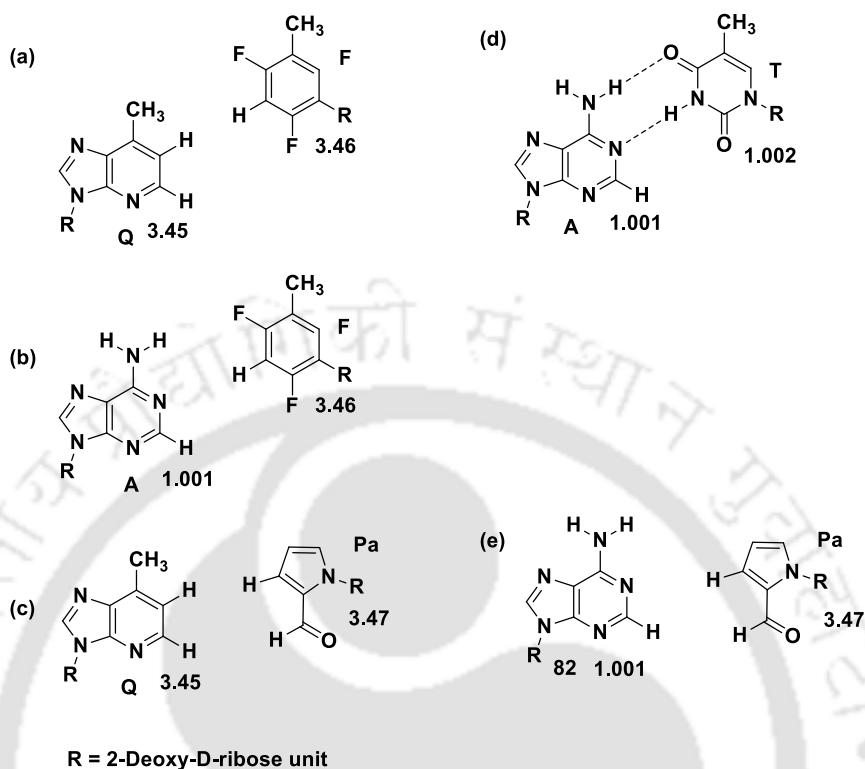


Figure 3.10: Q-F, A-T, A-F, Q-Pa, A-Pa base pairs

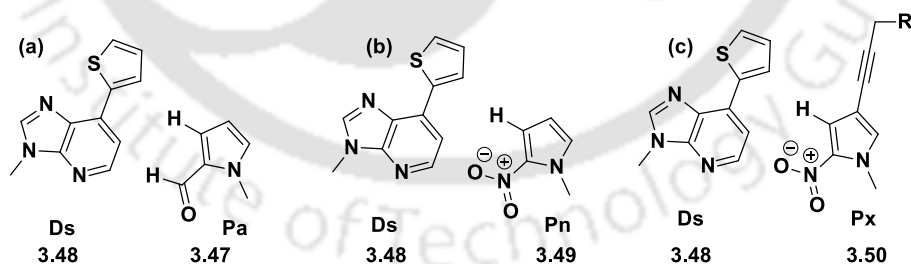


Figure 3.11: Hirao's **Ds-Pa**, **Ds-Pn** and **Ds-Px** unnatural base pair that function in PCR

Next the aldehyde group of **Pa** 3.47 was replaced by nitro group and they made **Pn** 3.49. **Ds** 3.48 was possible to efficiently and selectively incorporated opposite to **Pn** 3.49. 500-fold amplification with greater than 99% selectivity was achieved by 20 cycles of PCR for

oligonucleotide fragments containing **Ds-Pn** pair (**Figure 3.11b**).²¹ The hydrophobicity of **Px 3.50** was increased by attaching a propynyl group to **Pn**. The increased hydrophobicity enhanced the interaction of **Px** with polymerases as well as it also increased the stacking efficiency with neighboring bases and thus **Px** is proved to be superior than the **Pn** whenever incorporated opposite to **Ds**. It has been found that **Ds-Px** pair (**Figure 3.11c**) showed high fidelity in PCR amplification. It was confirmed by Hirao groups that the survival of **Ds-Px** pair was up to 100 cycles of PCR and in the 10²⁸-fold amplification 97% of the **Ds-Px** (**Figure 3.11c**) pair was retained after 100 cycles of PCR.²²

3.3. Background

Synthesis of non-H-bonding unnatural nucleobase surrogates is growing at a fast pace towards expanding the genetic alphabets since the inception of the concept of hydrophobic forces to stabilize a DNA duplex by Kool *et al.* in 1994.²³ Later on the concept was further elaborated by Romesberg group with their designed hydrophobic bases.²⁴ Since then, much effort has been put forth to develop non-natural, stable, hydrophobic base pairs of orthogonal recognition properties with a hope to translate an expanded genetic alphabet into an expanded genetic code creating a synthetic organism one day with ability to encode proteins with new physicochemical properties.³ Many of these studies have been aimed for expansion of nature's genetic alphabet. However, going beyond the purine–pyrimidine H-bonded framework of the natural system, the research effort expanded with new approach including metal-bridged base pairs,²⁵ nonpolar base pairs,^{23-24,26} and pairs containing more than the three different types of hydrogen bonds found in nature.²⁷ Furthermore, the lack of naturally occurring fluorescent bases has spurred the design of unnatural DNA bases/pairs with tuned photophysical properties and is also recently a rapidly growing research field towards the development of nucleic acid based diagnostics and sensing materials.²⁸ Toward this end, several unnatural nucleobases have been designed for the development of functional nucleic acids.²⁹ However, the rational design of non-hydrogen bonding base pairs remains a challenge. While in the design of unnatural nucleobases, a great deal of efforts have been paid in considering H-bonding mimicry or force like π - π stacking/hydrophobic interaction, the force like charge transfer complexation has not been considered in the context of DNA base design. Interactions among the unnatural donor-acceptor nucleobases via charge transfer complexation, however, might have potential impact on DNA duplex stabilization. We, recently, explored the Charge transfer (CT) interaction force as a new and efficient force to stabilize a DNA duplex by utilizing our newly designed triazolyl donor/acceptor nucleosides pair.³⁰ In our previous design we mainly focused on tuning the photophysical property of the triazole linked donor/acceptor aromatics via click reaction and establishing the CT force to stabilize the duplex without much considering the size and shape mimicry. Our results suggested that the triazolyl donor-acceptor unnatural bases have good selectivity to form hetero-pair and/or self-pair via charge transfer and/or π -stacking interactions and we are the first, to the best of our knowledge to explore the possibility of unnatural hetero-duplex stabilization via charge transfer interaction along with π -stacking interaction.³⁰

3.4. Objective

Now, we thought that if we could fuse the polyaromatic hydrocarbons with triazole we could end up with bases having more compact characteristic, close shape mimicry of natural purines with wider size as well as tuned photophysical property. In our earlier design the triazole was linked with the sugar through N-glycoside linkage and the donor acceptor aromatics were linked to the triazole creating a large size of the nucleobase. Moreover, the aromatic phenanthrene remained slightly out of plane of the triazole ring leading to π - π stacking interaction in part exposing a portion of phenanthrene ring to groove binding. However the stability of the DNA duplex was comparable to that of natural A-T pair and that of an abasic site comparable to other reported bases. Koll *et al.* reported that the nonpolar DNA base isosteres stack considerably more strongly than their natural counterparts indicating that the surface area alone is not the determining factor of stacking ability.³¹ It is also known that the stacking interactions become stronger with the increasing size of the base. However, a comparison between the molecules of same size revealed that the less polar molecules stack more strongly than the more polar ones. Moreover, it was observed that the stacking interactions increased with the increase in surface area of nonpolar bases of different sizes. Therefore, the base replacements by rigid polyaromatic hydrocarbons not only introduced the fluorescence property into DNA but also increased the thermal stability of modified duplexes. This approach was first introduced by Leonard *et al.* in their benzo-fused analogue of adenine ribonucleotide which is useful as a tool in studies of ATP-dependent enzymes.³² Later on Kool *et al.* elaborated the concept of size-expanded DNA bases via the design of many expanded (xDNA) and widened (y) DNA bases. While xDNA involved a linear extension of natural bases,^{33a} the design, of “yDNA” involves a vertical extension vector.³³ Both the theoretical and experimental studies showed that DNA single strand with x and yDNA base when paired against natural DNA strand the duplex was found to be more stable than natural B-DNA³⁴ due to enhanced stacking interaction. Moreover, these modified DNA exhibit increased charge-transfer³⁵ and fluorescent^{34a,36} properties. Furthermore, these bases were found to be replicated in *E. coli*, uncovering the opportunity for the design of new and novel class of widened hydrophobic bases.³⁷

3.5. Results and discussion

3.5.1. Design and synthesis

With this idea and inspiration from our previous result we report herein the design of a new class of triazolyl nucleobase. Our designed base is a triazole-fused phenanthrene **3.52** [**FTPhen**], a compact form of our earlier triazolylphenanthrene nucleoside 3.51 [^{TPhen}B_{Do}] (**Figure 3.12a**). This compact hydrophobic nucleobase surrogate **3.52** mimick the shape of purine bases with bi-conically enlarged size similar to the concept of Kool's widened base dyA (**Figure 3.12b**).^{33b}

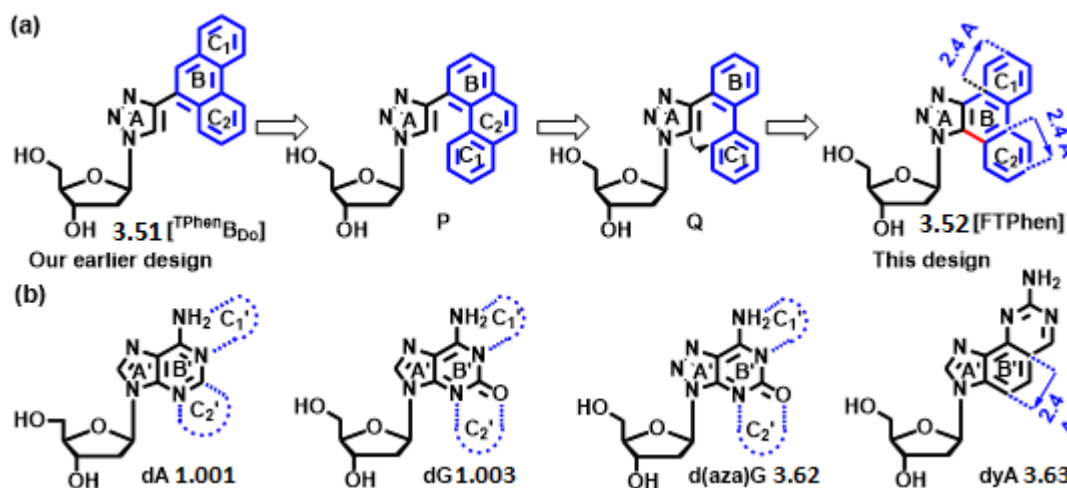
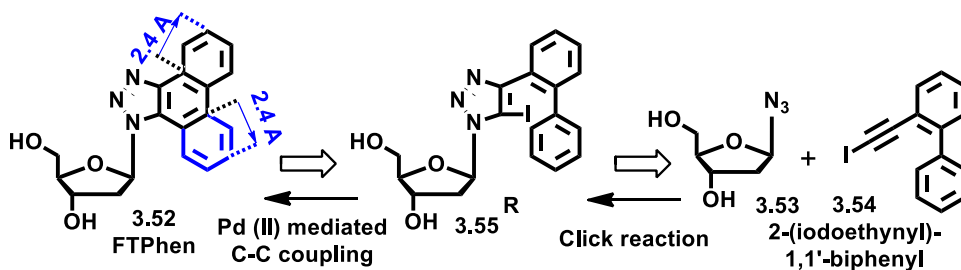


Figure 3.12. The chronological approaches for the design of our present base, **FTPhen** from our earlier reported base, **TPhenBDo**. (b) The chemical structures of dA, dG, d(aza)G and Kool's dyA bases.

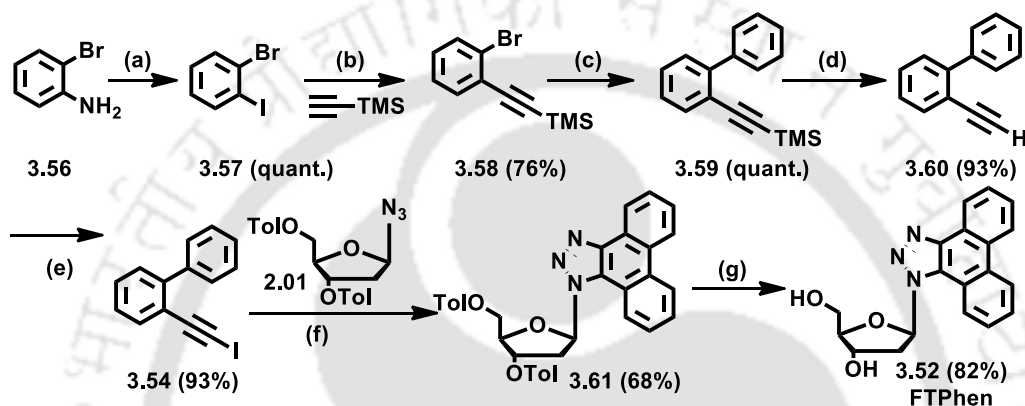
The design concept is depicted pictorially in **Figure 3.12**. The strategy to design this widened triazolyl fused phenanthrene nucleobase involves the concept of the blocking the heteroatomic functionalities (the H-bond donor-acceptor) of dA or dG by two hydrophobic benzene rings while the C-8 was replaced by "N" atom similar to d(aza)G (**Figure 3.12b**).³⁸ Therefore the base can also be treated as doubly widened hydrophobic base analogue of 8-azaguanosine.³⁸ In this initial study we synthesized fused triazolyl phenanthrene **3.52** [**FTPhen**] based on the above concept which is conceptually novel and new DNA base. The molecular design leads the vertical dimensions of the base 2.4 Å wider than the natural purines in both ways.^{33b}

It is now clear that the length of extension of the base is similar to the dyA i.e. by 2.4 Å with two-way extension vector of 60°. From a molecular model study it is expected that the doubly widened base would yield different groove depths in a putative DNA helix, a shallower major groove and deeper minor groove than the reported widened bases the xDNA helix does.^{33e} As the design does not involve linear expansion rather both way vertical expansion would expected not to perturb the pairing with oppositely placed natural bases but would enhance the stacking interaction with both the top and bottom bases in the same DNA strand. Therefore, geometrically **FTPhen** is expected to be compatible with the natural genetic system if incorporated into a DNA. Furthermore, such type of bases could be useful to study the structure and stability of DNA, to understand how genetic systems function and in various biotechnological and material science applications especially in self-assembling functional DNA based materials with interesting photophysics.

(a) Retrosynthesis



(b) Synthesis



Reagents and Condition: (a) i) NaNO_2 , HCl , 0°C , 30 min, ii) KI , 0°C to rt; (b) $\text{PdCl}_2(\text{PPh}_3)_2$, CuI , Et_3N , rt; (c) PhB(OH)_2 , $\text{Pd(PPh}_3)_4$, Toluene: H_2O (5:1), 120°C , 18h; (d) $\text{DCM}:\text{MeOH}$ (1:1), K_2CO_3 , rt, 2h; (e) N -iodomorpholine hydrogeniodide, CuI , Dry THF (f) i) CuI , TBTA , THF, ii) Pd(OAc)_2 , TBAB , K_2CO_3 , PPH_3 , 80°C , (g) NaOMe , MeOH

Scheme 3.1: (a) Retrosynthetic analysis and (b) the synthetic route to yield **FTPPhen** (3.52).

Before chalking out the synthetic plane we proceeded for a retrosynthetic analysis which suggested a click chemistry between β -azidosugar **3.53** and 2-(iodoethynyl)-1,1'-biphenyl **3.54** followed by Pd-catalysed C-C bond formation as the two key reactions to achieve our target **FTPPhen** **3.52**. The key intermediate 2-(iodoethynyl)-1,1'-biphenyl **3.54** was synthesized from commercially available 2-bromoaniline **3.56** following the route shown in **Scheme 3.1**. On the other hand, the other key intermediate bis-toluoyl-1-azido-2-deoxy- β -D-ribofuranose **2.01** was synthesized following our earlier reported protocol.³⁰ Finally, Cu(I)-catalysed Huisgen azide-alkyne cycloaddition between β -azidosugar **2.01** and 2-ethynyl-1,1'-biphenyl **3.54** yielded the intermediate 5-iodo-4-biphenyl triazolyl nucleoside **R** **3.55** which upon treatment with Pd(OAc)_2 generated the desired fused triazolylphenanthrene nucleoside **115** in diprotected form in 68 % yield in the same reaction pot (**Scheme 3.1**). NaOMe/MeOH mediated deprotection of the toluoyl groups ultimately yielded the desired nucleoside **3.52** [**FTPPhen**] in 82 % yield. All the isolated

intermediates and the final product were purified by silica-gel column chromatography and characterized by mass and NMR spectroscopy.

3.5.2. Crystallography

The β -anomeric configuration was confirmed from X-ray single crystal structure analysis (**Figure 3.13**).^{30,39} The X-ray single crystal analysis revealed that the unit cell of the fused triazolylphenanthrene nucleoside, **FTPhen**, contained four molecules with one H₂O in the unit cell with space group P1, cell length, $a = 7.19$, $b = 10.86$, $c = 20.87$ Å; cell angles $a = 82.43$, $b = 88.57$, $\gamma = 84.36^\circ$ and cell volume 1608.7 (**Figure 3.13a-b**). A pair of **FTPhen** unit is held together via aromatic stacking interaction forming a layer while second pair formed another layer opposite to the other plane (**Figure 3.13c-d**). The two aromatic units in a layer are separated by a distance of 3.81 Å. The axis passed through two sugar unit, one each from two layers and the water molecule. The sugar, triazole-N and the H₂O molecule are held together by H-bonding interaction making a tree like architecture expanded along crystal axis b/a . It was revealed that the planar **FTPhen** base would take part in intercalation/or in full stacking interaction within the nucleobases inside a DNA duplex after incorporation into DNA. Therefore, crystal packing suggested that the **FTPhen** can indeed engage in H-bonding via the utilization of triazole unit as well π -stacking interaction if incorporated in DNA.

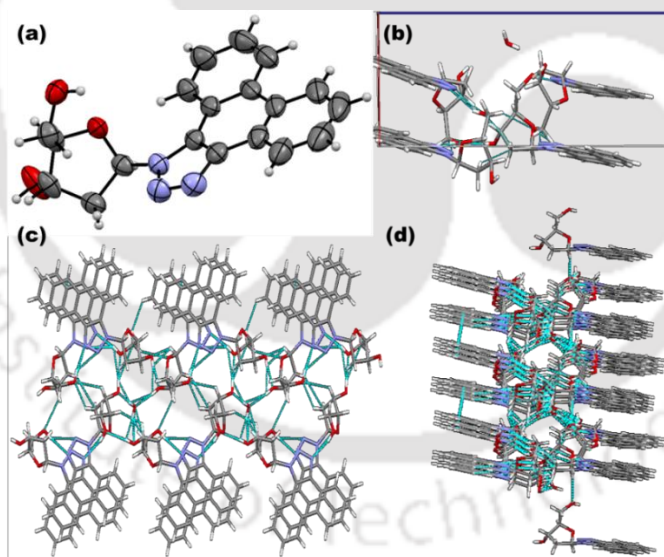


Figure 3.13 (a) ORTEP molecular diagram with thermal ellipsoid at 50% probability, (b) crystal packing and molecular arrangement along a axis (c) and along b axis of nucleoside **FTPhen** [CCDC 1415701].

Next, we studied the sugar and N-glycosidic conformation. Sugar puckers in nucleoside **FTPhen** is South (S) (2'-endo) and the fused triazolyl phenanthrene base displayed *anti*-

conformation with respect to the glycosyl torsion angle which was evidenced from a strong $^3J_{H1'-H2'}$ (7.8 Hz) coupling.³⁹ A theoretical calculation based on B3LYP/6-31G* functional using G09 program package⁴⁰ also supported the sugar conformations as S-locked nucleoside. From both the crystal and the DFT optimized geometry it was revealed that the glycosidic angle adopted *anti*-glycosyl conformation of the triazolylphenanthrene which is probably due to the less repulsion between the lone pair of triazole-N and O4'. This repulsive force restricts the rotation of the glycosyl bond and thus, fix the S-sugar conformation of nucleosides leading to the *anti*-disposition of fused triazolyl phenanthrene base unit. Therefore, the nucleoside **FTPhen** behaved almost the same way as the natural purine nucleosides. It is therefore anticipated that the introduction into short oligonucleotide sequence the **FTPhen** nucleoside with locked-S(*anti*) geometry could fix the conformational state and would help understanding the impact of conformational restrictions in DNA.³⁹

3.5.3. Space-filling model

Space-filling model (**Figure 3.14a**) illustrates the close relationship between natural guanosine base, Kool's widened bases and **FTPhen** which is enlarged biconically by addition of two benzo rings group. Electrostatic potentials mapped as colors on the surfaces shows its hydrophobic character with negative charge density onto the triazole ring indicating significantly less polar nucleoside. The triazolylphenanthrene unit in **FTPhen** is fully planar in contrary to our earlier reported base **T^{Phen}B_{Do}**. As surface area and hydrophobicity are the reflection in the stacking propensities of DNA bases and analogues, we calculated these parameters for **FTPhen** nucleoside.⁴¹ Our calculation based on Molinspiration property engine v2016.10 calculator showed topological polar surface area (TPSA)= 80.41 Å², volume = 291.34 Å³. Calculated log *P* (octanol-water) value was found to be 2.43 indicating the strongly hydrophobic character of **FTPhen** compared to natural nucleosides. Only base part without sugar unit exhibited TPSA= 30.721 Å², volume = 209.63 Å³ and log *P* (octanol-water) value of 3.63 (**Figure 3.14b**).⁴²

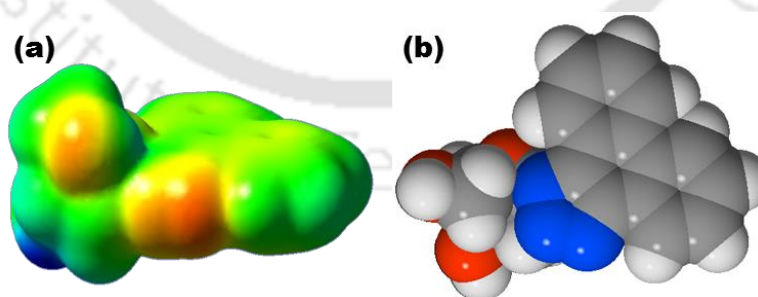


Figure 3.14: (a) The space-filling models of the **FTPhen** nucleoside with DFT-calculated electrostatic potentials (G09, B3LYP/6-31G*) mapped onto the van der Waals surfaces. (b) Molinspiration property engine v2016.10 calculated CPK model.

3.5.4. Photophysical properties

On the basis of the foregoing observations on nucleoside conformation and shape, we expected that the nucleoside **FTPhen** may exhibit favourable photophysical properties, useful for analytical and structural studies in DNA. Therefore, we first studied the photophysical property of the nucleoside in various organic solvents prior to show theoretically that the nucleoside if incorporated into DNA could able to stabilize a duplex with perturbing much the B-form DNA. Thus, photophysical study showed a chrysene type structured absorption maxima at 244 nm, 251, 290 and weak band at 321, 337 nm (**Figure 3.15a**). All the bands experienced a hypsochromic shift of 3 nm and hyperchromic effect as the solvent polarities increases which was also reflected from a dioxane water titration spectra (**Figure 3.16a**). The UV-vis absorption spectra thus revealed pronounced conjugation effects of phenanthrene on the absorption property upon fusion with triazole. The chrysene type weak bands between 321 and 337 were shown by the **FTPhen** nucleoside which maintained the relative heights of the 0–0 and the 0–1 vibronic bands in all solvents.⁴³ Upon excitation it showed structured emission bands at 340, 355, and 373 nm with middle band of higher intensity. In addition, it exhibits a low intensity band at about 393 nm (**Figure 3.15b**). The emission band experienced slight blue shift in wavelength with enhancement of fluorescence intensity as the solvent polarity increases in contrary to the natural DNA bases which was also supported from the spectra of dioxane-water titration (**Figure 3.16b**). This may be due to increased intermolecular H-bonding between triazolyl nucleoside **FTPhen** and water or polar protic solvent.^{36d}

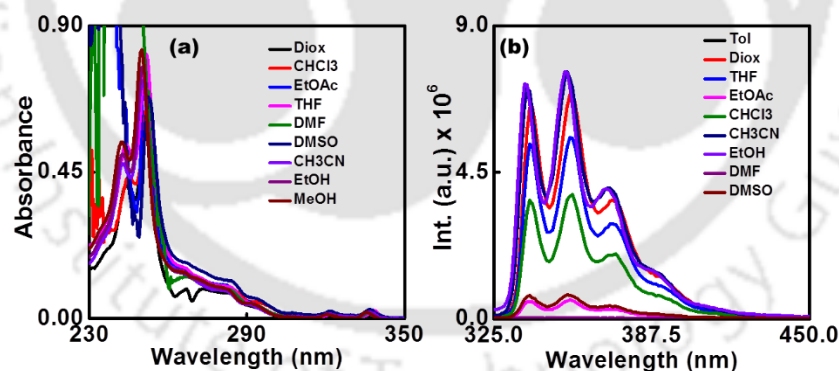


Figure 3.15: (a) UV-visible absorption and (b) fluorescence emission spectra of the nucleoside in various organic solvents (10 μ M).

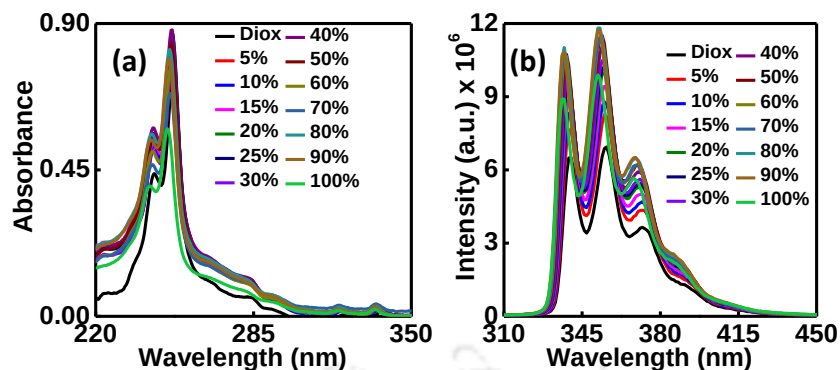


Figure 3.16: (a) UV-visible and (b) fluorescence under dioxane-water titration.

3.5.5. TDDFT calculation of FTPhen

The TD-DFT calculated transition at 307 nm (4.04 eV) indicated a transition from HOMO to LUMO with configuration interaction (CI) value of 0.49 and low transition oscillator strength $f = 0.0219$. Other feasible transition occurs at 261 nm with $CI = 0.18253$, $f = 0.1094$ (4.7556 eV); at 241 nm with $CI = 0.10307$, $f = 0.6974$ (5.1374 eV) and at 292 nm with $CI = 0.41482$, $f = 0.0204$ (4.2518 eV) which is HOMO $\rightarrow$ LUMO+1 (**Figure 3.17**). All the calculated transitions supported the experimental observation.

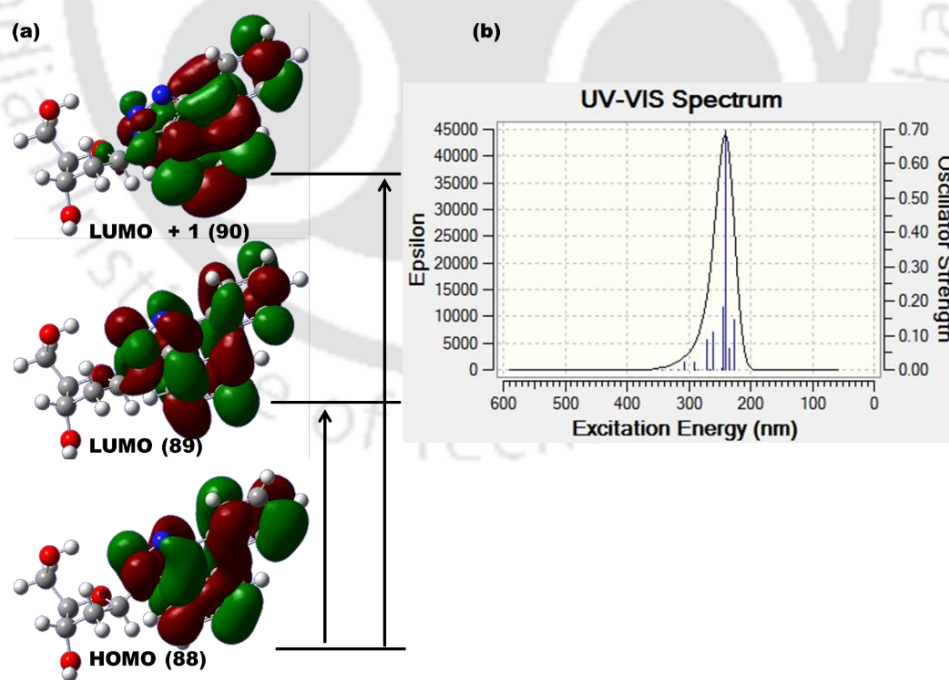


Figure 3.17 (a) HOMO-LUMO energy diagram. (b) TDDFT generated UV-VIS spectrum.

3.5.6. Macromodel calculation

To know the conformation and stacking stability which might be offered by our designed nucleoside if incorporated into a short DNA strand, we, first, carried out macromodel calculation using Schrodinger MacroModel.⁴⁴ Thus, the conformations of DNA duplexes [(5'-CGCAATXTAACGC-3'/3'-GCGTTANATTGCG-5')]; (**X** = **FTPhen**, **N** = A, G, C, T)] with fused triazolyl phenanthrene (**FTPhen**) nucleoside opposite to all four natural bases (A, G, C, T) were minimized with the Amber* force field using Maestro, version 9.0.

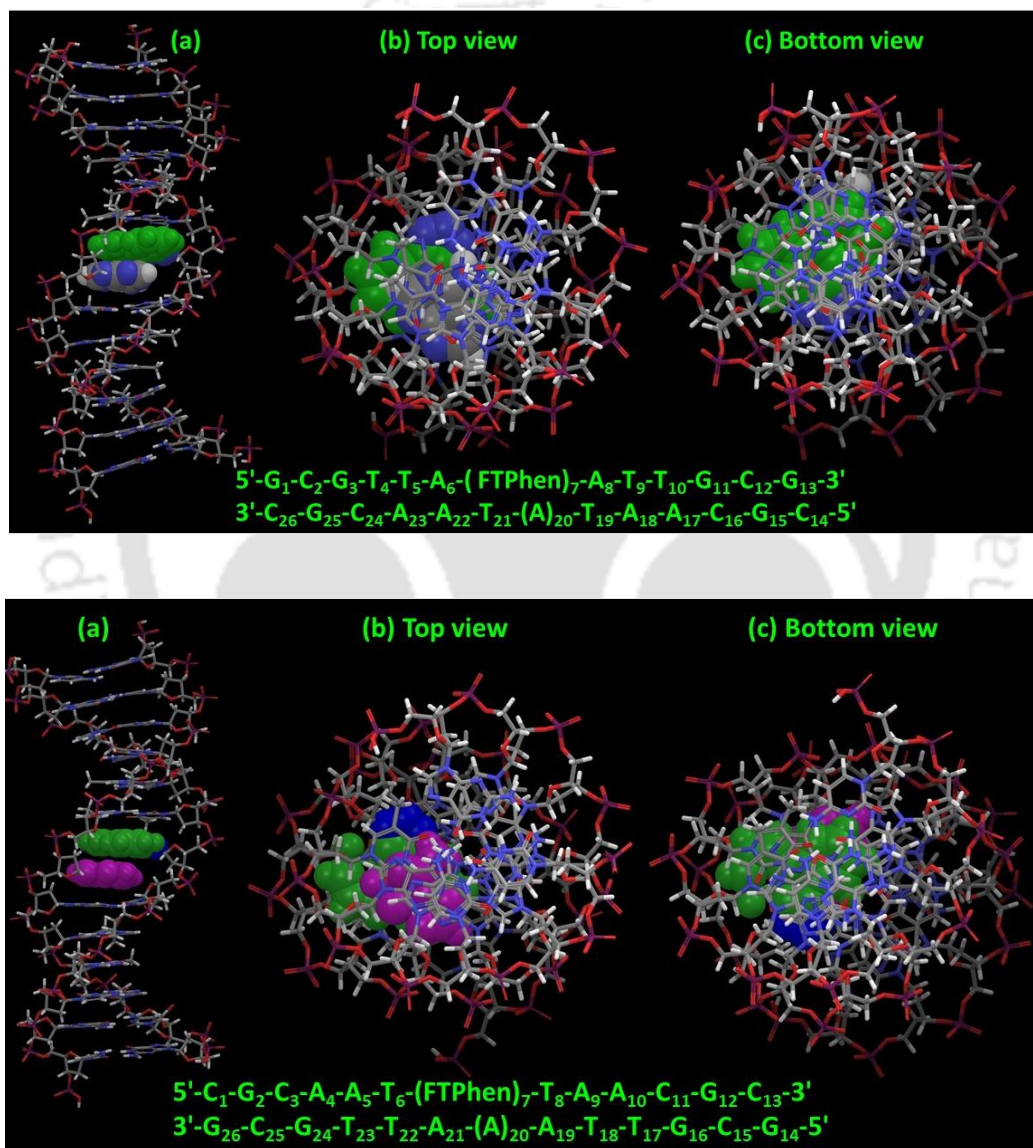


Figure 3.18: Representative Amber* force field optimized conformations of DNA containing **FTPhen** nucleoside paired against all four natural DNA bases.

The Amber* minimized structures of all the natural bases paired against **FTPhen** showed intrahelical conformation of both the **FTPhen** and natural bases wherein the **FTPhen** reaches deeper into the interior of the helix enabling an optimal positioning for both the inter- and intra-strand stacking interactions inside the duplex model DNA duplex. Both the inter-/intra strand stacking was found to be more in duplex **FTPhen : N(=A, G, C, T)** compared to its reverse sequence(**N(=A, G, C, T) : FTPhen**) (Section 3.10. for **Amber*** force field optimized conformation of **ODNs**). It was also revealed that regardless of the opposite natural base, the all the DNA duplex containing **FTPhen** showed right-handed B-form helix with all residues having *anti* glycosidic bond and C2'-*endo*/C3'-*exo* sugar conformations. Helical rise values 3.3–3.5 Å are close to those observed in B-form natural DNA (3.4 Å), with slight deviations at and near the purine bases.

3.5.7. DFT calculation in the context of DNA

Next, we have carried out DFT calculation to get a preliminary idea about the stacking propensity between the **FTPhen** base and natural nucleoside bases using Gaussian 09 programme package.⁴⁰ All the geometries of natural and unnatural bases were optimized by **B3LYP/6-31g(d,p)** and stacked bases were optimized using the **M05-2X** density functional developed by Truhlar and Zhao.⁴⁵ In the present preliminary calculation, the initial starting geometries of stacked dimers natural/unnatural hybrids in B-DNA conformation were generated using the Schrodinger Macromodel program. From the generated structures in B-DNA conformation, we removed the sugar, phosphate backbone for simplicity and optimized each individual pair. Basis set superposition error (**BSSE**) corrections were carried out to obtain the corrected interaction energy by using the counterpoise correction procedure (CP).⁴⁶ From the calculation, a considerable amount of stacking interactions was observed between **FTPhen** and natural nucleosides from DFT calculation using **M05-2X/6-31G(d,p)**⁴⁵ level of theory. Therefore, our preliminary observation revealed that a considerable amount of stacking interaction is operating between various pairing partners which are comparable to the stacking energies between natural bases. The BSSE corrected pairing energy calculation showed a strong stacking propensity with natural bases following the order **FTPhen-C** (-6.75 kcal/mol) > **FTPhen -A** (-4.59 kcal/mol) > **FTPhen -G** (-3.55 kcal/mol) > **FTPhen -T** (-3.29 kcal/mol) (Table 3.1). The more pairing propensity with C might be attributed to the involvement of stacking as well as possible H-bonding/electrostatic interaction.

Table: 3.1 BSSE corrected stacking energy of the **FTPhen**-natural nucleobase pairs.

Stacking energy of the single nucleobase pairs	
Single base pair	BSSE corrected stacking energy (kcal/mol)
FTPhen:A	-4.59
FTPhen:G	-3.55
FTPhen:C	-6.75
FTPhen:T	-3.29

3.5.8. ONIOM calculation in the context of DNA

Next, we performed calculation on trimeric DNA duplex containing **FTPhen** in the middle of the strand paired against all four natural bases in two sequence context. In case of trimeric B-DNA duplex, the initial structures generated from Schrodinger MacroModel were used for a **QM/MM** calculation wherein the aromatic bases were optimized with **M05-2X/6-31G(d,p)**⁴⁵ and sugar-phosphate backbones were treated with **UFF** force field embedded in **ONIOM** calculation in G09 programme package.

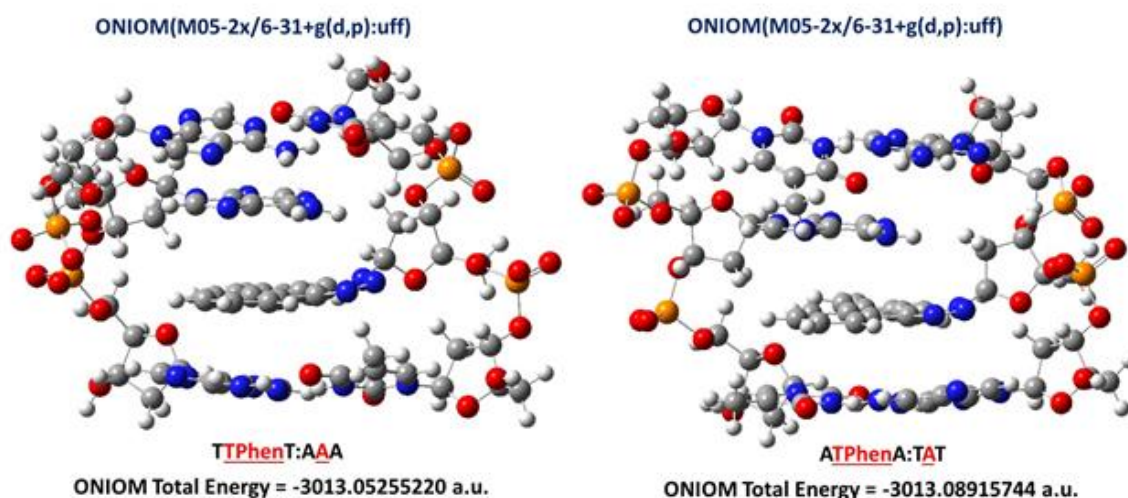


Figure 3.19: QM/MM calculated represented geometry of trimeric DNA duplexes applied for calculating stabilization energy of trimeric DNA duplexes.

Next, for a simplicity, the sugar phosphate backbones of two trimeric duplexes [(5'-Tp-**FTPhenp**-T-3')/(3'-Ap-**Xp**-A-5') and (5'-Ap-**FTPhenp**-A-3')/(3'-Tp-**Xp**-T-5'); X = A, G, C, T] were removed from the optimized geometries (See Section 3.11. for **QM/MM** calculated geometry) obtained from QM/MM calculation and the stack of aromatic bases of the trimeric duplexes were utilized for calculating the stabilization energies of the DNAs [(5'-T-**FTPhen**-T-3')/(3'-A-**X**-A-5') and (5'-A-**FTPhen**-A-3')/(3'-T-**X**-T-5'); X = A, G, C, T] at M05-2X/6-31G(d,p) level of theory. Our preliminary observation revealed a considerable amount of stabilization of the trimeric DNA duplexes in both the sequence contexts which followed the order as **FTPhen**-T > **FTPhen** -C > **FTPhen** -G > **FTPhen** -A in the normal sequence context wherein the **TPhen** base is flanked between T bases. On the other hand the order of stabilization in the reversed sequence context wherein the flanking bases were A was **FTPhen**-C > **FTPhen** -T > **FTPhen** -G > **FTPhen** -A (Table 3.2). The stabilization was mainly coming from a combination of both inter-/intra-strand stacking interaction as well as H-bonding interaction with the triazole unit, however, a more rigorous calculation is needed to arrive at the conclusion.

Table: 3.2 BSSE corrected Stabilisation energy (E_{SE}) for the systems T-FTPhen-T: A-X-A and A-FTPhenp-A:T-X-T.

Trimeric system	Stabilisation energy (E_{SE}) (kcal/mol) BSSE corrected
Stabilisation energy (E_{SE}) for the system TpFTPhenpT:ApXpA	
T-FTPhen-T:A-A-A	-10.00
T-FTPhen-T:A-T-A	-20.12
T-FTPhen-T:A-G-A	-10.34
T-FTPhen-T:A-C-A	-14.97
Stabilisation energy (E_{SE}) for the system ApFTPhenpA:TpXpT	
A-FTPhen-A:T-A-T	-9.63
A-FTPhen-A:T-T-T	-15.55
A-FTPhen-A:T-G-T	-13.58
A-FTPhen-A:T-C-T	-17.47

3.6. Conclusion

In conclusion, we have successfully synthesised a fused triazolyl phenanthrene nucleoside as a new generation of doubly widened hydrophobic base surrogate. It showed interesting photophysical property. Slightly blue shifted solvatochromicity was shown by the nucleoside with increasingly enhanced emission intensity as the polarity of the solvent increases. Though the gas phase DFT calculations do not take into account solvent interactions, our preliminary observations suggested a good stabilizing property of the designed base via pairing with natural bases in a DNA if incorporated. Our recent focus is to incorporate it into a short DNA and study the photophysical outcome upon hybridization.

3.7. Experimental section

3.7.1. General experimental

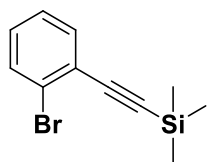
^1H NMR spectra were measured with a 600 MHz machine; ^{13}C NMR spectra were measured with 150 MHz spectrometer. Coupling constant (J value) is reported in hertz. The chemical shifts are shown in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in ^1H NMR, $\delta = 77.0$ in ^{13}C NMR) dimethyl sulfoxide ($\delta = 2.48$ in ^1H NMR, $\delta = 39.5$ in ^{13}C NMR), and methanol ($\delta = 3.30$ in ^1H NMR, $\delta = 49.0$ in ^{13}C NMR), as an internal standard. FAB masses were recorded on a mass spectrometer.

3.7.2. Characterization

Synthesis of 1-bromo-2-iodobenzene (3.57): 2-bromoaniline (2 g, 11.7 mmol) was dissolved in the HCl (37%, 6 mL) in H_2O (20 mL) and allowed to cool to 0°C . To this solution NaNO_2 (0.968 g, 14 mmol) in H_2O (5 mL) was added. The reaction mixture was allowed to stir for 10 minutes. KI (2.96 g, 17.438 mmol) was dissolved in H_2O (5 mL) and added drop wise to the reaction mixture. The colour of the reaction mixture was turned to blood red. The reaction mixture was further stirred for 30 minutes at room temperature and then allowed to heat at 70°C for another 1 h. The reaction mixture was then allowed to cool to room temperature and neutralised by $\text{Na}_2\text{S}_2\text{O}_3$ (0.43 mol/L). DCM was added portion wise to the reaction mixture and all the organic fraction was collected, washed with brine and dried over sodium sulphate. The organic part was then concentrated in reduced pressure and subjected to column chromatography with hexane to afford **1-bromo-2-iodobenzene** (pale yellow liquid, 3.3 g, 100%).

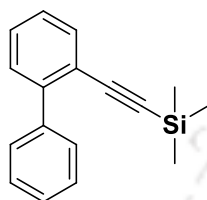
Synthesis of ((2-bromophenyl)ethynyl)trimethylsilane (3.58): 1-bromo-2-iodobenzene **3.57** was taken in a 2 neck round bottom flask fitted with magnetic stirring bar and septum. Triethyl amine was added to it. The reaction vessel was then purged with N_2 . $\text{PdCl}_2(\text{PPh}_3)_2$ (246 mg, 0.03 eq), CuI (133 mg, 0.06 eq) and ethynyltrimethylsilane (1.374 g, 14 mmol) were added to the reaction mixture and it is then allowed to stir for 3h. Completion of the reaction was checked by TLC. The reaction mixture was then diluted with Hexane and all the organic fraction was collected

and washed with NH_4Cl , brine, concentrated in vacuo. It is then purified by column chromatography with hexane to afford ((2-bromophenyl)ethynyl)trimethylsilane (3.58) (3.062 g,



98%) as a colorless oil. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.57 (dd, $J = 8.1, 1.2$ Hz, 1H), 7.49 (dd, $J = 7.7, 1.7$ Hz, 1H), 7.24 (td, $J = 7.6, 1.2$ Hz, 1H), 7.15 (td, $J = 7.8, 1.7$ Hz, 1H), 0.28 (s, 9H). $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 133.7, 132.5, 129.7, 127.0, 125.9, 125.3, 103.1, 99.7, -0.02. $^{13}\text{C NMR}$ (150 MHz, CDCl_3 , DEPT-135) δ 133.75, 132.51, 129.71, 127.03, -0.02.

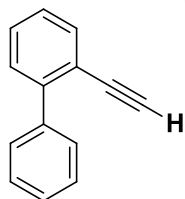
Synthesis of ([1,1'-biphenyl]-2-ylethynyl)trimethylsilane (3.59): ((2-bromophenyl)ethynyl)trimethylsilane (**110**) (400 mg, 1.6 mmol) and phenyl boronic acid (430 mg, 3.52 mmol) were dissolved in a 5:1(v/v) mixture of toluene (15 ml) and water (3 ml). K_2CO_3 (663



mg, 4.8 mg) was added to it followed by $\text{Pd}(\text{PPh}_3)_4$ (184 mg, 0.16 mg). The reaction mixture was subjected to three freeze-pump-thaw cycles and backfilled with a N_2 atmosphere. Now the reaction mixture was heated to 100°C for 6h. After 6 h the completion of the reaction was checked by TLC. Now ethyl acetate was added to the reaction mixture in 3 portion and the reaction mixture was washed with brine. The combined organic layer was evaporated and the crude

material was subjected to column chromatography to give ([1,1'-biphenyl]-2-ylethynyl)trimethylsilane (**3.59**) (300 mg, 76%) as a pale yellow oil. $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.61 (d, $J = 7.4$ Hz, 2H), 7.58 (d, $J = 7.8$ Hz, 1H), 7.44 – 7.34 (m, 5H), 7.30 – 7.26 (m, 1H), 0.13 (s, 9H). $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 144.4, 140.4, 133.3, 129.4, 128.8, 127.8, 127.5, 127.0, 121.5, 104.9, 97.6, -0.17. $^{13}\text{C NMR}$ (150 MHz, CDCl_3 , DEPT-135) δ 133.3, 129.4, 128.8, 127.8, 127.5, 127.0, -0.17.

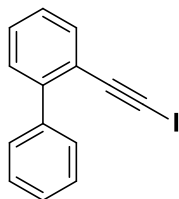
Synthesis of 2-ethynyl-1,1'-biphenyl (3.60): ([1,1'-biphenyl]-2-ylethynyl)trimethylsilane (**3.59**) (290 mg, 1.174 mmol) was dissolved in a 1:1 mixture of DCM (5 ml) MeOH (5 ml) and



and K_2CO_3 (1.62 g, 11.74 mmol) was added to it. The reaction mixture was allowed to stir for 2h. After 2h TLC shows the complete conversion of the starting material into product. The solvent of the reaction mixture was evaporated and diluted with hexane, wash it with brine. Now after evaporation of the hexane the crude product was subjected to column chromatography to give 2-ethynyl-1,1'-biphenyl (**3.60**) (163 mg, 93%). $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.63 (d, $J = 7.6$

Hz, 1H), 7.62 – 7.58 (m, 2H), 7.40 (ddt, $J = 9.4, 7.3, 6.4$ Hz, 5H), 7.31 (td, $J = 7.5, 1.5$ Hz, 1H), 3.04 (s, 1H). $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 144.5, 140.3, 134.0, 129.7, 129.3, 129.1, 128.1, 127.6, 127.1, 120.5, 83.2, 80.2. $^{13}\text{C NMR}$ (150 MHz, CDCl_3 , DEPT-135) δ 133.8, 129.6, 129.2, 128.9, 128.0, 127.5, 127.0, 83.1, 80.1.

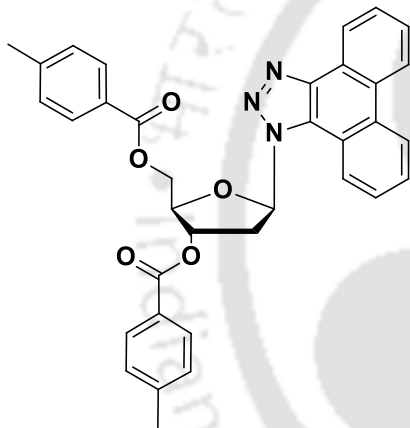
Synthesis of 2-(iodoethynyl)-1,1'-biphenyl (3.54): 2-ethynyl-1,1'-biphenyl (3.54) (163 mg, 1mmol) is dissolved in 5 ml THF, N-iodomorpholine Hydrogen iodide (408 mg, 1.1 mmol)



was added to it followed by CuI (11 mg, 0.05 mmol) and the reaction mixture was allowed to stir overnight. After that the reaction mixture was diluted with dichloromethane and excess iodine was washed with Na₂S₂O₃ and again the reaction mixture was washed with brine and the organic layer was collected and dried. The crude mass was subjected to column chromatography with hexane to give **2-(iodoethynyl)-1,1'-biphenyl (3.54)** (280 mg, 93%). ¹H

NMR (400 MHz, CDCl₃): δ 7.648-7.60 (m, 3H), 7.508-7.402 (m, 5H), 7.352-7.311 (td, *J* = 2.8, 5.2, 0.8, 4.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 144.7, 140.0, 134.1, 129.5, 129.2, 129.1, 128.1, 127.6, 127.0, 121.7, 93.8, 80.3, 77.1.

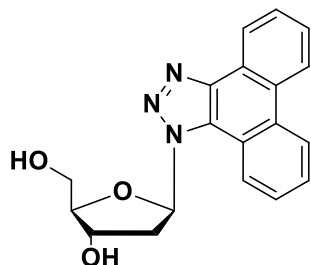
Synthesis of ((2R,3S,5R)-3-((4-methylbenzoyl)oxy)-5-(1H-phenanthro[9,10-d][1,2,3]triazol-1-yl)tetrahydrofuran-2-yl)methyl 4-methylbenzoate (3.61): Take a 2 neck



round bottom flask fitted with septum, coil condenser and magnetic stirring bar. Evacuate the round bottom flask and backfilled with nitrogen. THF (10 ml) was added to the reaction vessel. **2-(iodoethynyl)-1,1'-biphenyl (3.54)** (120 mg, 0.4332 mg) and Beta Sugar azide (**2.01**) (257.22 mg, 0.65 mmol) were added to THF followed by CuI (8.27 mg, 0.0433 mmol) and TBTA (23 mg, 0.0433 mmol). The reaction mixture was allowed to stir for 24 h. After that if starting material were remaining unreacted another TBTA (23 mg, 0.0433 mmol) were added to the reaction mixture and let the reaction mixture stir for another 24 h. Complete conversion of the starting material was checked by TLC. After that tetrabutylammonium bromide (140 mg, 0.4332 mmol),

Pd(OAc)₂ (10 mg, 0.0433 mmol), K₂CO₃ (60 mg, 0.4332 mmol), PPh₃ (24 mg, 0.09 mmol) were added to the reaction mixture and the reaction mixture was heated to 80°C for another 24 h. Complete conversion of the starting material was checked by TLC. After the completion of the reaction the reaction mixture was diluted with ethyl acetate and washed 2 times with ammonium hydroxide solution and brine. The organic layer was evaporated and the crude product was subjected to column chromatography which gives **3.61** (253 mg, 68%) as a white solid ¹H NMR (600 MHz, CDCl₃) δ 8.77 (dd, *J* = 7.8, 1.0 Hz, 1H), 8.74 – 8.70 (m, 1H), 8.63 – 8.59 (m, 2H), 8.01 (d, *J* = 8.1 Hz, 2H), 7.76 – 7.66 (m, 4H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 2H), 7.00 – 6.96 (m, 1H), 6.05 (dt, *J* = 6.9, 4.5 Hz, 1H), 4.80 (dd, *J* = 8.4, 4.8 Hz, 1H), 4.52 (dd, *J* = 12.0, 4.9 Hz, 1H), 4.41 – 4.30 (m, 2H), 2.99 (ddd, *J* = 14.1, 6.2, 4.9 Hz, 1H), 2.44 (s, 3H), 2.31 (s, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 166.1, 144.4, 143.7, 142.8, 131.2, 129.9, 129.6, 129.4, 129.1, 129.1, 129.0, 128.1, 127.9, 127.7, 127.2, 126.8, 126.6, 125.0, 124.3, 124.2, 123.3, 123.1, 120.5, 88.1, 83.9, 77.1, 75.3, 63.5, 36.2, 21.8, 21.7. ¹³C NMR (150 MHz, CDCl₃, DEPT-135) δ 129.9, 129.6, 129.4, 129.0, 128.1, 127.9, 127.7, 127.2, 124.3, 124.2, 123.3, 123.1, 88.1, 83.9, 75.3, 63.5, 36.2, 21.8, 21.7. HRMS Calculated 572.218 found [*M*+*H*]⁺ = 572.2189.

Synthesis of (2R,3S,5R)-2-(hydroxymethyl)-5-(1H-phenanthro[9,10-d][1,2,3]triazol-1-yl)tetrahydrofuran-3-ol (3.52): Protected sugar (3.52) was dissolved in MeOH and sodium methoxide was added to it. The reaction mixture was allowed to stir for 4h and completion of the



reaction was checked by TLC. The solvent was evaporated and the compound was dissolved in ethyl acetate washed with brine. The organic layer was evaporated and the crude was subjected to column chromatography to give compound **107** (130 mg, 82%). **¹H NMR** (600 MHz, MeOD): δ 8.821-8.806 (d, J = 9 Hz, 1H), 8.729-8.716 (d, J = 7.8 Hz), 8.671-8.657 (d, J = 8.4 Hz), 8.629-8.616 (d, J = 7.8 Hz, 1H), 7.786-7.694 (m, 4H), 7.024-7.006 (q, J = 4.8, 1.2 Hz, 1H), 4.816-4.789 (q, J = 6, 4.8, 5.4 Hz, 1H), 4.202-4.178 (q, J = 4.8 Hz, 1H), 3.771-3.731

(ddd, J = 4.2, 1.8, 4.8, 2.4, 1.2 Hz, 1H), 3.583-3.556 (dd, J = 4.2, 7.8, 4.2 Hz, 1H), 3.437-3.408 (dd, J = 5.4, 6.6, 5.4 Hz, 1H), 2.708-2.665 (m, 1H); **¹³C NMR** (150 MHz, MeOD) δ 143.0, 132.2, 130.5, 130.3, 129.2, 129.2, 128.9, 128.5, 125.6, 125.5, 125.3, 124.8, 123.3, 121.5, 89.9, 89.3, 72.7, 63.5, 39.4. **¹³C NMR** (150 MHz, MeOD, DEPT-135) δ 129.0, 128.9, 128.6, 128.2, 125.3, 125.0, 124.5, 123.0, 89.6, 89.0, 72.4, 63.2, 49.0, 39.1. **HRMS** Calculated 336.1343 found $[M+H]^+ = 336.1330$.

3.8. Studies on Photophysical Properties of the Nucleoside

UV-visible measurements: All the UV-visible spectra of the nucleoside monomers (10 μ M) were measured in different organic solvents using a UV-Visible spectrophotometer with a cell of 1 cm path length at 25 °C. Equal volume of each sample solution in different solvents were used. The measurements were taken in absorbance mode. The absorbance values of the sample solutions were measured in the wavelength regime of 200–550 nm. All the sample solutions were prepared just before doing the experiment.

Fluorescence experiments: All the sample solutions of the nucleoside monomers (10 μ M) were prepared as described in UV measurement experiments. Fluorescence spectra were obtained using a fluorescence spectrophotometer at 25 °C using 1 cm path length cell. The excitation wavelengths for monomers were set at λ_{abs}^{max} , and emission spectra were measured in the wavelength regime of 315–600 nm with an integration time of 0.1 sec .

3.9. Representative coordinates

TTPhenT:AAA

1 O1	8.0525	2.8897	-5.1840	O
2 C2	9.0147	3.3774	-4.2771	C
3 C3	8.4250	3.5505	-2.8673	C
4 O4	7.3488	4.4651	-2.8814	O
5 C5	7.9435	2.2345	-2.2751	C
6 O6	9.0031	1.6065	-1.5808	O
7 C7	6.8671	2.6931	-1.3315	C
8 C8	6.4311	4.0465	-1.8896	C
9 N9	5.0985	3.9627	-2.4537	N
10 C10	4.0109	4.3854	-1.6875	C
11 O11	4.1051	4.8294	-0.5548	O
12 N12	2.7980	4.2816	-2.3221	N
13 C13	2.5348	3.7402	-3.5584	C
14 O14	1.3918	3.7222	-4.0131	O
15 C15	3.6922	3.2154	-4.2667	C
16 C16	3.4657	2.5119	-5.5670	C
17 C17	4.8939	3.3593	-3.6880	C
18 H18	1.9854	4.5962	-1.7523	H
19 P19	-7.0515	2.3349	2.8102	P
20 O20	-8.2059	2.6657	3.7299	O
21 O21	-7.6234	2.3525	1.2168	O
22 O22	-5.8249	3.5082	2.9680	O
23 C23	-5.4594	3.5921	4.3269	C
24 C24	-4.3324	4.6150	4.5008	C
25 O25	-3.2405	4.2621	3.6875	O
26 C26	-4.7642	6.0316	4.1431	C
27 O27	-5.2734	6.6970	5.2692	O
28 C28	-3.4795	6.6538	3.6673	C
29 C29	-2.6364	5.4547	3.2361	C
30 N30	-2.5018	5.4021	1.8045	N
31 C31	-3.5164	5.1919	0.8917	C
32 N32	-3.0851	5.0200	-0.3261	N
33 C33	-1.7135	5.1060	-0.2100	C
34 C34	-0.6813	4.9435	-1.1591	C
35 N35	-0.9208	4.7302	-2.4566	N
36 N36	0.5836	5.0233	-0.7145	N
37 C37	0.8189	5.2527	0.5887	C
38 N38	-0.0616	5.4260	1.5618	N
39 C39	-1.3175	5.3309	1.1039	C
40 H40	-0.1560	4.4138	-3.0478	H
41 H41	-1.8602	4.4783	-2.7107	H
42 H42	9.8785	2.6744	-4.2105	H
43 H43	9.3892	4.3658	-4.6254	H
44 H44	9.2241	3.9614	-2.2089	H
45 H45	7.4981	1.6089	-3.0852	H
46 H46	6.0364	1.9577	-1.2883	H
47 H47	7.2849	2.8281	-0.3100	H
48 H48	6.4602	4.7984	-1.0630	H
49 H49	4.4157	2.2592	-6.0388	H

Chapter 3

50 H50	2.9066	1.5916	-5.3879 H
51 H51	2.8795	3.1343	-6.2425 H
52 H52	5.7821	3.0047	-4.1969 H
53 H53	-6.3215	3.9140	4.9542 H
54 H54	-5.0863	2.6082	4.6932 H
55 H55	-3.9948	4.5864	5.5623 H
56 H56	-5.5121	6.0291	3.3159 H
57 H57	-3.6614	7.3761	2.8410 H
58 H58	-2.9715	7.1851	4.5035 H
59 H59	-1.6306	5.5348	3.7057 H
60 H60	-4.5564	5.1929	1.1777 H
61 H61	1.8699	5.2920	0.8550 H
62 P62	8.6989	-0.0718	-1.4796 P
63 O63	9.8631	-0.7431	-0.7847 O
64 O64	8.5625	-0.7205	-3.0365 O
65 O65	7.2481	-0.3660	-0.6287 O
66 C66	7.4707	-0.1222	0.7418 C
67 C67	6.1458	-0.2044	1.5099 C
68 O68	5.2372	0.7558	1.0007 O
69 C69	5.5128	-1.6082	1.4204 C
70 O70	5.0414	-2.0112	2.6923 O
71 C71	4.3254	-1.3629	0.5304 C
72 C72	4.0107	0.0915	0.8301 C
73 P73	-8.2023	-0.2345	-1.6404 P
74 O74	-9.3799	0.4974	-1.0356 O
75 O75	-7.6582	0.6513	-2.9760 O
76 O76	-6.9371	-0.3545	-0.5019 O
77 C77	-7.4552	-0.9124	0.6843 C
78 C78	-6.3376	-1.0837	1.7167 C
79 O79	-5.3172	-1.9027	1.2015 O
80 C80	-5.7142	0.2401	2.1406 C
81 O81	-6.4369	0.7949	3.2245 O
82 C82	-4.3466	-0.1984	2.5876 C
83 C83	-4.1106	-1.4985	1.8167 C
84 N84	-3.0771	-1.3263	0.8265 N
85 C85	-3.2141	-1.4124	-0.5479 C
86 N86	-2.0811	-1.5288	-1.1777 N
87 C87	-1.1413	-1.5456	-0.1703 C
88 C88	0.2584	-1.6767	-0.1966 C
89 N89	0.9174	-1.8696	-1.3530 N
90 N90	0.9284	-1.6350	0.9632 N
91 C91	0.2233	-1.4835	2.0961 C
92 N92	-1.0867	-1.3770	2.2594 N
93 C93	-1.7247	-1.4236	1.0847 C
94 H94	1.9193	-1.7963	-1.3697 H
95 H95	0.4079	-1.7912	-2.2164 H
96 H96	8.1695	-0.8800	1.1616 H
97 H97	7.9013	0.8905	0.9100 H
98 H98	6.3570	0.0474	2.5732 H
99 H99	6.1716	-2.3681	0.9340 H
100 H100	4.6226	-1.5090	-0.5310 H
101 H101	3.4756	-2.0314	0.7723 H
102 H102	3.4596	0.1040	1.7926 H
103 H103	-8.2391	-0.2578	1.1261 H
104 H104	-7.8973	-1.9150	0.4905 H
105 H105	-6.7705	-1.5923	2.6095 H

.Synthesis and Study of Photophysical Properties of Fused Triazolyl Phenanthrene Nucleoside

106 H106	-5.5746	0.9106	1.2584 H
107 H107	-3.5850	0.5795	2.3689 H
108 H108	-4.3385	-0.4067	3.6811 H
109 H109	-3.8017	-2.2992	2.5258 H
110 H110	-4.1718	-1.3483	-1.0371 H
111 H111	0.8173	-1.4383	3.0035 H
112 P112	6.2086	-2.9891	3.4707 P
113 O113	7.5513	-2.3024	3.4932 O
114 O114	6.3462	-4.4646	2.6567 O
115 O115	5.7800	-3.3582	5.0852 O
116 C116	4.5325	-2.7875	5.4237 C
117 C117	3.3265	-3.6882	5.0684 C
118 O118	2.8194	-3.3961	3.7883 O
119 C119	3.6185	-5.1853	5.0964 C
120 O120	3.4955	-5.7071	6.3962 O
121 C121	2.5437	-5.7404	4.1953 C
122 C122	2.0171	-4.5088	3.4447 C
123 N123	2.0333	-4.7127	2.0093 N
124 C124	0.8254	-4.7258	1.3037 C
125 O125	-0.2737	-4.6794	1.8303 O
126 N126	0.9655	-4.8273	-0.0577 N
127 C127	2.1245	-4.8332	-0.7947 C
128 O128	2.0951	-4.8364	-2.0254 O
129 C129	3.3498	-4.8538	-0.0110 C
130 C130	4.6634	-4.9072	-0.7273 C
131 C131	3.2334	-4.7918	1.3272 C
132 H132	0.0663	-4.7669	-0.5807 H
133 O133	-7.5510	-2.3441	-5.8362 O
134 C134	-8.6544	-2.8624	-5.1412 C
135 C135	-8.2292	-3.4665	-3.7959 C
136 O136	-7.2550	-4.4681	-3.9819 O
137 C137	-7.6631	-2.4287	-2.8390 C
138 O138	-8.7106	-1.7884	-2.1369 O
139 C139	-6.8328	-3.2947	-1.9360 C
140 C140	-6.3984	-4.4355	-2.8545 C
141 N141	-5.0271	-4.2646	-3.2691 N
142 C142	-4.5453	-4.0890	-4.5521 C
143 N143	-3.2440	-4.0704	-4.6155 N
144 C144	-2.8416	-4.2559	-3.3119 C
145 C145	-1.5607	-4.3616	-2.7330 C
146 N146	-0.4337	-4.2347	-3.4442 N
147 N147	-1.4954	-4.5994	-1.4148 N
148 C148	-2.6257	-4.6784	-0.6954 C
149 N149	-3.8766	-4.5857	-1.1242 N
150 C150	-3.9234	-4.3874	-2.4499 C
151 H151	0.4616	-4.4513	-3.0121 H
152 H152	-0.5143	-4.1631	-4.4427 H
153 H153	4.5332	-2.6674	6.5286 H
154 H154	4.3947	-1.7520	5.0370 H
155 H155	2.5254	-3.4724	5.8151 H
156 H156	4.6133	-5.4214	4.6407 H
157 H157	2.9665	-6.5118	3.5151 H
158 H158	1.7272	-6.2050	4.7912 H
159 H159	0.9782	-4.3004	3.8045 H
160 H160	5.4887	-4.8921	-0.0119 H
161 H161	4.7405	-5.8086	-1.3352 H

Chapter 3

162 H162	4.7686	-4.0533	-1.3974 H
163 H163	4.1314	-4.7999	1.9325 H
164 H164	-9.3804	-2.0409	-4.9616 H
165 H165	-9.1768	-3.6370	-5.7496 H
166 H166	-9.1272	-3.9408	-3.3361 H
167 H167	-6.9957	-1.7352	-3.4043 H
168 H168	-5.9822	-2.7301	-1.5048 H
169 H169	-7.4556	-3.7036	-1.1093 H
170 H170	-6.4898	-5.4040	-2.3150 H
171 H171	-5.1917	-3.9776	-5.4070 H
172 H172	-2.4712	-4.8332	0.3675 H
173 N173	3.5252	0.5346	-1.5038 N
174 N174	2.5015	0.8607	-2.2123 N
175 C175	1.4999	1.2622	-1.3777 C
176 C176	1.9532	1.2202	-0.0745 C
177 N177	3.2241	0.7254	-0.2038 N
178 C178	0.1184	1.4951	-1.7071 C
179 C179	-0.7681	1.7132	-0.6296 C
180 C180	-2.1373	1.8375	-0.9308 C
181 C181	-2.5952	1.7403	-2.2311 C
182 C182	-1.7020	1.5163	-3.2856 C
183 C183	-0.3517	1.3980	-3.0241 C
184 H184	-2.8605	2.0080	-0.1466 H
185 H185	-3.6560	1.8355	-2.4285 H
186 H186	-2.0672	1.4338	-4.3014 H
187 H187	0.3650	1.2442	-3.8192 H
188 C188	-0.2477	1.8355	0.7428 C
189 C189	1.1318	1.6585	1.0314 C
190 C190	1.6184	1.9584	2.3154 C
191 C191	0.7556	2.2789	3.3424 C
192 C192	-0.6159	2.3444	3.0948 C
193 C193	-1.0959	2.1699	1.8127 C
194 H194	2.6827	2.0014	2.5045 H
195 H195	1.1460	2.5003	4.3271 H
196 H196	-1.2966	2.5673	3.9054 H
197 H197	-2.1496	2.3212	1.6297 H
198 H198	-7.1957	-3.0793	-6.4000 H
199 H199	4.3981	-5.6191	6.8111 H
200 H200	-5.6643	7.5479	4.9394 H
201 H201	8.5413	2.6470	-6.0153 H

ATPhenA:TAT

1 O1	8.5362	3.3775	-5.4807 O
2 C2	9.4591	3.3229	-4.4275 C
3 C3	8.7335	3.2274	-3.0807 C
4 O4	7.8238	4.2927	-2.9392 O
5 C5	7.9661	1.9214	-2.9180 C
6 O6	8.8238	0.9192	-2.4075 O
7 C7	6.8900	2.3209	-1.9404 C
8 C8	6.7760	3.8331	-2.1111 C
9 N9	5.5061	4.1875	-2.6859 N
10 C10	5.1595	4.1445	-4.0199 C
11 N11	3.9008	4.4105	-4.2322 N
12 C12	3.3859	4.6111	-2.9699 C
13 C13	2.0843	4.9172	-2.5255 C

.Synthesis and Study of Photophysical Properties of Fused Triazolyl Phenanthrene Nucleoside

14 N14	1.0592	5.0993	-3.3664 N
15 N15	1.8877	5.0185	-1.2018 N
16 C16	2.9180	4.8233	-0.3597 C
17 N17	4.1785	4.5546	-0.6589 N
18 C18	4.3554	4.4601	-1.9843 C
19 H19	0.1398	5.3376	-3.0076 H
20 H20	1.2350	5.0414	-4.3526 H
21 P21	-7.1944	2.6792	2.7861 P
22 O22	-8.1391	3.0810	3.8971 O
23 O23	-7.8927	3.1508	1.3173 O
24 O24	-5.7049	3.4917	2.9720 O
25 C25	-5.2169	3.2466	4.2724 C
26 C26	-3.8832	3.9756	4.4760 C
27 O27	-3.0075	3.6816	3.4161 O
28 C28	-4.0447	5.4900	4.5436 C
29 O29	-4.2661	5.9254	5.8595 O
30 C30	-2.7175	5.9864	4.0344 C
31 C31	-2.1444	4.7893	3.2686 C
32 N32	-1.9793	5.0935	1.8592 N
33 C33	-0.6964	5.0835	1.2892 C
34 O34	0.3298	4.8950	1.9143 O
35 N35	-0.6764	5.3229	-0.0641 N
36 C36	-1.7374	5.5461	-0.9029 C
37 O37	-1.5812	5.7143	-2.1110 O
38 C38	-3.0435	5.5694	-0.2551 C
39 C39	-4.2469	5.8062	-1.1126 C
40 C40	-3.0934	5.3359	1.0669 C
41 H41	0.2739	5.2509	-0.4953 H
42 H42	10.1277	2.4416	-4.5529 H
43 H43	10.0846	4.2433	-4.4348 H
44 H44	9.4957	3.3125	-2.2717 H
45 H45	7.5095	1.6389	-3.8945 H
46 H46	5.9410	1.7894	-2.1659 H
47 H47	7.2015	2.0955	-0.8982 H
48 H48	6.8787	4.3247	-1.1175 H
49 H49	5.8786	3.9226	-4.7927 H
50 H50	2.6579	4.8965	0.6911 H
51 H51	-5.9330	3.6021	5.0470 H
52 H52	-5.0400	2.1584	4.4241 H
53 H53	-3.4232	3.6084	5.4227 H
54 H54	-4.8555	5.8467	3.8651 H
55 H55	-2.8467	6.8884	3.3961 H
56 H56	-2.0424	6.2478	4.8803 H
57 H57	-1.1733	4.5144	3.7380 H
58 H58	-5.1627	5.7765	-0.5216 H
59 H59	-4.1774	6.7731	-1.6126 H
60 H60	-4.3023	5.0480	-1.8964 H
61 H61	-4.0551	5.3271	1.5648 H
62 P62	8.0376	-0.5999	-2.4547 P
63 O63	9.0741	-1.6920	-2.3105 O
64 O64	7.2365	-0.8191	-3.9298 O
65 O65	6.9021	-0.7100	-1.1858 O
66 C66	7.5973	-0.6783	0.0409 C
67 C67	6.6027	-0.5288	1.1971 C
68 O68	5.8458	0.6482	1.0112 O
69 C69	5.6541	-1.7407	1.3128 C

Chapter 3

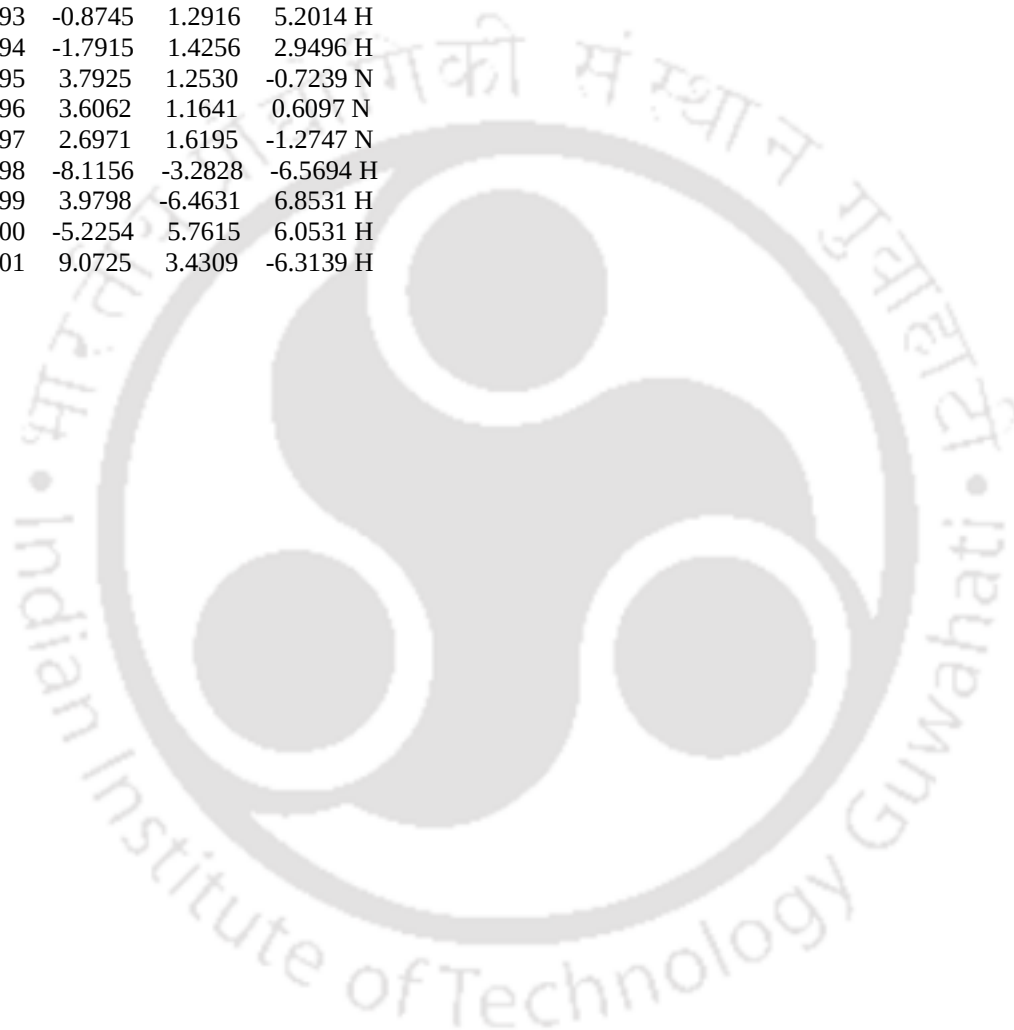
70 O70	5.6679	-2.2801	2.6189 O
71 C71	4.2966	-1.1348	1.0607 C
72 C72	4.5198	0.3372	1.3560 C
73 P73	-9.2110	-1.3597	-1.6000 P
74 O74	-10.5134	-1.1824	-0.8506 O
75 O75	-9.2807	-0.4373	-3.0168 O
76 O76	-7.8949	-0.8217	-0.6546 O
77 C77	-8.0692	-1.2735	0.6698 C
78 C78	-6.8015	-0.9966	1.4881 C
79 O79	-5.6957	-1.6341	0.8957 O
80 C80	-6.4911	0.4994	1.6078 C
81 O81	-6.9682	0.9831	2.8497 O
82 C82	-4.9804	0.5447	1.5292 C
83 C83	-4.5655	-0.9066	1.3078 C
84 N84	-3.5247	-1.0072	0.3192 N
85 C85	-3.6482	-0.8072	-1.0393 C
86 N86	-2.5114	-0.8785	-1.6742 N
87 C87	-1.5829	-1.1239	-0.6816 C
88 C88	-0.1724	-1.2095	-0.6923 C
89 N89	0.5436	-1.0801	-1.8172 N
90 N90	0.4702	-1.3826	0.4734 N
91 C91	-0.2568	-1.4719	1.5940 C
92 N92	-1.5746	-1.3939	1.7457 N
93 C93	-2.1854	-1.2066	0.5707 C
94 H94	1.5422	-1.1672	-1.7469 H
95 H95	0.0876	-1.1811	-2.7100 H
96 H96	8.1785	-1.6160	0.1814 H
97 H97	8.3001	0.1848	0.0919 H
98 H98	7.1881	-0.4115	2.1355 H
99 H99	5.8254	-2.5186	0.5345 H
100 H100	4.0109	-1.2877	-0.0045 H
101 H101	3.5036	-1.5668	1.7115 H
102 H102	4.4082	0.5050	2.4412 H
103 H103	-8.9207	-0.7414	1.1504 H
104 H104	-8.2620	-2.3704	0.7021 H
105 H105	-6.9485	-1.4329	2.5036 H
106 H106	-6.9219	1.0327	0.7307 H
107 H107	-4.6807	1.1922	0.6785 H
108 H108	-4.5180	0.9359	2.4595 H
109 H109	-4.2084	-1.3330	2.2735 H
110 H110	-4.6011	-0.6100	-1.5062 H
111 H111	0.3149	-1.6163	2.5048 H
112 P112	7.0137	-3.3179	2.8349 P
113 O113	8.1409	-2.5462	3.4831 O
114 O114	7.5672	-3.9660	1.3715 O
115 O115	6.5698	-4.6164	3.8510 O
116 C116	5.8843	-4.1332	4.9909 C
117 C117	4.4132	-4.5744	4.9613 C
118 O118	3.7828	-4.0486	3.8129 O
119 C119	4.2640	-6.1065	4.9723 C
120 O120	3.4310	-6.5134	6.0272 O
121 C121	3.6000	-6.4067	3.6558 C
122 C122	2.9855	-5.0731	3.2725 C
123 N123	2.8981	-4.9270	1.8435 N
124 C124	3.9486	-4.8287	0.9558 C
125 N125	3.5684	-4.6163	-0.2726 N

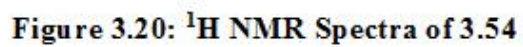
.Synthesis and Study of Photophysical Properties of Fused Triazolyl Phenanthrene Nucleoside

126 C126	2.1935	-4.5719	-0.1909 C
127 C127	1.2012	-4.3604	-1.1673 C
128 N128	1.4845	-4.1499	-2.4618 N
129 N129	-0.0784	-4.3933	-0.7666 N
130 C130	-0.3681	-4.5855	0.5311 C
131 N131	0.4741	-4.7696	1.5345 N
132 C132	1.7448	-4.7565	1.1138 C
133 H133	0.7557	-3.7748	-3.0604 H
134 H134	2.4498	-4.0416	-2.7170 H
135 O135	-7.6538	-3.3920	-5.6979 O
136 C136	-8.4731	-4.2267	-4.9260 C
137 C137	-7.8577	-4.4537	-3.5406 C
138 O138	-6.5454	-4.9482	-3.6631 O
139 C139	-7.8040	-3.1802	-2.7067 C
140 O140	-9.0181	-3.0085	-2.0016 O
141 C141	-6.6547	-3.4681	-1.7758 C
142 C142	-5.8506	-4.5476	-2.4998 C
143 N143	-4.5299	-4.0607	-2.8473 N
144 C144	-3.4450	-4.3925	-2.0263 C
145 O145	-3.5414	-5.0117	-0.9833 O
146 N146	-2.2283	-3.9699	-2.5043 N
147 C147	-1.9868	-3.1304	-3.5585 C
148 O148	-0.8356	-2.8041	-3.8596 O
149 C149	-3.1670	-2.6813	-4.2836 C
150 C150	-2.9762	-1.6670	-5.3655 C
151 C151	-4.3584	-3.1720	-3.9035 C
152 H152	-1.4178	-4.1874	-1.8771 H
153 H153	6.3653	-4.5708	5.8918 H
154 H154	5.9316	-3.0282	5.1376 H
155 H155	3.9147	-4.1399	5.8570 H
156 H156	5.2412	-6.6419	5.0327 H
157 H157	4.3715	-6.7202	2.9182 H
158 H158	2.8282	-7.2037	3.7363 H
159 H159	1.9701	-5.0019	3.7258 H
160 H160	4.9735	-4.9381	1.2702 H
161 H161	-1.4275	-4.5941	0.7637 H
162 H162	-9.4827	-3.7709	-4.8138 H
163 H163	-8.5849	-5.2099	-5.4353 H
164 H164	-8.4713	-5.2196	-3.0114 H
165 H165	-7.5424	-2.3201	-3.3689 H
166 H166	-6.0595	-2.5501	-1.5903 H
167 H167	-7.0219	-3.8650	-0.8036 H
168 H168	-5.7791	-5.4414	-1.8390 H
169 H169	-3.9156	-1.4538	-5.8758 H
170 H170	-2.5897	-0.7445	-4.9276 H
171 H171	-2.2436	-2.0174	-6.0930 H
172 H172	-5.2524	-2.8621	-4.4297 H
173 C173	1.7463	1.7413	-0.3057 C
174 C174	2.2991	1.4548	0.9268 C
175 C175	0.3535	2.0039	-0.5015 C
176 C176	-0.4782	1.8932	0.6283 C
177 C177	-1.8556	2.1135	0.4352 C
178 C178	-2.3685	2.3988	-0.8151 C
179 C179	-1.5231	2.4870	-1.9296 C
180 C180	-0.1684	2.2949	-1.7703 C
181 H181	-2.5278	2.0828	1.2785 H

Chapter 3

182 H182	-3.4316	2.5690	-0.9334 H
183 H183	-1.9307	2.7247	-2.9033 H
184 H184	0.5142	2.3595	-2.6069 H
185 C185	0.1037	1.6277	1.9502 C
186 C186	1.5088	1.5229	2.1392 C
187 C187	2.0158	1.5085	3.4525 C
188 C188	1.1756	1.4542	4.5454 C
189 C189	-0.2073	1.3962	4.3552 C
190 C190	-0.7224	1.4957	3.0809 C
191 H191	3.0727	1.6105	3.6438 H
192 H192	1.5935	1.4519	5.5439 H
193 H193	-0.8745	1.2916	5.2014 H
194 H194	-1.7915	1.4256	2.9496 H
195 N195	3.7925	1.2530	-0.7239 N
196 N196	3.6062	1.1641	0.6097 N
197 N197	2.6971	1.6195	-1.2747 N
198 H198	-8.1156	-3.2828	-6.5694 H
199 H199	3.9798	-6.4631	6.8531 H
200 H200	-5.2254	5.7615	6.0531 H
201 H201	9.0725	3.4309	-6.3139 H





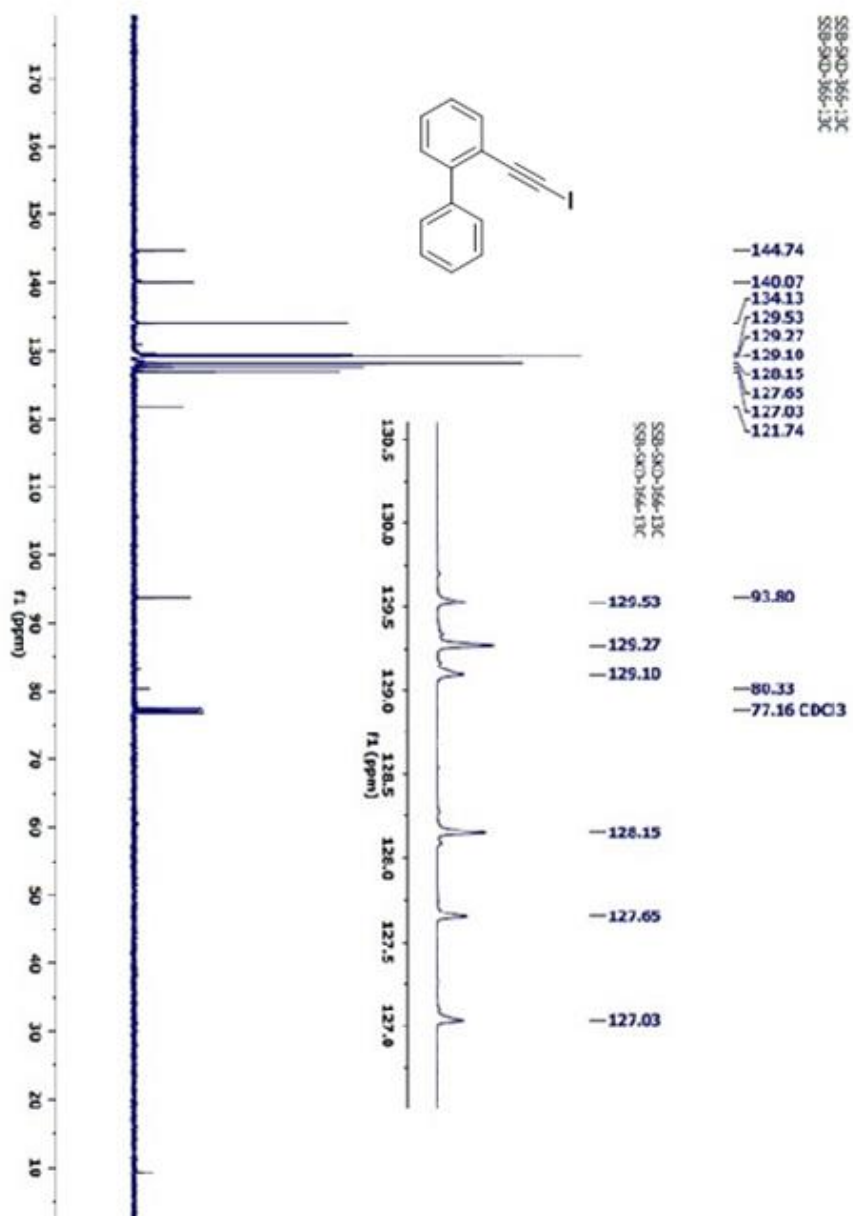


Figure 3.21: ¹³C NMR Spectra of 3.54

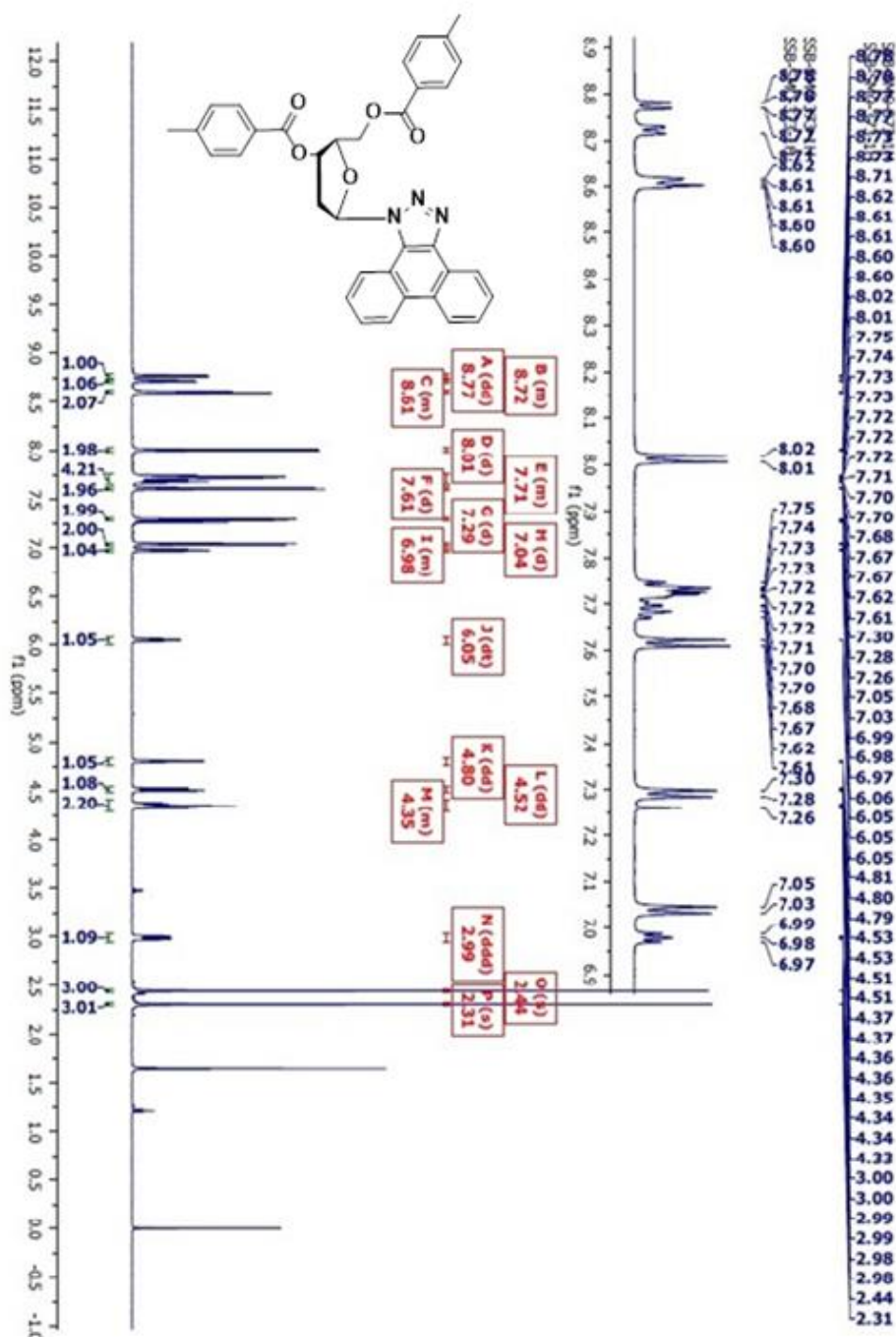
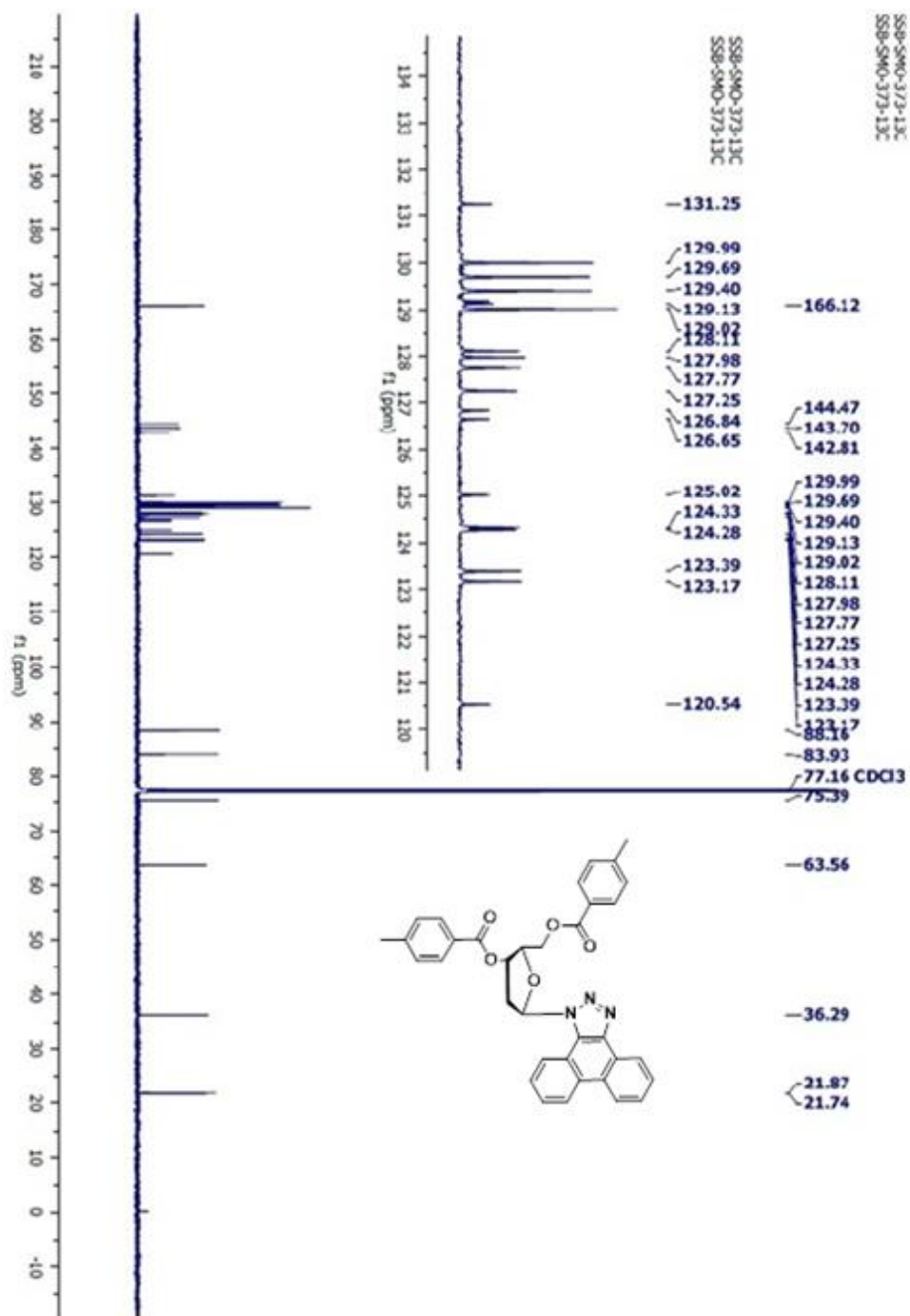


Figure 3.22: ¹H NMR Spectra of 3.61

Figure 3.23: ^{13}C NMR Spectra of 3.61

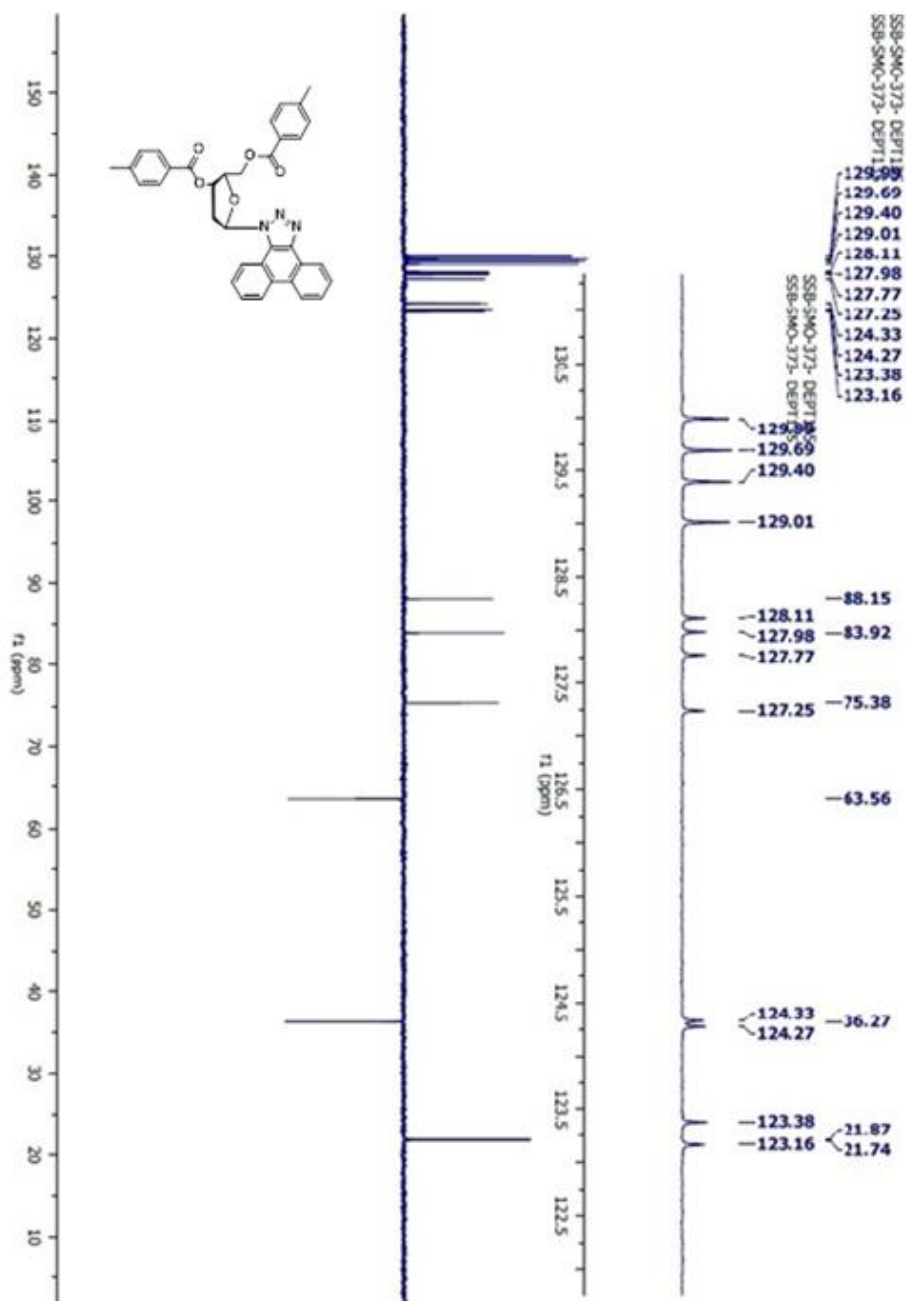
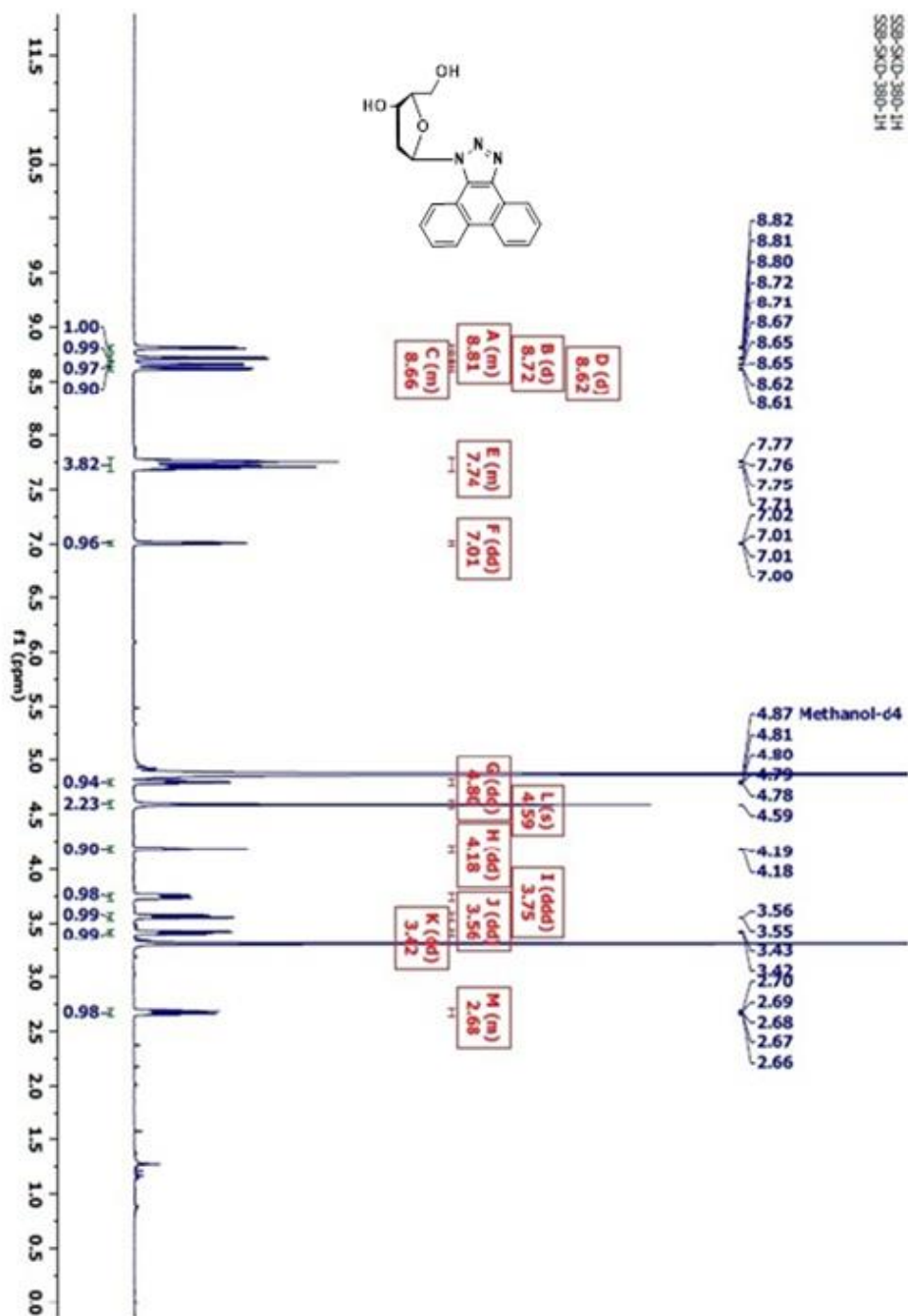


Figure 3.24: DEPT 135 of 3.61

Figure 3.25: ^1H NMR Spectra of 3.52

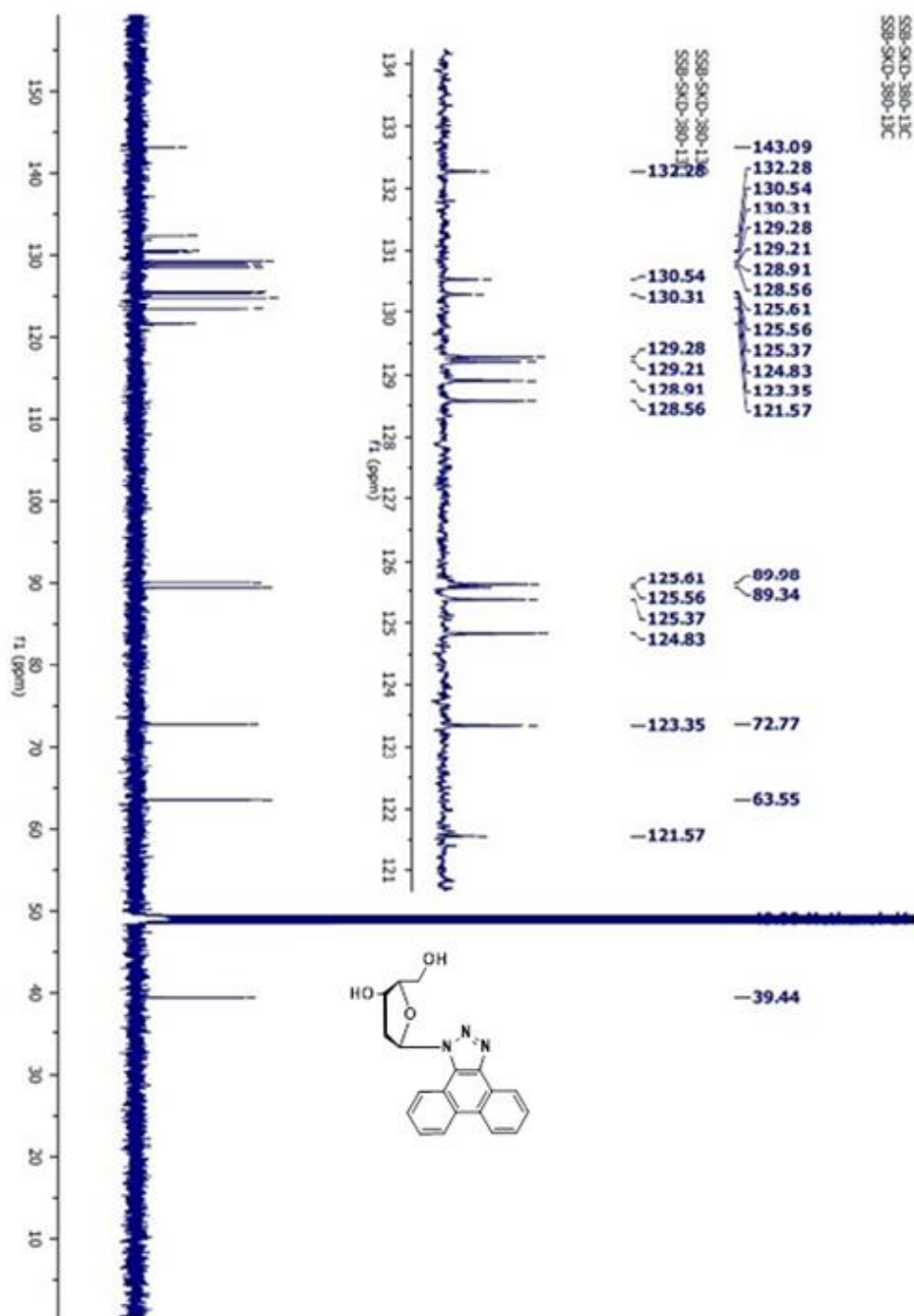


Figure 3.26: ^{13}C NMR Spectra of 3.52

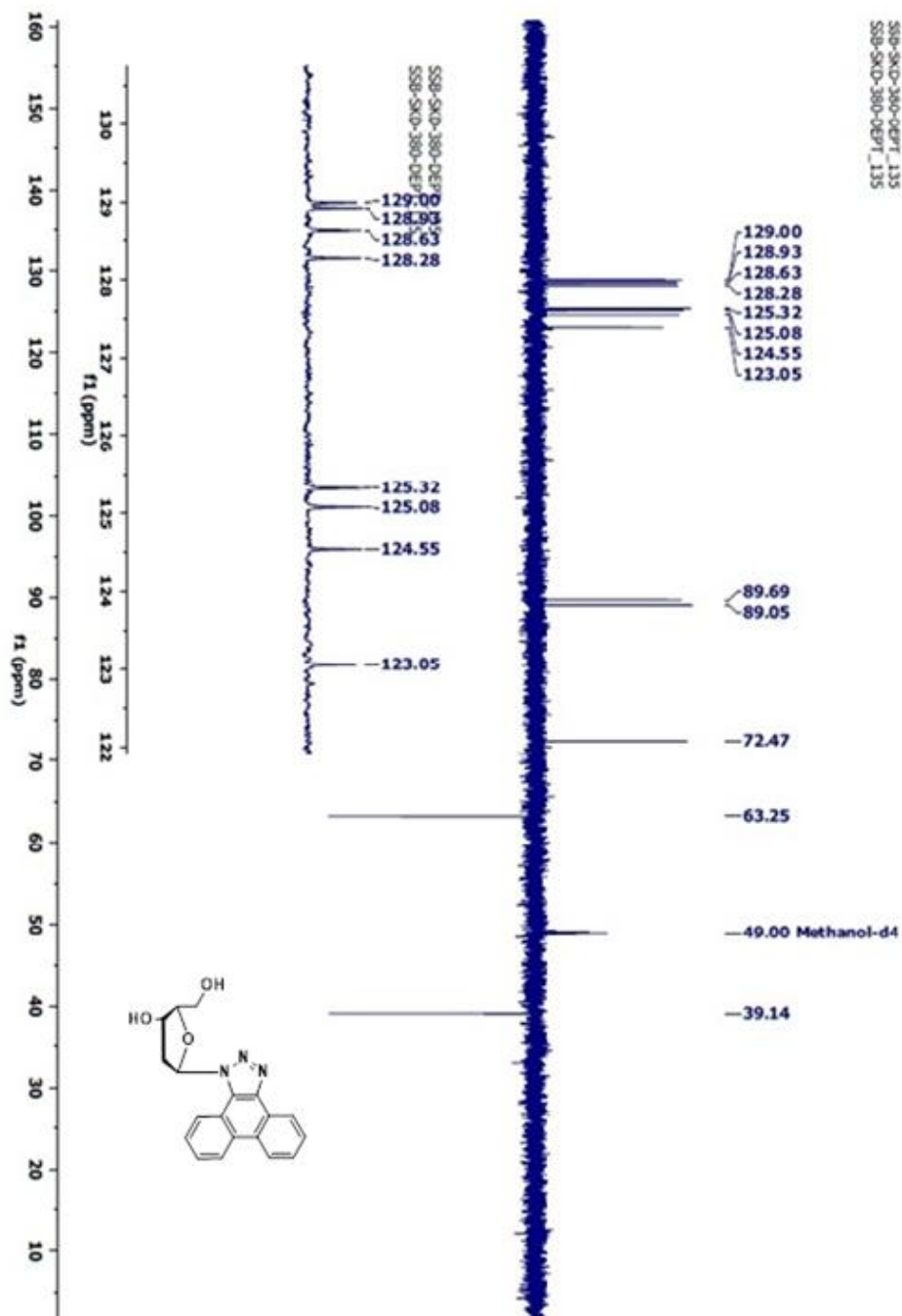


Figure 3.27: DEPT 135 of 3.52

3.11. Reference

1. Morales, J. C.; Kool, E. T. *Nat. Struct. Biol.* **1998**, 5 (11), 950.
2. McMinn, D. L.; Ogawa, A. K.; Wu, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, 121, 11585.
3. Ogawa, A. K.; Wu, Y.; McMinn, D. L.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, 122, 3274.
4. Wu, Y.; Ogawa, A. K.; Berger, M.; McMinn, D. L.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, 122, 7621.
5. Henry, A. A.; Olsen, A. G.; Matsuda, S.; Yu, C.; Geierstanger, B. H.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, 126, 6923.
6. Berger, M.; Luzzi, S. D.; Henry, A. A.; Romesberg, F. E. *J. Am. Chem. Soc.* **2002**, 124, 1222.
7. Leconte, A. M.; Matsuda, S.; Romesberg, F. E. *J. Am. Chem. Soc.* **2006**, 128, 6780.
8. Matsuda, S.; Leconte, A. M.; Romesberg, F. E. *J. Am. Chem. Soc.* **2007**, 129, 5551.
9. Matsuda, S.; Fillo, J. D.; Henry, A. A.; Rai, P.; Wilkens, S. J.; Dwyer, T. J.; Geierstanger, B. H.; Wemmer, D. E.; Schultz, P. G.; Spraggon, G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2007**, 129, 10466.
10. Leconte, A. M.; Hwang, G. T.; Matsuda, S.; Capek, P.; Hari, Y.; Romesberg, F. E. *J. Am. Chem. Soc.* **2008**, 130, 2336.
11. Seo, Y. J.; Hwang, G. T.; Ordoukhanian, P.; Romesberg, F. E. *J. Am. Chem. Soc.* **2009**, 131, 3246.
12. Malyshev, D. A.; Young, J. S.; Ordoukhanian, P.; Romesberg, F. E. *J. Am. Chem. Soc.* **2009**, 131, 14620.
13. Malyshev, D. A.; Pfaff, D. A.; Ippoliti, S. I.; Hwang, G. T.; Dwyer, T. J.; Romesberg, F. E. *Chem. - A Eur. J.* **2010**, 16, 12650.
14. Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, 59, 7238.
15. Guckian, K. M.; Morales, J. C.; Kool, E. T. *J. Org. Chem.* **1998**, 63, 9652.
16. Ren, R. X. F.; Chaudhuri, N. C.; Paris, P. L.; Rumney IV, S.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, 118, 7671.
17. (a) Strässler, C.; Davis, N. E.; Kool, E. T. *Helv. Chim. Acta* **1999**, 82, 2160. (b) Godde, F.; Tolume, J. J.; Moreau, S. *Biochemistry* **1998**, 37, 13765.

18. Mitsui, T.; Kitamura, A.; Kimoto, M.; To, T.; Sato, A.; Hirao, I.; Yokoyama, S. *J. Am. Chem. Soc.* **2003**, *125*, 5298.
19. Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc.* **1999**, *121*, 2323.
20. Hirao, I.; Kimoto, M.; Mitsui, T.; Fujiwara, T.; Kawai, R.; Sato, A.; Harada, Y.; Yokoyama, S. *Nat. Methods* **2006**, *3*, 729.
21. Hirao, I.; Mitsui, T.; Kimoto, M.; Yokoyama, S. *J. Am. Chem. Soc.* **2007**, *129*, 15549.
22. Kimoto, M.; Kawai, R.; Mitsui, T.; Yokoyama, S.; Hirao, I. *Nucleic Acids Res.* **2009**, *37*, e14.
23. (a) Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863. (b) Matray, T. J.; Kool, E. T. *J. Am. Chem. Soc.*, **1998**, *120*, 6191. (c) Matray, T. J.; Kool, E. T. *Nature*, **1999**, *399*, 704.
24. (a) McMinn, D. L.; Ogawa, A. K.; Wu, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, *121*, 11585. (b) Tae, E. L.; Wu, Y.; Xia, G.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2001**, *123*, 7439.
25. (a) Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714. (b) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212.
26. (a) Mitsui, T.; Kitamura, A.; Kimoto, M.; To, T.; Sato, A.; Hirao, I.; Yokoyama, S. *J. Am. Chem. Soc.* **2003**, *125*, 5298. (b) Kool, E. T. *Acc. Chem. Res.*, 2002, *35*, 936 and references therein. (c) Harcourt, E. M.; Kool, E. T.; Chapter 1 : Designer bases, base pairs, and genetic sets: biochemical and biological activity, in *Synthetic Biology* **2014**, *1*, 1-30.
27. Minakawa, N.; Kojima, N.; Hikishima, S.; Sasaki, T.; Kiyosue, A.; Atsumi, N.; Ueno, Y.; Matsuda, A. *J. Am. Chem. Soc.*, **2003**, *125*, 9970.
28. (a) Wojciechowski, F.; Leumann, C. J. *Chem. Soc. Rev.* **2011**, *40*, 5669. (b) Grigorenko, N. A.; Leumann, C. J.; *Chem.-Eur. J.* **2009**, *15*, 639. (c) Wilson, J. N.; Teo, Y. N.; Kool, E. T. *J. Am. Chem. Soc.* **2007**, *129*, 15426. (d) Robertson, N.; McGowan, C. A. *Chem. Soc. Rev.*, **2003**, *32*, 96. (e) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yoseph, G.; Sivan, U.; Braun, E. *Science* **2002**, *297*, 72. (f) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731.

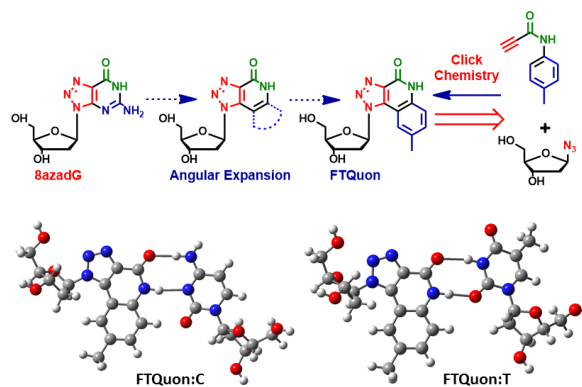
29. (a) Stambasky, J.; Hocek, M.; Kouovsky, P. *Chem. Rev.* **2009**, *109*, 6729 (b) E. T. Kool, *Acc. Chem. Res.*, 2002, *35*, 936. (c) Y. Tor, P. B. Dervan, *J. Am. Chem. Soc.*, 1993, *115*, 4461. (d) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem., Int. Ed.*, 2007, *46*, 6226.
30. Bag, S. S.; Talukdar, S.; Matsumoto, K.; Kundu, R. *J. Org. Chem.* **2013**, *78*, 278.
31. (a) Guckian, K. M.; Schweitzer, B. A.; Ren, R. X.-F.; Sheils, C. J.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.*, **2000**, *122*, 2213. (b) Kool, E. T. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 1.
32. (a) Scopes, D. I. C.; Barrio, J. R.; Leonard, N. J. *Science* 1977, **195**, 296. (b) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128. (c) Lessor, R. A.; Gibson, K. J.; Leonard, N. J. *Biochemistry* **1984**, *23*, 3868. (d) Stevenson, T. M.; Leonard, N. J. *J. Org. Chem.* **1984**, *49*, 2158. (e) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128. (f) Leonard, N. J.; Scopes, D. I. C.; Van der Lijn, P.; Barrio, J. R. *Biochemistry* **1978**, *17*, 3677.
33. (a) Liu, H.; Gao, J.; Lynch, S. R.; Saito, Y. D.; Maynard, L.; Kool, E. T. *Science* **2003**, *302*, 868. (b) Lu, H.; He, K.; Kool, E. T. *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 5834. (c) Lee, A. H.; Kool, E. T. *J. Org. Chem.* **2005**, *70*, 132. (d) Lu, H.; Lynch, S. R.; Lee, A. H.; Kool, E. T. *ChemBioChem.* **2009**, *10*, 2530. (e) Lee, A. H. F.; Kool, E. T. *J. Am. Chem. Soc.* **2005**, *127*, 3332. (f) Liu, H.; Gao, J.; Kool, E. T. *J. Org. Chem.* **2005**, *70*, 639. (g) Krueger, A. T.; Lu, H.; Lee, A. H. F.; Kool, E. T. *Acc. Chem. Res.* **2007**, *40*, 141. (h) Liu, H.; Lynch, S. R.; Kool, E. T.; *J. Am. Chem. Soc.* **2004**, *126*, 6900. (i) Liu, H.; Gao, J.; Kool, E. T. *J. Am. Chem. Soc.* **2005**, *127*, 1396.
34. (a) Krueger, A. T.; Kool, E. T. *J. Am. Chem. Soc.* **2008**, *130*, 3989. (b) Winnacker, M.; Kool, E. T. *Angew. Chem., Int. Ed. Engl.* **2013**, *52*, 12498.
35. (a) Fuentes-Cabrera, M.; Zhao, X.; Kent, P. R. C.; Sumpter, B. G. *J. Phys. Chem. B* **2007**, *111*, 9057. (b) Migliore, A.; Corni, S.; Varsano, D.; Klein, M. L.; Di, F. R. *J. Phys. Chem. B* **2009**, *113*, 9402. (c) Blas, J. R.; Huertas, O.; Tabares, C.; Sumpter, B. G.; Fuentes-Cabrera, M.; Orozco, M.; Ordejon, P.; Luque, F. J. *J. Phys. Chem. A* **2011**, *115*, 11344.
36. (a) Jarchow-Choy, S. K.; Krueger, A. T.; Liu, H.; Gao, J.; Kool, E. T. *Nucleic Acids Res.* **2011**, *39*, 1586. (b) Fuentes-Cabrera, M.; Sumpter, B. G.; Lipkowski, P.; Wells, J. C. *J. Phys. Chem. B*, **2006**, *110*, 6379. (c) Fuentes-Cabrera, M.; Sumpter, B. G.; Wells, J. C. *J. Phys. Chem. B* **2005**, *109*, 21135. (d) Fuentes-Cabrera, M.; Lipkowski, P.; Huertas, O.; Sumpter, B. G.; Orozco, M.; Luque, F. J.; Wells, J. C.; Leszczynski, J. *Int. J. Quantum Chem.* **2006**, *106*, 2339.
37. (a) Chelliserrykattil, J.; Lu, H.; Lee, A. H.; Kool, E. T. *ChemBioChem.* **2008**, *9*, 2976. (b) Krueger, A. T.; Peterson, L. W.; Chelliserry, J.; Kleinbaum, D. J.; Kool, E. T. *J. Am. Chem. Soc.* **2011**, *133*, 18447. (c) Delaney, J. C.; Gao, J.; Liu, H.; Shrivastav, N.; Essigmann, J. M.; Kool, E. T. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 4524.

Chapter 3

38. (a) Wierzchowski, J. *Curr. Top. Biophys.* **2010**, 33, 9 (b) Wierzchowski, J.; Antosiewicz, J. M.; Shugar, D. *Mol. Biosyst.* **2014**, 10, 2756. (c) Lim, F. P. L.; Dolzhenko, A. V. *Eur. J. Med. Chem.* **2014**, 85, 371e390.
39. Bag, S. S.; Talukdar, S.; Anjali, S. J. *Bioorg. Med. Chem. Lett.* **2016**, 26, 2044.
40. Bag, S. S.; Talukdar, S.; Das, S. K.; Pradhan, M. K.; Mukherjee, S. *Org. Biomol. Chem.* **2016**, 14, 5088.
41. M. J. Frisch, *et al.* Gaussian 09, revision A.1, Gaussian, Inc., Wallingford, CT, 2009.
42. Kim, T. W.; Kool, E. T. *J. Org. Chem.* **2005**, 70, 2048.
43. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, 43, 3714.
44. (a) Yamaji, M.; Maeda, H.; Nanai Y.; Mizuno, K. *ISRN Phys. Chem.* **2012**, 1. (b) Khorev, O.; Bosch, C. D.; Probst, M.; Haner, R. *Chem. Sci.* **2014**, 5, 1506.
45. MacroModel, Version 9.9 Schrodinger, LLC, New York, NY, 2012.
46. (a) Zhao, Y.; Schultz, N. E.; Truhlar, D. G.; *J. Chem. Theory Comput.* **2006**, 2, 364. (b) Zhao, Y.; Truhlar, D. G. *Acc. Chem. Res.* **2008**, 41, 157. (c) Zhao, Y.; Truhlar, D. G.; *J. Chem. Phys.* **2006**, 125, 194101. (d) Zhao, Y.; Truhlar, D. G. *J. Chem. Theory Comput.* **2007**, 3, 289.

Chapter 4

DESIGN, SYNTHESIS AND PHOTOPHYSICAL PROPERTIES OF FUSED TRIAZOLYL 2-QUINOLINONE NUCLEOSIDE



4.1. Introduction

A:T and **G:C** are the two base pairs that are provided by nature in constructing a DNA helix where the backbone of the DNA extended as usually by phosphodiester linkage. Now the whole DNA is nothing but the sequential arrangement of **A:T** and **G:C** base pairs in different combinations. This different pattern of the two base pair in constructing DNA generally includes different characteristic into a gene. Apart from these two combinations there are no other combinations available within DNA. So the genetic alphabet is restricted to number 4 i.e. **A**, **T**, **G** and **C**. From a top down level of approach we can conclude easily that the sequential arrangement of nature provided **A**, **T**, **G** and **C** are the key of the genetic information that are encoded to a gene. With the variation of these sequential arrangement of these base pairs, the encoded information in a gene will be changed quickly. So according to that, knowing a gene sequence it will be easy to synthesize particular targeted ODN. It is possible to successfully synthesize a pair of nucleosides that could pair up via hydrogen bonding between them like the natural base pairs and then they might be successfully replicated and transcribed. With that bottom up view chemist have started mimicking hydrogen bonding pattern of the natural base pairs and tried to construct base pairs to increase the number of genetic alphabet. Benner was the first person who took the responsibility and put the first step to show the scientific community how modified nucleobase pairs **isoG:isoC** developed by A. Rich¹ behave within DNA.² He claimed to extend genetic alphabet up to number 6 by this **isoG:isoC** base pair (**Figure 4.1e**).⁴ Although the evaluation of the Benner's developed genetic alphabet suffers a lot due to tautomerisation problem but surprisingly his **P:Z** pair¹⁸ (**Figure 4.1i**) was successfully recognized by DNA polymerase I (*E Coli*) and then it was possible to be further amplified in PCR. Not only that after successful translation the synthesized **mRNA** contains nonstandard nucleoside and with the help of Ribosome the **mRNA** further produced protein that contains nonstandard amino acid.¹⁸ Benner's work inspired others to synthesis more base pairs to put further effort towards increasing genetic alphabet. Today the science is not restricted up to double or triple hydrogen bonded Watson-Crick base pairs, it was touched to four hydrogen bonded base pairs by Minakawa and Mastuda *et al.*^{24,25} Apart from that, size expanded base pairs were developed by Kool group where they looked towards expanding the aromatic ring size by keeping a pattern of hydrogen bonding to make them a stable pair within DNA helix and thereby installing also fluorescence properties within these multichromophoric systems.²⁰⁻²²

4.2. Extension of genetic alphabet

The first example of unnatural base pair was introduced by Benner's group by designing different hydrogen bonding pattern by mimicking Watson-Crick hydrogen bonding pattern (**Figure 4.1**). In the year 1989 Benner's group experimentally proved that **isoG** **4.05** and **isoC** **4.06** unnatural base pair first developed by A. Rich¹ can be possible to successfully incorporate into DNA and RNA and they have shown how it is possible to replicate them and translated them.² Then xanthine(**X**) and 2,6-diaminopyrimidine (**κ**) were introduced by them for further extension of the concept (**Figure 4.1a-f**).³ In the year 1992 a new codon-anticodon **isoG-isoC** (**Figure 4.1e**) pair was reported by them which was then in vitro translated to a peptide through Ribosome to

synthesis a nonstandard protein containing nonstandard amino acid through the synthesis of **mRNA** which contains nonstandard unnatural nucleoside.⁴

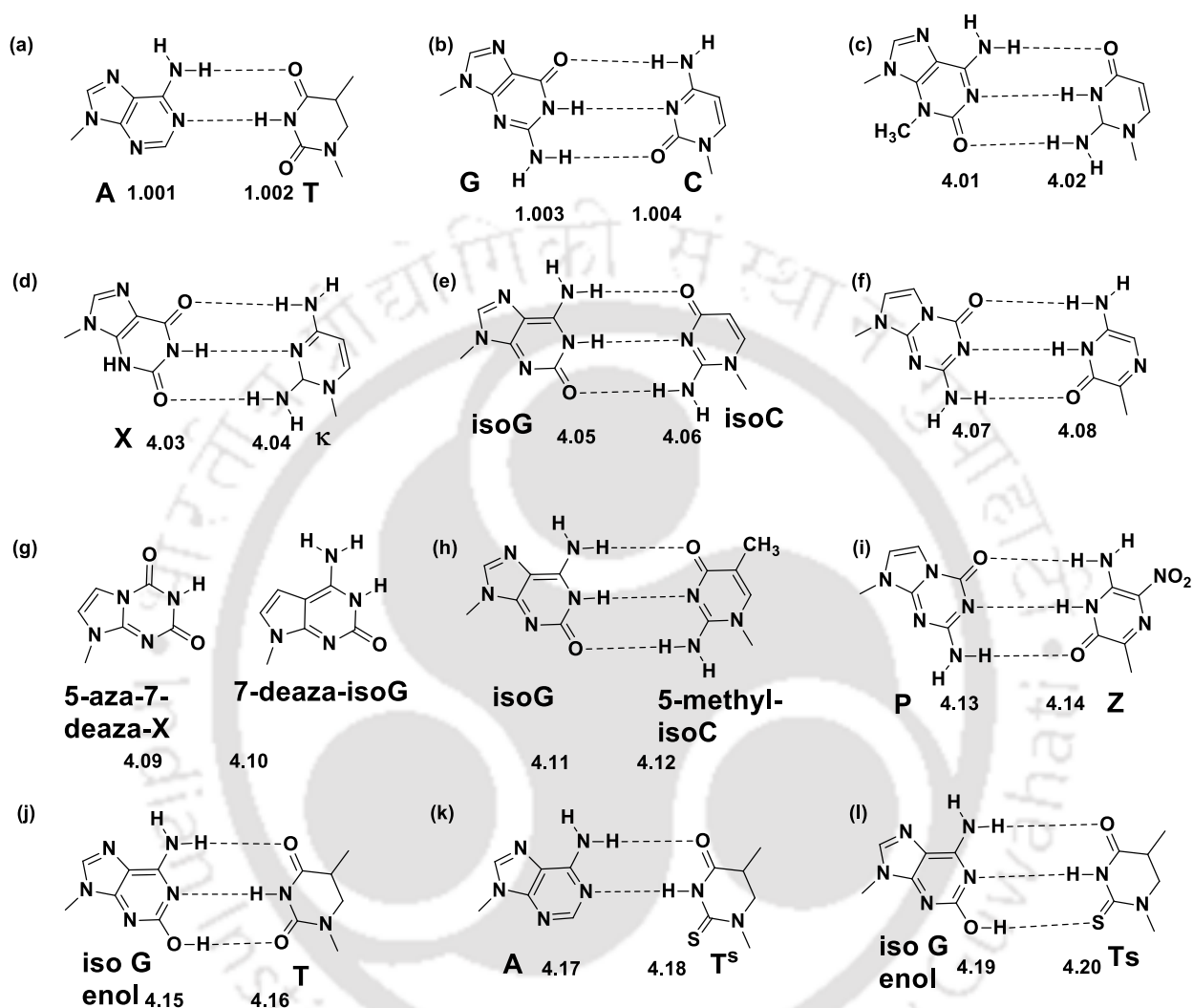


Figure 4.1: Benner's developed hydrogen bonded base pairs.

However, this exceptional work pioneered by Benner was suffered due to having some of the significant short comings because of base pairing. Instead of proton acceptor residues, **isoC**, **κ** and **X** unnatural nucleobases contained proton-donor amino or imino groups which were incorporated into DNA enzymatically with low efficiency.^{5,6} **X** is anionic in physiological condition ($pK_a \sim 5.6$),⁷ leading to destabilize DNA duplex.⁸ This problem was tackled by developing and introducing a new unnatural nucleobase, 5-aza-7-deazaxanthine (**Figure 4.1g**).⁹ The third problem was the chemical instability of **isoC** (**Figure 4.1e**) and pyrazine-scaffold-base (**Figure 4.1f**) nucleosides.^{8,2} Subsequent investigation revealed that the substitution at 5-position

such as **5-methyl-isoC** (**Figure 4.1h**), is leading to enhance stability.^{10,11} Lastly the major drawback in **isoG** was the keto-enol tautomerisation under physiological pH condition.^{5,12} In an aqueous solution 10% of the enol tautomer of **isoG** was present which paired with **T(U)**¹³ was the reason of low fidelity of **isoG-isoC** base pair and during PCR amplification it was mutated to **A-T** pair.

The problem was solved by Benner's group by employing two strategies. The first strategy was favoring the keto form over enol form (ca. 1000:1) of **isoG** which was done by employing **7-deaza-isoG** (**Figure 4.1g**),¹³ and the next one is employing a replacement of oxygen of the keto group of thymine by sulphur, thus representing the thymine analogue **2-thio-T(T^s)** instead of **T** (**Figure 4.1k**).¹⁴ The first variation is dominating keto form over enol form of **isoG** was supported by second variation, 2-thione group of sulphur substituted thymine analogue reducing its hydrogen bonding ability in turn decreasing the pairing with enol form of **isoG** leading to predominating keto form of **isoG** analogue (**Figure 4.1l**). **isoG-5-methyl-isoC**, **A-T_s**, and **G-C** are the three types of base pairs by the use of which the selectivity of unnatural base pairs reached up to 98% by *TitaniumTaq* DNA polymerase in PCR. The most selective latest unnatural base pairs are formed between **P** (2-aminoimidazo[1,2-*a*]-1,3,5-triazin-4(8*H*)-one) and **Z** (6-amino-5-nitro-2(1*H*)-pyridone) (**Figure 4.1i**).^{15,16,17} Both the bases contained proton acceptor sites in the minor groove region to enhance their interaction with DNA polymerase. The newly developed **P** and **Z** bases are completely free from any chemical instability and tautomerisation problem. They have recently reported their most successful **P-Z** pair which shows high fidelity in PCR amplification by using Taq DNA polymerase.¹⁸

4.3. Size expanded DNA

Leonard was the pioneer to introduce the concept of size expansion in synthetic nucleosides where a benzene ring was introduced in between the two rings i.e. one five membered and one six membered ring of adenine and thus the size of the modified adenine was expanded.¹⁹ This unnatural nucleoside was employed as a substrate for many ATP dependent enzymes in order for probing the active sites of enzyme. However, the size expansion could not be explored by incorporating into DNA due to not having been invented DNA synthesizer. Although it was predicted by Leonard that the stretched adenine because of having large size compare to the natural adenine turned out to be the reason of destabilization within a natural DNA. The concept of size expansion was further extended by E. T. Kool by introducing **xDNA** and **yDNA**.

4.3.1. Benzo fused size expanded DNA bases: xDNA and yDNA

Kool's group was the first to introduce benzo fused size expanded DNA and incorporated it to the DNA helix through automated DNA synthesizer. It was found that after the incorporation of size expanded nucleoside the regular helicity of the DNA was still retained although the diameter was expanded.²⁰ The size of the natural nucleoside was expanded by **2.4 Å** due to the fusion of a benzene ring with parallel extension vectors. Theoretical modeling as well as experimental evidence revealed that the size expansion does not need to adjust bond angle in a

larger extent to sugar conformation to adjust the expansion of the nucleobase within the DNA helix. However, the size expanded DNA differs from the natural B-DNA by having larger number of base pairs per turn creating larger outer edge in comparison with natural B-DNA. The theoretical modeling suggested that a number of 14 base pair could be existed per turn in the size expanded DNA whereas it is 10.5 per turn for the natural B-DNA.

While natural DNA composed of 4 components i.e. **A**, **T**, **G** and **C** but the size expanded DNA composed of 8 components where there are 4 **xDNAs** bringing up the linear expansion and 4 **yDNAs** bringing up the angular expansion. In **xDNA** the number of combination made by Kool's group are 4. They are two size expanded purine with natural pyrimidine and the another two size expanded pyrimidine with natural purine (**Figure 4.2a-d**).

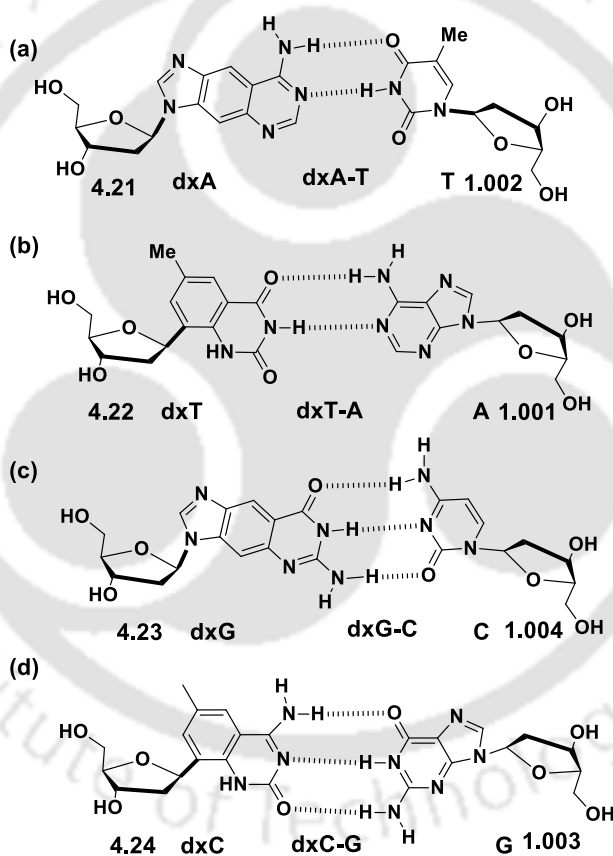


Figure 4.2: Structures and H-bonding pattern of **x-DNA** bases developed by Kool's group.

While linear expansion produced 4 combinations, the same number of combinations were also possible for angular expansion in **yDNA** (**Figure 4.3a-d**).²¹ The angularly expanded

pyrimidines make a pair with natural purine and thus forming two combinations. Then the further combinations were made within natural pyrimidine and angularly expanded purine and thus they formed again two combinations. So total 4 combinations for angular expansion and another 4 combinations for linear expansion made over all 8 combinations of size expanded base pairs.

The size expansion was found to be the cause of DNA backbone distortion. For the linear expansion the amount of distortion was found to be from 0.3 to 1.7 kcal/mol whereas for angular expansion the distortion parameter ranged from 0.6 kcal/mol and it is up to 2.2 kcal/mol, somewhat higher than the linear expansion. Although it might appear that either linear or angular expansion is the cause of DNA backbone distortion but indeed they formed a stable sequence selective double helix.²¹

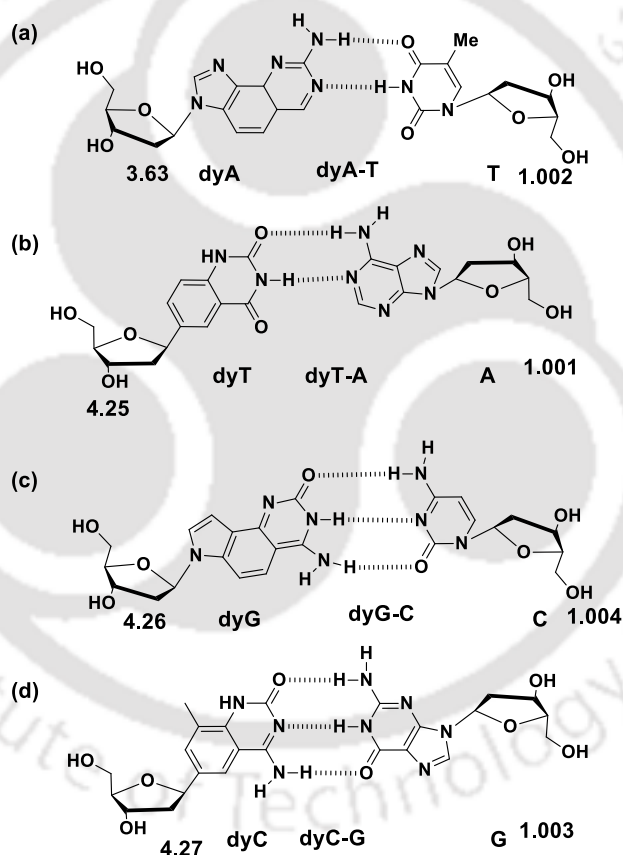


Figure 4.3: Structures and H-bonding pattern of **y-DNA** bases developed by Kool's group.

4.3.2. Naptho fused size expanded DNA bases

The another widened base pair was introduced by Kool's group. They used naphthalene fusion strategy to widen the nucleobase more compare to the previous benzo expansion. **dydT 4.28** and **dydC 4.29** thus were synthesized and incorporated into DNA duplex (**Figure 4.4**). It was found that naphthohomologues are **4.8 Å** widened as compare to their natural analogues. The expansion of size by introducing further benzene led them to exhibit modulated photophysical properties. The another interesting feature of the doubly widened **yyDNAs** is their complex denaturation behavior suggesting strong intrastand stacking with the complementary bases.²²

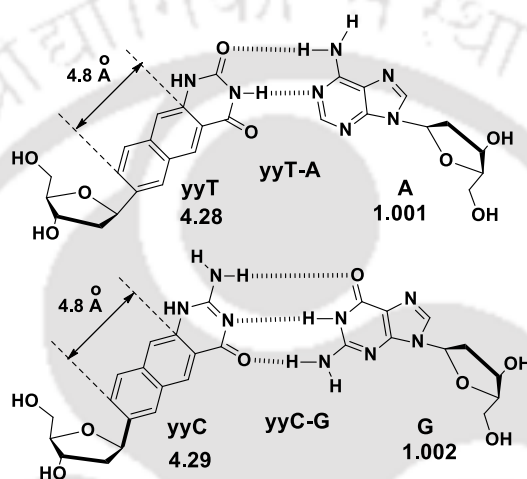


Figure 4.4: Structures and H-bonding pattern of naphthowidened bases developed by Kool's group.

4.3.3. Ethynyl extended DNA bases: Inoyue's base pair

Two base pair were designed and developed by Inoyue *et al.* which were appeared to form similar types of hydrogen bonds comparable with Watson-Crick hydrogen bonds, whenever incorporated into DNA and moreover they form right handed duplex with comparable stability as that of natural one (**Figure 4.5**).²³

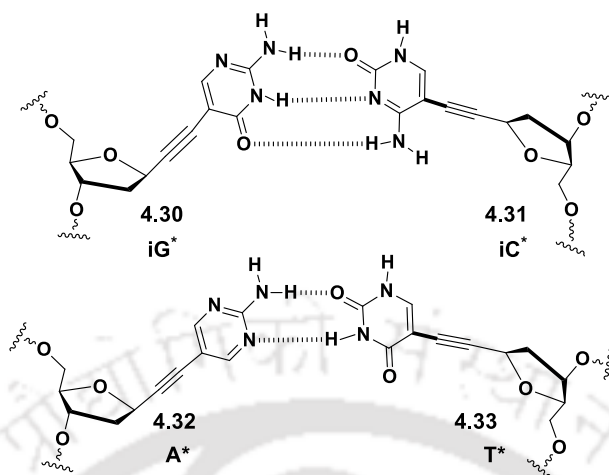


Figure 4.5: Structures and H-bonding pattern of ethynyl extended base pairs developed by Inoyue.

4.3.4. Unnatural base pairs with four hydrogen bonding

Minakawa and Matsuda *et al.* have reported two base pairs which are comparable to form four hydrogen bonds (**Figure 4.6**). Thus, these base pairs provide extra stability to the duplex DNA by +8-9 °C. These base pairs have successfully recognized and extended by DNA polymerase I (*E. coli*).²⁴⁻²⁵

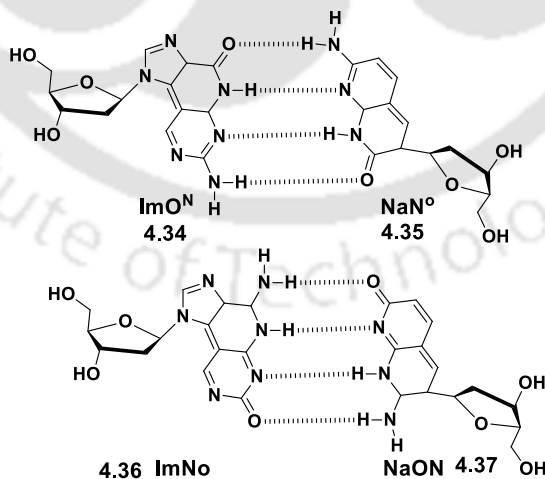


Figure 4.6: Structures and four hydrogen bonded base pairs developed by Minakawa and Mastuda.

4.3.5. 8azaG:C pair

Just like Guanine, **8-azaguanine 4.39** also capable to form three hydrogen bonds with cytosine as expected from the same Watson-Crick hydrogen bonding pattern. Replacement of **G** by **8azaG** does not alter the melting temperature within DNA duplex. Moreover, it is evidenced from the study that π -stacking and hydrogen bonding also remained intact and the DNA helicity is not disrupted (**Figure 4.7**).²⁶

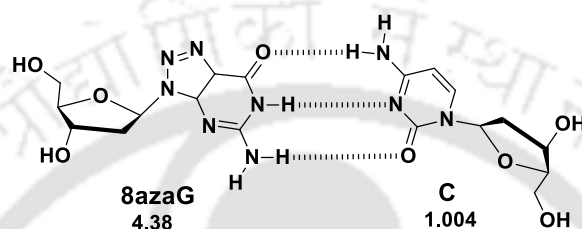


Figure 4.7: 8azaG:C hydrogen bonding pattern.

4.4. Background

The attractive forces like H-bonding, aromatic stacking, hydrophobic or CH- π interactions between the unnatural nucleobases and isomorphous nucleobase design has been explored in details in the context of unnatural nucleobase design. Several studies in DNA reflected that in the design of isomorphous base, the structural modifications of purines in the five-membered ring, especially of 2'-deoxyisoguanosine are tolerated as long as the Watson-Crick face of the nucleoside is not perturbed. With this line several analogs have been reported utilizing glycosylation strategy and their photophysical and biophysical properties in the context of DNA have been evaluated. However, till the date, in the design of isomorphous purines, the concept of size expansion or widening the shape in an approach taken by Kool *et al.*, which might provide several advantages of more effective π -stacking or arresting the inherent tautomerisation, were not considered. Furthermore, unnatural bases with the above ability and novel chemical functionalities expectedly would expand the functional potential of DNA. Such an expanded genetic alphabet with increased information might eventually be capable of expanding the genetic code of a living organism.

4.5. Objective

As a part of our ongoing research effort toward generation of molecules with tuned photophysical properties *via* click reaction and the recent development of click chemistry derived triazolyl-N/C-nucleosides containing donor/acceptor aromatics, widened aryl fused hydrophobic triazolyl N-nucleosides, we thought that it would be worthwhile to synthesize aryl fused triazolyl N-nucleoside with H-bonding ability and widened shape with strong stacking ability which

expectedly could produce modulated photophysical response into the unnatural nucleosides (UNNs) and render strong π -stacking interaction inside DNA if incorporated.

The logic behind our choice of triazole fused H-bonded nucleobase is: (a) the nucleoside would be the widened version of isomorphous base analogue of 8-azaguanosine, (b) Fusion of **C2-N3** bond by benzene would convert it with ability to form 2-H-bonds keeping the tautomeric **>CO-NH** part intact which would possibly show interesting photophysical property such as pH and/or microenvironment sensitive fluorescence emission, (c) the vertically (60°) widened fused benzene expectedly would provide more stacking interaction with possibly widening the groove size, (d) it could form H-bonding pair with **dT/dU** or **dC** as well as render good stacking stabilization of DNA if incorporated, (e) the microenvironment sensitive (H-bonding network, solvent polarity, isotope substitution, pH, even buffer concentration) intrinsic fluorescence properties of such nucleoside could especially be useful for biophysical research in the context of DNA, DNA-protein interactions and to study the mode of interaction with the enzyme. The interesting photophysical outcome could be utilized in studying biophysical interactions with protein either in the base state or in a DNA, (f) fused triazolyl isosteres can also improve charge transfer interaction properties if incorporated into a DNA.

Utilizing this concept, we framed our objective as below:

- (a) Design and synthesis of fused triazolyl 2-quinolinone (**FTQuon**) nucleoside with two H-bonding face-one H-bond acceptor and another donor via click chemistry
- (b) Study the photophysical properties.
- (c) Theoretical study in the context of DNA
- (d) Study of interaction with BSA

4.6. Results and Discussion

4.6.1. Design and synthesis of fused triazolyl 2-quinolinone (FTQuon)

As revealed from the early study **8azaG** can be a good replacement of **G** and indeed incorporation of **8azaG** in place of **G** doesn't disrupt the DNA helix by keeping the melting temperature unaffected, so we thought to angularly expand **8azaG** just like Kool's angularly widened **dyA 3.63** and **dyG 4.26**.²¹ The design concept is explained in **Figure 4.8**. The expansion of **8azaG** was designed in such a way that we can block the amine group so that the hydrogen bond made by this amine group can be replaced with a hydrophobic benzene moiety to explore the effect of hydrophobicity if incorporated into a short oligonucleotide against cytosine and thymine. As amide moiety in **8azaG** remained unaffected with hydrophobic benzene expansion so according to our expectations it can be still paired with **C/T**.

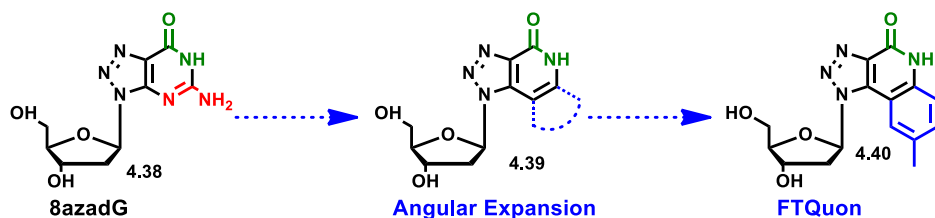
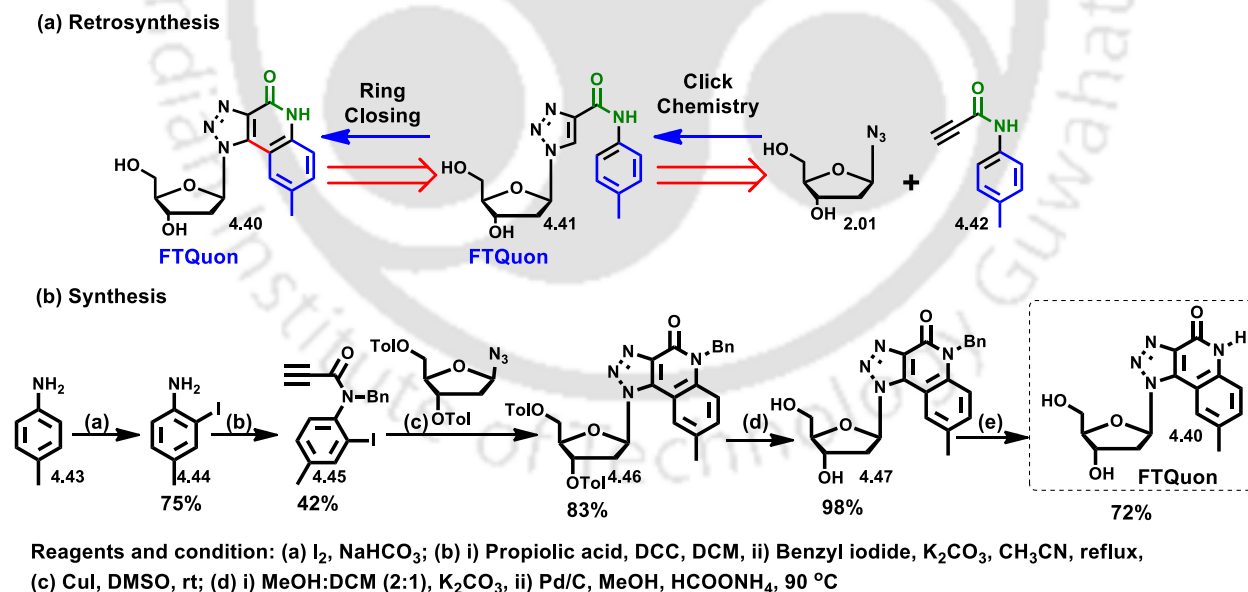


Figure 4.8: Design concept of **FTQuon**.

A retrosynthetic analysis suggested that click chemistry between β -azido-deoxyriboside **2.01** and *N*-(*p*-tolyl)propiolamide **4.43** followed by ring closing are the two key steps to achieve our targeted **FTQuon 4.41**. β -azido-deoxyriboside **2.01** synthesized by following our earlier work.²⁷ The alkyne partner *N*-(*p*-tolyl)propiolamide **4.43** was synthesized from *o*-iodo-*p*-toluidine **4.44** as shown in the **Scheme 4.1**. Finally, click reaction between β -azido-deoxyriboside **2.01** and *N*-(*p*-tolyl)propiolamide **4.43** followed by intramolecular C-C coupling afforded the bistoluoyl-protected nucleoside in 83% yield. Toluoyl deprotection followed by deprotection of *N*-benzyl yielded the desired fused triazolyl nucleoside **FTQuon** (**Scheme 4.1**).



Scheme 4.1: (a) Retrosynthesis analysis of **FTQuon**. (b) Synthesis of **FTQuon**.

4.6.2. Structural Characterization

The β -configuration of **FTQuon** was confirmed from the NOESY spectra (**Figure 4.9**). It revealed that there is no interaction between $1'H$ - $3'H$ and $1'H$ - $5'H$. Whereas there is interaction between $3'H$ - $5'H$, $3'H$ - $2'H$ and there is another interaction between $1'H$ and the **aromatic hydrogen** of **FTQuon** as shown in the **Figure 4.9**. All these interactions suggested the β -configuration of **FTQuon**.

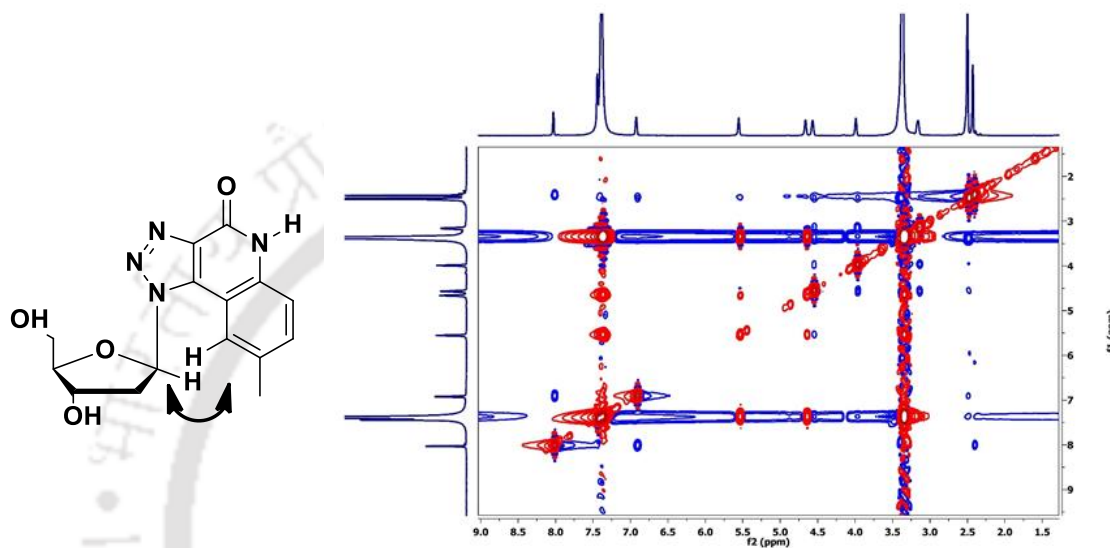


Figure 4.9: NOESY interaction of FTQuon

4.6.3. Study of photophysical properties

All the UV-visible spectra were recorded with a 10 μ M sample concentration at 25 $^{\circ}$ C with a cell of 1cm path length and 1nm slit width. The same 10 μ M sample was used to measure its fluorescence emission property with a cell of 1 cm path length and 3nm emission/excitation slit width at 25 $^{\circ}$ C. The nucleoside **FTQuon** is showed an interesting UV-Visible absorption spectra as the solvent polarity increased from dioxane to buffer. In dioxane, chloroform and ethyl acetate it showed structureless absorption maxima at 315 nm. But as the solvent polarity increased the structurless absorption band changed to structured absorption band and in buffer it was blue shifted by 14 nm. In buffer two bands appeared with slight hyperchromicity, one at 300 nm and another at 313 nm. The absorption spectra of **FTQuon** in acidic environment exhibited a structurless absorption band at 315 nm. In basic environment it showed structureless absorption band at 325 nm with hyperchromic effect (**Figure 4.10a**). Upon excitation at 250-320 nm **FTQuon** exhibited structureless emission spectra in different solvent. In dioxane structureless emission band was appeared at 374 nm. In methanol a large amount of hyperchromicity with a slight (4 nm) blue shift was observed and the emission band appeared at 370 nm. On the other hand, in buffer a

hypochromic effect was observed at 428 nm. In acidic medium structured emission spectra was appeared at 358 nm and 426 nm. In basic medium the fluorescent intensity of the emission band was further decreased and it was further red shifted and appeared at 448 nm as a structureless emission band (**Figure 4.10b**).

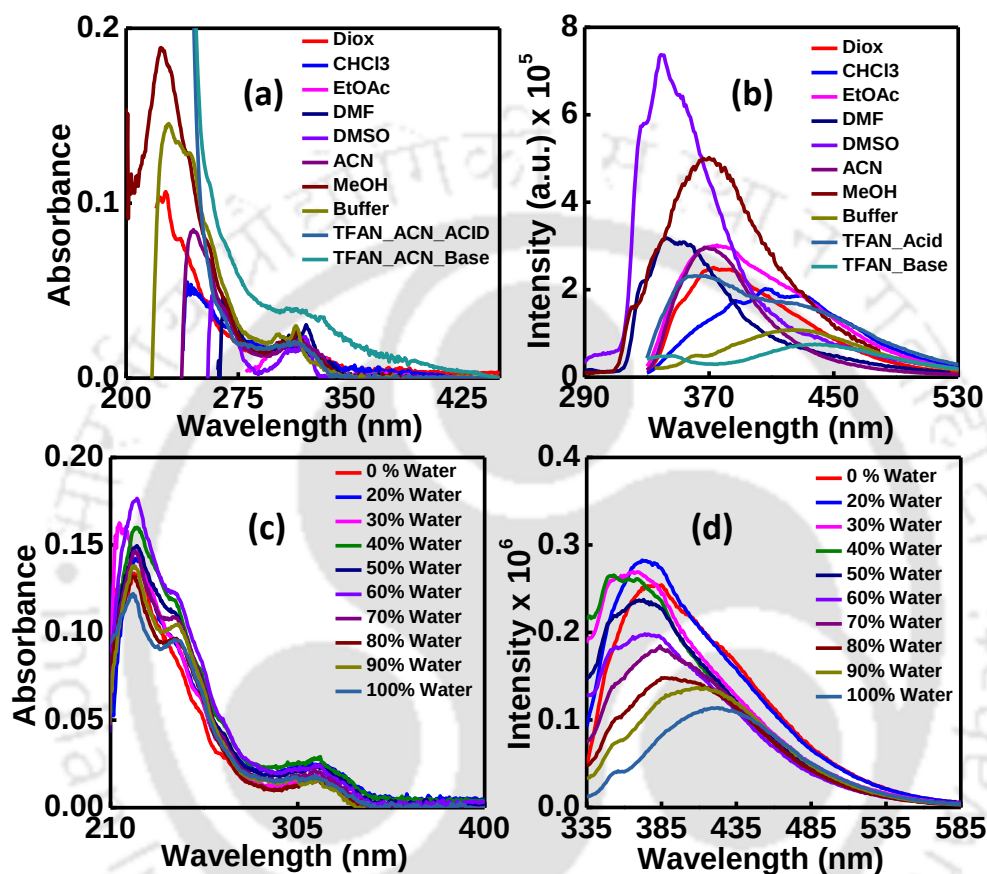


Figure 4.10: UV-visible and fluorescence spectra of the fused triazolyl 2-quinolinone nucleoside (**FTQuon**) (a-b) in various organic solvents (c-d) in different concentration of water and dioxane (Nucleoside concentration = 10 μ M).

In dioxane **FTQuon** showed structured absorption band at 243 and 316 nm. With increasing the volume of water a slight hyperchromic shift at 243 nm was observed (**Figure 4.10c**). Upon excitation at 330 nm the emission band in dioxane was appeared at 380 nm. Increase of the volume of water exhibited a hypochromicity in the emission maxima and with a red shifted of 42 nm in 100% water (**Figure 4.10d**).

Next we have studied photophysical properties of **FTQuon** in the solution of different pH. **FTQuon** exhibited a structured short wavelength absorption band at 250 nm and a long

wavelength absorption band at 320 nm. At pH 12 **FTQuon** exhibited absorption at 290 nm and 242 nm indicating a tautomerization (**Figure 4.11a**).

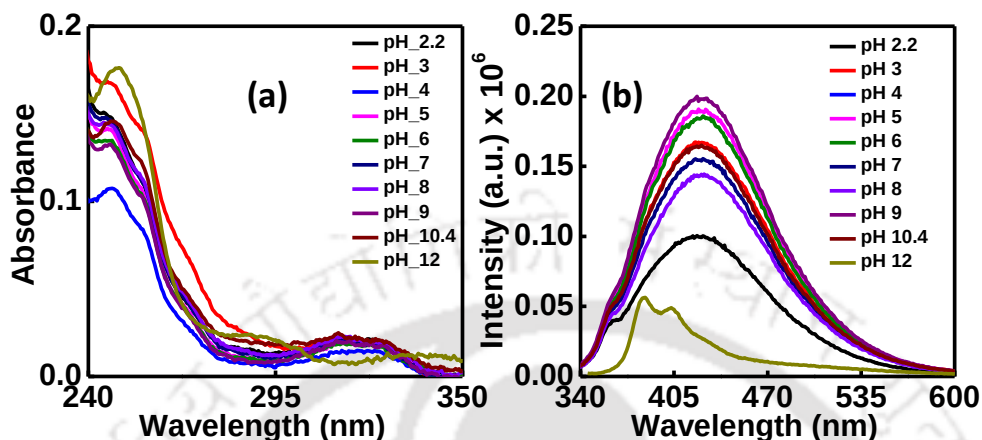


Figure 4.11: (a) UV-visible and (b) fluorescence spectra of the fused triazolyl 2-quinolinone nucleoside (**FTQuon**) in different pH (Nucleoside concentration = 10 μ M).

Upon excitation at 335 nm **FTQuon** exhibited structureless absorption band at 423 nm in the pH ranging from 2.2 to 10.4 nm. As the pH increased, a hypochromic shift has been observed after pH 5. At pH 12 the emission band for slightly blue shifted and exhibited a structured emission bands at 404 nm and 383 nm (**Figure 4.11b**).

4.6.4. Macromodel Study in the context of DNA

Next we used Schrodinger Macromodel calculation²⁸ to see how our synthesized nucleoside would behave within a DNA duplex if incorporated. The **Amber*** force field was utilized to optimize the structure of the theoretical DNA duplex [(5'-CGCAATXTAACGC-3'/3'-GCGTTANATTGCG-5'); (X = **FTQuon**, N = A, G, C, T)] where **FTQuon** was placed opposite to all the natural bases A, T, G and C to observe its interaction with natural bases.

It was clear from the structural investigation that in the duplex DNA, **FTQuon** resided deeply into the interior of the helix optimizing the helicity and the groove properties whenever present opposite to thymine and cytosine. The base **FTQuon** was able to maintain a B-form DNA conformation and held both via interstand and intrastand stacking interaction with the natural bases having an average base-base rise comparable to the natural bases. Most interestingly **FTQuon** made hydrogen bonds with **C** which is within 1.81-1.88 Å. This result is comparable with the distance 1.8 Å which is the distance of hydrogen bonds in natural **A:T** and **G:C** pair. Although **FTQuon** did not fit well as an opposite pairing partner with **A** and **G** due to its unmatched hydrogen bonding sites as revealed by **Amber*** optimized geometry but its stacking efficiency

because of having angularly expanded benzene π -cloud could stabilize the duplex DNA by π - π stacking interaction (**Figure 4.12**).

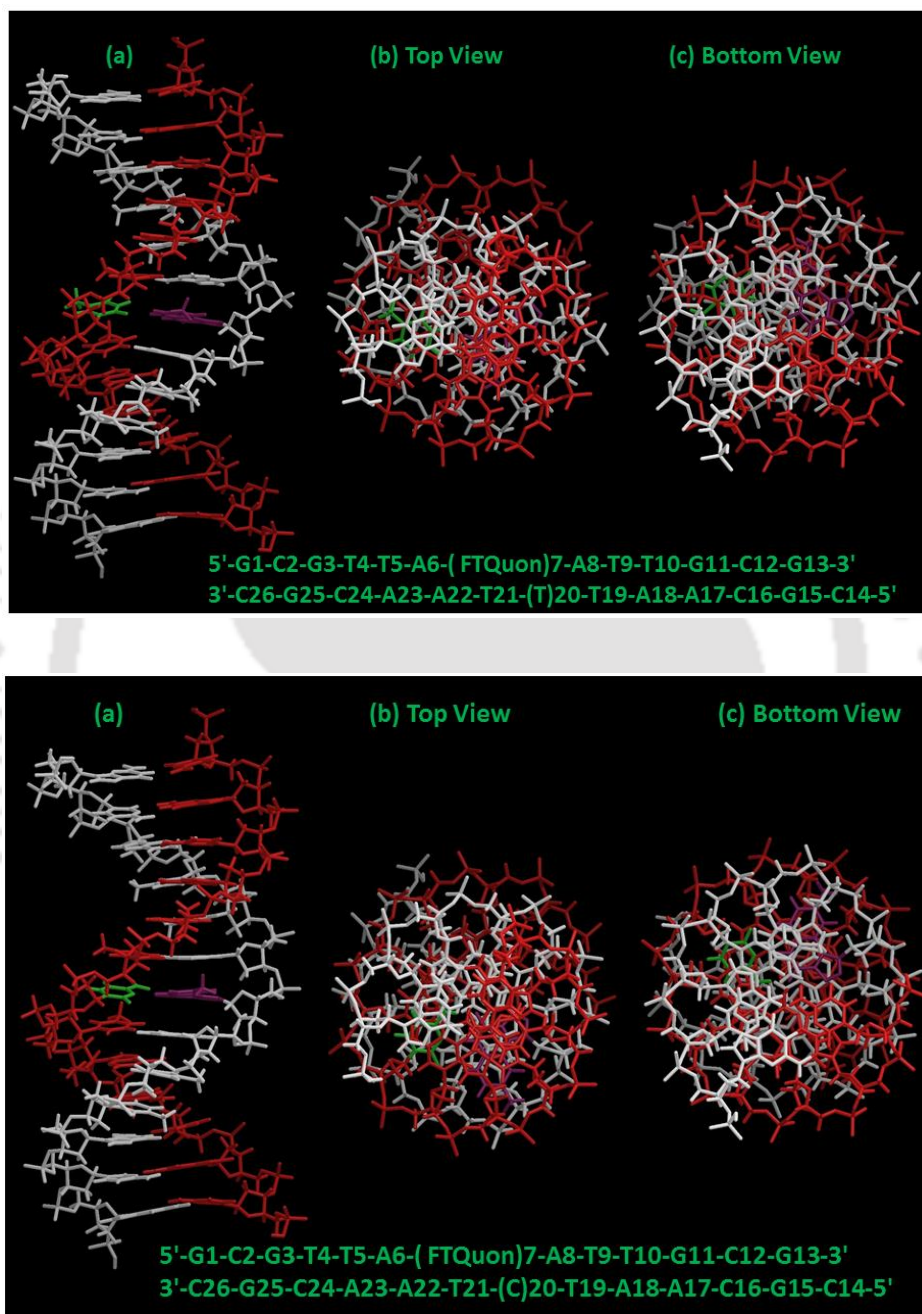


Figure 4.12: Representative Macromodel of the **Amber*** optimized Duplex DNA where **FTQuon** is placed opposite to **T** and **C**.

4.6.5. DFT Calculation in the context of DNA

Next to be more precise we carried out DFT calculation to get a better idea about the pairing efficiency of **FTQuon** with **C** and **T** in a theoretical DNA sequence using G09 program.²⁹ So for that we have taken out natural base pair **A:T** and **G:C** from the Amber* optimized geometry of Schrodinger Macromodel and optimized their geometry with the **B3LYP/6-31g(d,p)** level of theory. Then to get the interaction energy we have first removed the sugar part for simplicity and then carried out **Basis set superposition error (BSSE)** corrections using counterpoise correction procedure (**CP**).³⁰ By following the same procedure **FTQuom:C** and **FTQuon:T** base pairs are segmented from Amber* optimised geometry and then optimised through **B3LYP/6-31g(d,p)** level of theory. Then by using counter poise correction method we have calculated **BSSE** corrected interaction energy for **FTQuom:C** and **FTQuon:T** base pairs. **BSSE** corrected interaction energy suggested **G:C** is the most stable base pair due to having its highest interaction energy i.e. **-31.48 kcal/mol**. The hydrogen bonding lengths within the **G:C** pair were found to be **1.77 Å**, **1.93 Å**, **1.89 Å**. Now interaction energy of another natural pair **A:T** was found to be **-15.46 kcal/mol** and its hydrogen bonding lengths were **1.97 Å** and **1.77 Å**. It is interesting to note that the energy of **FTQuon:C** pair is found to be **-22.00 kcal/mol** and the hydrogen bonding lengths were **1.86 Å** and **2.012 Å** suggesting it to be more stable than **A:T** pair and less stable than **G:C** pair which is also supported by the hydrogen bond lengths which are close to natural value **1.8 Å**. So interaction energy order of these three pair is **G:C > FTQuom:C > A:T**.

While both **A:T** pair and **FTQuon:C** pair contain two hydrogen bonds but **FTQuon:C** contains extra benzene ring which provided extra stability through π -stacking interaction leading to more stable **FTQuon:C** pair than natural **A:T** pair.

The BSSE corrected interaction energy of the another two pair **FTQuon:A** and **FTQuon:G** were found to be **-7.09 kcal/mol** and **-8.10 kcal/mol**. These values suggested very weak interaction of **FTQuon** with **A** and **G**. It might be because of the large volume of **FTquon** and **A/G**. The large base pairs probably could not be accommodated within the space available inside a helix and more over they structurally mismatched to be paired through hydrogen bonding.

The interaction energy of **FTQuon:T** was found to be **-14.28 kcal/mol** which was close to the interaction energy of natural **A:T** pair and the length of the hydrogen bonds were found to be **1.7 Å** and **1.97 Å**.

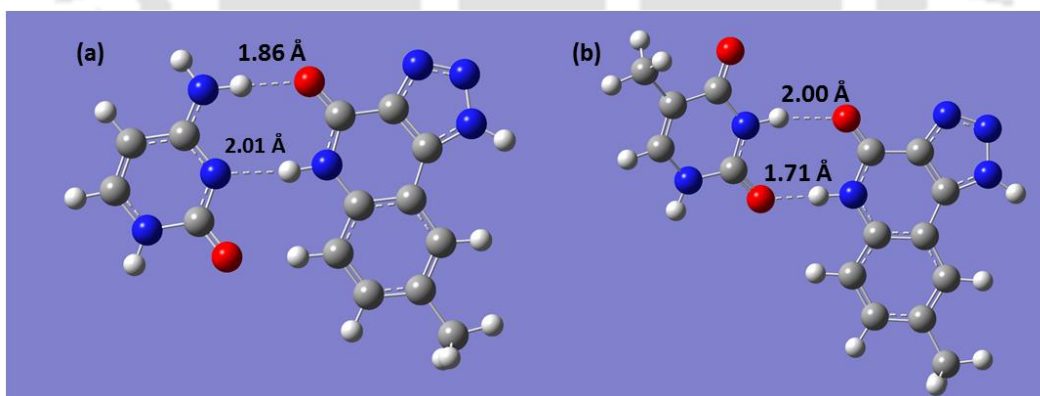
By looking at this close proximity of the hydrogen bonds forming pattern of **FTQuon** with **T** and **C**, we decided to explore **FTQuon:C/T** base pairs in a trimeric duplex with **ONIOM** calculation using G09 program.

So here the order of stability based on the **BSSE** corrected interaction energy of the base pairs were found to be as follows:



Table 4.1: List of base pairs and BSSE corrected interaction energy.

Stacking energy of the single nucleobase pairs		
Single base pair	BSSE corrected stacking energy (kcal/mol)	Hydrogen bonding distance(Å)
FTQuon:A	-7.09	
FTQuon:G	-8.10	
FTQuon:C	-22.00	1.86, 2.012
FTQuon:T	-14.28	1.7, 2.0
A:T	-15.46	1.97, 1.77
G:C	-31.48	1.77, 1.93, 1.89

**Figure 4.13:** (a) FTQuon:C base pair. (b) FTQuon:T base pair.

4.6.6. QM/MM calculation in the context of DNA

We next carried out QM/MM calculation for the trimeric duplex sequence **AFTQuonA:TXT** (X = T and C). The **Amber*** optimized geometry was taken and optimized it again by using G09 program package. We have only chosen here C and T because of having their highest interaction energy (-22.0 and -14.28 kcal/mol respectively) as was observed before. A and G were excluded from this study because of having their low fidelity to form base pairs as revealed from the interaction energy calculation (-7.09 and -8.10 kcal/mol respectively). Except the phosphodiester backbone and ribose the rest of the aromatic system of the trimeric duplex were chosen to be the **QM** region for quantum mechanics calculation using **M05-2X/6-31G(d,p)** level of theory. Phosphodiester back bones and ribose sugar part were treated under **UFF** force field and chosen as **MM** region for molecular mechanics calculation. After having the optimized geometry of the trimeric duplex containing **FTQuon:C** and **FTQuon:T** pairs we have removed the sugar and phosphodiester backbone to calculate interaction energy among the base pairs of the respective trimer for simplicity. The **BSSE** corrected interaction energy calculation using counter poise correction method illustrated that trimeric duplex containing **FTQuon:C** pair has interaction energy -15.61 kcal/mole. Whereas trimeric duplex containing **FTQuon:T** base pair is having interaction energy -15.31 kcal/mole which suggested the close proximity of interaction energy of **FTQuon:C** and **FTQuon:T** base pair whenever incorporated into duplex DNA.

For both the trimer **AFTQuonA:TXT** (X = T and C), containing **FTQuon:T** and **FTQuon:C** the rise values were close to the natural rise value. More over **FTQuon** is formed two hydrogen bonds with both C and T. The distance of hydrogen bonding was also within 1.8-2.0 Å for **FTQuon:C** base pair and 1.7-2.0 Å for **FTQuon:T** base pair which is comparable with the natural hydrogen bonding distance (1.8 Å) formed within **A:T** and **G:C** pair. As **FTQuon** is able to form two hydrogen bonds with both C and T and as the distance of formed hydrogen bonds within **FTQuon:C/T** were close enough to each other and comparable with the distance of hydrogen bond within natural **A:T** and **G:C** pair, it should be compelling to accept that their interaction energy will be close enough to each other. Indeed, the interaction energy for both the trimer is very close and they are -15.61 kcal/mole for **AFTQuonA:TCT** and -15.31 kcal/mole for **AFTQuonA:TTT**.

So **ONIOM** optimized geometry of these trimer suggested that **FTQuon** can be treated as a complementary of both C and T.

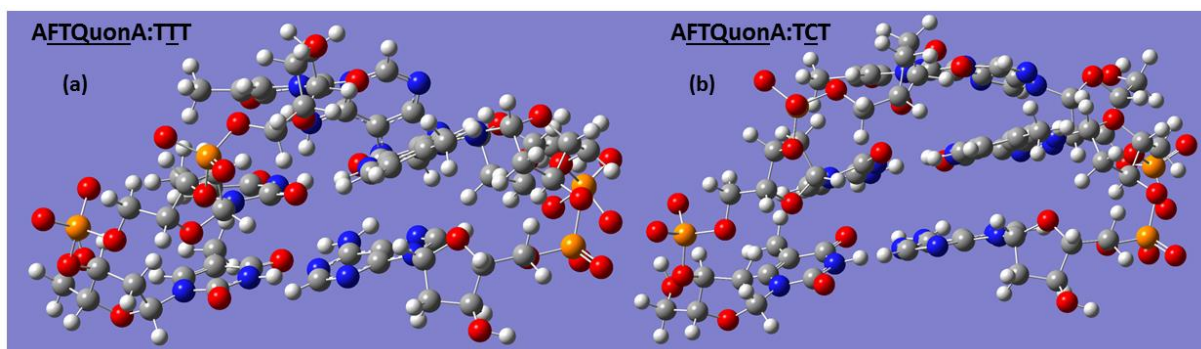


Figure 4.14: (a) ONIOM optimized AFTQuonA:TIT duplex; (b) ONIOM optimized AFTQuonA:TCT duplex.

4.6.7. Study of interaction with BSA

Next we studied the interaction of **FTQuon** with model protein BSA. With the gradual increase of BSA concentration the absorption band at 320 nm showed a slight hyperchromicity (**Figure 4.15a**). Excitation at 320 nm the emission spectra of **FTQuon** exhibited structurless broad emission band at 420 nm which showed hyperchromic effect upon gradual increase of BSA concentration (**Figure 4.15b**). This hyperchromicity at 420 nm with the increase of BSA concentration illustrated the interaction of the **FTQuon** with the hydrophobic pocket of BSA.

The excitation spectra exhibited a long wavelength band at 355 nm which showed a prominent hyperchromic effect upon addition of increasing concentration of BSA (**Figure 4.15c**). The interaction of **FTQuon** with the hydrophobic pocket of BSA is also supported from steady stated anisotropy recorded for 320 nm excitation maxima and 420 nm emission maxima where it showed an increase of anisotropy with the increase of BSA concentration (**Figure 4.15d**).

Upon excitation at 355 nm, which is prominent in fluorescence excitation spectra, a broad structured emission band appeared at 430 nm which with gradual addition of BSA became structurless and showed a prominent hyperchromic shift (**Figure 4.15e**). It is also confirmed from the steady state anisotropy experiment at 430 nm emission maxima and 355 nm excitation maxima. Here also steady state anisotropy supports the interaction of **FTQuon** with hydrophobic pocket of BSA (**Figure 4.15f**).

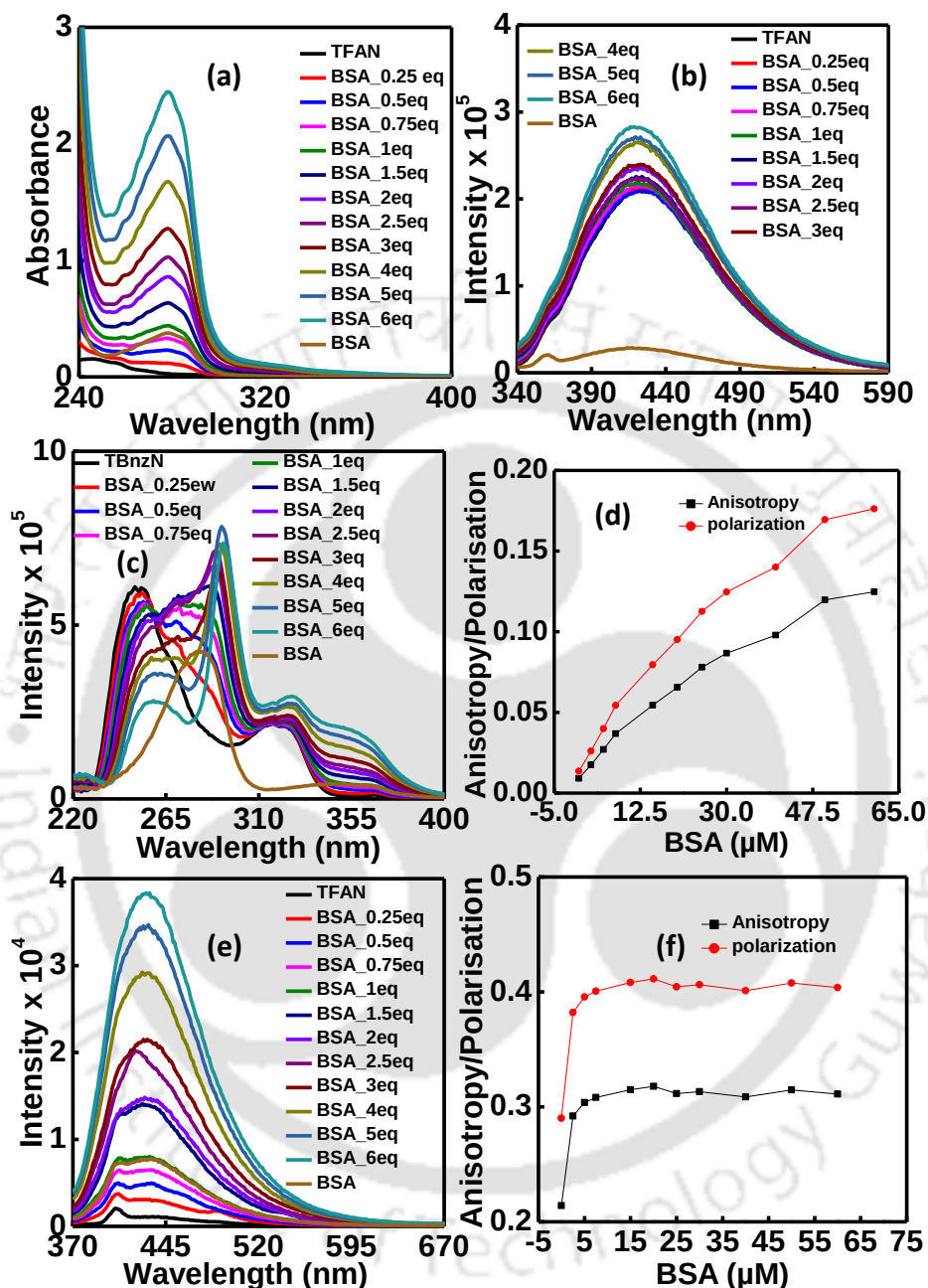


Figure 4.15: (a) UV-visible titration of **FTQuon** (b) Fluorescence Emission spectra of **FTQuon** ($\lambda_{\text{ex}} = 320$ nm) (c) Excitation spectra of **FTQuon** ($\lambda_{\text{ex}} = 420$ nm) (d) Steady-state anisotropy and polarisation plot ($\lambda_{\text{em}} = 420$ nm) of **FTQuon** (e) Fluorescence Emission spectra of **FTQuon** ($\lambda_{\text{ex}} = 355$ nm) (f) Steady-state anisotropy and polarisation plot ($\lambda_{\text{em}} = 430$ nm) of **FTQuon** in presence of increasing BSA concentration. [**FTQuon**]= $10\mu\text{M}$ and BSA added from 0.25eq to 6eq.

Benesi-Hildebrand Plot to Evaluation Binding Constant: The association constant (**K**) of the nucleoside **FTQuon** with BSA was determined by a **Benesi-Hildebrand** plot using the following equation 1.

$$\frac{1}{(I - I_0)} = \frac{1}{(I_\infty - I_0)} + \frac{1}{(I_\infty - I_0)K[BSA]} \quad \dots\dots\dots (1)$$

where I_0 , I and I_∞ are the emission intensities of **FTQuon** in the absence of BSA, in the presence of an intermediate and at infinite concentration of BSA respectively. From the slop of the $1/(I - I_0)$ vs. $1/[BSA]$ plot of equation 1, binding constant K was determined and its value is $7.5519 \times 10^{-9} \text{ M}^{-1}$ (**Figure 4.16a**) for emission maxima at 420 nm and 7.60061×10^{-9} for emission maxima at 430 nm (**Figure 4.16b**).

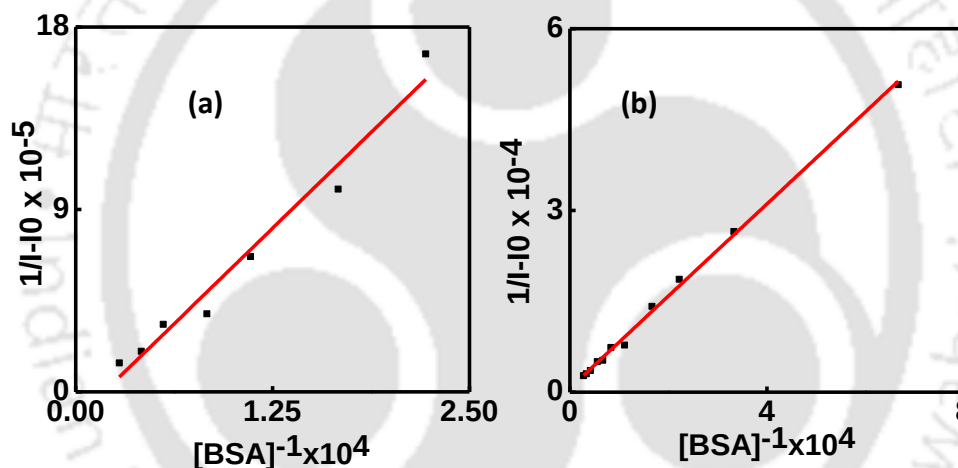


Figure 4.16: (a) The Benesi-Hildebrand plot. $[FTQuon] = 10 \mu\text{M}$ and BSA added from 0.25eq to 6eq.

Molecular Docking

Docking calculations were carried out using Autodock4 (Bikadi, Hazai, 2009). The amino acid sequence of BSA protein was observed from the NCBI website, <http://www.ncbi.nlm.nih.gov/protein/CAA76847.1>. The 3D model of the BSA protein was built using the 3D structure 1AO6 chain 'A' as template using the ESyPred3D11 web server. This template shares 72.4% identities with the BSA sequence. Following is the BSA sequence which was used to generate the 3D model. >gi|3336842|emb|CAA76847.1| bovine serum albumin [Bos taurus]

MKWVTFISLLLLFSSAYSIRGVFRDTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQC
PFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLFGDELCKVASLRETYGDMADCCE

KQEPERNECFLSHKDDSPDLPKLKPDPTLCDEFKADEKKFWGKYLYEIARRHPYFYAP
ELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLTSARQRLRCASIQKFGERAL
KAWSVARLSQKFPKAEFVEVTKLVDTLTKVHKECCHGDLLECADDRADLAKYICDNQ
DTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLG
SFLYEYSRRHPEYAVSVLLRLAKEYEATLEECCA KDDPHACYSTVFDKCLKHLVDEPQNL
IKQNC DQFEKLGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTKPESERM
PCTEDYLSLILNRLCVLHEKTPVSEKVT KCCTESLVNRRPCFSALTPDETYVPKAFDEKL
FTFHADICTLPDTEKQIKKQTALVELLKHKPKATEEQ LKTVMENFVAFVDKCCAADDKE
ACFAVEGPKLVVSTQTALA.

The binding of **FTQuon** inside the hydrophobic pocket of BSA was also supported from the docking study as is shown in **Figure 4.17a-b**.

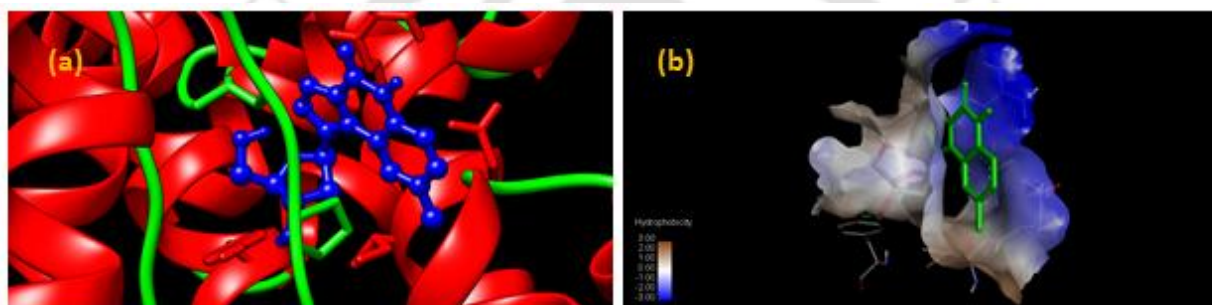


Figure 4.17: (a) Complex structure of **FTQuon** with BSA. (b) Interaction of pyrenayl triazolyl moiety of **FTQuon** with the hydrophobic pocket of BSA.

Estimated Free Energy of Binding	= -5.82 kcal/mol [(1)+(2)+(3)-(4)]
Estimated Inhibition Constant, K_i	= 54.39 μ M (micromolar) [Temperature = 298.15 K]
(1) Final Intermolecular Energy	= -7.01 kcal/mol
vdW + Hbond + desolv Energy	= -6.72 kcal/mol
Electrostatic Energy	= -0.29 kcal/mol
(2) Final Total Internal Energy	= -1.93 kcal/mol
(3) Torsional Free Energy	= +1.19 kcal/mol
(4) Unbound System's Energy [(2)]	= -1.93 kcal/mol

4.7. Conclusion

In conclusion, we have successfully synthesised 1'-(8-methyl-1H-[1,2,3]triazolo[4,5-c]quinolin-4(5H)-one)-2'-deoxyribose which we designate as fused triazolyl 2-quinolinone (**FTQuon**) nucleoside with two H-bonding face-one H-bond acceptor and another donor via click chemistry. **BSSE** corrected interaction energy of the individual **FTQuon:C** and **FTQuon:T** pair and the hydrogen bonds formed within **B3LYP/6-31g(d,p)** level optimized **FTQuon:C/T** pair suggested **FTQuon** can be capable to be paired with both cytosine and thymine whenever incorporated into DNA duplex. Further **QM/MM** calculation with **M05-2X/6-31G(d,p)** level optimized geometry of **AFTQuonA:TXT** (**X = T and C**) resulted the interaction energy **-15.61 kcal/mole** for **AFTQuonA:TCT** and **-15.31 kcal/mole** for **AFTQuonA:TTT** which were very much comparable to each other suggested that **FTQuon** can be capable to be equally paired with cytosine and thymine. Apart from that interesting microenvironment sensitive fluorescence and good interaction with BSA with the enhancement of fluorescence intensity would allow this nucleoside for further exploration in biophysical research in the context of DNA, DNA-protein interactions and to study the mode of interaction with the enzyme.

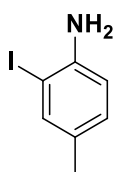
4.8. Experimental section

4.8.1. General experimental

All reactions were carried out under a nitrogen atmosphere. Organic extracts were dried over anhydrous sodium sulphate. Solvents were removed in a rotary evaporator under reduced pressure. Silica gel (60-120 mesh size) was used for the column chromatography. Reactions were monitored by TLC on silica gel 60 F254 (0.25mm). ¹H NMR spectra were measured with Bruker 600 (600 MHz) and ¹³C NMR spectra were measured with Bruker 600 (150 MHz) spectrometer. Coupling constant (*J* value) was reported in hertz. The chemical shifts were shown in ppm downfield from tetramethylsilane, using residual chloroform ($\delta = 7.24$ in ¹H NMR, $\delta = 77.23$ in ¹³C NMR), methanol ($\delta = 3.34$ in ¹H NMR, $\delta = 49.2$ in ¹³C NMR), dimethyl sulfoxide ($\delta = 2.48$ in ¹H NMR, $\delta = 39.5$ in ¹³C NMR), as an internal standard. Mass spectra were recorded using WATERS MS system, Q-tof premier and data analyzed using Mass Lynx 4.1. IR spectra were recorded in KBr or neat on a Perkin Elmer Spectrum one FT-IR spectrometer.

4.8.2. Synthesis and characterisation

Synthesis of 2-iodo-4-methylaniline (4.44): p-toluidine (2g, 18.7 mmol), NaHCO₃ (28 mmol) and water (20 ml) are introduced in a 100 ml round bottom flask. The reaction mixture was allowed to cool to 0° C and with stirring iodine was added in portion of 0.96 g at an interval of 15 min. After completion of the addition iodine the reaction mixture was allowed to stir at room temperature for 12 h. Completion of the reaction was checked by TLC. The reaction mixture was diluted with ethyl acetate added portion wise for 3 times and the organic fraction was washed with Na₂S₂O₃ and I₂. The organic fraction

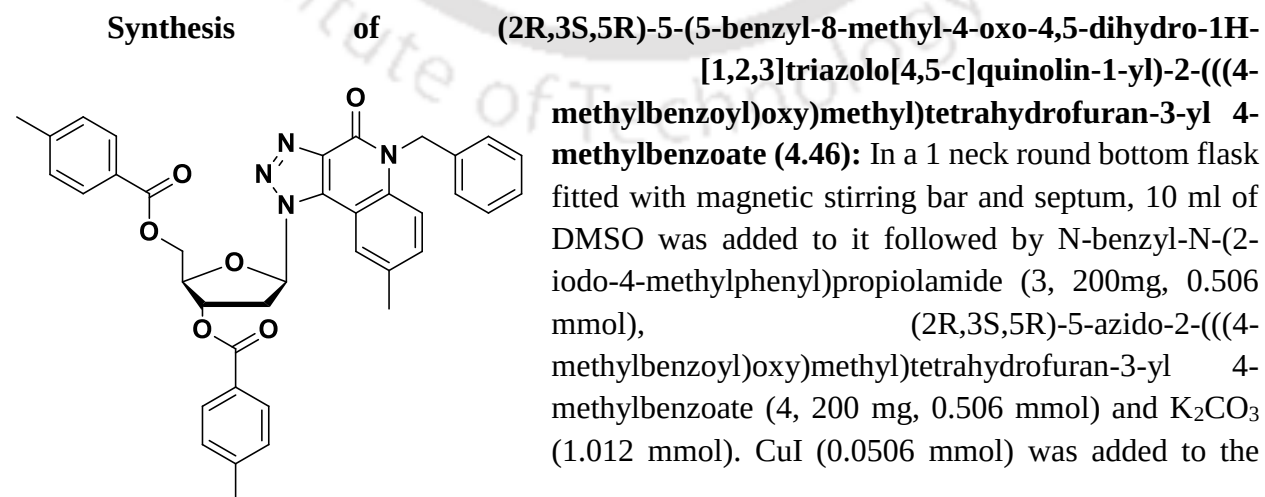


was collected and evaporated to dryness to get the crude reaction mixture which was purified by 15:1 Hex:EtOAc to get blackish brown solid of 3.21 g (Yield=74.65%).

¹H NMR (600 MHz, CDCl₃) δ 7.48 (s, 1H), 6.95 (d, J = 7.7 Hz, 1H), 6.66 (d, J = 8.1 Hz, 1H), 3.95 (s, 2H), 2.21 (s, 3H). **¹³C NMR** (150 MHz, CDCl₃) δ 144.41, 139.12, 130.16, 129.63, 114.78, 84.44, 77.16, 19.96.

Synthesis of N-(2-iodo-4-methylphenyl)propiolamide (4.45): A 2 neck round bottom flask fitted with septum, adapter and magnetic stirring bar was evacuated and backfilled with nitrogen and the process was repeated for 3 times. DCC (2.86 mmol) was added to it and the above process was repeated again by heating the outer surface of the round bottom flask to remove the moisture from the reaction vessel. In nitrogen atmosphere DCM (10 ml) was injected and the reaction mixture was allowed to come to 0⁰ C. At that temperature propiolic acid (0.71 mmol) was added and the reaction mixture was allowed to stir for half an hour ensuring the formation of the white precipitate in the transparent reaction mixture. After half an hour aniline was added to it and the reaction mixture was allowed stir for overnight. Completion of the reaction mixture was checked by TLC. The reaction mixture was filtered through celite and washed with 3M HCl and saturated NaHCO₃. Evaporation of the organic layer gives crude product which was purified by column chromatography with 10:1 Hex:EtOAc to get pure product as brown gummy material (yield = 46%). This material then treated with benzyl iodide and K₂CO₃ in acetonitrile at 80⁰ C for 12 h and completion of the reaction was checked by TLC. The reaction mixture was then evaporated to dryness and diluted with DCM and washed with water and brine. Evaporation of the organic residue flowed by purification through column chromatography gives pure product (Yield=42%).

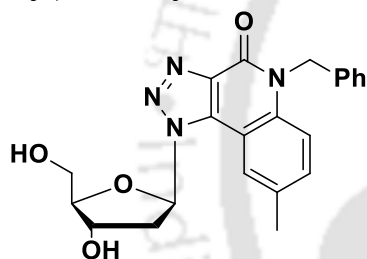
¹H NMR (600 MHz, CDCl₃) δ 7.75 (s, 1H), 7.28 – 7.25 (m, 3H), 7.22 – 7.19 (m, 2H), 6.99 (d, J = 7.8 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 5.59 (d, J = 14.3 Hz, 1H), 4.04 (d, J = 14.3 Hz, 1H), 2.76 (s, 1H), 2.31 (s, 3H). **¹³C NMR** (150 MHz, CDCl₃) δ 153.49, 140.85, 140.44, 140.34, 136.02, 130.92, 129.77, 129.69, 128.64, 128.00, 100.04, 79.46, 77.16, 76.38, 51.43, 20.76.



reaction mixture and the reaction was allowed stir at ambient temperature. After 12 h completion of the reaction was checked by TLC. The reaction mixture was diluted with ethyl acetate and washed with water and brine. Evaporation of the organic solvent followed by purification of the crude reaction mixture through column chromatography by 2:1 Hexane: Ethyl acetate gives a white solid. Yield= 280 mg (83.4%).

¹H NMR (400 MHz, CDCl₃) δ 8.03 (s, 1H), 7.98 – 7.93 (m, 2H), 7.61 – 7.57 (m, 2H), 7.25 – 7.17 (m, 6H), 7.16 – 7.07 (m, 4H), 6.79 (dd, J = 6.5, 4.4 Hz, 1H), 5.98 – 5.92 (m, 1H), 5.56 (d, J = 69.0 Hz, 2H), 4.75 (q, J = 4.5 Hz, 1H), 4.45 (dd, J = 12.1, 4.4 Hz, 1H), 4.32 (dd, J = 12.2, 5.2 Hz, 1H), 4.29 – 4.23 (m, 1H), 2.97 (dt, J = 14.4, 5.9 Hz, 1H), 2.40 (s, 3H), 2.38 (s, 4H), 2.31 (s, 3H). **¹³C NMR** (100 MHz, CDCl₃) δ 21.1, 21.8, 21.9, 36.2, 45.9, 63.4, 74.9, 77.2, 84.9, 88.6, 110.5, 116.9, 126.5, 126.6, 127.4, 128.9, 129.1, 129.3, 129.4, 129.6, 129.9, 130.3, 132.4, 132.9, 135.6, 136.3, 136.9, 137.4, 144.0, 144.6, 156.5, 166.1. **IR (KBr)** 2923.67, 1712.98, 1681.37, 1610.63, 1454.41, 1411.95, 1384.18, 1271.79, 1178.82, 1093.75, 1020.14, 805.15, 752.70, 691.89 cm⁻¹. **HRMS [M+H]⁺** calculated 643.2551, found 643.2558.

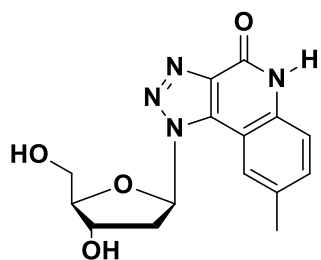
Synthesis of 5-benzyl-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-methyl-1H-[1,2,3]triazolo[4,5-c]quinolin-4(5H)-one (4.47): (2R,3S,5R)-5-(5-benzyl-8-methyl-4-oxo-4,5-dihydro-1H-[1,2,3]triazolo[4,5-c]quinolin-1-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate (5, 100 mg, 0.16 mmol) was taken in a round bottom flask fitted with magnetic stirring bar and septum. Methanol (10 ml) was added to it followed by DCM (5 ml) and K₂CO₃ (86 mg, 0.63 mmol). The reaction mixture was allowed to stir at room temperature for 6 h. Completion of the reaction was checked by



TLC. The organic part was evaporated to dryness and the crude reaction mixture was purified by column chromatography by 10:1 CHCl₃: MeOH to yield a white solid of 62 mg (Yield= 98%)

¹H NMR (400 MHz, CD₃OD) δ 8.23 (s, 1H), 7.45 – 7.38 (m, 2H), 7.28 (d, J = 7.2 Hz, 2H), 7.20 (d, J = 7.9 Hz, 3H), 6.93 (dd, J = 6.9, 3.7 Hz, 1H), 5.65 (d, J = 16.2 Hz, 2H), 4.79 (q, J = 5.8 Hz, 1H), 4.16 (q, J = 4.8 Hz, 1H), 3.69 (ddt, J = 14.0, 7.8, 3.6 Hz, 1H), 3.58 (dd, J = 12.1, 4.3 Hz, 1H), 3.43 (dd, J = 12.0, 5.4 Hz, 1H), 2.68 (dt, J = 13.3, 6.4 Hz, 1H), 2.46 (s, 3H), 2.40 (s, 1H). **¹³C NMR** (100 MHz, CD₃OD) δ 20.8, 30.7, 39.3, 46.6, 49.0, 63.1, 72.3, 89.4, 90.1, 118.3, 126.1, 127.5, 128.4, 129.8, 130.0, 130.7, 133.4, 134.7, 137.6. **IR (KBr):** 2924.02, 2853.26, 1676.35, 1657.79, 1566.73, 1520.85, 1456.56, 1384.22, 1369.85, 1265.65, 1206.06, 1158.49, 1087.16, 1048.36, 1011.35, 993.58, 807.71, 696.84. **HRMS [M+H]⁺** calculated 407.1714, found 407.1701.

Synthesis of 1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-methyl-1H-[1,2,3]triazolo[4,5-c]quinolin-4(5H)-one (4.40): 5-benzyl-1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-8-methyl-1H-[1,2,3]triazolo[4,5-c]quinolin-



4(5H)-one (7, 50 mg, 0.13 mmol) was taken in a two neck round bottom flask fitted with magnetic stirring bar and septum. The reaction vessel was evacuated and back filled with nitrogen. The process was repeated for 3 times to make it ensure that there is no tress of air present in the reaction vessel. Pd/C was added 5 times of the weight of the compound (7) followed by ammonium formate (34 mg, 0.52 mmol). Methanol was added to the reaction mixture. The reaction mixture was then allowed to heat at 90 °C. After 4h hours completion of the reaction was checked by TLC. The reaction mixture was then filtered through whatman 42 filter paper. Evaporation of the solvent followed by coloumn chromatography with 10:1 CHCl₃:MeOH yield 72% white solid.

¹H NMR (400 MHz, DMSO) δ 8.03 (s, 1H), 7.44 (s, 3H), 6.93 (dd, J = 6.7, 3.8 Hz, 1H), 5.57 (d, J = 4.9 Hz, 1H), 4.68 (t, J = 5.3 Hz, 1H), 4.61 – 4.53 (m, 1H), 3.99 (q, J = 4.9 Hz, 1H), 3.44 (s, 1H), 3.43 (s, 1H), 3.33 (t, J = 4.7 Hz, 1H), 3.20 – 3.12 (m, 1H), 2.43 (s, 3H). **¹³C NMR** (150 MHz, DMSO) δ 155.72, 136.95, 136.19, 135.73, 131.99, 131.66, 123.81, 116.56, 108.79, 88.76, 87.49, 70.50, 61.47, 39.52, 38.07, 20.79. **¹³C NMR, DEPT135** (150 MHz, DMSO) δ 131.73, 123.55, 88.50, 87.23, 70.24, 61.21, 39.52, 37.82, 20.53. **IR (KBr):** 3434.39, 3198.62, 3145.2, 1687.17, 1633.24, 1401.35, 1119.90, 1084.37, 1059.79, 1025.59, 948.34, 889.75, 859.00, 706.23. **HRMS [M+H]⁺** calculated 317.1244, found 317.1194.

4.8.3. Study of Photophysical property of FTQuon

UV-visible measurements: All the UV-visible spectra of the nucleoside monomers (10 μ M) were measured in different organic solvents using a UV-Visible spectrophotometer with a cell of 1 cm path length at 25 °C. Equal volume of each sample solution in different solvents were used. The measurements were taken in absorbance mode. The absorbance values of the sample solutions were measured in the wavelength regime of 200–550 nm. All the sample solutions were prepared just before doing the experiment.

Fluorescence experiments: All the sample solutions of the nucleoside monomers (10 μ M) were prepared as described in UV measurement experiments. Fluorescence spectra were obtained using a fluorescence spectrophotometer at 25 °C using 1 cm path length cell. The excitation wavelengths for monomers were set at λ_{abs}^{max} , and emission spectra were measured in the wavelength regime of 315–600 nm with an integration time of 0.1 sec .

4.8.4. Studies On the Interaction of FTQuon with BSA

Preparation of BSA solution: Phosphate buffer of pH 7.02 was used to prepare the solution of BSA (Merck). A 1200 μ M of stock BSA solution was prepared by dissolving 0.08 gm of BSA in 1 mL phosphate buffer (20 mM) of pH 7.0. From that stock solution sub stock of 1000 μ M BSA

was prepared. The compound stock solution was prepared in DMF because of the poor solubility in water. 1.0 mg of nucleoside **FTQuon** was dissolved in 1.5 mL DMF to make a stock probe solution of concentration 910.3 μM .

General experimental on interaction study of BSA by photophysical study: All the spectral measurements were carried out at room temperature. To study the interaction of compound with BSA, an aqueous solution of nucleoside **FTQuon** (10 μM) was titrated with different concentrations of BSA (ranging from 0, 0.25, 0.5, 7.5, 1.0, 1.5, 2, 2.5, 3.0, 4, 5, 6, 10, 14, 18, 20 equivalent). The total volume of the final solution for each sample was 3 mL. The % of DMF content was 2%. The presence of 2% DMF does not induce structural changes to biomolecules. Each sample solution was mixed well before spectral measurements.

UV-Visible study with BSA: The UV–Visible absorbance measurements were performed using UV-visible spectrophotometer with a cell of 1 cm path length at 298 K. All the UV-Visible studies were carried out in 20 mM phosphate buffer of pH 7.02 containing solution at 298 K. 2 % DMF was used to solubilize the probe. The measurements were taken in absorbance mode and the absorbance values of the sample solutions were measured in the wavelength regime of 200– 700 nm. All the experiments were carried out with freshly prepared sample solutions.

Fluorescence study with BSA: All fluorescence and steady state anisotropy experiments were performed using a steady state fluorescence spectrophotometer with a cell of 1 cm path length at 298 K. The excitation wavelength for probe nucleoside **FTQuon** was set at 220 nm, 420 nm and emission spectra were measured in the wavelength regime of 290–650 nm. Steady state anisotropy of the solutions was measured using steady state fluorescence spectrophotometer. The fluorescence lifetime experiment was carried out as described above.

4.8.5. Cartesian coordinates of ONIOM optimized trimer

AFTQuonA:TTT

ONIOM Total Energy = -2917.64599807 a.u.

1 O1	7.5226	-6.7944	-1.4912 O
2 C2	8.3318	-5.6734	-1.7218 C
3 C3	7.5059	-4.3851	-1.6347 C
4 O4	6.3839	-4.4627	-2.4797 O
5 C5	6.9528	-4.1282	-0.2314 C
6 O6	7.8320	-3.6483	0.7812 O
7 C7	5.7294	-3.2836	-0.4852 C
8 C8	5.4724	-3.5000	-1.9912 C
9 N9	4.1210	-3.9316	-2.2511 N
10 C10	3.4324	-4.9284	-1.5895 C
11 N11	2.1514	-4.9228	-1.8314 N
12 C12	1.9833	-3.8671	-2.7031 C
13 C13	0.8255	-3.2836	-3.2587 C
14 N14	-0.4074	-3.7440	-3.0184 N
15 N15	0.9869	-2.2178	-4.0608 N
16 C16	2.2288	-1.7608	-4.3030 C

Design, Synthesis and Photophysical Properties of Fused Triazolyl 2-Quinolinone Nucleoside

17 N17	3.3751	-2.2112	-3.8324 N
18 C18	3.1903	-3.2427	-2.9944 C
19 H19	-1.2030	-3.1984	-3.3269 H
20 H20	-0.5485	-4.3377	-2.2193 H
21 P21	-5.3828	5.5599	-0.6565 P
22 O22	-5.0885	6.8476	0.0830 O
23 O23	-6.8581	5.7434	-1.4655 O
24 O24	-4.1623	5.2117	-1.8024 O
25 C25	-2.9449	5.7742	-1.3670 C
26 C26	-1.7776	5.2355	-2.2018 C
27 O27	-1.6489	3.8499	-2.0107 O
28 C28	-1.9353	5.4845	-3.7006 C
29 O29	-1.3289	6.6912	-4.0829 O
30 C30	-1.2204	4.3061	-4.3200 C
31 C31	-0.9659	3.3609	-3.1417 C
32 N32	-1.4054	2.0107	-3.4353 N
33 C33	-0.4560	0.9881	-3.5778 C
34 O34	0.7471	1.1742	-3.5812 O
35 N35	-0.9929	-0.2685	-3.7230 N
36 C36	-2.3216	-0.6228	-3.7419 C
37 O37	-2.6748	-1.7982	-3.7891 O
38 C38	-3.2612	0.4944	-3.7137 C
39 C39	-4.7162	0.1892	-3.8999 C
40 C40	-2.7600	1.7309	-3.5481 C
41 H41	-0.2775	-1.0252	-3.8733 H
42 H42	9.1550	-5.6371	-0.9735 H
43 H43	8.7814	-5.7444	-2.7373 H
44 H44	8.1290	-3.5442	-2.0007 H
45 H45	6.5658	-5.1015	0.1461 H
46 H46	4.8862	-3.6146	0.1591 H
47 H47	5.8977	-2.2185	-0.2609 H
48 H48	5.6721	-2.5466	-2.5296 H
49 H49	3.9311	-5.6318	-0.9400 H
50 H50	2.2774	-0.8972	-4.9566 H
51 H51	-2.9779	6.8811	-1.4761 H
52 H52	-2.7355	5.5170	-0.3019 H
53 H53	-0.8440	5.7261	-1.8410 H
54 H54	-3.0045	5.4661	-4.0164 H
55 H55	-1.8505	3.8415	-5.1105 H
56 H56	-0.2528	4.6103	-4.7783 H
57 H57	0.1227	3.3885	-2.9073 H
58 H58	-5.3123	1.1023	-3.8965 H
59 H59	-4.8715	-0.3265	-4.8487 H
60 H60	-5.0725	-0.4776	-3.1134 H
61 H61	-3.4326	2.5804	-3.5099 H
62 P62	8.8153	-2.3081	0.3500 P
63 O63	9.9137	-2.7443	-0.5946 O
64 O64	9.5433	-1.7695	1.7797 O
65 O65	7.9195	-1.0203	-0.3292 O
66 C66	8.7822	0.0772	-0.5444 C
67 C67	7.9775	1.3803	-0.6634 C
68 O68	7.0576	1.3098	-1.7298 O
69 C69	7.2084	1.7134	0.6085 C
70 O70	8.0252	2.4332	1.5115 O
71 C71	6.1141	2.5820	0.0663 C
72 C72	5.8989	2.0289	-1.3408 C

Chapter 4

73 N73	4.7478	1.1631	-1.3700 N
74 N74	3.7653	-0.7296	-1.3814 N
75 C75	2.8348	0.2296	-1.1679 C
76 C76	1.4300	0.0193	-0.8698 C
77 O77	0.8174	-1.0450	-0.9365 O
78 N78	0.8300	1.1662	-0.4283 N
79 C79	3.4391	1.4753	-1.1409 C
80 H80	-0.1485	1.0543	-0.1510 H
81 P81	-9.4489	-0.4773	0.4923 P
82 O82	-10.7459	0.2435	0.2037 O
83 O83	-8.8325	-1.1060	-0.9561 O
84 O84	-8.3539	0.6080	1.2209 O
85 C85	-7.8372	1.4867	0.2514 C
86 C86	-6.6073	2.1848	0.8459 C
87 O87	-5.7000	1.1990	1.3004 O
88 C88	-5.9130	3.0987	-0.1718 C
89 O89	-5.4944	4.2857	0.4782 O
90 C90	-4.7237	2.2779	-0.5871 C
91 C91	-4.4889	1.3613	0.5986 C
92 N92	-3.9616	0.0813	0.1599 N
93 C93	-2.5930	-0.0387	-0.0210 C
94 O94	-1.8235	0.8946	0.1992 O
95 C95	-2.9372	-2.3653	-0.7672 C
96 C96	-4.3758	-2.1022	-0.7693 C
97 C97	-4.8092	-0.9220	-0.3031 C
98 H98	9.5136	0.2252	0.2830 H
99 H99	9.3480	-0.0816	-1.4886 H
100 H100	8.6940	2.2046	-0.8860 H
101 H101	6.7596	0.7774	1.0241 H
102 H102	5.2079	2.5283	0.7039 H
103 H103	6.4525	3.6407	0.0027 H
104 H104	5.7462	2.8575	-2.0644 H
105 H105	-7.5159	0.9567	-0.6728 H
106 H106	-8.6050	2.2483	-0.0104 H
107 H107	-6.9462	2.7827	1.7214 H
108 H108	-6.5839	3.2872	-1.0437 H
109 H109	-4.9888	1.6899	-1.4904 H
110 H110	-3.8235	2.8894	-0.7981 H
111 H111	-3.7790	1.8544	1.3021 H
112 H112	-5.8693	-0.7132	-0.2978 H
113 P113	7.6171	2.0059	3.1135 P
114 O114	8.4115	2.8635	4.0738 O
115 O115	8.0320	0.3872	3.3745 O
116 O116	5.9403	2.1874	3.3861 O
117 C117	5.6403	3.5588	3.5129 C
118 C118	4.1331	3.7462	3.7342 C
119 O119	3.4087	3.1917	2.6610 O
120 C120	3.6477	3.0974	5.0260 C
121 O121	3.7249	3.9846	6.1106 O
122 C122	2.2186	2.7712	4.7066 C
123 C123	2.2089	2.6541	3.1840 C
124 N124	2.0595	1.2793	2.7790 N
125 C125	3.0075	0.4673	2.1884 C
126 N126	2.5467	-0.7075	1.8637 N
127 C127	1.2255	-0.6677	2.2466 C
128 C128	0.1899	-1.6186	2.1151 C

Design, Synthesis and Photophysical Properties of Fused Triazolyl 2-Quinolinone Nucleoside

129 N129	0.3782	-2.7933	1.5188 N
130 N130	-1.0152	-1.2899	2.6247 N
131 C131	-1.1894	-0.0739	3.1716 C
132 N132	-0.3121	0.9092	3.3003 N
133 C133	0.8893	0.5522	2.8202 C
134 H134	-0.3786	-3.4672	1.4576 H
135 H135	1.2140	-2.9194	0.9723 H
136 O136	-8.6927	-5.4886	0.8476 O
137 C137	-9.5864	-4.9328	1.7766 C
138 C138	-8.9297	-3.7885	2.5609 C
139 O139	-7.7353	-4.2264	3.1683 O
140 C140	-8.6157	-2.5781	1.6928 C
141 O141	-9.7556	-1.7437	1.5979 O
142 C142	-7.4856	-1.9502	2.4666 C
143 C143	-6.8400	-3.1286	3.1944 C
144 N144	-5.5552	-3.4757	2.6026 N
145 C145	-4.4791	-2.5937	2.8009 C
146 O146	-4.5993	-1.4823	3.2875 O
147 N147	-3.2564	-3.0741	2.4011 N
148 C148	-2.9710	-4.3147	1.8686 C
149 O149	-1.8220	-4.6725	1.6522 O
150 C150	-4.1364	-5.1342	1.5648 C
151 C151	-3.9042	-6.4017	0.8037 C
152 C152	-5.3427	-4.6889	1.9505 C
153 H153	-2.4528	-2.4271	2.5524 H
154 H154	6.1747	4.0041	4.3816 H
155 H155	5.9280	4.1116	2.5904 H
156 H156	3.9187	4.8391	3.7653 H
157 H157	4.1941	2.1499	5.2466 H
158 H158	1.8947	1.8380	5.2186 H
159 H159	1.5486	3.6040	5.0184 H
160 H160	1.3588	3.2426	2.7768 H
161 H161	4.0274	0.7796	2.0294 H
162 H162	-2.1934	0.1110	3.5406 H
163 H163	-10.5171	-4.5708	1.2799 H
164 H164	-9.8841	-5.7287	2.4919 H
165 H165	-9.6325	-3.4844	3.3708 H
166 H166	-8.2502	-2.9129	0.6952 H
167 H167	-6.7829	-1.4285	1.7860 H
168 H168	-7.8820	-1.2247	3.2121 H
169 H169	-6.6801	-2.8558	4.2621 H
170 H170	-4.8414	-6.9268	0.6176 H
171 H171	-3.4235	-6.1676	-0.1482 H
172 H172	-3.2281	-7.0574	1.3533 H
173 H173	-6.2054	-5.2955	1.7121 H
174 C174	1.3795	2.4273	-0.3898 C
175 C175	2.7061	2.6767	-0.7981 C
176 C176	3.1729	4.0019	-0.7780 C
177 C177	2.3903	5.0483	-0.3218 C
178 C178	1.0920	4.7634	0.1318 C
179 C179	0.5874	3.4811	0.0937 C
180 H180	4.1662	4.2401	-1.1339 H
181 H181	0.4737	5.5684	0.5131 H
182 H182	-0.4124	3.2545	0.4459 H
183 C183	2.9103	6.4626	-0.3021 C
184 H184	2.8818	6.8756	0.7083 H

Chapter 4

185 H185	3.9400	6.5069	-0.6561 H
186 H186	2.3063	7.1113	-0.9397 H
187 H187	4.6738	3.9913	6.4010 H
188 H188	-8.8953	-5.0804	-0.0340 H
189 H189	8.1218	-7.5831	-1.5513 H
190 H190	-1.9427	7.4165	-3.7962 H
191 N191	4.9063	-0.1689	-1.5077 N
192 O192	-2.4446	-3.4485	-1.0183 O
193 C193	-5.2707	-3.1494	-1.3543 C
194 H194	-5.1092	-4.1088	-0.8640 H
195 H195	-6.3193	-2.8661	-1.2539 H
196 H196	-5.0301	-3.2741	-2.4124 H
197 N197	-2.1523	-1.2558	-0.4604 N
198 H198	-1.1344	-1.3438	-0.5637 H

AFTQuonA:TC

ONIOM Total Energy = -2976.83364822 a.u.

1 O1	9.6567	-3.6470	-4.8495 O
2 C2	10.1997	-2.7528	-3.9165 C
3 C3	9.0956	-2.1551	-3.0372 C
4 O4	8.0809	-1.5993	-3.8388 O
5 C5	8.4147	-3.2043	-2.1445 C
6 O6	9.0494	-3.5674	-0.9217 O
7 C7	6.9969	-2.7006	-2.0025 C
8 C8	6.9340	-1.5442	-3.0182 C
9 N9	5.7460	-1.6116	-3.8325 N
10 C10	5.3013	-2.7147	-4.5342 C
11 N11	4.0737	-2.6023	-4.9543 N
12 C12	3.6849	-1.3576	-4.5079 C
13 C13	2.4317	-0.7118	-4.5346 C
14 N14	1.3482	-1.2752	-5.0806 N
15 N15	2.3501	0.5138	-3.9900 N
16 C16	3.4500	1.0636	-3.4468 C
17 N17	4.6564	0.5349	-3.3165 N
18 C18	4.7072	-0.7064	-3.8276 C
19 H19	0.4342	-0.8677	-4.9014 H
20 H20	1.4203	-2.2411	-5.3464 H
21 P21	-6.7105	5.1542	-0.9522 P
22 O22	-7.2636	6.4268	-0.3504 O
23 O23	-7.5239	4.8626	-2.4085 O
24 O24	-5.0474	5.3512	-1.2922 O
25 C25	-4.3858	5.7340	-0.1076 C
26 C26	-2.8731	5.8389	-0.3538 C
27 O27	-2.3543	4.5662	-0.6704 O
28 C28	-2.5247	6.8269	-1.4822 C
29 O29	-1.6493	7.8205	-1.0146 O
30 C30	-1.8227	5.9688	-2.5035 C
31 C31	-1.4109	4.7467	-1.7003 C
32 N32	-1.3392	3.5599	-2.5333 N
33 C33	-0.1007	2.9564	-2.7762 C
34 O34	0.9735	3.4355	-2.4593 O
35 N35	-0.1776	1.7515	-3.4373 N
36 C36	-1.3026	1.0817	-3.8562 C
37 O37	-1.2311	-0.0108	-4.4155 O

38 C38	-2.5560	1.7787	-3.6178 C
39 C39	-3.8226	1.1230	-4.0717 C
40 C40	-2.5059	2.9572	-2.9754 C
41 H41	0.7428	1.3127	-3.6406 H
42 H42	10.9422	-3.2764	-3.2732 H
43 H43	10.7185	-1.9278	-4.4539 H
44 H44	9.5425	-1.3252	-2.4536 H
45 H45	8.3414	-4.1442	-2.7362 H
46 H46	6.2783	-3.5166	-2.2340 H
47 H47	6.7626	-2.3632	-0.9774 H
48 H48	6.9579	-0.5769	-2.4682 H
49 H49	5.9326	-3.5742	-4.7018 H
50 H50	3.3019	2.0646	-3.0535 H
51 H51	-4.7494	6.7275	0.2396 H
52 H52	-4.5497	4.9830	0.6991 H
53 H53	-2.4015	6.1706	0.5990 H
54 H54	-3.4241	7.3009	-1.9418 H
55 H55	-2.5363	5.6978	-3.3124 H
56 H56	-0.9405	6.4747	-2.9542 H
57 H57	-0.4284	4.9644	-1.2222 H
58 H58	-4.6757	1.7888	-3.9385 H
59 H59	-3.7509	0.8441	-5.1234 H
60 H60	-3.9966	0.2029	-3.5081 H
61 H61	-3.4238	3.4965	-2.7862 H
62 P62	9.5263	-2.2489	0.0678 P
63 O63	10.8892	-1.7659	-0.3808 O
64 O64	9.6581	-2.8150	1.6561 O
65 O65	8.3898	-0.9743	0.0493 O
66 C66	8.9925	0.1797	0.5856 C
67 C67	7.9371	1.2706	0.8047 C
68 O68	7.0772	1.3621	-0.3102 O
69 C69	7.0715	1.0256	2.0316 C
70 O70	7.7093	1.4910	3.2040 O
71 C71	5.8777	1.8684	1.6946 C
72 C72	5.8122	1.7742	0.1760 C
73 N73	4.7906	0.8329	-0.2024 N
74 N74	4.0265	-1.0558	-0.8348 N
75 C75	2.9796	-0.2239	-0.6020 C
76 C76	1.5710	-0.5356	-0.7573 C
77 O77	1.1300	-1.5927	-1.2102 O
78 N78	0.7623	0.4822	-0.3316 N
79 C79	3.4405	1.0062	-0.1630 C
80 H80	-0.2446	0.3189	-0.4499 H
81 P81	-10.1053	-1.4024	0.3354 P
82 O82	-11.4490	-0.7114	0.3816 O
83 O83	-9.7731	-1.8483	-1.2656 O
84 O84	-8.9124	-0.3453	0.9412 O
85 C85	-8.6349	0.6341	-0.0295 C
86 C86	-7.5522	1.5721	0.5168 C
87 O87	-6.4163	0.8007	0.8653 O
88 C88	-7.1476	2.6454	-0.5111 C
89 O89	-6.9349	3.8727	0.1654 O
90 C90	-5.8559	2.0805	-1.0378 C
91 C91	-5.3209	1.3426	0.1674 C
92 N92	-4.3778	0.3182	-0.2362 N
93 C93	-3.0143	0.6955	-0.4078 C

Chapter 4

94 O94	-2.6729	1.8469	-0.1585 O
95 N95	-2.1379	-0.2442	-0.8353 N
96 C96	-2.5380	-1.4630	-1.1771 C
97 N97	-1.6238	-2.3398	-1.5699 N
98 C98	-3.9335	-1.8281	-1.1460 C
99 C99	-4.7989	-0.9129	-0.6653 C
100 H100	-0.6407	-2.0646	-1.5282 H
101 H101	-1.8654	-3.3133	-1.6591 H
102 H102	9.4965	-0.0262	1.5590 H
103 H103	9.7445	0.5725	-0.1348 H
104 H104	8.4677	2.2448	0.9196 H
105 H105	6.7842	-0.0548	2.0606 H
106 H106	4.9620	1.4847	2.1847 H
107 H107	6.0474	2.9260	1.9980 H
108 H108	5.5845	2.7626	-0.2755 H
109 H109	-8.2513	0.1649	-0.9639 H
110 H110	-9.5483	1.2316	-0.2528 H
111 H111	-7.9503	2.0572	1.4362 H
112 H112	-7.9198	2.7381	-1.3079 H
113 H113	-6.0736	1.3719	-1.8675 H
114 H114	-5.1402	2.8446	-1.3887 H
115 H115	-4.8326	2.0707	0.8564 H
116 H116	-4.2737	-2.7923	-1.4926 H
117 H117	-5.8569	-1.1348	-0.6152 H
118 P118	7.1304	0.6089	4.5490 P
119 O119	7.7323	1.1833	5.8125 O
120 O120	7.6288	-1.0014	4.4055 O
121 O121	5.4224	0.6462	4.6289 O
122 C122	5.0110	1.9167	5.0817 C
123 C123	3.4781	2.0176	5.0576 C
124 O124	2.9836	1.7358	3.7693 O
125 C125	2.8092	1.0606	6.0351 C
126 O126	2.6794	1.6309	7.3108 O
127 C127	1.4617	0.8530	5.4051 C
128 C128	1.7080	1.1442	3.9248 C
129 N129	1.6198	-0.0673	3.1495 N
130 C130	2.6587	-0.8461	2.6804 C
131 N131	2.2565	-1.9155	2.0530 N
132 C132	0.8835	-1.8427	2.1134 C
133 C133	-0.1202	-2.6778	1.5832 C
134 N134	0.1647	-3.7743	0.8682 N
135 N135	-1.3978	-2.3517	1.8450 N
136 C136	-1.6679	-1.2318	2.5425 C
137 N137	-0.8153	-0.3505	3.0364 N
138 C138	0.4551	-0.7090	2.7957 C
139 H139	-0.5925	-4.2449	0.3879 H
140 H140	1.0924	-3.8248	0.4797 H
141 O141	-9.2413	-6.3525	-0.1274 O
142 C142	-9.9676	-5.9544	1.0061 C
143 C143	-9.1858	-4.9219	1.8296 C
144 O144	-7.9027	-5.4094	2.1427 O
145 C145	-9.0264	-3.5881	1.1134 C
146 O146	-10.1693	-2.7849	1.3369 O
147 C147	-7.7861	-3.0397	1.7739 C
148 C148	-7.0473	-4.2878	2.2604 C
149 N149	-5.8153	-4.4853	1.5137 N

Design, Synthesis and Photophysical Properties of Fused Triazolyl 2-Quinolinone Nucleoside

150 C150	-4.7095	-3.6844	1.8477 C
151 O151	-4.7609	-2.7755	2.6542 O
152 N152	-3.5472	-4.0035	1.1875 N
153 C153	-3.3930	-4.8973	0.1615 C
154 O154	-2.3128	-5.0291	-0.4201 O
155 C155	-4.5857	-5.6458	-0.2058 C
156 C156	-4.4807	-6.6305	-1.3295 C
157 C157	-5.7180	-5.4057	0.4808 C
158 H158	-2.7224	-3.3875	1.4316 H
159 H159	5.3586	2.0932	6.1239 H
160 H160	5.4172	2.7222	4.4295 H
161 H161	3.1959	3.0647	5.3148 H
162 H162	3.3437	0.0822	6.0846 H
163 H163	1.0871	-0.1781	5.5910 H
164 H164	0.7236	1.5824	5.8085 H
165 H165	0.9460	1.8645	3.5543 H
166 H166	3.6949	-0.5836	2.8250 H
167 H167	-2.7240	-1.0505	2.7118 H
168 H168	-10.9665	-5.5441	0.7270 H
169 H169	-10.1454	-6.8509	1.6372 H
170 H170	-9.7360	-4.7616	2.7860 H
171 H171	-8.8305	-3.7668	0.0315 H
172 H172	-7.1864	-2.4397	1.0552 H
173 H173	-8.0570	-2.4023	2.6453 H
174 H174	-6.8064	-4.1750	3.3422 H
175 H175	-5.4350	-7.1269	-1.5048 H
176 H176	-4.1674	-6.1325	-2.2484 H
177 H177	-3.7251	-7.3836	-1.1031 H
178 H178	-6.6100	-5.9501	0.2026 H
179 C179	1.1774	1.6905	0.2103 C
180 C180	2.5396	2.0460	0.2884 C
181 C181	2.8720	3.2986	0.8359 C
182 C182	1.9118	4.1657	1.3204 C
183 C183	0.5670	3.7693	1.2609 C
184 C184	0.1990	2.5601	0.7148 C
185 H185	3.8984	3.6293	0.8982 H
186 H186	-0.2007	4.4279	1.6524 H
187 H187	-0.8406	2.2665	0.6582 H
188 C188	2.2890	5.5042	1.8994 C
189 H189	3.3716	5.6124	1.9691 H
190 H190	1.9094	6.3175	1.2774 H
191 H191	1.8696	5.6309	2.8995 H
192 H192	3.5613	1.5309	7.7547 H
193 H193	-9.5874	-5.8280	-0.8956 H
194 H194	10.4239	-3.9994	-5.3710 H
195 H195	-2.2015	8.4549	-0.4870 H
196 N196	5.1080	-0.4162	-0.5867 N

4.9. ^1H , ^{13}C NMR and NOESY spectra

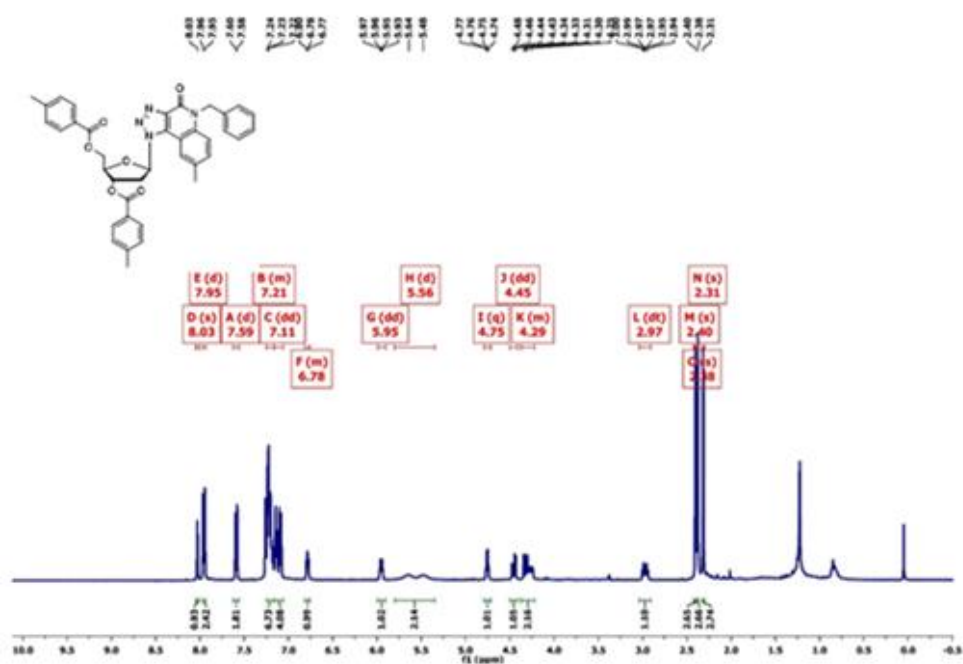
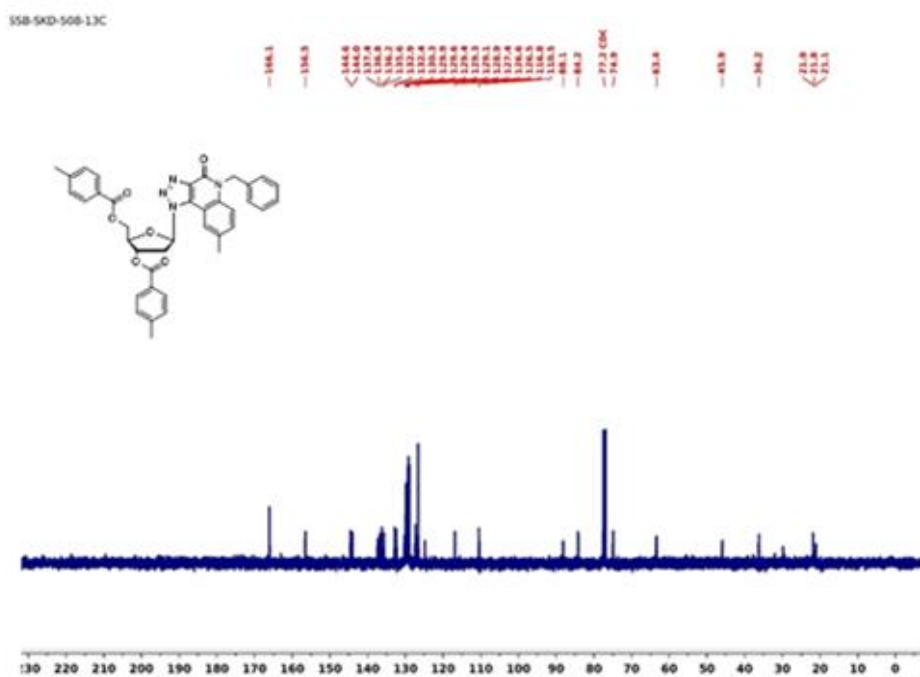


Figure 4.18: ^1H NMR of 4.46



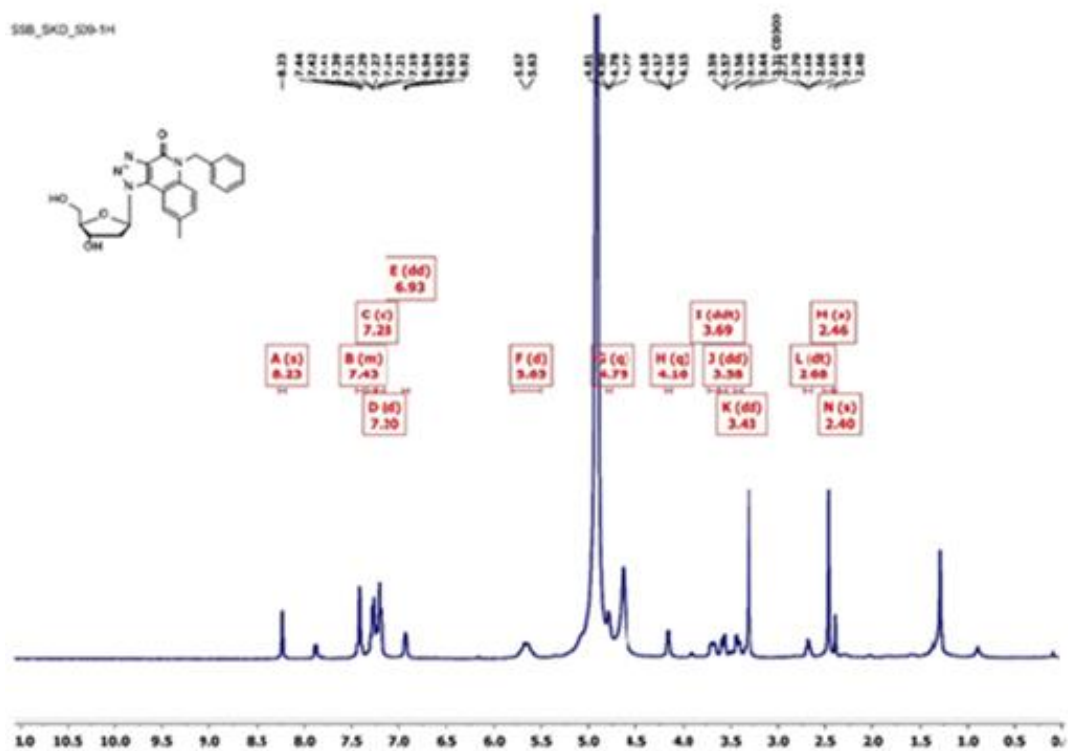


Figure 4.20: ^1H NMR of 4.47

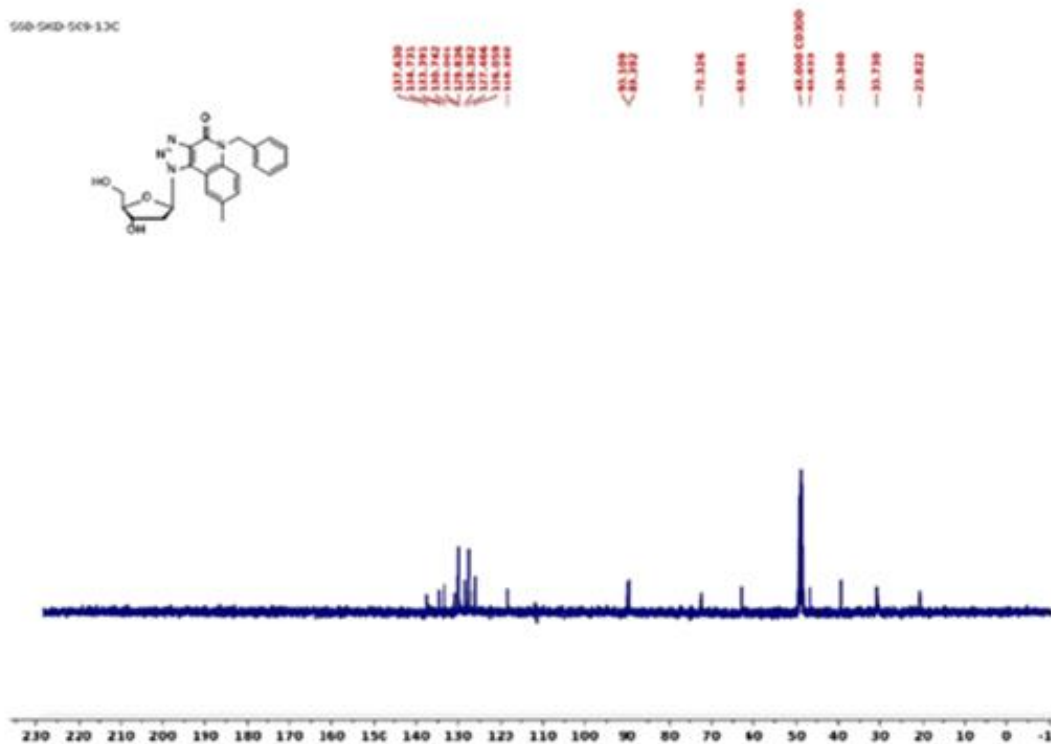


Figure 4.21: ^{13}C NMR of 4.47

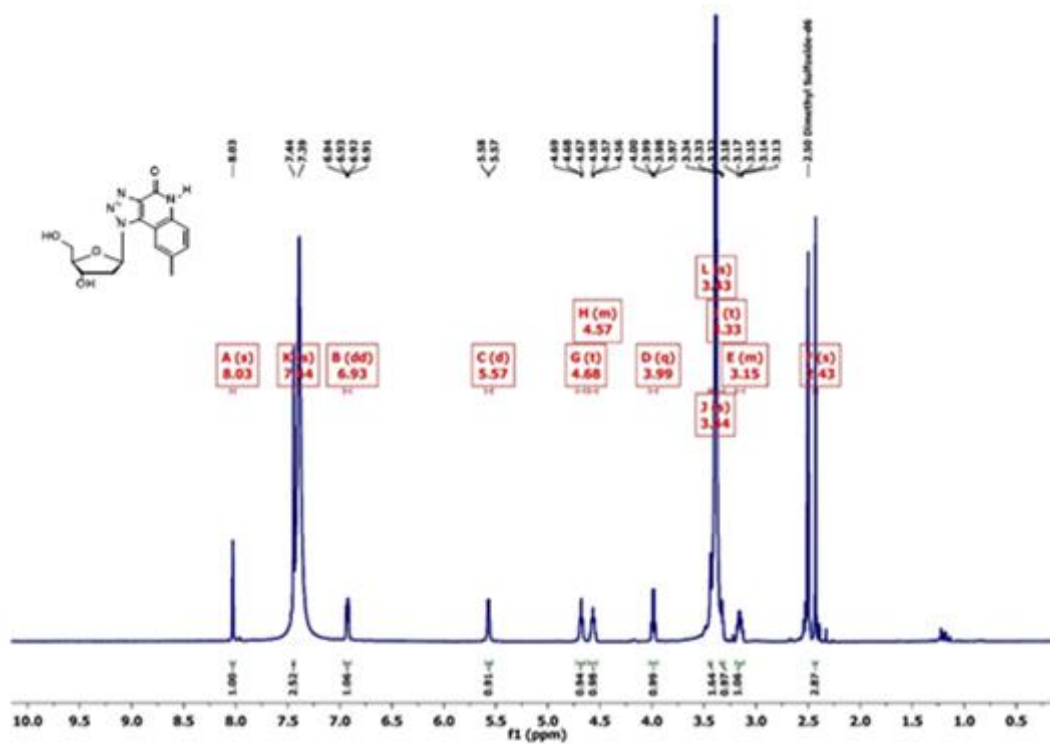


Figure 4.22: ¹H NMR of 4.40

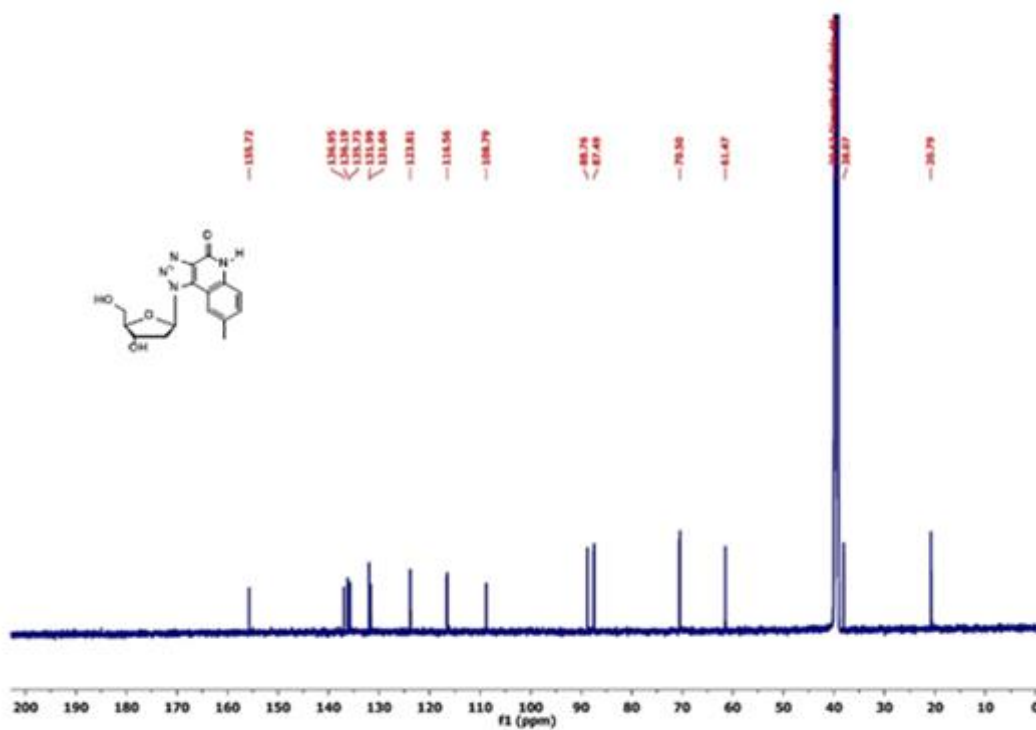
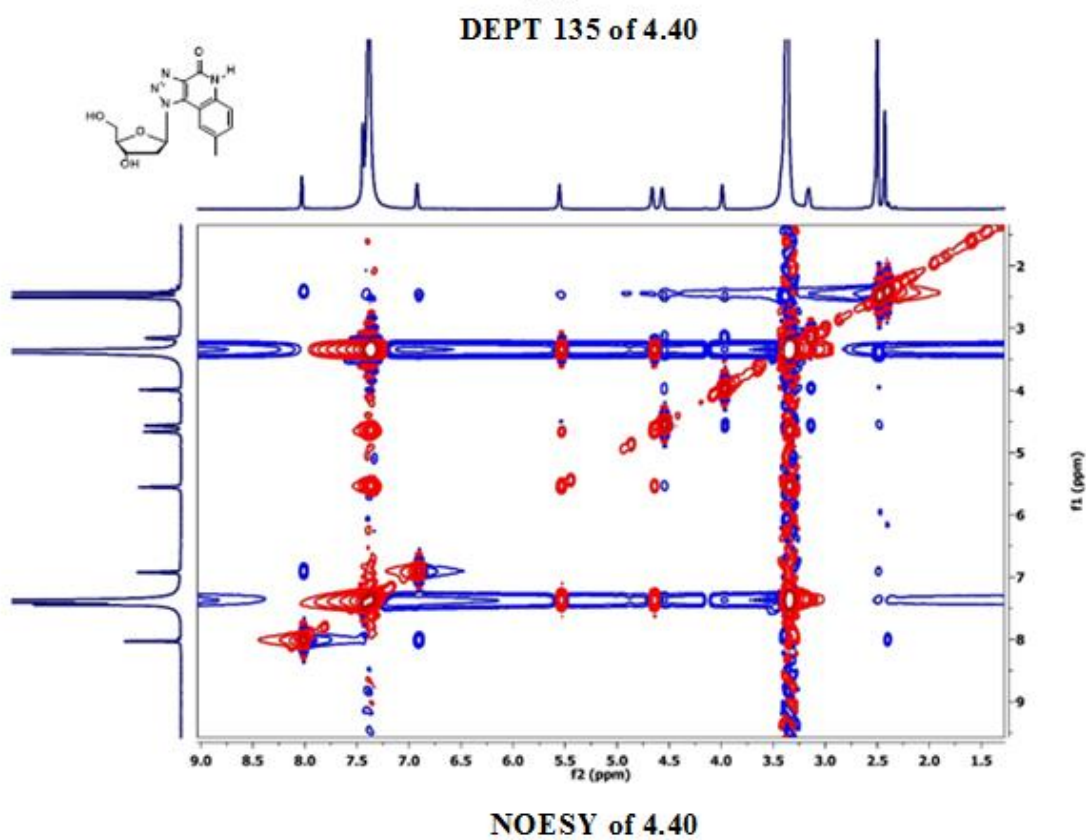
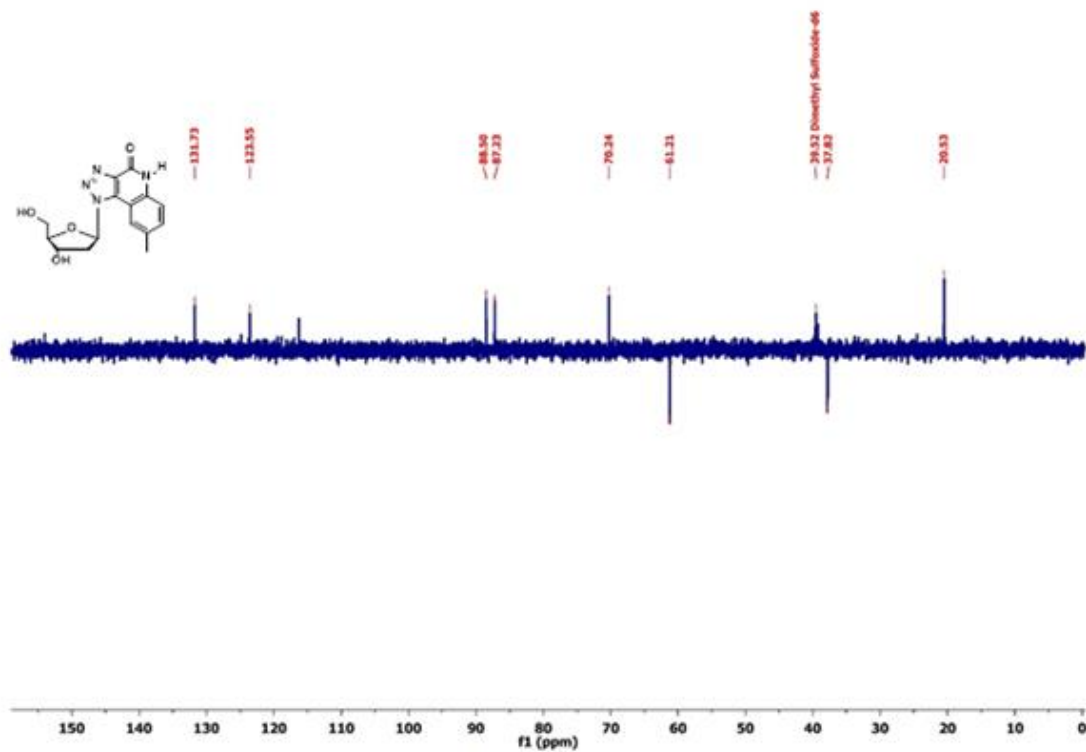


Figure 4.23: ¹³C NMR of 4.40

Design, Synthesis and Photophysical Properties of Fused Triazolyl 2-Quinolinone Nucleoside



4.10. Reference

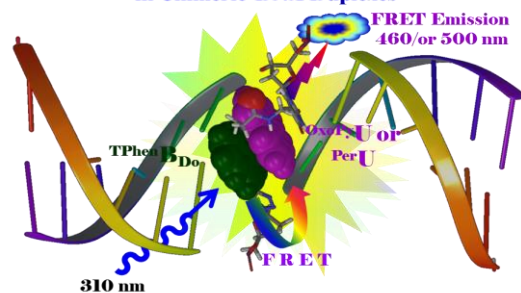
1. Rich, A.; Kasha, M.; Pullmann, B. *Horizons in Biochem.* **1962**, 103.
2. Switzer, C.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, 111, 8322.
3. Sanchez, R. A.; Ferris, J. P.; Orgel, L. E. *J. Mol. Biol.* **1968**, 38 (1), 121 128.
4. Story, R. M.; Steitz, T. A. Benner, S. A. *Nature* **1992**, 355, 242–244.
5. Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, 32, 10489–10496.
6. Horlacher, J.; Hottiger, M.; Podust, V. N.; Hubscher, U.; Benner, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, 92, 6329–6333.
7. Kulikowska, E.; Kierdaszuk, B.; Shugar, D. *Acta. Biochim. Pol.* **2004**, 51, 493.
8. Benner, S. A. *Acc. Chem. Res.* **2004**, 37, 784.
9. Rao, P.; Benner, S. A. *J. Org. Chem.* **2001**, 66, 5012.
10. Roberts, C.; Bandaru, R.; Switzer, C. *J. Am. Chem. Soc.* **1997**, 119, 4640.
11. Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, 115, 4461.
12. Sepiol, J.; Kazimierczuk, Z.; Shugar, D. *Z. Naturforsch.* **1976**, 31, 361.
13. Martinot, T. A.; Benner, S. A. *J. Org. Chem.* **2004**, 69, 3972.
14. Sismour, A. M.; Benner, S. A. *Nucleic Acids Res.* **2005**, 33, 5640.
15. Yang, Z.; Sismour, A. M.; Sheng, P.; Puskar, N. L.; Benner, S. A. *Nucleic Acids Res.* **2007**, 35, 4238.
16. Yang, Z.; Chen, F.; Alvarado, J. B.; Benner, S. A. *J. Am. Chem. Soc.* **2011**, 133, 15105.
17. Yang, Z.; Chen, F.; Chamberlin, S. G.; Benner, S. A. *Angew. Chem., Int. Ed.* **2010**, 49, 177.
18. Yang, Z.; Chen, F.; Alvarado, J. B.; Benner, S. A. *J. Am. Chem. Soc.* **2011**, 133, 15105.
19. (a) Scopes, D. I.; Barrio, J. R.; Leonard, N. J. *Science* **1977**, 195, 296. (b) Lessor, R. A.; Gibson, K. J.; Leonard, N. J. *Biochemistry* **1984**, 23, 3868.
20. (a) Liu, H.; Gao, J.; Maynard, L.; Saito, Y. D; Kool, E. T. *J. Am. Chem. Soc.* **2004**, 126, 1102. (b)) Fuentes-Cabrera, M.; Sumpter, B. G.; Wells, J. C. J. *Phys. Chem. B* **2005**, 109, 21135.
21. (a) Lu, H.; He, K.; Kool, E. T. *Angew. Chem., Int. Ed.* **2004**, 43, 5834. (b) Lee, A. H. F.; Kool, E. T. *J. Am. Chem. Soc.* **2005**, 127, 3332. (c) Lee, A. H. F.; Kool, E. T. *J. Org. Chem.* **2005**, 70, 132.

22. Gao, J.; Liu, H.; Kool, E. T. *Angew. Chem., Int. Ed.* **2005**, 44, 3118.
23. Doi, Y.; Chiba, J.; Morikawa, T.; Inouye, M. *J. Am. Chem. Soc.* **2008**, 130, 8762.
24. (a) Minakawa, N.; Kojima, N.; Hikishima, S.; Sasaki, T.; Kiyosue, A.; Atsumi, N.; Ueno, Y.; Matsuda, A. *J. Am. Chem. Soc.* **2003**, 125, 9970. (b) Hikishima, S.; Minakawa, N.; Kuramoto, K.; Ogata, S.; Matsuda, A. *ChemBioChem* **2006**, 7, 1970.
25. Minakawa, N.; Ogata, S.; Takahashi, M.; Matsuda, A. *J. Am. Chem. Soc.* **2009**, 131, 1644.
26. Da Costa, C. P.; Fedor, M. J.; Scott, L. G. *J. Am. Chem. Soc.* **2007**, 129, 3426.
27. Bag, S. S.; Talukdar, S.; Matsumoto, K.; Kundu, R. *J. Org. Chem.* **2013**, 78, 278.
28. MacroModel, Version 9.9 Schrodinger, LLC, New York, NY, 2012.
29. Frisch M. J. *et al.* Gaussian 09, revision A.1, Gaussian, Inc., Wallingford, CT, **2009**.
30. (a) Zhao, Y.; Schultz, N. E.; Truhlar, D. G.; *J. Chem. Theory Comput.* **2006**, 2, 364. (b) Zhao, Y.; Truhlar, D. G. *Acc. Chem. Res.* **2008**, 41, 157. (c) Zhao, Y.; Truhlar, D. G.; *J. Chem. Phys.* **2006**, 125, 194101. (d) Zhao, Y.; Truhlar, D. G. *J. Chem. Theory Comput.* **2007**, 3, 289.

Chapter 5

HYBRIDISATION ACCOMPANYING FRET EVENT IN LABELLED NATURAL NUCLEOSIDE-UNNATURAL NUCLEOSIDE CONTAINING CHIMERIC DNA DUPLEXES

Hybridization Accompanying FRET Event
in Chimeric DNA Duplexes



5.1. Introduction

Förster resonance energy transfer (FRET) has got immense application in chemistry, biology, optical imaging and in DNA analysis including medicinal sciences.¹ FRET is a process in which excitation energy is transferred from a donor fluorophoric unit (**D**) from its excited state to an acceptor fluorophoric unit (**A**) (**Figure 5.1**). The absorption spectrum of the donor overlaps with the fluorescence emission spectrum of the acceptor, indicates the possibility of FRET process to occur. The excited state energy transfer can take place through a number of different type of interaction mechanisms which might be *coulombic* and/or *intermolecular orbital overlap*. The *coulombic* interaction includes short-range multi-polar interactions and long range dipolar-interactions. On the other hand, *intermolecular orbital overlap* includes *electron exchange* and *charge resonance* interactions where both are short range interactions. It is to be noted that both the *coulombic* and *intermolecular orbital overlap* interactions are singlet-singlet energy transfer interaction ($^1\text{D}^* + ^1\text{A} \rightarrow ^1\text{D} + ^1\text{A}^*$) whereas *intermolecular orbital overlap* also involves triplet-triplet ($^3\text{D}^* + ^1\text{A} \rightarrow ^1\text{D} + ^3\text{A}^*$) energy transfer interaction. On the other hand, *coulombic* interaction is the process where the excited electron on the donor returns to its ground state while simultaneously an electron on the acceptor promoted to its excited state. The exchange term involves to an energy transfer process which is associated with an exchange of two electrons between acceptor (**A**) and donor (**D**).²

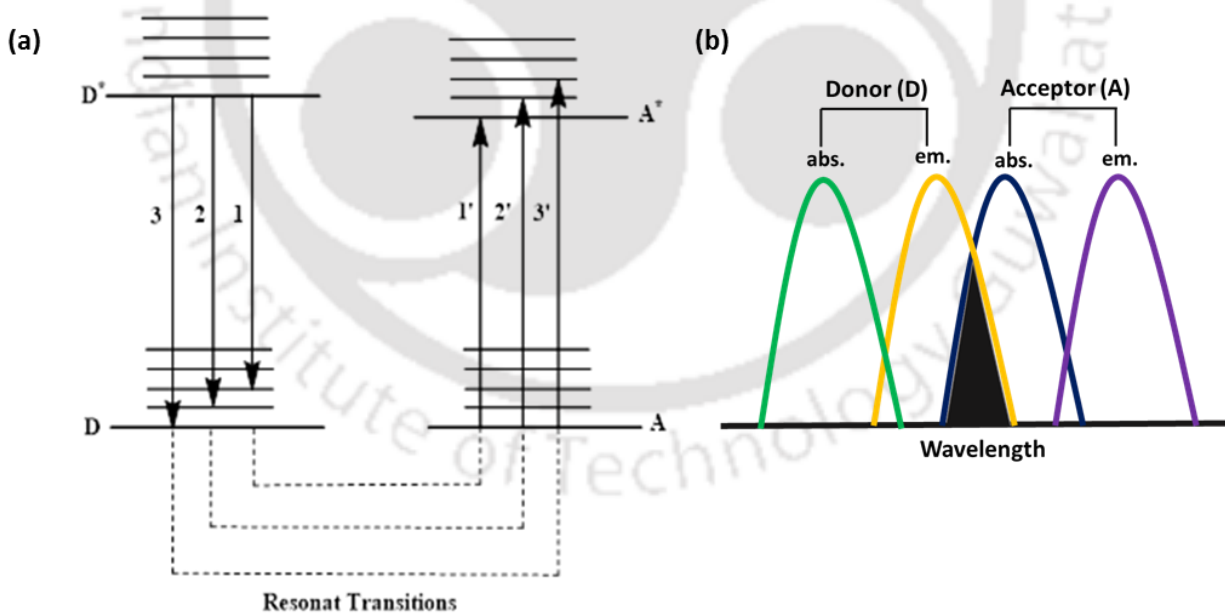


Figure 5.1: (a) Schematic representation of Förster resonance energy transfer (FRET). (b) Overlapping region of the emission spectrum of the donor (**D**) with the absorption spectrum of the acceptor (**A**).

The nonradiative energy transfer occurs only when the donor and acceptor will be within a certain distance i.e. the characteristic Förster distance and that is why FRET is a distance dependent nonradiative energy transfer phenomena. Due to this reason FRET is used to measure distance in molecular assemblies. The intermolecular distance between **D** and **A** defines the rate of energy transfer between these two moieties.^{1,3} Generally in a FRET process as the energy is transferred from donor to acceptor thereby fluorescence intensity and quantum efficiency of the donor decreased and that of the acceptor increased.

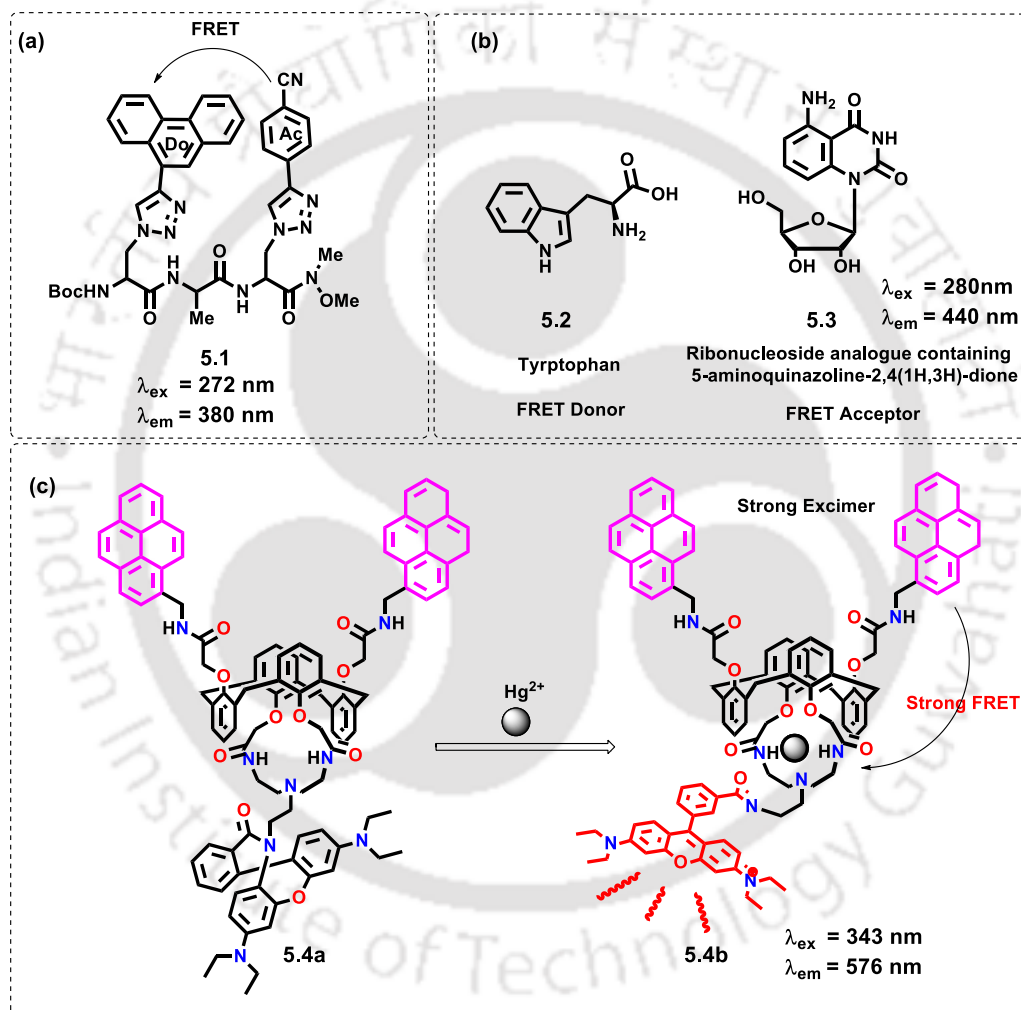


Figure 5.2: (a) FRET in determining peptide conformation. (b) RNA analogues can be selective towards protein containing native tryptophan. (c) Metal ion sensing through FRET process.

FRET is a spectroscopic ruler, and thus can be effectively utilized in determining molecular proximity at angstrom level in many biological process with much accuracy compare to the other

methods.⁴ There are many biological events that require its usage e.g. in the study of cellular process where FRET imaging technique is used extensively.^{5,4c} It is also used widely for probing cell structure,^{5b} in determining protein folding/unfolding status,^{4c} for glucose detection in clinical diagnostic^{5d} and studying the antigen-antibody interaction.^{5e}

Recently our group reported a tripeptide **5.1** where FRET process have been used to carry out its conformational analysis. As FRET is a distance dependent phenomenon, it helps to draw a conclusion about the solution conformation of the tripeptide. The proof of the two triazolyl moieties i.e. phenanthrene triazole and 4-benzonitrile triazole are coming closer is evidenced by the FRET event operating within them helping us to conclude that the solution conformational orientation of this tripeptide is β -turn (**Figure 5.2**).^{6a}

Tor *et al.* reported that a fluorescent RNA analogue **5.3** comprised 5-aminoquinazoline-2,4(1*H*,3*H*)-dione can be capable to act as a FRET acceptor of natural amino acid tryptophan **5.2** (**Figure 5.2**). So this FRET pair is capable in monitoring protein-RNA interactions in proteins and peptides with native tryptophan residues.^{6b}

Besides Lee *et al.* used it in metal ion sensing. They developed a calix[4]arene derivative **5.4a** consist of two pyrene and one rhodamine fluorophore can be capable to act as a selective sensor for Hg^{2+} ion (**Figure 5.2**).^{6c}

5.2. FRET based DNAs and its applications

Förster resonance energy transfer (FRET) has been widely used in monitoring the hybridization of the duplex DNA containing fluorescent dye. It is the process through which it is possible to get a glimpse of the structure and dynamics of the supramolecules containing chromophoric unit.⁷ The last two decades have been involved in the process of determination and the analysis of duplex DNA conformation.

In the pioneering work of Clegg *et al.*⁸ it was reported that the oligonucleotide containing donor acceptor fluorophore in the appropriate position led to the FRET event. This study provided an excellent opportunity in exploring FRET techniques for the investigation of structure of the hybridized duplex DNA. Latter on this technique has been extended by Tor *et al.*⁹ and several such studies showed that the DNA duplex can be an excellent platform in studying the FRET process due to its predictable nature of arraying nucleobases in a probable manner. After the invention of DNA as a sculpture in exploring FRET process with much accuracy, quite a lot of FRET based DNA system have been explored. As for example in an electrostatic complex of a dye-labelled anionic DNA strand and a cationic conjugated polymer, in situ demonstration of optical DNA detection has been carried out via FRET techniques through the measurement of amplified fluorescence of DNA which is labelled with fluorescent dye.¹⁰

A FRET system was designed consisting of gold nanoparticles (**GNPs**), oligonucleotides, and chromophores, utilised for the detection of DNA complementary to G-quadruplex. It was found that the hybridisation with targeted DNA leading to the enhancement of fluorescence

resulted because of the stretching of G-quadruplex. The detection limit by using this FRET system can be as low as 40 pM ($S/N=3$). This result led to a conclusion that the bigger **GNPs** had the ability of higher fluorescent enhancement after hybridisation.¹¹ Luca *et al.* applied FRET techniques in a suitable donor-acceptor system which revealed DNA sequences that is specific to genetically correlated disease.¹² They employed this strategy and able to identify an individual, even heterozygote which was carrying the DQB1 gene sequence confers the exposure for developing insulin-dependent diabetes mellitus without having been performed any PCR amplification, DNA extraction or purification.¹³ So this discussion suggests over the year development of FRET-DNA system which are extensively used in the field of monitoring DNA hybridization and detection of genetic mutations.¹⁴

In another example fluorescein **5.5** and rhodamine **5.6** family were used respectively as a donor/acceptor pairs which are often linked with DNA nucleobase through a flexible linker which allow them to move freely in space to interact with each other in taking part to the FRET phenomenon (**Figure 5.3**).¹⁵

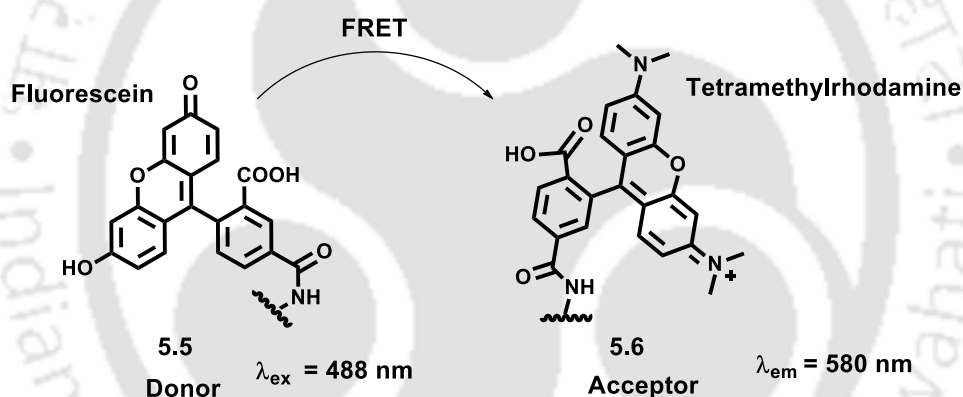


Figure 5.3: DNA labelled with fluorescein-rhodamine FRET donor-acceptor pair and their FRET involvement.

Pyrene and perylene based aromatic hydrocarbon can be chosen as a FRET pair because of their spectral characteristic. As perylene has high quantum yield its absorption spectrum overlaps with the fluorescence emission spectrum of pyrene and thus perylene can be designated as an FRET acceptor whereas pyrene can be designated as a FRET donor.^{16,17} Masuko and coworkers are the first to introduce pyrene and perylene as a donor/acceptor FRET pair in the study of the DNA duplex hybridisation.¹⁷ So after hybridisation according to their report the FRET efficiency was 100%.

Saito *et al.*¹⁸ reported dual-labeled oligonucleotide probe was used to sense adenosine through FRET interaction. Pyrene **5.7** and anthracene **5.8** containing fluorescent nucleoside were

incorporated into the middle of the DNA, were acted as a FRET donor. Nucleoside consisting of fluorescein **5.9** is incorporated into the 5' end of the oligonucleotide acted as a FRET acceptor. The FRET phenomenon was observed between the donor and acceptor nucleoside. Upon excitation at the donor, a selective emission from the acceptor was observed only when adenine would place opposite to the base discriminating fluorescence (**BDF**) nucleoside i.e. ^{Py}U and/or ^{2-Ant}U. Thus it was possible to detect the presence or absence of adenine whenever placed as a complementary pair opposite to **BDF** labeled probe DNA through the generation of a discriminating fluorescence signal via FRET (**Figure 5.4**).

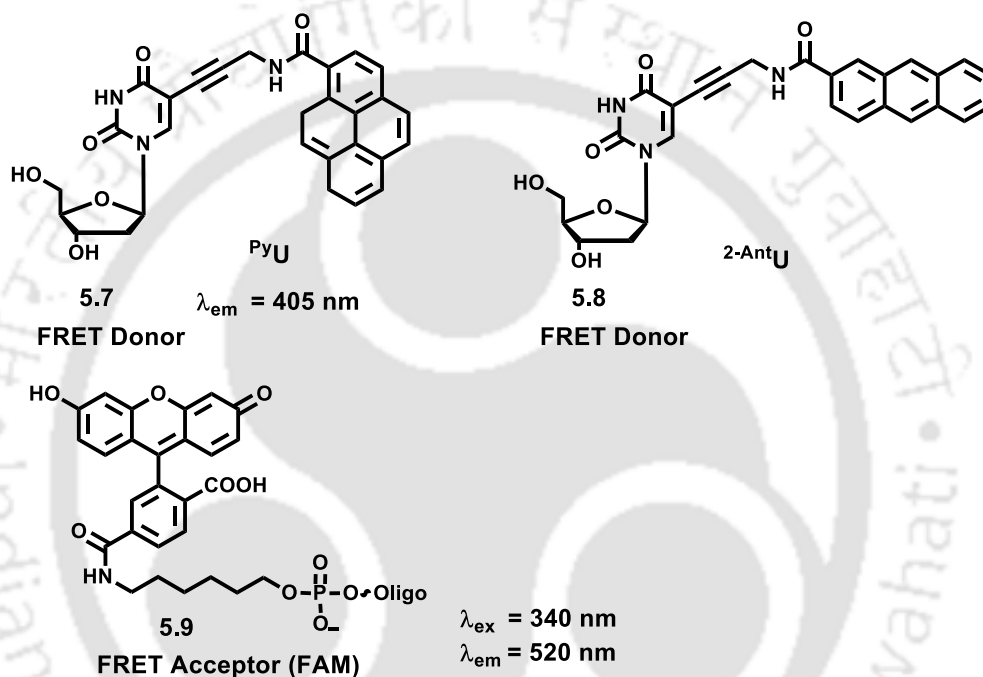


Figure 5.4: Structures of the two **BDF** nucleosides ^{Py}U and ^{2-Ant}U as donors and the **FAM** (F) as the FRET acceptor used in probe designing for DNA detection.

Fluorophores tethered with nonnucleosidic moieties was incorporated into DNA and then FRET was explored through DNA hybridization. To explore the structure, dynamics and hybridization with the non-nucleosidic base incorporation into the DNA, Asanuma and co-workers¹⁹ have chosen two non nucleosidic moiety based on D-threoninol where D-threoninol is labelled with pyrene **5.10** and perylene **5.11**. This incorporation followed by hybridization of two single strand ODNs where pyrene and perylene modified D-threoninol placed just opposite to each other make them understand to draw a conclusion that the pyrene-peryene is forming as a FRET donor-acceptor pair (**Figure 5.5**).

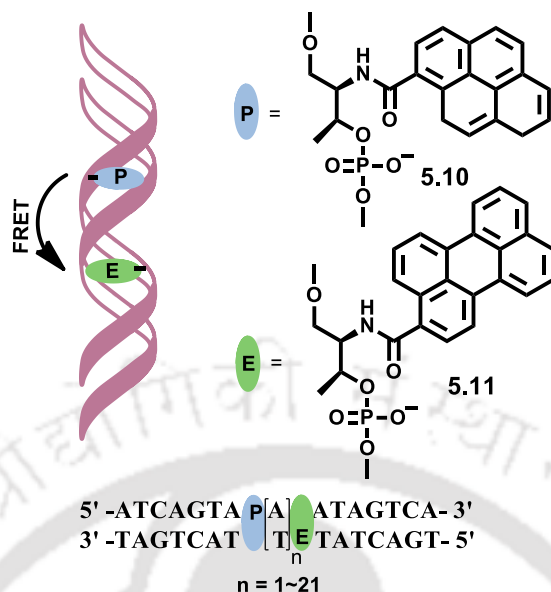


Figure 5.5: Incorporated of pyrene and perylene modified D-threoninol non-nucleosidic base into DNA which are behaving as a FRET pair.

Wilhelmsson *et al.*²⁰ presented first time a detailed a precise investigation on the structure and dynamics of the nucleic acid and the interaction of them with other biomacromolecules through the exploitation of FRET interaction. The doubly labelled DNA duplex was designed contained two nucleobases in two strand acting as the donor acceptor FRET pair. They designed one strand contains an energy donor unnatural nucleoside labelled with 1,3-diaza-2-oxophenoxazine **5.12** and another strand modified with a nucleoside labelled with an energy acceptor 7-nitro-1,3-diaza-2-oxophenothiazine **5.13**. In the designed duplex both the nucleobases are rigidly located within the base stack confirming a high control of orientation factor in the FRET event. They observed the FRET pair is able to monitor distances which successfully covering up more than one turn of DNA duplexes. As the number of bases are separating the two FRET pair, they are able to generate different type of distinguishable FRET signal. This study through the interaction of the FRET pair revealed that the distance dependent FRET event can efficiently able to provide the information about the varying interaction between the differently placed nucleic acids and other molecules and biomacromolecules (**Figure 5.6**).

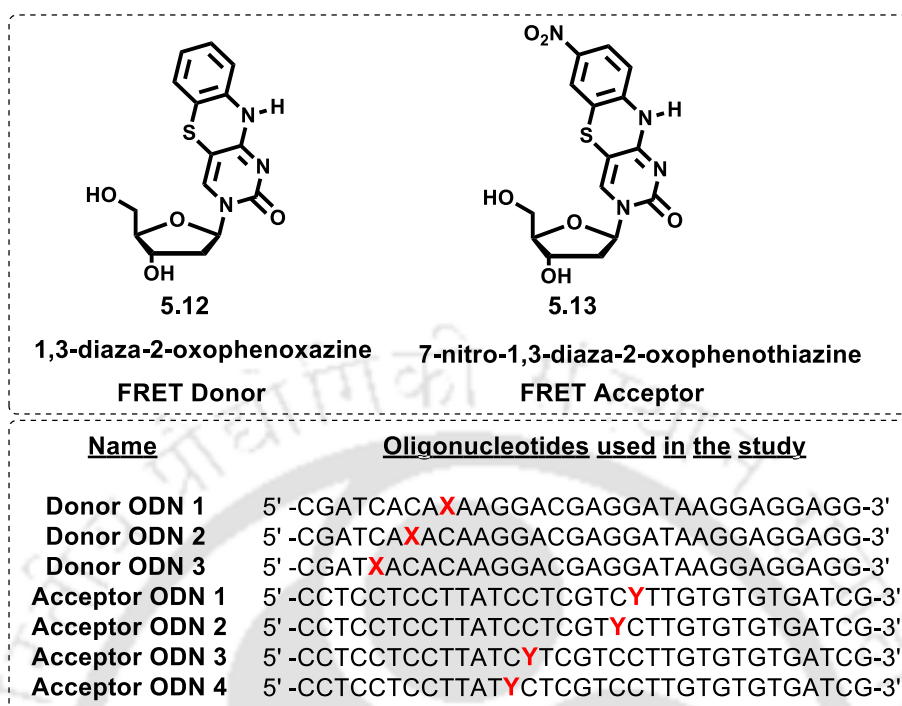


Figure 5.6: 1,3-diaza-2-oxophenoxazine and 7-nitro-1,3-diaza-2-oxophenoxazine modified FRET pair and oligonucleosides used in FRET interaction.

5.3. Background

The transfer of excited electronic state energy non-radiatively of a donor fluorescent molecule (**D**) to an acceptor molecule (**A**) via long range dipole-dipole interaction is known as Förster resonance energy transfer (FRET).¹ The rate of energy transfer is dependent on the intermolecular distance between the **D** and **A**.¹ In general, the fluorescence intensity and the quantum efficiency of the acceptor increases and that of the donor decreases in a FRET event.¹ FRET as a spectroscopic ruler, thus, can probe the molecular proximity quantitatively in many biological events at angstrom distances with much more accuracy than other methods can provide.⁴ Rigid structure of double helical DNA allows the opportunity for studying distance dependence of FRET process.²¹ Many FRET-based DNA systems have, therefore, been developed and widely utilized in the genetic analysis such as the detection of genetic mutations and monitoring the DNA hybridization events.¹⁴ Recently, several backbone modified multichromophoric DNA systems have been developed for studying various photophysical interactions among the chromophores.²² A number of DNA scaffolds such as non-nucleosidic base surrogates,^{23a} labels of nucleobases,^{23b} unnatural nucleobase^{23c} and fluorescent nucleic acids mimics^{23d} with multiple chromophores have been developed and their photophysical properties investigated. A number of laboratories have

also explored FRET interactions in multichromophoric DNA using traditional monomeric fluorescent dyes in quest of optoelectronic devices, light harvesting materials and clinical diagnostics.²⁴ Therefore, it is clear that FRET have been widely employed in biological and biophysical studies. Because of the broad utility, the addition of new classes of chromophores/labeled DNA for studying hybridization accompanying FRET event is currently an attractive research area in nucleic acid system.

5.4. Objective

Recently, we have reported a dual door entry system to exciplex emission wherein we observed that the FRET is one of the two paths through which exciplex emission is observed in a chimeric DNA duplex.²⁵ Herein, we want to report two novel FRET based DNA systems in which fluorescent unnatural nucleoside, triazolyphenanthrene (**5.15**, $\text{T}^{\text{Phen}}\text{B}_{\text{Do}}$) paired against either γ -oxopyrene labeled-2'-deoxyuridine (**5.14**, OxoPyU) or perylene labeled 2'-deoxyuridine (**5.16**, PerU) in the central position of the formed two chimeric DNA duplexes (**Figure 5.7**).

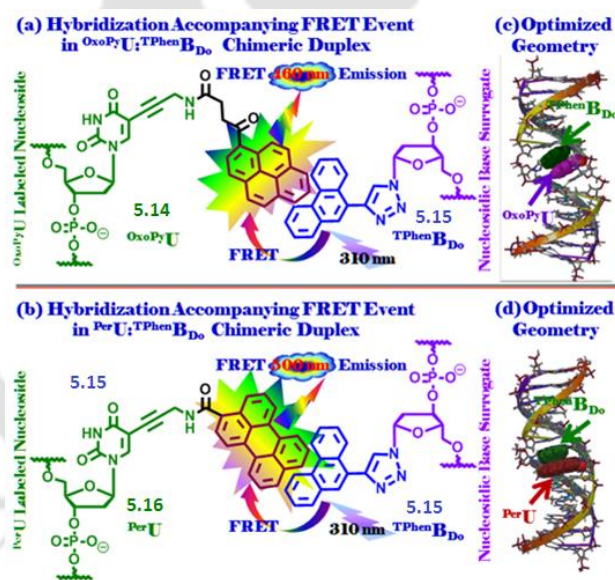


Figure 5.7: (a-b) Schematics of FRET process and the structures of the oxopyrene and perylene labelled natural uridine (OxoPyU and PerU) and unnatural nucleoside, triazolyphenanthrene ($\text{T}^{\text{Phen}}\text{B}_{\text{Do}}$). (c-d) Amber* optimized geometry of the chimeric base pairs $\text{OxoPyU}:\text{T}^{\text{Phen}}\text{B}_{\text{Do}}$ and $\text{PerU}:\text{T}^{\text{Phen}}\text{B}_{\text{Do}}$ held by inter-strand π -stacking interaction.

The idea behind our choice of a pair of fluorescent chimeric nucleobases is the flexible long chain of OxoPyU and/or rigid propynylcarboxamide linkage in PerU which might allow oxopyrene and/or perylene chromophore of the probe DNAs to come closer to the oppositely spaced triazolyphenanthrene ($\text{T}^{\text{Phen}}\text{B}_{\text{Do}}$) nucleoside of the target DNA in the formed chimeric DNA duplexes and lead to FRET interaction process. To the best of our knowledge, the design of

such chimeric DNA duplex wherein a fluorescent unnatural nucleoside ($^{TPhen}B_{D0}$) paired against a fluorescently labeled natural nucleoside ($^{OxoPy}U$ or ^{Per}U) involved in dipolar interaction is not known.

5.5. Results and discussion

The γ -oxopyrene labeled 2'-deoxyuridine (5.14, $^{OxoPy}U$), perylene labeled 2'-deoxyuridine (5.16, ^{Per}U) and the unnatural nucleoside (5.15, $^{TPhen}B_{D0}$) were synthesized following our published protocol.²⁶

5.5.1. Study of UV-visible and Fluorescence Photophysical Properties of Nucleoside Monomers.

The UV-visible spectra of $^{OxoPy}U$ (Figure 5.8a) nucleoside in various organic solvents showed a red shifted absorbance (~ 10 nm) with decreased intensity of the band at 345-355 nm as the solvent polarity increased and it showed a highly micropolarity sensitive fluorescence emission with a large solvatochromicity (Figure 5.8b).

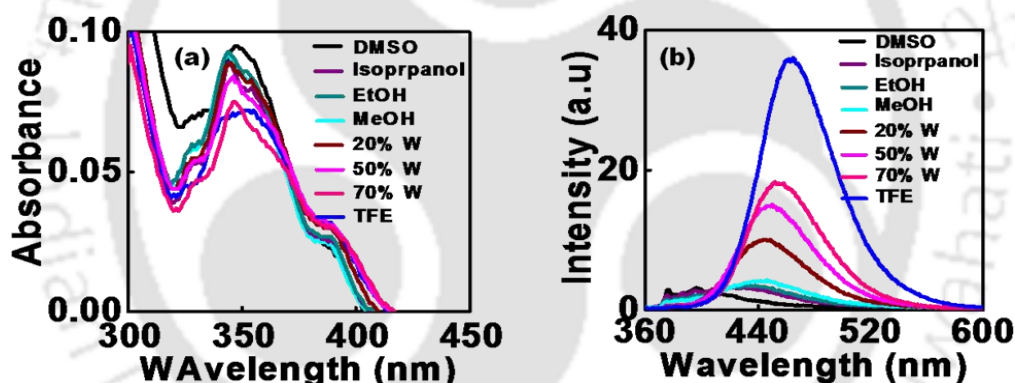


Figure 5.8: (a) UV-visible and (b) fluorescence spectra of $^{OxoPy}U$ nucleoside in different solvents.

On the other hand, ^{Per}U exhibited a strong and long wavelength absorption at 440-445 nm (Figure 5.9a). Upon excitation at 443 nm ^{Per}U showed strong fluorescence emission (Figure 5.9b) at around 478-480 nm in all organic solvents. The $^{TPhen}B_{D0}$ nucleoside showed blue shifted absorbance as the polarity of the solvents was increased ($\lambda_{max} = 302$ nm in dioxane $\rightarrow$ 298 nm in MeOH (Figure 5.10a). Upon excitation at 300 nm, $^{TPhen}B_{D0}$ showed structured bands at 363 and 380 nm (Figure 5.10b) with similar intensities and low quantum yields in all solvents.

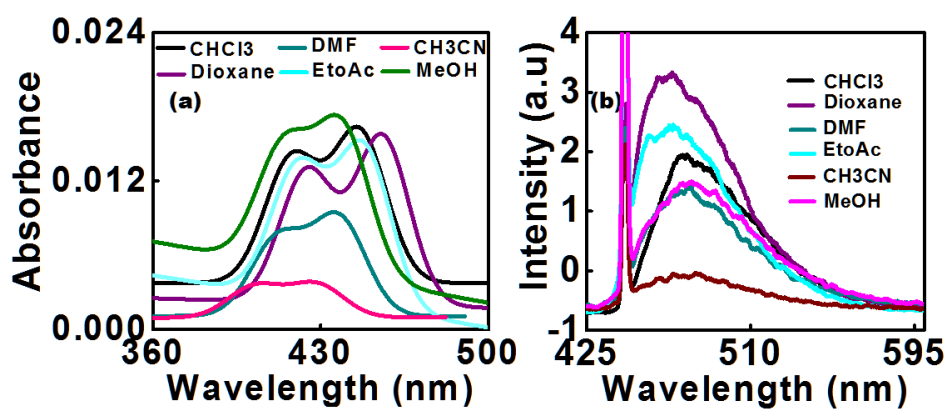


Figure 5.9: (a) UV-visible and (b) fluorescence spectra of **PerU** nucleoside in different solvents.

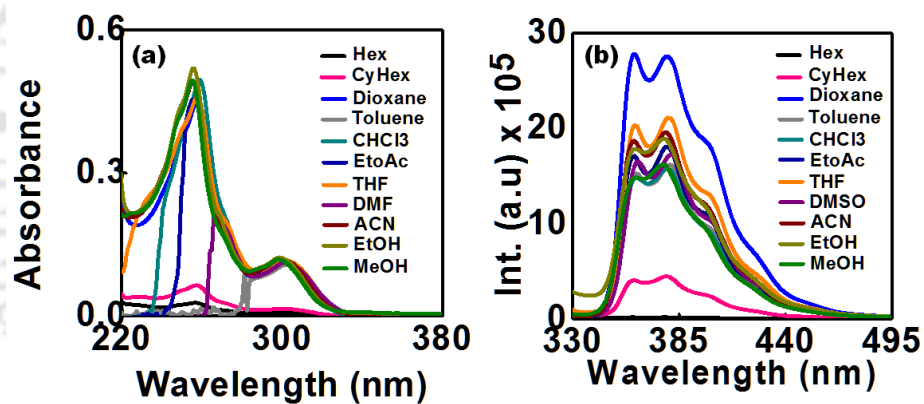


Figure 5.10: (a) UV-visible and (b) fluorescence spectra of **TPhenB_{D0}** nucleoside in different solvents.

5.5.2. Sequence of ODNs Used in This Study

Table 5.1: Sequence of ODNs

5'-CGCAAT X TAACGC-3'	
3'-GCGTTA Y ATTGCG-5'	
ODNs	Sequence
ODN 1	5'-CGCAAT OxoPyU TAACGC-3'
ODN 2	5'-CGCAAT PerU TAACGC-3'
ODN 3	5'-GCGTTA T^{Phen}B_{Do} ATTGCG-3'
ODN 4-7	5'-GCGTTA N ATTGCG-3' [N = A, G, C, T]
ODN 8-11	5'-CGCAAT N TAACGC-3' [N = A, G, C, T]

5.5.3. Synthesis and Characterization of Modified Oligonucleotides (ODN 1-3)

The synthesis of the oligonucleotide probes containing the labeled nucleodides, **OxoPydU** (ODN 1), **PerdU** (ODN 2) or the unnatural triazolylphenanthrene nucleoside, **T^{Phen}B_{Do}** (ODN 3), at the centre of each sequence was performed by a conventional phosphoramidite method using an automated DNA/RNA synthesizer. Synthesis was performed on a controlled pore glass (CPG) supported cartridge containing the 3'-nucleoside (dC for ODN 1-2 and dG for ODN 3) attached to CPG support. Synthesis was carried out automatically by sequential addition of natural and modified β -cyanoethyl phosphoramidite monomers from 3'- to 5'- direction. The monomers and most of the other reagents were prepared with 10 ppm acetonitril (p.a) and left for overnight on pre dried molecular sieves (4Å) in dark. Extended coupling (10 min, 4,5-dicyanoimidazole as activator) and oxidation (60 sec) times were used during incorporation of modified phosphoramidite monomers for achieving in very good coupling yields (>90%). The unmodified phosphoramidites- DMTr-dA^{Bz}, DMTr-dC^{Bz}, DMTr-dG^{iBu}, and DMTr-T, controlled pore glass (CPG) supported cartridges, TCA deblock, activator (4,5-dicyanoimidazole (DCI), Cap A and Cap B solutions were purchased and used. A 1 μ mol scale-solid phase synthesis of the desired ODNs was carried out in trityl-on mode using modified-phosphoramidite as the extra (fifth) phosphoramidite and programmed to cleave the trityl group from the last unmodified nucleoside. The advancement of the synthesis was monitored with a 'trityl viewer' on the basis of the outgoing trityl group produced during detritylation. The coupling yield is 90%.

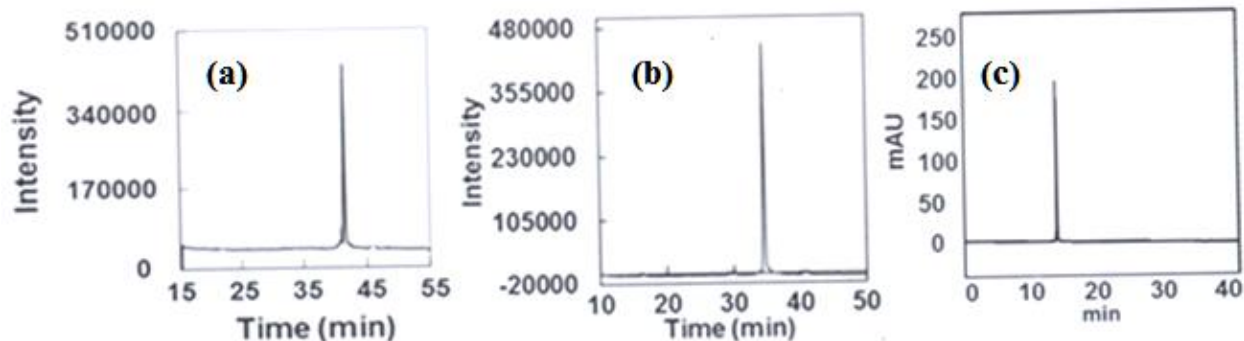


Figure 5.11: HPLC Trace of pure (a) ODN 1: 5'-CGCAAT ^{OxoPyU} TAACGC-3'; (b) ODN 2: 5'-CGCAAT ^{PerU} TAACGC-3' and (c) ODN 3: 3'-GCGTTA ^{TPhenBDo} ATTGCG-5'.

After the DNA synthesis was over, the cartridges from the synthesizer were taken out and transferred into an eppendorf (2 mL). Then the oligonucleotide was cleaved from the solid support under standard conditions (conc. aq. NH_3 , 55 °C, 12 h) by incubating the solution for at least 12 hr at 55°C in a temperature-controlled incubator. After deprotection, the ammonia was evaporated in a *speed vac*, the ODN was diluted with double distilled water (0.5 mL) and the glass beads were filtered 'off' through a Whatman 0.2 μm syringe filter. Next the solution was concentrated in a *speed vac* and made it a final solution of 1 mL. The deprotected DNA was purified from the unsuccessfully coupled sequences by reversed-phase HPLC on a 5-ODS-H column (10×150 mm, elution with 50 mM ammonium formate buffer (AF), pH 7.0, with a linear gradient over 45 min from 3% to 40% acetonitrile at a flow rate 2.0 ml/min) (**Figure 5.11**). The desired fractions were collected in 10 mL eppendorf tubes, evaporated and finally transferred into 2 mL eppendorf. The purified ODN was dissolved in 1 mL double-distilled water in an eppendorf tube and characterized by its m/z value obtained from *MALDI-TOF* using 2,4,6-trihydroxyacetophenone (0.5 M in EtOH)/Diammoniumcitrate (0.1 M in water) in 1:1 v/v as matrix. The *MALDI-TOF* was measured in normal positive mode with 19 KV acceleration voltage **Table 5.2**. The characterized and pure DNA stock solution (1-1.5 ml) was then kept in refrigerator at -80 °C for further studies.

The concentration of the DNA stock solution was then determined applying *Lamberts-Beer's* equation: $A_{260} = \log I/I_0 = c \times \epsilon_{260} \times l$ where $\epsilon_{260} = \sum \epsilon_i$; A_{260} is the absorbance of the probe at 260 nm which is determined from the intensity of the transmitted light (I) compared to the intensity of the emerging light (I_0), c is the concentration of the probe DNA, ϵ_{260} is the algebraic sum of extinction-coefficients of the individual nucleosides at 260 nm (for natural nucleosides, this is calculated with Oligo Analyser (<http://eu.idtdna.com>)) and l is the pathlength of the light through the sample.

The concentration of stock ODN 1, ODN 2 and ODN 3 were 338 μM (1.5 ml), 375 μM (1.5 ml), 138.5 μM (2.0 ml). All the natural complementary ODNS (ODN 4-7) were purchased and used as supplied.

Table 5.2: MALDI-TOF-MS of synthesized ODNs.

ODNs	Sequences	m/z found [M + H] ⁺	m/z calcd. [M + H] ⁺
ODN 1	5'- CGC AAT X TAA CGC -3' [X = OxoPyU]	4241.89	4241.92
ODN 2	5'- CGC AAT X TAA CGC -3' [X = PerU]	4235.99	4235.98
ODN 3	5'- GCG TTA Y ATT GCG -3' [Y = T ^{Phen} B _{Do}]	4097.56	4098.00

5.5.4. Thermal Melting Temperature (T_m) Measurements of the Oligonucleotides.

The probe ODNs containing oxopyrene (OxoPyU, ODN 1) and/or perylene (PerU, ODN 2) labeled natural 2'-deoxy uridine were hybridized separately with the target ODN 3 containing triazolyphenanthrene unnatural nucleoside (T^{Phen}B_{Do}) to form two separate chimeric duplexes, ODN 1•3 and ODN 2•3, respectively.

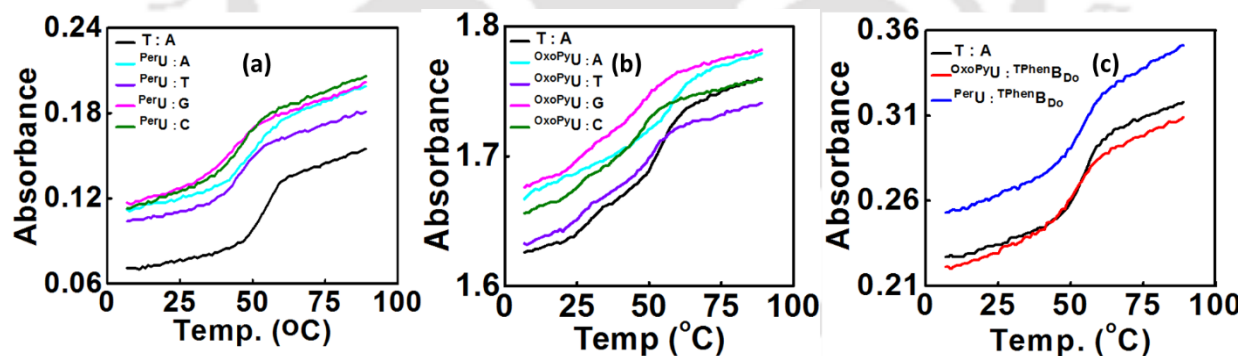


Figure 5.12: Thermal melting profiles of the various duplexes shown. The concentration of each single stranded ODN in each set = 1.0 μM in 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0.

From the thermal melting temperature, it was observed that the stability of the chimeric duplex ODN 1•3 (Figure 5.12) is comparable to that of a natural A-T or a T-A pair. On the other hand, the chimeric duplex ODN 2•3 was found to be more stable than any of the natural A-T (by 2.9 °C) or T-A (by 1.2 °C) pair (Table 5.3). Moreover, the duplex ODN 1•3 was more stable (by

0.7-3.5 °C) compared to the duplexes of ODN **1** with natural bases (ODN **1•5-7**) except when paired against adenosine (ODN **1•4**) (Table 5.3).

Therefore, $^{TPhen}B_{D0}$ is the second most selective base of $OxoPyU$. On the other hand, the thermal melting stability of duplex ODN **2•3** was found to be more (by 4.2-11 °C) compared to the duplexes wherein ^{Per}U of ODN **2** paired against any of the natural bases (Table 5.3). The highest stability and pairing selectivity of ^{Per}U for $^{TPhen}B_{D0}$ might be because of strong intercalative stacking interaction of the two chromophores (^{Per}U and $^{TPhen}B_{D0}$) inside the duplex.

5.5.5. UV-visible and Fluorescence Measurements of the Oligonucleotides.

The intercalative stacking interaction was also supported from a bathochromic shift of absorption wavelength and hypochromic effect in UV-visible spectra of the corresponding duplex ODN **1•3** and ODN **2•3** in comparison to the single strand ODN **1** and **2**, respectively (Figure 5.13a-b and 5.13c-d).²⁷

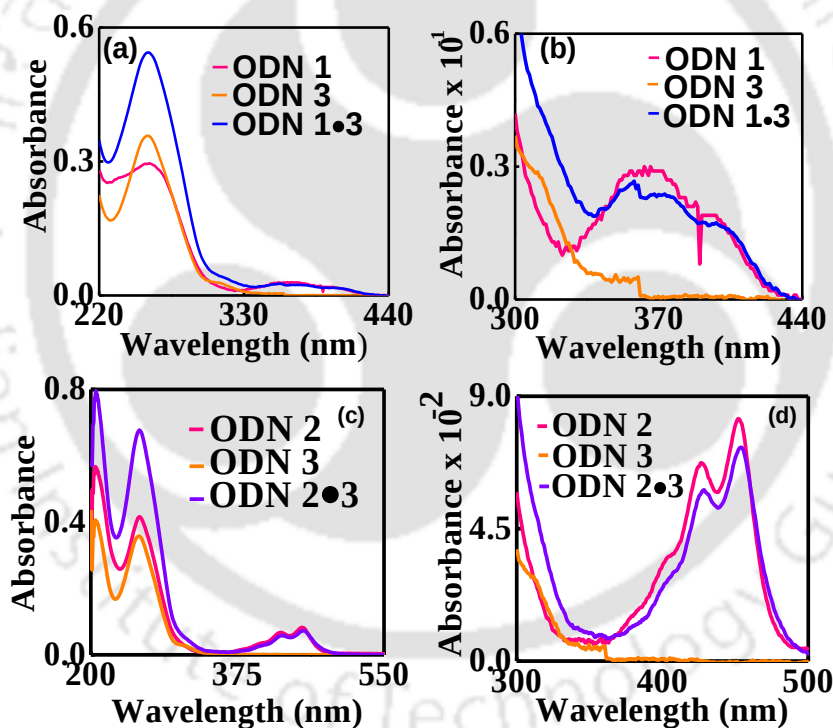


Figure 5.13: UV-visible spectra and expansion of chromophoric region of (a-b) ODN **1** (-T- $OxoPyU$ -T-), ODN **3** (-A- $^{TPhen}B_{D0}$ -A-) and their hybridized ODN **1•3** ($OxoPyU \cdot ^{TPhen}B_{D0}$), and of (c-d) ODN **2** (-T- ^{Per}U -T-), ODN **3** (-A- $^{TPhen}B_{D0}$ -A-) and their hybridized ODN **2•3** ($^{Per}U \cdot ^{TPhen}B_{D0}$). The concentration of each single stranded ODN in each set = 2.5 μ M in 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature.

Upon excitation of the chimeric duplex ODN **1•3** at the absorbance wavelength maximum (310 nm) of the donor **^{TPhen}B_{Do}**, the emission from the acceptor **OxoPyU** was observed at 475 nm. It was observed that the fluorescence intensity of the acceptor **OxoPyU** in chimeric duplex ODN **1•3** increased from that of the single stranded ODN **1** by almost three times in presence of donor **^{TPhen}B_{Do}**. On the other hand, the fluorescence intensity of the donor **^{TPhen}B_{Do}** in the chimeric duplex ODN **1•3** decreased almost by half of that of the single stranded ODN **3** (**Figure 5.14a**).

Similar observations were noted for the case of chimeric duplex ODN **2•3** which upon excitation at 310 nm showed an emission from the acceptor **^{Per}U** at 500 nm. It was also observed that the fluorescence intensity of the acceptor **^{Per}U** in chimeric duplex ODN **2•3** increased from that in the single stranded ODN **2** by almost two times in presence of donor **^{TPhen}B_{Do}**. On the other hand, the fluorescence intensity of the donor **^{TPhen}B_{Do}** in the chimeric duplex ODN **2•3** decreased to a negligible intensity in comparison to that of the single stranded ODN **3** (**Figure 5.14b**).

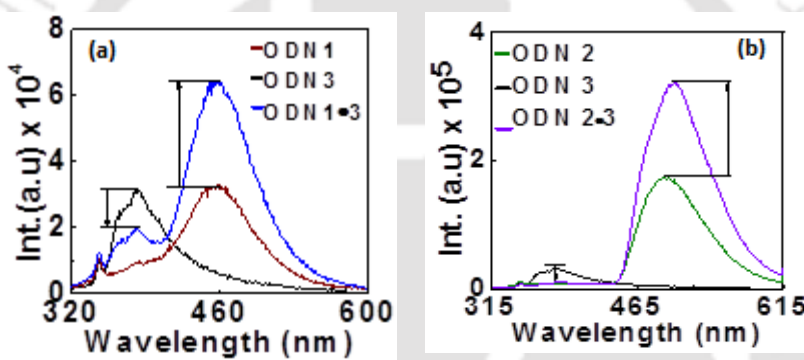


Figure 5.14: (a) fluorescence emission spectra ($\lambda_{\text{ex}} = 310$ nm) of ODN **1** (-T-**OxoPyU**-T-), ODN **3** (-A-**^{TPhen}B_{Do}**-A-) and their hybridized ODN **1•3** (**OxoPyU** • **^{TPhen}B_{Do}**). (b) fluorescence emission spectra ($\lambda_{\text{ex}} = 310$ nm) of ODN **2** (-T-**^{Per}U**-T-), ODN **3** (-A-**^{TPhen}B_{Do}**-A-) and their hybridized ODN **2•3** (**^{Per}U** • **^{TPhen}B_{Do}**). Concentration of each single stranded ODN = 1.0 μM in 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature.

Table 5.3: Summary of Photophysical Properties of ODNs

ODNs	X : Y	λ_{abs} (nm)	$\epsilon(\times 10^3 \text{ M}^{-1} \text{ cm}^{-1})$	λ_{fl} (nm)	T_m ($^{\circ}\text{C}$) (1.0 μM)	Φ
ODN 1	-T- OxoPyU-T-	360	12	463	---	0.046
ODN 2	-T-PerU-T-	405,426,451	32	501	---	0.29
ODN 3	-A-TPhenB _{Do} -A-	310	11	384	---	0.06
ODN 1•3	OxoPyU : TPhenB _{Do}	316, 370	16, 10	382, 456	52.1	0.17
						0.12 ^[a] 0.03 ^[b]
ODN 2•3	PerU : TphenB _{Do}	313, 429,	20, 29	388, 505	54.3	0.52
		455				0.43 ^[c] 0.005 ^[d]
ODN 1•4	OxoPyU : A	365	12	462	56.8	0.22
ODN 1•5	OxoPyU : G	365	12	460	49.6	0.03
ODN 1•6	OxoPyU : C	365	10	462	48.6	0.17
ODN 1•7	OxoPyU : T	365	10	460	51.4	0.14
ODN 2•4	PerU : A	403,426,452	33	472, 492	50.1	0.22
ODN 2•5	PerU : G	403,426,452	33	489	43.3	0.27
ODN 2•6	PerU : C	402,425,450	32	485	46.6	0.38
ODN 2•7	PerU : T	403,425,451	34	488	46.6	0.23
ODN 8•3	A : TPhenB _{Do}	314	7	384	45.2	0.06
ODN 9•3	G : TPhenB _{Do}	314, 317	9, 8	383	46.6	0.03
ODN 10•3	C : TPhenB _{Do}	316, 321	11, 10	384	46.7	0.04
ODN 11•3	T : TPhenB _{Do}	315, 322	10, 8	384	45.1	0.04
ODN 11•4	T : A	--	--	--	53.1	---
ODN 8•7	A:T	--	--	--	51.4	---
[a] For OxoPyU component and [b] for TphenB _{Do} component. [c] For PerU component and [d] for TphenB _{Do} component.						

The anisotropy (r) value (15.0×10^{-2}) of the oxopyrene-modified DNA in the double-stranded form ODN **1•3** ($\lambda_{\text{ex}} = 365 \text{ nm}$) was higher than that (13.0×10^{-2}) of the single-stranded form ODN **1**. Also, the duplex ODN **2•3** exhibited an enhanced anisotropy (10.0×10^{-2}) compared to the single stranded ODN **2** (4.0×10^{-2}) at 450 nm. These fluorescence anisotropy measurements indicate that the oxopyrene (in ODN **1•3**) and/or perylene (in ODN **2•3**) are strongly stacked between the bases or the base pairs in the DNA duplex compared with the single-stranded form, and mostly they remained intercalated inside the duplexes. Fluorescent anisotropy findings, therefore, are consistent with the observations from the UV-visible spectroscopic studies (Table 5.4).

Table 5.4. Steady State Fluorescence Anisotropy of ODNs

ODNs	X : Y	Anisotropy			Polarization		
ODN 1	(-T-OxoPyU-T-)	0.13 ($\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$); -0.06 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 460$)			0.18 ($\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$); -0.10 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 460$)		
ODN 2	(-T-PerU-T-)	0.04 ($\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$); -0.02 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 500$)			0.06 ($\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$); -0.03 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 500$)		
ODN 3	(-T- T ^{Phen} B _{D0} -T-)	-0.07 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$)			-0.11 ($\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$)		
ODN 1•3	(OxoPyU • T ^{Phen} B _{D0})	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 460$	$\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 460$	$\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$
		0.14	0.05	0.15	0.19	0.07	0.21
ODN 1•4	OxoPyU • A	0.12 ($\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$)			0.17 ($\lambda_{\text{ex}} = 365$; $\lambda_{\text{em}} = 460$)		
ODN 2•3	(PerU • T ^{Phen} B _{D0})	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 500$	$\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 380$	$\lambda_{\text{ex}} = 310$; $\lambda_{\text{em}} = 500$	$\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$
		0.043	0.16	0.10	0.06	0.23	0.15
ODN 2•4	PerU•A	0.10 ($\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$)			0.15 ($\lambda_{\text{ex}} = 450$; $\lambda_{\text{em}} = 500$)		

5.5.6. Förster Resonance Energy Transfer

It was observed that the absorption spectra of ODN **1** and/or ODN **2** and the emission spectra of ODN **3** overlapped in a considerable range of wavelength indicating a high possibility of FRET process to occur from donor triazolyphenanthrene of T^{Phen}B_{D0} to the acceptor oxopyrene chromophore of OxoPyU and/or perylene chromophore of PerU in the chimeric duplexes, ODN **1•3** (Figure 5.15a) and ODN **2•3** (Figure 5.15b), respectively.

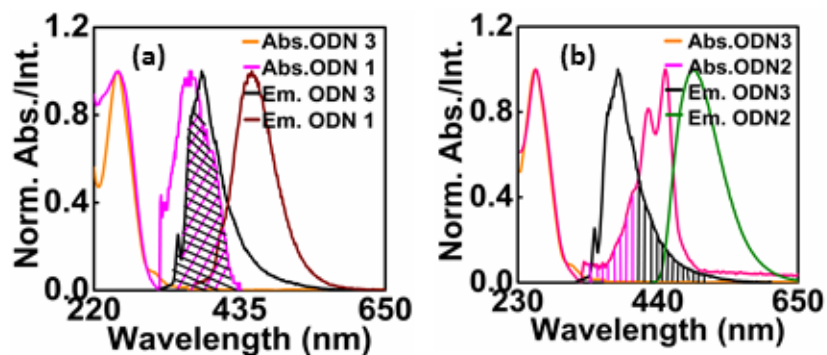


Figure 5.15: (a) Normalized absorbance, emission ($\lambda_{\text{ex}} = 375$ nm) spectra of ODN 1 and normalized absorbance, fluorescence emission ($\lambda_{\text{ex}} = 310$ nm) of ODN 3, respectively. (b) Normalized absorbance, emission ($\lambda_{\text{ex}} = 445$ nm) spectra of ODN 2 and normalized absorbance, fluorescence emission ($\lambda_{\text{ex}} = 310$ nm) of ODN 3, respectively, ([ODN 1] = [ODN 2] = [ODN 3] = 1 μ M, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). [**Em** = Emission spectra, **Abs** = Absorption spectra].

5.5.7. Study of Time Resolved Fluorescence of ODNs

This observation was also supported by time resolved fluorescence. Thus, the donor life time was found to decrease from 1.0 ns (in ODN 3) to 0.3 ns (in ODN 1•3) in presence of acceptor and the acceptor lifetime was increased from 2.8 ns (in ODN 1) to 3.3 ns (in ODN 1•3) in presence of donor (Table 5.5) (**Figure 5.16**).

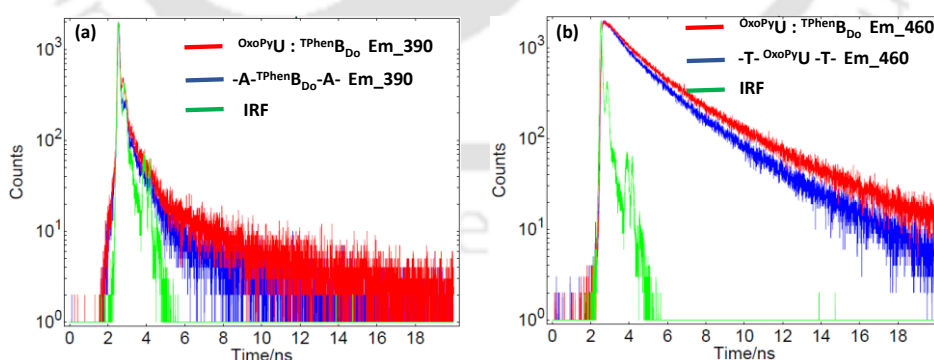


Figure 5.16: Time resolved fluorescence decay spectra of ODN 1 (-T- OxoPyU - T -), ODN 3 (-A-TPhenB_{Do}-A-), and ODN 1•3 (OxoPyU •TPhenB_{Do}) [(a) $\lambda_{\text{ex}} = 375$ nm, $\lambda_{\text{em}} = 390$ nm; (b) $\lambda_{\text{ex}} = 375$ nm, $\lambda_{\text{em}} = 460$ nm].

Likewise, time resolved spectroscopic study showed a decrease in life time of donor from 1.0 ns (in ODN 3) to 0.4 ns (in ODN 2•3) in presence of acceptor and an increase in life time of acceptor from 7.4 ns (in ODN 2) to 8.0 ns (in ODN 2•3) in presence of donor (Table 5.5) (**Figure 5.17**).²⁸

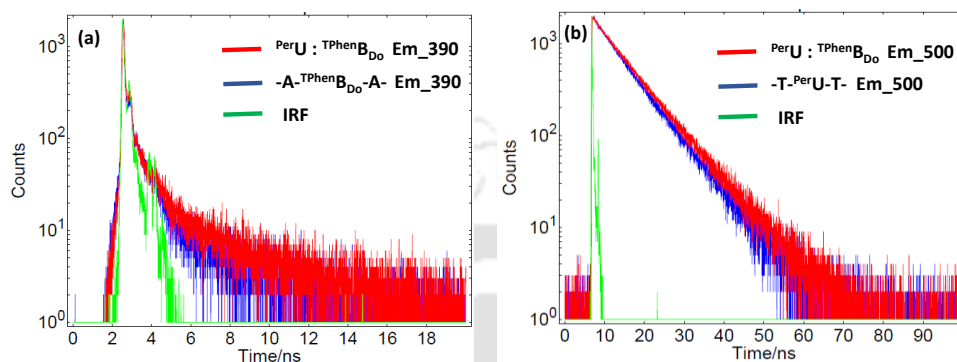


Figure 5.17: Time resolved fluorescence decay spectra of ODN 2 (-T-^{Per}U-T-), ODN 3 (-A-T^{Phen}B_{D0}-A-), and ODN 2•3 (^{Per}U•T^{Phen}B_{D0}) [(a) $\lambda_{ex} = 375$ nm, $\lambda_{em} = 390$ nm; (b) $\lambda_{ex} = 445$ nm, $\lambda_{em} = 500$ nm].

Table 5.5: Summary of Time Resolved Fluorescence of ODNs

ODNs	λ_{ex} and λ_{em}	Φ_f	τ_1 [ns]	τ_2 [ns]	$\langle\tau\rangle$ [ns]	k_f [$10^8 s^{-1}$]	k_{nr} [$10^8 s^{-1}$]	χ^2
ODN 1 (-T-OxoPyU-T)	375, 460	0.08	0.7 (66%)	2.8 (34%)	0.37	2.29	4.28	1.10
ODN 2 (-T- ^{Per} U-T)	445, 500	0.295	7.4 (100%)	-----	7.38	0.34	0.99	0.93
ODN 3 (-T-T ^{Phen} B _{D0} -T)	375, 390	0.06	1.0 (100%)	-----	0.98	0.61	9.55	1.02
ODN 1•3 (OxoPyU • T ^{Phen} B _{D0})	375, 390	0.03	0.3 (95%)	3.3 (5%)	1.28	0.23	7.56	1.04
ODN1•3 (OxoPyU • T ^{Phen} B _{D0})	375, 460	0.12	0.8 (64%)	3.3 (36%)	2.55	0.47	3.44	1.05
ODN 2•3 (^{Per} U • T ^{Phen} B _{D0})	375, 390	0.005	0.4 (92%)	3.0 (8%)	1.37	0.04	7.21	0.99
ODN 2•3 (^{Per} U • T ^{Phen} B _{D0})	445, 500	0.43	8.0 (100%)	-----	8.00	0.54	0.70	0.88

5.5.8. Study of Time Resolved Fluorescence Anisotropy of ODNs

To study nanosecond fluorescence anisotropy decay, the analyzer was rotated at regular intervals to get perpendicular ($I_{\perp}$) and parallel ($I_{\parallel}$) components of fluorescence decay. The anisotropy function $r(t)$ was calculated from the following formula:

$$r = \frac{(I_{VV} - I_{VH}G)}{(I_{VV} + 2I_{VH}G)}; \quad G = \frac{I_{HV}}{I_{HH}}$$

where, I_{VV} and I_{VH} are the emission intensities when the excitation polarizer is vertically oriented and the emission polarizer is oriented vertically and horizontally respectively.

G is the correction factor. The terms I_{HV} and I_{HH} are the emission intensity when the excitation polarization is horizontally oriented and the emission polarization is oriented vertically and horizontally, respectively. A system-dependent factor, G , accounting for differences in sensitivity in the detection channels $I_{\perp}$ and $I_{\parallel}$, was determined. G is for single stranded ODN **1** = 1.1 and duplex ODN **1•3** = 1.9. G is for single stranded ODN **2** = 0.41 and duplex ODN **2•3** = 0.35.

Time Resolved fluorescence anisotropy measurement revealed that the anisotropies of the acceptor, **OxoPyU** and **PerU** in the duplexes ODN **1•3** and ODN **2•3** were very low ($r = 0.05$ and 0.08 , respectively) when the acceptors were excited via FRET ($\lambda_{\text{ex}} = 310$) in comparison to the anisotropies observed ($r = 0.15$ and 0.16 , respectively) upon excitation at long wavelength ($\lambda_{\text{ex}} = 365$ and 450 , respectively).^{29a-c} These results clearly indicated a FRET process in the chimeric duplexes. The anisotropy of the acceptor, **OxoPyU** ($\lambda_{\text{ex}} = 375$ nm, $\lambda_{\text{em}} = 460$ nm) in the duplex ODN **1•3** decays from a high initial value (of 0.35) to 0.1 with a time scale of 6.3 ns (decay time) and then to 0.06 with a time scale of 13 ns (**Figure 5.18a**). On the other hand, the anisotropy of the acceptor, **PerU** ($\lambda_{\text{ex}} = 375$ nm, $\lambda_{\text{em}} = 460$ nm) in the duplex ODN **2•3** decays from a high initial value (0.3) to 0.07 with a time scale of only 2.9 ns corresponds to the decay time of the chromophore, **PerU** (**Figure 5.18b**). The initial very fast decay of the anisotropy indicated the occurrence of a FRET process in the chimeric duplexes.^{29d-e}

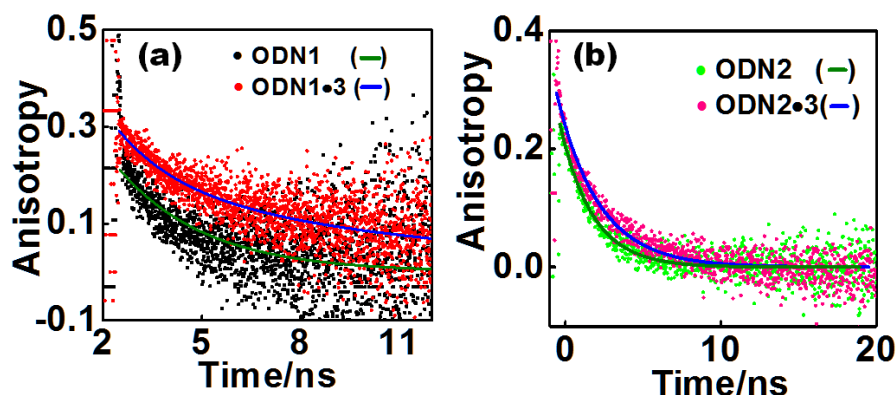


Figure 5.18: Fluorescence anisotropy decay of ODN 1 (-T- ^{OxoPyU} - T -) and ODN 1•3 (^{OxoPyU} •^{TPhenBD₀}); $\lambda_{ex} = 375$ nm, $\lambda_{em} = 460$ nm (a) and of ODN 2 (-T-^{PerU}-T-) and ODN 2•3 (^{PerU}•^{TPhenBD₀}); $\lambda_{ex} = 445$ nm, $\lambda_{em} = 500$ nm (b). The points denote the actual values of anisotropy, and the solid lines denote the best fit to the experimental data as a guide for the eye.

5.5.9. Calculation of Förster Distance and the FRET Efficiency

The fluorescence resonance energy transfer (FRET) and the Förster distance for the FRET were calculated using the following three equations. The efficiency of energy transfer, E , was calculated using the equation (5.1)

$$E = \frac{R_0^6}{R_0^6 + r^6} = 1 - \frac{F}{F_0} \dots\dots\dots (5.1)$$

where F and F_0 are the fluorescence intensity of donor in the presence and absence of acceptor, r is the distance between donor and the acceptor and R_0 is the critical distance when the energy transfer efficiency is 50%. The Förster distance R_0 (Å) was calculated by the following equation (5.2)

$$R_0 = [8.79 \times 10^{-5} \kappa^2 n^{-4} \Phi_D J(\lambda)]^{1/6} \dots\dots\dots (5.2)$$

where κ^2 is the orientation, n is the refractive index of the medium, Φ_D is the quantum yield of the donor in the absence of acceptor $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor given by the following equation (5.3)

$$J = \frac{\int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \dots\dots\dots (5.3)$$

where $F_D(\lambda)$ is the fluorescence intensity of the donor in the wavelength range λ to $\lambda + \Delta \lambda$ with the total intensity normalized to unity. $\varepsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor as a function of wavelength (λ).

To calculate the fluorescence resonance energy transfer (FRET) and the Förster distance for the FRET, the UV-visible and the fluorescence experiments of **1** (-T-^{OxoPyU}-T-), ODN **2** (-T-^{PerU}-T-), **3** (-A-^{TPhenB_{Do}}-A-) and the hybridized ODN **1 • 3** (^{OxoPyU} • ^{TPhenB_{Do}}) and ODN **2 • 3** (^{PerU} • ^{TPhenB_{Do}}) were carried out in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride with 1 μ M single strand concentration each.

The efficiency of energy transfer (E) from donor to acceptor was calculated using the equation 1, and it was found that there was 70% efficiency of energy transfer from donor residue (^{TPhenB_{Do}}) to acceptor residue (^{PerU}) for the hybrid ODN **2 • 3** (^{PerU} • ^{TPhenB_{Do}}) and was 45% efficiency of energy transfer from donor residue (^{TPhenB_{Do}}) to acceptor residue (^{OxoPyU}) for the hybrid ODN **1 • 3** (^{OxoPyU} • ^{TPhenB_{Do}}).

Using the values of $\kappa^2 = 2/3$, $n = 1.33$, $\Phi_D = 0.06$, and the obtained overlap integral, $J(\lambda) = 2.65782 \times 10^{14}$ for the hybrid ODN **2•3** (^{PerU} • ^{TPhenB_{Do}}), the R_0 and r values were calculated which were found as $R_0 = 24$ Å and $r = 21$ Å. Similarly, for the hybrid ODN **1•3** (^{OxoPyU} • ^{TPhenB_{Do}}) with obtained overlap integral, $J(\lambda) = 5.21805 \times 10^{13}$, the R_0 and r values were calculated which were found as $R_0 = 18$ Å and $r = 19$ Å.

5.5.10. Study of Circular Dichroism Spectroscopy of ODNs

Furthermore, the spatial disposition of the chromophoric moieties inside the chimeric duplexes was examined using circular dichroism (CD) spectroscopy. Thus, all spectra showed a stabilized B-form conformation of the chimeric DNA duplexes.

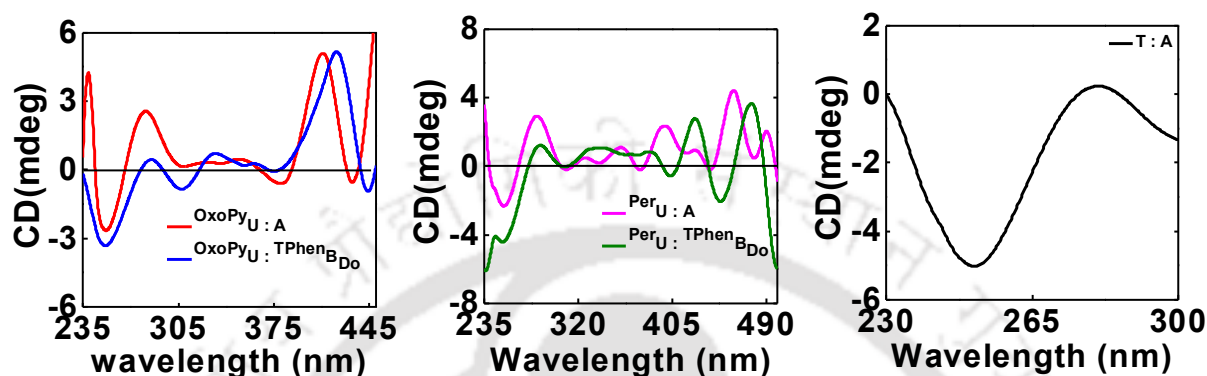


Figure 5.19: Circular dichroism Spectra of ODN 1 • 3 (OxoPyU • TPhenBDo), ODN 2 • 3 (PerU • TPhenBDo) and the natural ODN 11 • 4 (T • A).

The induced CD signal between 300-500 nm corresponding to the chromophores' absorption band supporting that the conformational freedom of **OxoPy** (in ODN 1•3) and **Per** (in ODN 2•3) moieties are restricted inside each chimeric duplex (**Figure 5.19**).³⁰

5.5.11. Macromodel Calculation

5.5.11.1. Optimization of B-form DNA structure for the Duplexes

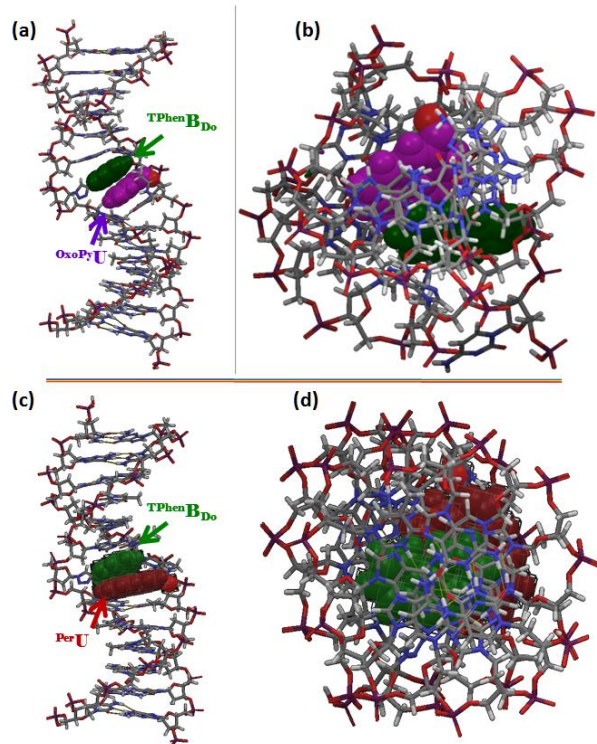


Figure 5.20: OPLS 2005 force field energy minimized conformations in water of the duplexes, (a-b) for ODN **1 • 3** (Side view and Top view) and (c-d) are for ODN **2 • 3** (Side view and Top view).

Molecular modeling study of the duplexes (fluorescently labeled ODNs and the complementary ODNs) is carried out using Schrodinger Macro Model (Maestro vs. 9.0) software with OPLS 2005 force field in water.³¹ The optimized geometry of the B-form duplexes showed an intercalated stacked structure of oxopyrene (**OxoPy**), perylene (**Per**), and triazolyphenanthrene (**TPhen**) chromophores in the corresponding duplexes, ODN **1 • 3** (**OxoPyU • TPhenBD₀**) and ODN **2 • 3** (**PerU • TPhenBD₀**), respectively. A conjugate gradient minimization scheme [PRCG (Polak-Ribiere Conjugate Gradient)] that uses the Polak-Ribiere first derivative method with restarts every 3N iterations was employed for the minimization of the duplexes.

5.5.11.2. Conformational Search of Optimized B-form DNA structure for the Duplexes

We carried out conformational search using OPLS 2005 force field at constant dielectric in water with “large scale low-frequency-mode conformational search” (Mixed torsional/Large scale low-mode sampling = MCMM/LMCS) method using Schrodinger Macromodel (Maestro vs. 9.0) software package. It is a hybrid functional method wherein Low-Mode Conformational Search Methods is hybridized with Monte Carlo Multiple Minimum (MCMM) global searching. This method uses a combination of the random changes in torsion angles and/or molecular position from the MCMM method, together with the low-mode steps from the LLMOD method used in Large scale low-mode.

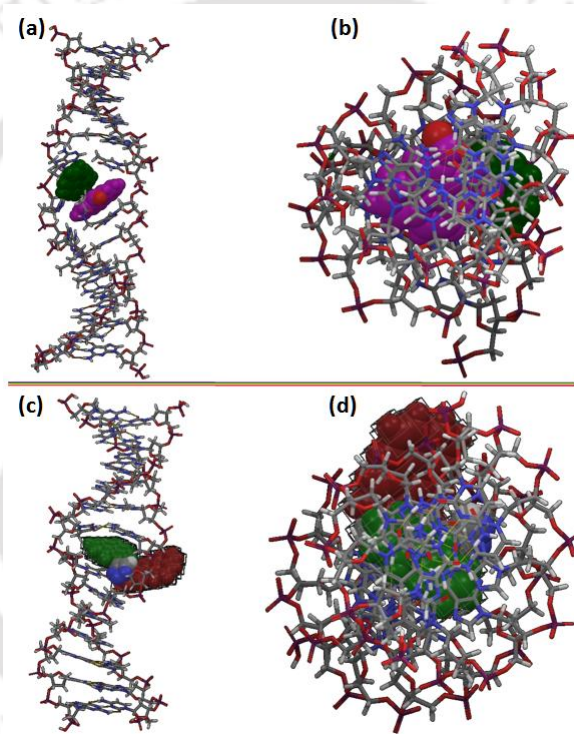


Figure 5.21 The conformer, obtained after conformational search, with 1.00 k.cal/mole (4.18 kJ/mole) global minimum. (a-b) for ODN 1 • 3 (Side view and Top view) and (c-d) are for ODN 2 • 3 (Side view and Top view).

A total of 500 structures were processed with 500 maximum no. of steps iteration. A global search analysis eliminates redundant conformers using RMS deviation for all compared atoms exceed the threshold Cutoff of 0.5 Å. An optimal minimization method was chosen for minimizing the generating conformers.

A total of 20 minimized and well converged conformers were generated out of which the one conformer appear 26 times which remains within 1.00 k.cal/mole (4.18 kJ/mole) global minimum with a convergence threshold of 0.017 RMSD (threshold cutoff = 0.05). The relative potential energy is zero and force field energy of -20,999.943 k J/mole for this conformer of the duplex ODN **1 • 3** (**OxoPyU • TPhenB_{Do}**) (**Figure 5.21 a-b**).

A total of 20 minimized and well converged conformers were generated out of which the one conformer appear 7 times which remains within 1.00 k.cal/mole (4.18 kJ/mole) global minimum with 0.24 (cutoff = 0.5). The relative potential energy is zero and force field energy of -21110.29 k J/mole for this conformer of the duplex ODN **2 • 3** (**PerU • TPhenB_{Do}**) (**Figure 5.21 c-d**).

The conformers thus obtained showed an intercalated stacked structure of triazolyphenanthrene (**TPhen**) chromophores in both the chimeric duplexes. Oxopyrene (**OxoPy**) remained intercalated in the duplex ODN **1 • 3** (**OxoPyU • TPhenB_{Do}**) while perylene (**Per**) extruded outside along the groove in the duplex ODN **2 • 3** (**PerU • TPhenB_{Do}**).

5.5.11.3. Molecular Dynamics Simulation of Optimized B-form DNA structure for the Duplexes

We carried out MD simulations for the duplexes ODN **1 • 3** (**OxoPyU • TPhenB_{Do}**) and ODN **2 • 3** (**PerU • TPhenB_{Do}**) using an OPLS 2005 force field. The starting structures for the duplexes were the global minimum conformers obtained in a conformational search as stated above.

The MD simulations were performed using Schrodinger Macromodel (Maestro vs. 9.0) software package with OPLS 2005 force field in which the systems were subjected to 100 ps simulations time (with time step of 1.5 fs and equilibrium time 1.0 ps) at constant temperature (300 K) and pressure (1 atm) with shaking bonds to hydrogens.

The triazolyphenanthrene, Oxopyrene [in duplex ODN **1 • 3** (**OxoPyU • TPhenB_{Do}**)] and perylene [in ODN **2 • 3** (**PerU • TPhenB_{Do}**)] with the linker units were choosen as freely moving moieties during the simulation. An optimal minimization method was chosen for minimizing the generating structure (with maximum iteration of 1000) with gradient convergence threshold of 0.05.

Figure 5.22 a and **c** shows the final structures of the duplexes, ODN **1 • 3** (**OxoPyU • TPhenB_{Do}**) and ODN **2 • 3** (**PerU • TPhenB_{Do}**), respectively obtained after the MD simulations. As shown in **Figure 5.22 a-b**, the structure of the duplexes retained their original B-form double helix in which the oxopyrene/phenanthrene (in **OxoPyU • TPhenB_{Do}**) units extended prominently to inside the helix. In the duplex ODN **2 • 3** (**PerU • TPhenB_{Do}**) while the phenanthrene unit involved in intercalative stacking the perylene come out of the helix and involved in stacking along groove side (**Figure 5.22c-d**). The MD simulations showed that the oxopyrene and phenanthrene are intercalated in the double helix. **Figure 5.22b** and **d** showed the top view of the duplexes. It can be seen that the phenanthrene/oxopyrene (in **OxoPyU • TPhenB_{Do}**) and the phenanthrene (in **PerU •**

T^{Phen}B_{Do}) aromatic rings are engaged in interstrand π -stacking interactions with the nucleobases of the duplexes.

The MD simulations show that, while the oxopyrene/phenanthrene (in **OxoPyU** • **T^{Phen}B_{Do}**) and phenanthrene (in **PerU** • **T^{Phen}B_{Do}**) are indeed intercalated into the base pairs of the helix, the chimeric DNA duplexes were not significantly distorted and retained the global conformation of the B-form structure. This finding is consistent with the other spectroscopic studies.

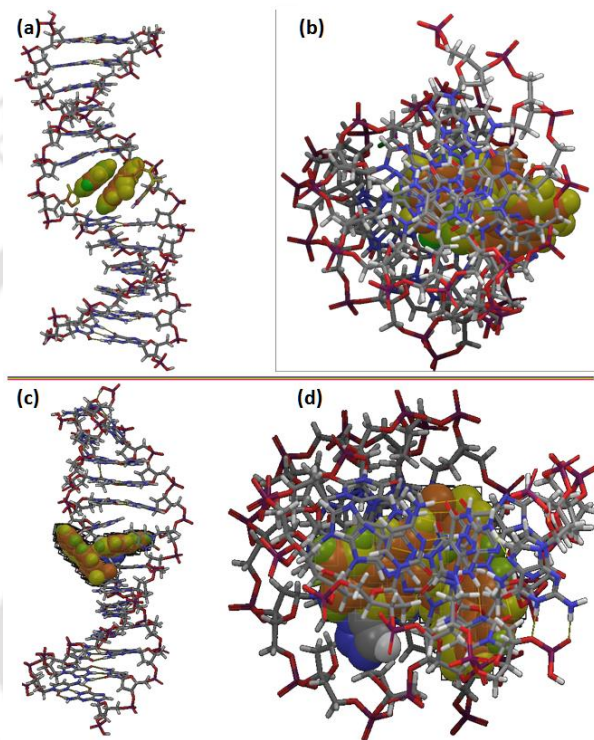


Figure 5.22 The structures obtained from molecular dynamics simulation for the duplexes, (a-b) ODN 1 • 3 (Side view and Top view) and (c-d) ODN 2 • 3 (Side view and Top view).

5.6. Conclusion

In conclusion, we established the FRET process in two chimeric DNA duplexes wherein a fluorescently labeled natural nucleoside (**OxoPyU** or **PerU**) paired against an unnatural nucleoside (**T^{Phen}B_{Do}**) without sacrificing the duplex stability and B-DNA conformation. The hybridization accompanying FRET event in these classes of interacting fluorophores is new. Our observed phenomenon can potentially be exploited in designing more of such efficient FRET pairs for useful application in the detection and analysis of biomolecular interactions and in material science application.

5.7. Material and methods

5.7.1. Procedure for oligonucleotide synthesis

The reagents for DNA synthesis were purchased from ChemGenes, USA and used. ODNs were synthesized by a conventional phosphoramidite chemistry by using an automated DNA/RNA synthesizer and purified by reverse phase HPLC on a 5-ODS-H column (10×150 mm, elution with 50 mM ammonium formate buffer (AF), pH 7.0, linear gradient over 45 min from 3% to 40% acetonitrile at a flow rate 2.0 ml/min). The concentration of each ODN was determined from molar extinction coefficient at 260 nm at 80 °C. Mass spectra of purified ODNs were determined with a MALDI-TOF mass spectrometer (See SI for details).

5.7.2. Studies on photophysical properties of the oligonucleotides

All the DNA solutions of desired concentrations were prepared in 50 mM sodium phosphate buffer, 100 mM NaCl, pH 7.0 for melting point (T_m) determination and all other spectroscopic studies. The two complementary DNA strands were hybridized just by mixing and sonicating for 5 minutes to get various double stranded ODNs (See SI for details).

5.7.2.1. UV-visible and thermal melting temperature (T_m) measurements of the oligonucleotides

All UV-visible and T_m s of the ODNs (1 μ M, final duplex concentration) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. The measurements were carried out in absorbance mode. The absorbance values of the sample solutions were measured in the wavelength regime of 200–650 nm. All the sample solutions were prepared just before doing the experiment. Total volume of 120 μ L from a stock solution of 700 μ L of 1 μ M concentration for each set was used for UV and T_m experiments in 8-micro cell using a UV-visible spectrophotometer. Absorbance vs. temperature profiles were measured at 260 nm using a UV-visible spectrophotometer equipped with a Peltier temperature controller unit using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 20 to 90 °C with a heating rate of 1 °C/min. From these profiles, average method was used to determine T_m values.

5.7.2.2. Steady state fluorescence study of the oligonucleotides

ODNs solutions were prepared as described in UV-visible and T_m measurement experiments. Fluorescence spectra were obtained using a fluorescence spectrophotometer at 25 °C using 1 cm path length cell. The excitation wavelengths for single strand duplex ODN were set at ~310 and ~450 nm, and emission spectra were measured in the wavelength regime of 300–700 nm with an integration time of 0.2 sec. All the sample solutions were prepared just before doing the experiment. Total volume of 500 μ L from a stock solution of 700 μ L of 1 μ M concentration for each set was used for fluorescence experiment in 1 ml cell. Fluorescence emissions were collected exciting the ODNs at the wave length corresponding to their absorption maxima. Steady-state

fluorescence emission spectra were recorded at room temperature as an average of five scans using an excitation slit of 3.0 nm, emission slit 3.0 nm, and scan speed of 120 nm/min. The fluorescence quantum yields (Φ_f) were determined using quinine sulphate as a reference with the known Φ_f (0.55)³ in 0.1 molar solution in sulphuric acid.

The steady state anisotropy experiment was performed with the same fluorescence spectrophotometer at 25 °C using 1 cm path length cell.

5.7.2.3. Time resolved fluorescence and anisotropy decay study of the oligonucleotides

The fluorescence lifetime experiment was carried out using a time resolved fluorescence spectrophotometer at 25 °C using 1 cm path length cell in 50 mM sodium phosphate buffer (pH 7.0) containing 100 mM sodium chloride. 375 nm and 445 nm laser were used as excitation light source. The lifetime data were calculated by software with fixed fitting range. The time correlated single photon counting (TCSPC) method was used to calculate the lifetime data. The life time data (Global Analysis) were calculated by the FAST software package (FAST Version 1.9.1. Edinburgh Instruments Ltd.) with fitting range 205 – 4000 channels.

To study nanosecond fluorescence anisotropy decay, the analyzer was rotated at regular intervals to get perpendicular ($I_{\perp}$) and parallel ($I_{\parallel}$) components of fluorescence decay.

5.7.3. Study of circular dichroism spectroscopy of the oligonucleotides

CD experiments were performed with a CD spectropolarimeter (concentration of each strand 1 μ M in 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). The data were collected using a 1 cm path length quartz cuvette with scanning from 500 to 200 nm (See SI for details).

5.7.4. Macromodel calculation

Molecular modeling study of the duplexes (fluorescently labeled ODNs and the complementary ODNs) was carried out using Schrodinger Macro Model (Maestro vs. 9.0) software with OPLS 2005 force field in water (See SI for details).

5.7.4.1. Conformational search of optimized B-form DNA structure for the duplexes

We carried out conformational search using OPLS 2005 force field at constant dielectric in water with “large scale low-frequency-mode conformational search” (Mixed torsional/Large scale low-mode sampling = MCMM/LMCS) method using Schrodinger Macromodel (Maestro vs. 9.0) software package. It is a hybrid functional method wherein Low-Mode Conformational Search Methods is hybridized with Monte Carlo Multiple Minimum (MCMM) global searching. This method uses a combination of the random changes in torsion angles and/or molecular position from the MCMM method, together with the low-mode steps from the LLMOD method used in Large scale low-mode.

Chapter 5

A total of 500 structures were processed with 500 maximum no. of steps iteration. A global search analysis eliminates redundant conformers using RMS deviation for all compared atoms exceed the threshold Cutoff of 0.5 Å. An optimal minimization method was chosen for minimizing the generating conformers.

5.7.4.2. Molecular dynamics simulation of optimized B-form DNA structures for the duplexes

The MD simulations were performed using Schrodinger Macromodel (Maestro vs. 9.0) software package with OPLS 2005 force field in which the systems were subjected to 100 ps simulations time (with time step of 1.5 fs and equilibrium time 1.0 ps) at constant temperature (300 K) and pressure (1 atm) with shaking bonds to hydrogens.



5.8. Reference

1. Lakowicz, J. R. Principles of Fluorescence Spectroscopy, 3rd. ed.; Springer: New York, **2006**.
2. *DNA Technology*; Lakowicz, J. R., Ed.; Kluwer Academic/Plenum Publishers: New York, **2003**; Vol. 7.
3. (a) Förster, T. *Naturwissenschaften* **1946**, 6, 166. (b) Valeur, B.; Berberan-Santos, M. *Molecular Fluorescence: Principles and Applications*, 2nd ed. Weinheim: Wiley-VCH. **2012**.
4. (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, 47, 819. (b) Selvin, P. R. *Methods Enzymol.* **1995**, 246, 300. (c) Sapsford, K. E.; Berti, L.; Medintz, I. L. *Angew. Chem. Int. Ed.* **2006**, 45, 4562. (d) Kato, T.; Kashida, H.; Kishida, H.; Yada, H.; Okamoto, H.; Asanuma, H. *J. Am. Chem. Soc.* **2013**, 135, 741.
5. (a) Peter, M.; Ameer-Beg, S. M.; Hughes, M. K. Y.; Keppler, M. D.; Prag, S.; Marsh, M.; Vojnovic, B.; Ng, T. *Biophys. J.* **2005**, 88, 1224. (b) Petty, H. R.; Poo, H.; Fox, B. *J. Cell. Biol.* **1994**, 159, 176. (c) Ge, X. D.; Tolosa, L.; Rao, G. *Anal. Chem.* **2004**, 76, 1403. (d) Ohiro, Y.; Ueda, H.; Shibata, N.; Nagamune, T. *Anal. Biochem.* **2007**, 360, 266.
6. (a) Bag, S. S.; Jana, S.; Yashmeen, A.; Kumar, S.; Bag, R. *Chem. Commun.* **2014**, 50, 433. (b) Xie, Y.; Maxson, T.; Tor, Y. *J. Am. Chem. Soc.* **2010**, 132, 11896. (c) Lee, Y. H.; Lee, M. H.; Zhang, J. F.; Kim, J. S. *J. Org. Chem.* **2010**, 75, 7159.
7. (a) Stryer, L. *Annu. Rev. Biochem.* **1978**, 47, 819. (b) Selvin, P. R. *Nat. Struct. Biol.* **2000**, 7, 730.
8. (a) Clegg, R. M. *Methods Enzymol.* **1992**, 211, 353. (b) Clegg, R. M.; Murchie, A. I. H.; Zechel, A.; Lilley, D. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, 90, 2994.
9. Hurley, D. J.; Tor, Y. *J. Am. Chem. Soc.* **2002**, 124, 13231.
10. (a) Epstein, J. R.; Biran, I.; Walt, D. R. *Anal. Chim. Acta* **2002**, 469, 3. (b) Pinto, M. R.; Schanze, S. *Proc. Nat. Acad. Sci. U.S.A* **2004**, 101, 7505. (c) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Nat. Acad. Sci. U.S.A* **2002**, 99, 10954 (d) Wang, S.; Liu, B.; Gaylord, B. S.; Bazan, G. C. *Adv. Funct. Mater.* **2003**, 13, 463. (e) Xu, Q. -H.; Gaylord, B.; Wang, S.; Bazan, G. C.; Moses, D.; Heeger A. J. *Proc. Nat. Acad. Sci. U.S.A* **2004**, 101, 11634. (f) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, 16, 4467. (g) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, 126, 5446 (h) Wang, S.; Bazan, G. C. *Chem. Commun.* **2004**, 2508. (i) Liu, B.; Bazan, G. C. *J. Am. Chem. Soc.* **2006**, 128, 1188. (j) Woo, H. Y.; Vak, D.; Korystov, D.; Mikhailovsky, A.; Bazan, G. C.; Kim, D- Y. *Adv. Funct. Mater.* **2007**, 17, 290. (k) Kyhm, K.; Kim, I.; Kang, M.; Woo, H. Y. *J. Nanosci. Nanotechnol.* **2012**, 12, 7733. (l) Kim, I.; Kyhm, K.; Kang, M.; Woo, H. Y. *JOL* **2014**, 149, 185.

11. Qiaoli, Y.; Tongfei, S.; Changna, W.; Lei, W.; Haibo, L.; Shuling, X.; Huaisheng, W.; Jifeng, L. *Biosens. Bioelectron.* **2013**, *40*, 75.
12. Alessandra, A.; Maria, B.; Marco, L.; Luca, N. *IEEE J. Sel. Top. Quant. Electron.* **2014**, *20*, 7100207.
13. Alessandra, A.; Maria, B.; Marco, L.; Luca, N. *IEEE J. Sel. Top. Quant. Electron.* **2014**, *20*, 7100207.
14. (a) Liu, C. -W.; Lin, Y. -W.; Huang, C. -C.; Chang, H. T. *Biosens. Bioelec.* **2009**, *24*, 2541. (b) Malinovskii, V. L.; Wenger, D.; Haner, R. *Chem. Soc. Rev.* **2010**, *39*, 410. (c) Teo, Y. N.; Kool, E. T. *Chem. Rev.* **2012**, *112*, 4221.
15. (a) Rist, M. J.; Marino, J. P. *Curr. Org. Chem.* **2002**, *6*, 775. (b) Marras, S. A. E.; Kramer, F. R.; Tyagi, S. *Nucleic Acids Res.* **2002**, *30*, e122.
16. Malakhov, A. D.; Skorobogaty, M. V.; Prokhorenko, I. A.; Gontarev, S. V.; Kozhich, D. T.; Stetsenko, D. A.; Stepanova, I. A.; Shenkarev, Z. O.; Berlin, Y. A.; Korshun, V. A. *Eur. J. Org. Chem.* **2004**, 1298.
17. Masuko, M.; Ohuchi, S.; Sode, K.; Ohtani, H.; Shimadzu, A. *Nucleic Acids Res.* **2000**, *28*, e34.
18. Saito, Y.; Bag, S. S.; Kusakabe, Y.; Nagai, C.; Matsumoto, K.; Mizuno, E.; Kodate, S.; Suzuka, I.; Saito, I. *Chem. Commun.*, **2007**, *43*, 2133.
19. (a) Kashida, H.; Liang, X. G.; Asanuma, H. *Curr. Org. Chem.* **2009**, *13*, 1065. (b) Kashida, H.; Takatsu, T.; Sekiguchi, K.; Asanuma, H. *Chem. Eur. J.* **2010**, *16*, 2479. (c) Kato, T.; Kashida, H.; Kishida, H.; Yada, H.; Okamoto, H.; Asanuma, H. *J. Am. Chem. Soc.* **2013**, *135*, 741.
20. Borjesson, K.; Preus, S.; El-Sagheer, A. H.; Brown, T.; Albinsson, B.; Wilhelmsson, L. M. *J. Am. Chem. Soc.* **2009**, *131*, 4288.
21. (a) Flehr, R.; Kienzler, A.; Bannwarth, W.; Kumke, M. U. *Biocon. Chem.* **2010**, *21*, 2347. (b) Kupstat, A.; Ritschel, T.; Kumke, M. U. *Biocon. Chem.* **2011**, *22*, 2546.
22. (a) Cuppoletti, A.; Cho, Y.; Park, J. S.; Strassler, C.; Kool, E. T. *Biocon. Chem.* **2005**, *16*, 528. (b) Teo, Y. N.; Wilson, J. N.; Kool, E. T. *J. Am. Chem. Soc.* **2009**, *131*, 3923.
23. (a) Malinovskii, V. L.; Samain, F.; Haner, R.; *Angew. Chem., Int. Ed. Engl.* **2007**, *46*, 4464. (b) H. A. Wagenknecht *Ann. N.Y. Acad. Sci.* **2008**, *1130*, 122. (c) Chiba, J.; Takeshima, S.; Mishima, K.; Maeda, H.; Nanai, Y.; Mizuno, K.; Inouye, M. *Chem. Eur. J.* **2007**, *13*, 8124. (d) Börjesson, K.; Preus, S.; El-Sagheer, A. H.; Brown, T.; Albinsson, B.; Wilhelmsson, L. M. *J. Am. Chem. Soc.* **2009**, *131*, 4288.

24. (a) Neelakandan, P. P.; Pan, Z.; Hariharan, M.; Zheng, Y.; Weissman, H.; Rybtchinski, B.; Lewis, F. D. *J. Am. Chem. Soc.* **2010**, *132*, 15808. (b) Trkulja, I.; Haner, R. *J. Am. Chem. Soc.* **2007**, *129*, 7982. (c) Garo, F.; Haner, R. *Angew. Chem. Int. Ed.* **2012**, *51*, 916.
25. Bag, S. S.; Talukdar, S.; Kundu, R.; Saito, I.; Jana, S. *Chem. Commun.* **2014**, *50*, 829.
26. (a) Bag, S. S.; Kundu, R.; Matsumoto, K.; Saito, Y.; Saito, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3227-3230. (b) Bag, S. S.; Saito, Y.; Hanawa, K.; Kodate, S.; Suzuka, I.; Saito, I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6338. (c) Bag, S. S.; Talukdar, S.; Matsumoto, K.; Kundu, R. *J. Org. Chem.* **2013**, *78*, 278.
27. Nakamura, M.; Fukunaga, Y.; Sasa, K.; Ohtoshi, Y.; Kanaori, K.; Hayashi, H.; Nakano H.; Yamana, K. *Nucleic Acids Res.* **2005**, *33*, 5887.
28. Nedumparaa, R. J.; Manua, P. J.; Vallabhanb, C. P. G.; Nampooria, V. P. N.; Radhakrishnana, P. *Opt. Laser Tech.* **2008**, *40*, 953.
29. (a) Piston, D. W.; Rizzo, M. A.; *Methods Cell Biol.* **2008**, *85*, 415. (b) Rizzo, M. A.; Piston, D. W. *Biophysical J.* **2005**, *88*, L14. (c) Jares-Erijman, E. A.; Jovin, T. M.; *Nat. Biotech.* **2003**, *21*, 1387. (d) Xu, Q.-H.; Wang, S.; Korystov, D.; Mikhailovsky, A.; Bazan, G. C.; Moses, D.; Heeger, A. J. *PNAS* **2005**, *102*, 530. (e) Zal, T.; Gascoigne, N. R. J. *Curr. Opin. Immun.* **2004**, *16*, 418.
30. (a) Kypr, J.; Kejnovska, I.; Renciuik D.; Vorlickova, M. *Nucleic Acids Res.* **2009**, *37*, 1713. (b) Kashida, H.; Asanuma H.; Komiyama, M. *Angew. Chem. Int. Ed.* **2004**, *43*, 6684.
31. Maestro, version 9.0, Schrödinger, LLC, New York, NY 2009.



SUMMARY AND OUTLOOK

Summary and Outlook

This thesis contains five chapters. The first chapter describes the need to modify nucleosides. This chapter also profoundly describes the synthetic protocol to modify C-nucleosides. The second chapter mainly focused on the synthetic protocol to synthesize a versatile precursor bistoluoylated 1'-ethynylriboside in β form, which is a challenging task. β -1'-ethynylriboside's application in the synthesis of C-triazolyl nucleosides is also described herein. The third chapter delineates the design and synthesis of a more compact hydrophobic doubly widened fused triazolyl nucleoside, **FTPhen**, the structure of which was established from X-ray crystallography. Its behavior within a theoretical trimeric duplex and its photophysical properties were also demonstrated. The fourth chapter describes the design of hydrophobic angularly expanded 8-azaguanosine analogue and its synthesis. It ultimately produced **FTQuon** which according to the theoretical study can be paired with both cytosine and thymine. The fifth chapter describes FRET event in two chimeric DNA duplex involving ^TPhen^BD₀ as an unnatural nucleoside and a FRET donor with ^{Oxo}PyU or ^{Per}U as a fluorescently labelled natural nucleoside and a FRET acceptor.

In brief in the first chapter we described the synthesis of unnatural C-nucleoside. Prof. Benner's work on the synthesis of protein containing nonstandard amino acid with the aid of ribosome is the remarkable one and inspired to develop the synthetic protocol for modified unnatural nucleoside. However, the N-nucleoside suffers from enzymatic and acid catalyzed N-glycosidic bond cleavage. On the other hand, as C-glycosides are stable towards enzymatic and acid catalyzed bond cleavage, the synthetic protocol to modify C-nucleosides have been developed. C-nucleoside synthesis involves with two major ideas. One is the construction of nucleobase and the other is the construction of ribose sugar. Keeping the ribose part unchanged and with the introduction of a nitrile group to the anomeric position can lead to a versatile precursor and a lot of C-nucleoside were synthesized from the 1'-cyanoriboside. Wittig reaction and cycloaddition are the other two ways to construct nucleobase upon ribose sugar. Though, building of glycoside upon nucleobase is tough but a few examples were reported. Use of organometallic reagent to the riboside is a versatile approach to synthesize C-nucleosides. Organolithium, organocadmium, Organocuprate, Organozinc are the reagents used in the synthesis of C-nucleoside successfully. Lactone formation onto the anomeric center and the use of halogenose are the other two versatile approach of making C-nucleosides. Palladium catalyzed coupling and the use of Lewis acid are also explored successfully for the synthesis of C-nucleoside.

In the second chapter we successfully established the synthetic route to convert α -1'-ethynylriboside to the desired β -1'-ethynylriboside. We have used Cobalt catalyzed Nicholas reaction to make the alkyne part bulky as well as this cobalt complex stabilize the electrophilic anomeric carbon and thus led to the β -anomer. β -1'-ethynyl riboside was then used in click reaction catalyzed by Cu(I)-Et₃N or Cu(I)-TBTA to synthesize a number of donor/acceptor C-triazolyl nucleoside. The β -configuration of C-nucleoside was established from X-ray crystallography. X-ray crystallography also displayed the C2'-endo/C3'-exo sugar pucker conformation of our synthesized C-glycoside. ^{cT}Anth^BD₀ and ^{cT}Py^BD₀ are fluorescent nucleoside and can be used as a

nucleoside probe. Photophysical study revealed the solvatochromic nature of ${}^{\text{cTDMB}}\text{B}_{\text{D}_0}$ and ${}^{\text{cTBMA}}\text{B}_{\text{Ac}}$. These nucleosides interacted well with BSA as was revealed from the photophysical and docking study of ${}^{\text{cTAnth}}\text{B}_{\text{D}_0}$ and ${}^{\text{cTPy}}\text{B}_{\text{D}_0}$ with BSA.

The third chapter deals with the design of a doubly widened hydrophobic nucleobase. The concept of widened base by Koll *et al.* was extended up to double expansion and resulted hydrophobic **FTPhen**. The β configuration and the fused form of triazolyl phenanthrene moiety was verified from X-ray crystal structure. Theoretical study revealed stable base pair formation with cytosine and thymine by this base analogue.

The fourth chapter describes the design of widened base **FTQuon** which can be treated as 8-azaguanosine analogue wherein the amine (H-bonding donor) was replaced by hydrophobic benzene. Its β -anomeric form was supported by NOESY spectra. Theoretical study suggested it to be an effective complementary base of cytosine. Not only cytosine but it can pair with thymine also. For both the cases the number of hydrogen bonds found in between **FTQuon:C/T** base pair are two and the distance of these hydrogen bonds are comparable with the distance of natural hydrogen bonds. Replacement of one hydrogen bond with one benzene ring although made it to be less potent to be paired with cytosine as expected, but on contrary **FTQuon:C** base pair is rather more stable than A:T base pair as suggested by theoretical study.

The fifth chapter describes a FRET event within chimeric DNA duplexes. ${}^{\text{TPhen}}\text{B}_{\text{D}_0}$ is the unnatural nucleoside act as a FRET donor. The other two fluorescently labelled natural nucleoside ${}^{\text{OxoPy}}\text{U}$ and ${}^{\text{Per}}\text{U}$ act as a FRET acceptor. Hybridization ensures the formation of chimeric DNA duplexes without sacrificing the duplex stability resulted FRET event between ${}^{\text{TPhen}}\text{B}_{\text{D}_0}$ with ${}^{\text{OxoPy}}\text{U}$ or ${}^{\text{Per}}\text{U}$.

Back in 1970s' Zamecnik and Stephenson first introduced targeted gene silencing using synthetic oligonucleotides to control the expression of a specific gene. This is called the antisense approach. An exogenous synthetic antisense oligonucleotide (AON) complementary to the targeted mRNA was introduced into the cell with the intent to block a specific gene expression through either translational inhibition or enzymatic cleavage of the targeted mRNA. The new age development of posttranscriptional gene expression was then introduced by Andrew Fire Mello and Craig C. Mello. They introduced RNA interference (RNAi) pathway and small interfering RNAs (siRNA) for the modulation of gene expression. It's well established that properly designed AON or siRNAs are potent to modulate the gene expression by the cleavage of a specific mRNA by taking the advantage of endogenous cellular pathways. But the most significant obstacles in AON or RNAi include their poor intracellular and extracellular stability, low efficiency of their intracellular delivery to target tissues or cells, their potential for off target gene silencing etc. Fortunately, a vast chemical modifications have been made to overcome the therapeutic limit of synthetic DNA and RNA. These nucleic acid analogs are often designed by allowing specific modification of the ingrained properties like nucleoside/nucleotide duplex conformation, hydrophobicity, targeted binding affinity etc. which are affecting their biological application.

Keeping the aim of these view we have synthesized unnatural modified nucleosides which could be utilized for the development of therapeutic oligonucleotides. Modified oligonucleotides with unnatural nucleobases have been reported and many more successful candidates are yet to come. Our design might shed light both in respect of synthetic point of view as well as interesting interaction forces for DNA duplex stabilization.

