6-28-2017

Novel nucleoside analogues with bases modified with (β-halo)vinyl sulfone or β-keto sulfone as probes to study RNA/DNA-Proteins interactions

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DOI: 10.25148/etd.FIDC001985

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NOVEL NUCLEOSIDE ANALOGUES WITH BASES MODIFIED WITH (*β*-HALO)VINYL SULFONE OR *β*-KETO SULFONE AS PROBES TO STUDY RNA/DNA-PROTEINS INTERACTIONS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Sk Md Sazzad Hossain Suzol

2017

To: Dean Michael R. Heithaus
College of Arts, Sciences, and Education

This dissertation, written by Sk Md Sazzad Hossain Suzol, and entitled Novel Nucleoside Analogues with Bases Modified with (β-Halo)vinyl Sulfone or β-Keto Sulfone as Probes to Study RNA/DNA-Proteins Interactions, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Joong Ho Moon

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Anthony De Caprio

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Deodutta Roy

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Stanislaw F. Wnuk, Major Professor

Date of Defense: June 28, 2017

The dissertation of Sk Md Sazzad Hossain Suzol is approved.

_____
Dean Michael R. Heithaus
College of Arts, Sciences and Education

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Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2017

DEDICATION

I dedicate this work to my parents and brother who are always inspiring to me. Without their affection and support, the completion of this work would have been impossible.

ACKNOWLEDGMENTS

I want to express my humble acknowledgment to the people who will be remembered for their their guidance, co-operation and encouragement.

First of all I want to thank my honorable adviser Dr. Stanislaw F. Wnuk for his outstanding mentorship, and guidance. As a person he was very friendly, however professional in the research activities. I have learnt a lot from him which will be faciliatory in my professional life. I will always remember my professor for his personal care and enormous affection to me!

I want to thank to all my committee members: Dr. Kevin O'Shea, Dr. Joong Ho Moon, Dr. Anthony DeCaprio, Dr. Deodutta Roy for giving me your valuable time and ample support throughout my graduate life. I will be grateful to all of you.

I want to convey my special gratitude to Dr. Rafael Raptis of FIU for providing data of X-ray analysis, Dr. Yuan Liu from FIU for providing details of DNA incorporation, and Dr. Sandra Liekens of Rega Institute for Medical Research, Belgium; for collaborative studies for exploring antiproliferative properties of my analogues. Without their help it wouldn't be possible to progress in my research so smoothly.

I would like to mention my gratitude to Mr. Yong Liang, my senior labmate who had trained me in the lab and was always with me in the lab to help. I would like to thank to all my family members and relative specifically my brother for their encouragement and unwavering support throughout my whole life.

Finally, I would like to thank all of my lab mates and friends for their co-operation throughout my PhD life. You guys have made my life easier!

ABSTRACT OF THE DISSERTATION

NOVEL NUCLEOSIDE ANALOGUES WITH BASES MODIFIED WITH (*β*-HALO)VINYL SULFONE OR *β*-KETO SULFONE AS PROBES TO STUDY RNA/DNA-PROTEINS INTERACTIONS

by

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Florida International University, 2017

Miami, Florida

Professor Stanislaw F. Wnuk, Major Professor

The C-5 modified pyrimidine analogues are well-known anticancer and antiviral drugs which underscore further development of novel probes to study their physical, chemical, and biological properties. In my dissertation the syntheses and properties of (β-halo)vinyl sulfone and/or (β-keto)sulfone analogues of C-5 modified pyrimidine have been discussed. In the first part of the dissertion, the synthesis of 5-(β-halo)vinyl sulfones either by transition metal-catalyzed or iodine-mediated halosulfonylation reaction of 5-acetylene pyrimidine nucleosides have been explored. The novel (β-chloro/bromo/iodo)vinyl sulfones efficiently undergo addition-elimination reaction with different nucleophiles such as thiols, amines, amino acid, peptides to provide (β-substituted)vinyl sulfone analogues. The rate of these substitution reactions depends on the nature of halogen atom presents at the β-position and increases with the order of I ≥ Br > Cl. (β-chloro/bromo/iodo)vinyl sulfones possess exclusively *E* stereochemistry while their β-substitued analogues possess either *E* (for β–thio analogues) or *Z* (for β–amino analogue) stereochemistry. It has been observed that the vinylic proton of (β-

chloro) or (β-amino)sulfone analogue undergoes exchanges with deuterium in polar protic deutorated solvents. The antiproliferative activities of those analogues have been explored and was found that protected 5-(*E*)-(1-chloro-2-tosylvinyl)-2'-deoxyuridine inhibited the growth of L1210, CEM and HeLa cells in lower micromolar range.

In the second part of the dissertation the syntheses and reactivities of 5-(β-keto) sulfone of pyrimidine nucleosides were investigated. Thus, 5-(β-halovinyl)sulfone of uracil and cytosine nucleosides have been efficiently converted into corresponding 5-(β-keto) sulfone analogues by displacement of halogen with ammonia followed by acid-catalyzed hydrolysis of the resulting (β-amino)sulfone analogues. A number of electrophiles were trapped at the acidic α-carbon of the 5-(β-keto)sulfones by treatment with electrophiles such as methyl, benzyl, or allyl halide in the presence of base. The 5-(α-iodo-β-keto)sulfone analogues of uracil nucleosides have been tested as an alternative substrates to probe the incorporation of nucleophiles at α-carbon.

In the third part of the dissertation, the synthesis of 5'-phosphates of 5-(β-chloro) and 5-(β-keto) sulfones of 2'-deoxyuridine and their polymerase-catalyzed incorporation into DNA were evaluated. Thus, 5'-*O*-phosphorylated analogues have been efficiently incorporated into the DNA by human DNA repair polymerase (pol β) or bacterial polymerase (pol I).

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LIST OF ABBREVIATIONS

5'-araCTP	5'-arabinofuranosylcytosine triphosphate
5-FdU	5-fluoro-2'-deoxyuridine
5-FU	5-fluorouracil
Abs	absorbance
Ar	aromatic (NMR)
AZT	3'-Azido-3'-deoxythymidine
α	alpha
β	beta
Bn	benzyl
br	broad (NMR)
BVDU	(*E*)-5-(2-bromovinyl)-2′-deoxyuridine
t-Bu	*tert*-butyl
calcd	calculated (HRMS)
CID	collision-induced dissociation (HRMS)
^{o}C	degrees Celsius
δ	delta
d	doublet (NMR)
DCE	1,2 dichloro methane
DCM	dichloromethane
dCK	deoxycytidine kinase

dCTP	deoxycytidine triphosphate
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DTBP	Di-tert-butyl peroxide
ESI	electrospray ionization
Et	ethyl
FDA	U.S. Food and Drug Administration
FUra	5-fluorouracil
λ	lambda
g	gram(s)
h	hour(s)
HBV	hepatitis B virus
HIV	human immunodeficiency virus
HSV	herpes simplex virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	hertz
J	coupling constant in Hz (NMR)
L	liter(s)

M	milli; multiplet (NMR)
μ	micro
M	moles per liter
Min	minute(s)
Mol	mole(s)
MS	mass spectrometry
m/z	mass to charge ratio (MS)
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NT	nucleoside transporter
p	para
$Pd_2(dba)_3$	tris(dibenzylideneacetone)dipalladium(0)
$Pd(OAc)_2$	palladium(II) acetate
%	percentage
q	quartet (NMR)
quin	quintet (NMR)
RNA	ribonucleic acid
rt	room temperature
s	second(s); singlet (NMR)
SAR	structure activity relationship

SET	single electron transfer
sex	sextet
T	triplet (NMR)
TBAF	tetra-*N*-butylammonium fluoride
TBHP	*t*-Bu hydroperoxide
TEA	triethylamine
THF	tetrahydrofuran
TK	thymidine kinase
TLC	thin layer chromatography
TMS	trimethylsilyl
*t*R	retention time (HPLC)
UV-VIS	ultraviolet visible
vs	versus
VV	vaccinia virus
VZV	varicella zoster virus

1. INTRODUCTION

1.1. Importance of nucleosides and nucleotides

Nucleosides are the glycosyamines which possess a β-glycosidic bond between the base and the sugar moiety while nucleotides additionally have one or more phosphate groups at the sugar moiety. Nucleosides can be converted into nucleotides inside the cell by nucleoside kinases which are the basic building blocks of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both DNA and RNA are the source of life in living cells where DNA carries the message. The discovery of the double helix structure of DNA by Watson and Crick is the milestone for the development of modern nucleoside and nucleotide chemistry. Both nucleosides and nucleotides play key roles in DNA and RNA replication which is critical for cell proliferation. Specific nucleoside transporters transport nucleoside analogues inside the cells where they are phosphorylated by nucleoside kinases.[1,2] The phosphorylated nucleoside analogues are incorporated into DNA by DNA polymerase catalyzed reaction and thus induce the termination of chain elongation,[3] the accumulation of mutation[4-6] as well as apoptosis.[7,8] So, through the modification of either the base or sugar moiety, nucleosides can inhibit the carcinogenic cell proliferation and viral DNA and RNA replication thus captivate attention to study as a precursor molecule in chemistry and biology.

1.1.1. Nucleoside analogues as anticancer drugs

Both pyrimidine and purine nucleoside analogues serve as potent anticancer drugs. For example cytarbine contributes to cure amyeloid leukeamia[9], while 6-mercaptapurine is efficacious against acute lymphoblastic leukaemia.[10] Gemcitabine, a bifluorinated pyrimidine nucleoside derivative is very active against haematological malignancy and different solid tumors.[11] So far 14 nucleoside analogues have been approved by the US Food and Drug

Administration (FDA) for the treatment of cancer which is 10% of the total cancer drugs available in the market. In addition a couple of nucleoside analogues such as Sapacetabine,[12] 8-chloro-adenosine,[13,14] 8-amino adenosine[15] are waiting for the approval of FDA.

Table 1. FDA approved anticancer purine and pyrimidine nucleoside analogues[16]

Drug	**Category**	**Year**
6-mercaptopurine (Purinethol)	Purine	1953
5-fluorouracil (Adrucil)	Uracil	1962
6-thioguanine (Lanvis)	Guanine	1966
arabinofuranosylcytosine (Cytarabine)	Cytidine	1969
5-fluoro-2'-deoxyuridine (Floxuridine)	2′-Deoxyuridine	1970
arabinofuranosyl-2-fluoroadenine (Fludarabine)	Purine analogue	1991
2'-deoxycoformycin (Pentostatin)	Adenosine	1991
2-chloro-2'-deoxyadenosine (Cladribine)	2′Deoxyadenosine	1992
2,2-difluoro-2'-deoxycytidine (Gemcitabine)	2′-Deoxycytidine	1996
N^4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine (Capecitabine)	Cytidine	1998
2'-fluoro-2'-deoxyarabinofuranosyl-2-chloroadenine (Clofarabine)	Cytidine	2004
5-aza-cytidine (Azacitidine)	2′-Deoxyadenosine	2004
O^6-methylarabinofuranosyl guanine (Nelarabine)	Guanosine	2005
5-aza-2'-deoxycytidine (Decitabine)	2′-Deoxycytidine	2006

1.1.2. Nucleoside analogues as antiviral drugs

Nucleoside analogues are well-known drugs because of their capability to treat a variety of viral infected patients including Hepatitis C virus (HCV), Hepatitis B virus (HBC), Human immunodeficiency virus (HIV), Herpes simplex virus (HSV), Varicella zoster virus (VZV), and Influenza virus. Several 2'-deoxy-2'-fluorocytidine (FdC) derivatives are well-known anti-HCV compounds such as methylated form of FdC (PSI-6130) can effectively inhibit the

genotype 1b HCB subgenomic replicon system.[17] Mericitabine in combination with pegylated interferon and ribavirin and or a first –generation protease inhibitor is very active to inhibit hepatitis C viral replication.[18,19] Elvucitabine and Apricitabine are potent inhibitor of human immunodeficiency viral (HIV) replication.[20,21] Festinavir which is also known as BMS-986001 is a chemically modified first generation anti-HIV nucleoside analogue stavudine increased potency and reduced mitochondrial toxicity.[22,23] Cyclopropavir which as a guanosine derivative and structurally related to actyclovir and ganciclovir is active against many herpesveuses including human cytomegalovirusm human herpesvirus 6A, human herpesvirus 6B and human herpesvirus 8.[24,25] Because of the potency against a wide range of herpes simplex virus, many protides of cyclopropavir have been synthesized and their antiviral activities are under investigation.[26]

Table 2. Antiviral properties of purine and pyrimidine nucleoside analogues[27]

Drug	**Category**	**Year**
5-iodo-2'-deoxyuridine (Idoxuridine	Uridine	1962
5-ethyl-2'-deoxyuridine (Edoxudine)	Thymidine	1969
9-*β*-D-arabinofuranosyladenine (Vidarabine)	Adenosine	1976
(*E*)-5-(2-bromovinyl)-2'-deoxyuridine (Brivudine)	Uridine	1980
2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one	Guanosine	1982
3'-azido-3'-deoxythymidine (Zidovudine)	Thymidine	1987
2',3'-didehydro-2',3'-dideoxythymidine (Stavudine)	Thymidine	1994
2',3'-dideoxy-3'-thiacytidine (Lamivudine)	Cytidine	1998
2',3'-didehydro-2',3'-dideoxythymidine (Stavudine)	Thymidine	2001
4-Hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentylguanosine	Thymidine	2005

5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine	Cytidine	2006
Tenofovir disoproxil fumarate, mixture of purine analogues (Tenofovir)	Purine	2008
2'-deoxy-2'-fluoro-2'-methyluridine-5'-phosphate (Sofosbuvir)	Thymidine	2013

1.2. 5-Modified pyrimidine nucleosides as reactive probes for bioconjugation

Considering the importance of bioconjugation in medicinal chemistry, chemical biology, nanotechnology, and material sciences, a considerable amount of effort has been given to connect new probes of nucleotides with peptides, proteins, and other biomolecule.[28] The bioconjugation strategy enables us to link important modified biomolecules to study chemical reactions on living cells, which can be crucial to verify the credibility of the anticancer or antiviral properties of the corresponding compound.[29] Bioconjugation is a useful chemical strategy to study DNA-protein interactions.[30] Attachment of reactive functionalities such as alkyne, alkene, azide, diene, or aldehyde at the base moiety of nucleosides/nucleotides and their incorporation into DNA offer good programmability to connect the modified DNA with important biomolecules. These functionalities allowed the modified DNA to tether with other biomolecules via click chemistry,[31] Straudinger ligation,[32] Diels-Alder reactions[33] etc.

Some of the notable works in bioconjugation have been done by Hocek's lab who introduced aldehyde functionality at 5 position of cytidine nucleotide and incorporated them into DNA and subsequently stained with 2,4-dintrophenyl hydrazine (2,4-DNPH) or nitrobenzofurazanehydrazine via hydrazone formation.[34] He also tethered reactive aldehyde functional group containing formaryl moieties at 5 position of 2'-deoxycytidine nucleotides which incorporated via polymerase incorporation. The reactive aldehyde functional group containing modified DNA was connected with amino acid lysine and peptides.[35] Recently Dr.

Hocek attached acrylamide and vinylsulfonamide to the 5 position of cytidine triphosphate and incorporated them into DNA via DNA polymerase. Later he did bioconjugation of these vinylsulfonamide and acrylamide modified DNA with the tumor-suppressor protein p53.[36] These bioconjugations have been taken place via Michael addition reactions where the modified DNA acts as a Michael acceptor while the protein p53 acts as a Michael donor.

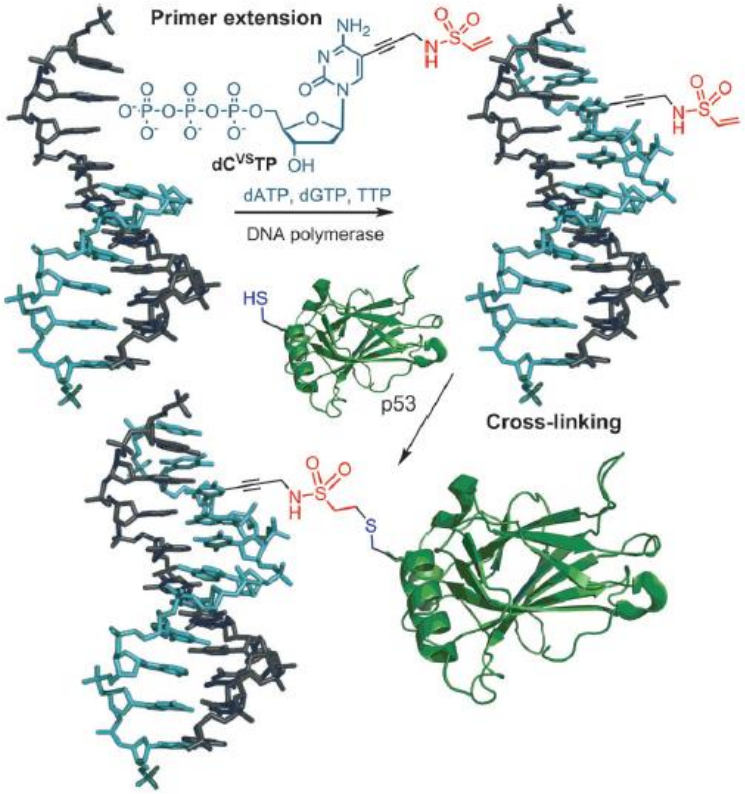


Figure 1. Synthesis of vinylsulfonamide modified DNA by PEX and its cross-linking with p53 protein[36]

From all of the examples of bioconjugation, we have been inspired to link the (β-halo)vinyl sulfone modified DNA with amino acid cysteine or proteins having thiol or amine residues. The 5-modified 2'-deoxypyrimidine nucleoside analogues modified with (β-halo)vinyl sulfone will be an excellent probe in bioconjugate chemistry as they are very susceptible to different nucleophiles (amine, cysteine etc.) and readily give product via

addition-elimination reaction. So, our first goal is to synthesize triphosphorylated pyrimidine nucleoside analogues having (β-halo)vinyl sulfone tethered at 5 postion of the nucloebase to polymerase catalyzed incorporation into DNA. Next, we want to link this modified DNA with amino acid cysteine or protein having amino or thiol residue which will efficiently react with (β-halo)vinyl sulfone through addition-elimination reactions. Note that Michal Hocek did similar types of bionconjugation via Michael addition reaction but our conjugation is totally different and occurs via an addition-elimination process. The 5' position of pyrimidine nucleoside analogues modified with (β-halo)vinyl sulfone, polymerage mediated incorporation in DNA and subsequent treatment of this modified DNA with suitable protein (having amino or thiol residues) will efficiently give bio-conjugated product via addition-elimination mechanism. In summary, phosphorylation at 5' position of pyrimidine nucleoside analogues modified with (β-halo)vinyl sulfone, polymerase mediated incorporation in DNA and subsequent treatment of this modified DNA with suitable protein will give our desired bio-conjugated product.

1.3. Overview of (*β*-halo)vinyl sulfone

The (β-halo)vinyl sulfones having halogen atom at the *β*-positions of the vinyl functional group are important precursurs due to their capabilities of serving as synthetic intermediate in organic chemistry.[37,38] Moreover, owing to the presence of reactive functional groups, and biologically important C-S bond, it has drawn considerable amount of attention to explore their biological activities.[39]

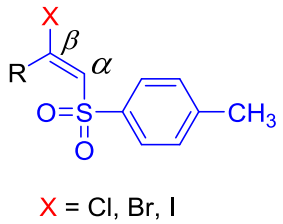
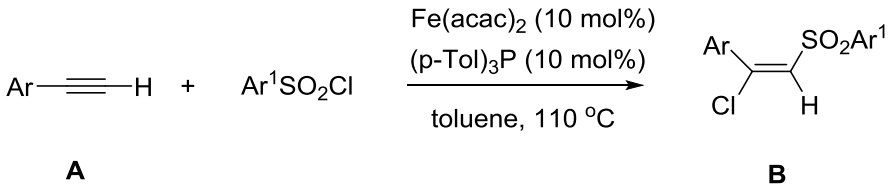


Figure 2. General structure of (*β*-halo)vinyl sulfones

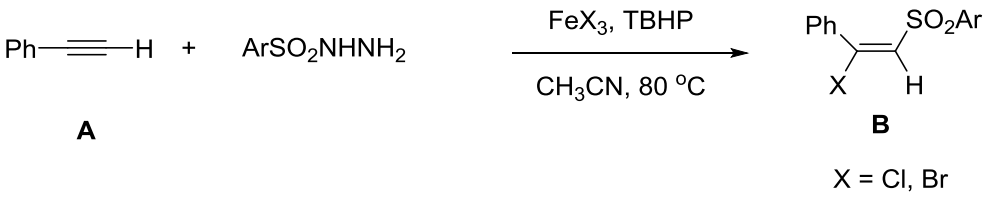
1.3.1. Synthesis

Terminal alkyne functional group offers unique opportunity to synthesize regio and stereoselective (β-halo)vinyl sulfone.[40] The Eiichi Nakamura group developed Iron-catalyzed and phosphine ligand-mediated regio and stereoselective chlorosulfonylation reaction. Thus, treatment of terminal alkyne substrate **A** with aryl sulfonyl chloride in the presence of iron (II) acetate and tri(*p*-tolyl)phosphine and affords (*E*)-(*β*-chloro)vinyl sulfone **B** with excellent yield (Scheme 1).[41]



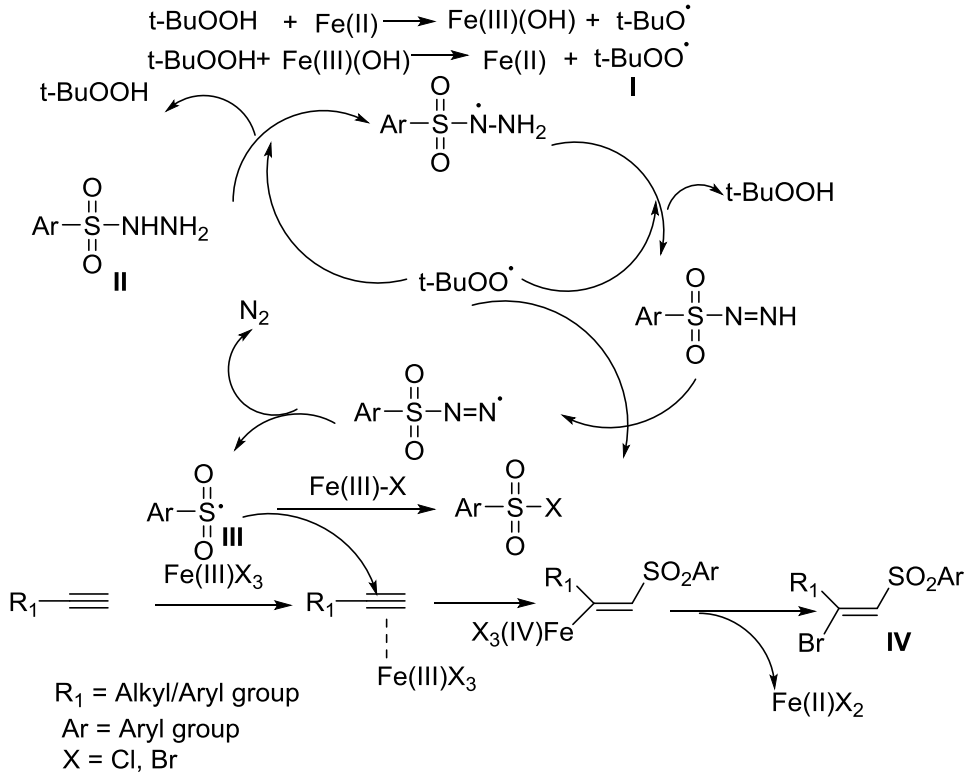
Scheme 1. Synthesis of (*E*)-(*β*-chloro)vinyl sulfone analogue from aryl acetylene using aryl sulfonyl chloride by Eiichi Nakamura's group

Sulfonyl hydrazide is a well-known source of sulfonyl moiety and widely used reagent during the halovinylsulfonylation reaction.[42] Xiangsheng Xu's group developed transition metal catalyzd regio and stereoselective halosulfonylation reaction to synthesize (*E*)-(*β*-chloro/bromo)vinyl sulfone **B** by the treatment of phenyl acetylene **A** with p-tolyl sulfonyl hydrazide in the presence of iron(III) halides and TBHP afford the halovinyl sulfone **B** (Scheme 2).[43]



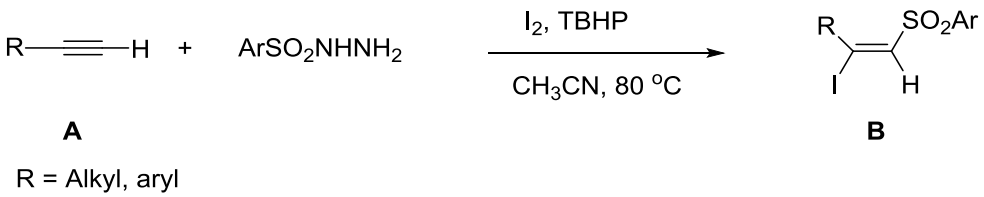
Scheme 2. Synthesis of (*E*)-(*β*-chloro/bromo)vinyl sulfone by transition metal catalyzed halosulfonylation reaction by Xiangseng Xu's group

The plausible mechanism of the reaction can be explained by the interaction of tertiary butyl hydroperoxide with iron(III) halide generates tertiary butyl peroxide radical **I** (Scheme 3). The resulting peroxide radical generates tosyl radical **III** from tosyl hydrazide through the removal of nitrogen gas. Attack on the alkyne group by the reactive tosyl radical and subsequent interaction with iron (III) halide generates regio and stereoselective products (*E*)-*β*-chloro/bromovinyl sulfone **IV** (Scheme 3).



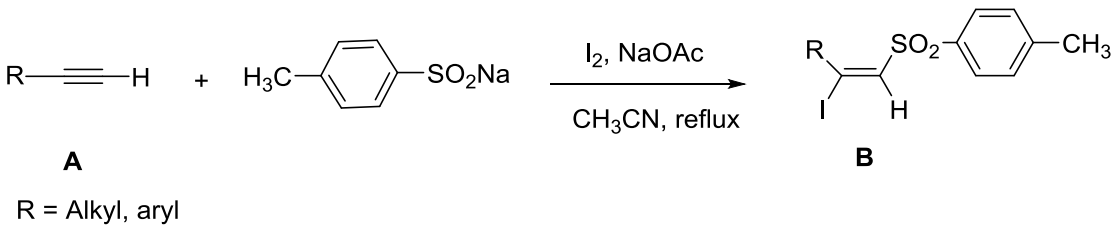
Scheme 3. Plausible mechanism of iron(III) halide-mediated halosulfonylation reactions to afford (*E*)-β-(chloro/bromo)vinyl sulfones [43]

Xiaoqing Li's group successfully synthesized (*E*)-(*β*-iodo)vinyl sulfone using I_2 instead of iron halides[44] as depicted below in Scheme 4. In this reaction interaction of tertiary butyl hydroxyl radical with tosyl hydrazide generates tosyl radical. Subsequent interaction of tosyl radical and iodine with alkyne moiety gives (*E*)-(β-iodo)vinyl sulfone (Scheme 4).



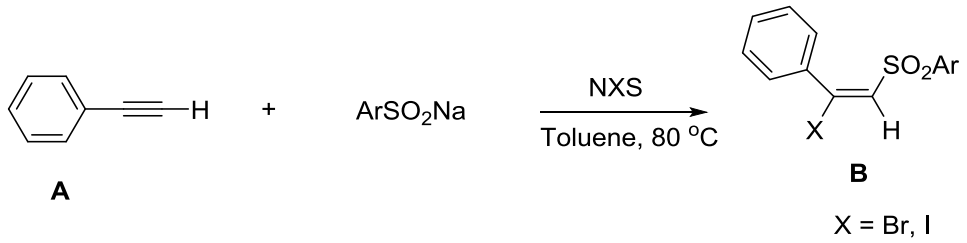
Scheme 4. Synthesis of (*E*)-(*β*-iodo)vinyl sulfone *via* iodine mediated halosulfonylated reaction by Xiaoqing Li's group

Treatment of terminal alkyne probes with sodium salt of p-toluene sulfinic acid and iodine in the presence of sodium acetate afford (*E*)-(*β*-iodo)vinyl sulfone as shown in Scheme 5.[45]



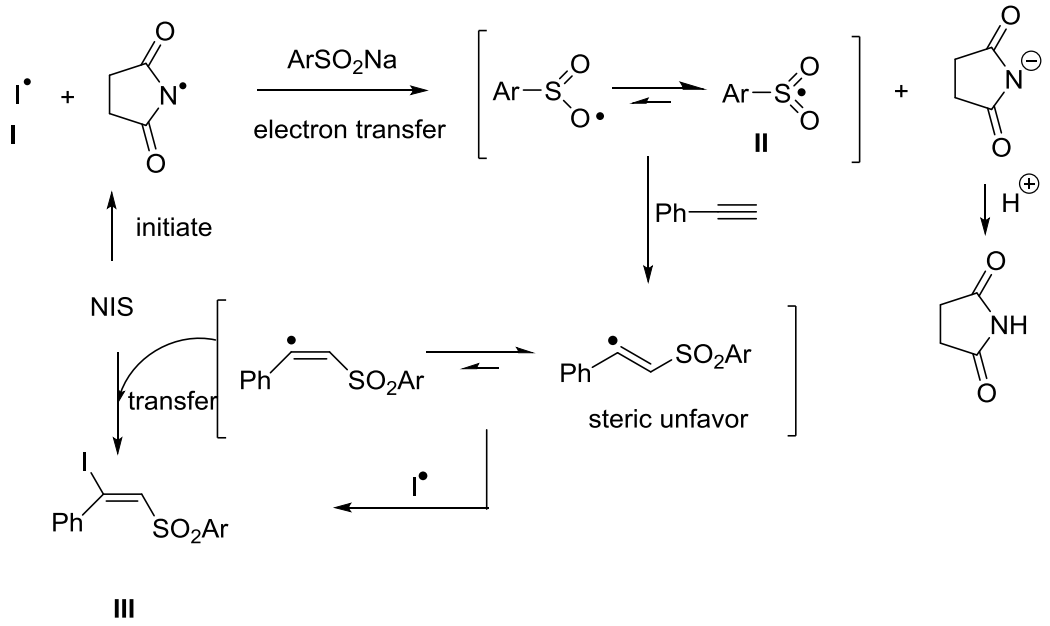
Scheme 5. Synthesis of (*E*)-(*β*-iodo)vinyl sulfone using sodium salt of p-toluenesulfinic acid

N-halosuccinimide (NXS) promoted halosulfonylation of terminal alkyne give (*E*)-(*β*-bromo/iodo)vinyl sulfones. Treatment of aryl acetyle **A** with sodium tosylate and *N*-halo succinamides (NXS) affords *E*)-(*β*-bromo/iodo)vinyl sulfones **B** as depicted in Scheme 6.[46]



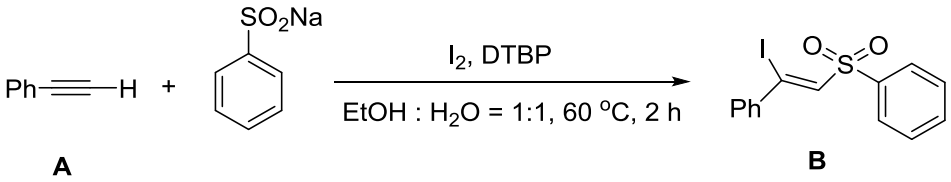
Scheme 6. Synthesis of (*E*)-(*β*-bromo/iodo)vinyl sulfones *via* N-bromo/iodo succinamide mediated halosulfonylation reaction

The plausible mechanism of the reaction can be explained by the formation of the iodide radical **I** and succinamide radical by the hemolytic cleavage of N-I bond of NIS in the initiating step (Scheme 6). Later, tosyl radical **II** is formed by single electron transfer with the help of succinamide radical and subsequent regio and stereoselective interaction of these tosyl radical **II** and iodide radical **I** with the terminal alkyne moiety gives (*E*)-*β*-iodovinylsulfone compound **III** (Scheme 7).



Scheme 7. Plausible mechanism of NIS promoted iodosulfonylation of aryl alkynes[44]

Iodine and di-tert-butyl peroxide (DTBP) promototed difunctionalization of terminal alkyne **A** with sodium benzene sulfinates afforded (*E*)-*β*-iodovinylsulfone **B** with excellent yield (Scheme 8).[47]



Scheme 8. Synthesis of (*E*)-*β*-iodovinylsulfone *via* iodine and DTBP promoted reaction by Zhao *et al.*

1.3.2. Biological activity

As vinyl sulfones are organosulfur compounds, the investigation of their biological activities is always fascinating to the medicinal chemists and biologists. Research shows that certain type of vinyl sulfone inhibits the replication of human immunodeficiency syndrome virus type 1 (HIV-1).[48] Vinyl sulfones also inhibit the protein degrading enzyme cathepsins L and B.[49] It has been also reported that vinyl sulfones inhibits the activities of cysteine protease by binding the thiol residue of the enzymes *via* Michael addition reaction.[50]

1.4. Overview of *β*-keto sulfone

The β-keto sulfones also known as 2-oxo-sulfones is a class of organosulfur compound having active methylene group at the *α*-position and carbonyl group at the *β*-position serves as a versatile synthetic site that can be used to synthesize different classes of organic compounds.[51] Important natural products have been synthesized where *β*-keto sulfones acts a structural feature of the target substrate. In addition, certain types of *β*-keto sulfones exhibit important biological activities. Considering these diverse applicability, *β*-keto sulfones have drawn significant attention in chemistry in last few decades and occupied major parts in organosulfur chemistry.[52]

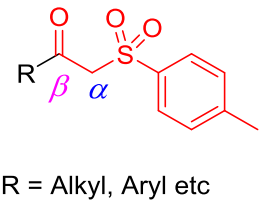
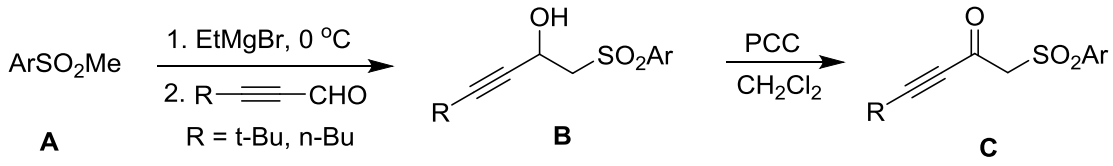


Figure 3. General structure of (*β*-keto)sulfone

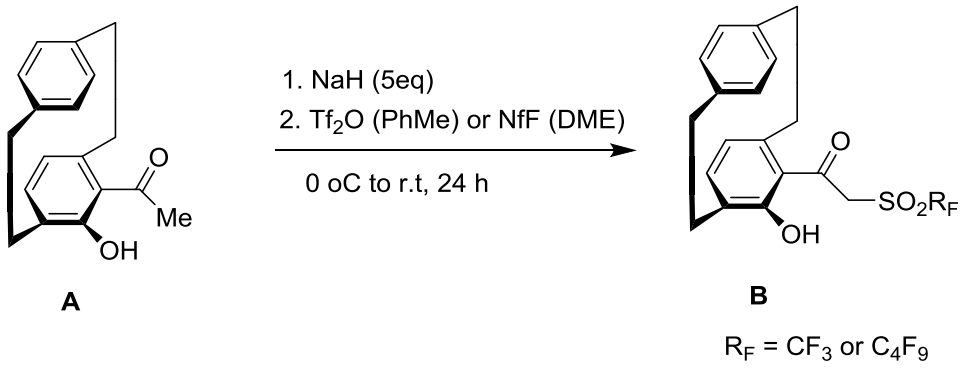
1.4.1. Synthesis

β-Keto sulfones have been synthesized from various precursor molecules by using a wide varieties of reagents.[53] Treatment of arylmethyl sulfone **A** with Grignard reagents generates arylsulfonyl methylene anion which attack at the carbonyl carbon of prop-2-ynals to give *β*-hydroxy sulfones **B**. Oxidation of these *β*-hydroxy sulfones by PCC give *β*-keto sulfones **C** with alkyne moiety at the carbonyl group (Scheme 9).[54]



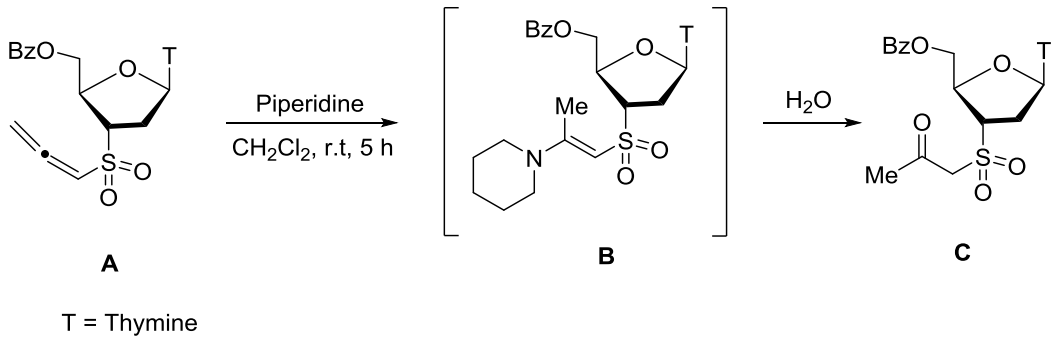
Scheme 9. Synthesis of *β*-keto sulfones with alkyne moiety at the carbonyl carbon

Treatment of 4-hydroxy-5-acyl[2.2]paracyclophane **A** with NaH and triflic anhydride or nonafluorobutanesulfonyl fluoride gives chiral[2.2]paracyclophane derivatives of *β*-keto sulfone **B** (Scheme 10) which is a potential ligand for asymmetric catalysis.[55]



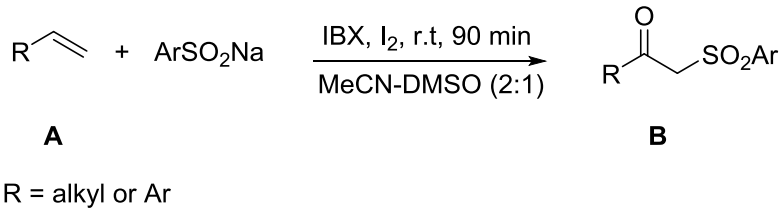
Scheme 10. Synthesis of *β*-keto sulfone with paracyclophane moiety

The *β*-keto sulfone moiety attached at 3' position of thymidine analogue has been reported.[56] Treatment of the allene sulfone **A** with piperidine at rt gives enamine sulfone intermediate **B** which convert to the β-keto sulfone **C** by hydrolysis (Scheme 11).



Scheme 11. Synthesis of *β*-keto sulfone from sulfonyl allene attached at 3' postion of 2'-deoxythymidine nucleoside

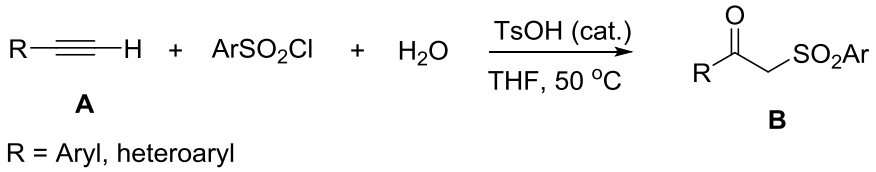
The *β*-keto sulfone has been synthesized from aliphatic or aromatic alkenes by IBX/I_2 mediated reactions with sodium arene sulfinates.[57] Treatment of the alkene substrates **A** with sodium salt of p-toluenesulfinic acid efficiently affords the β-keto sulfone **B** (Scheme 12).



Scheme 12. Synthesis of *β*-keto sulfone from alkene via IBX/I_2 mediated reaction

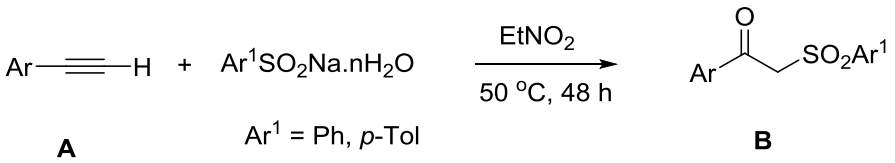
Terminal alkyne is a convenient functional group for the synthesis of *β*-keto sulfone. Different research groups have synthesized *β*-keto sulfones reacting terminal alkyne probes with sulfonyl moiety containing precursors.

Hua's group successfully synthesized *β*-keto sulfones **B** by reacting aryl/heteroaryl acetylene substrates **A** with sulfonyl chloride and water in the presence of catalytic amount of *p*-toluenesulfonic acid as depicted in scheme 13.[58]



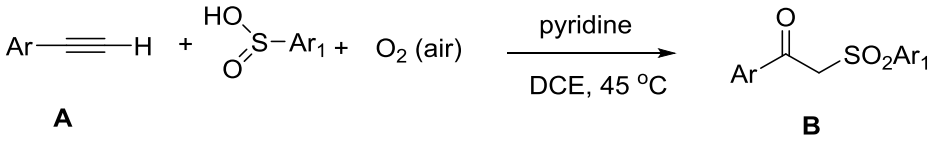
Scheme 13. Synthesis of *β*-keto sulfones by *p*-toluenesulfonic acid catalyzed reaction

The *β*-keto sulfones anologues **B** from acid sensitive acetylene precursors **A** have been synthesized using sodium salt of *p*-toluenesulfinic acid as shown in scheme 14. In this sulfonylation reaction nitroethane is used as solvent.[59]

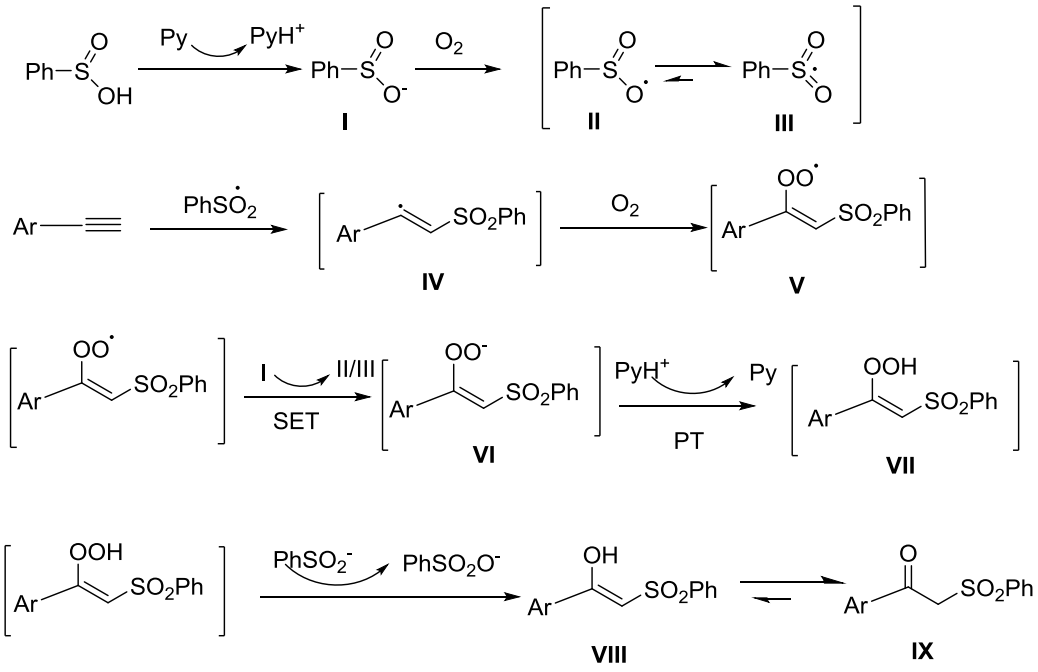


Scheme 14. Synthesis of *β*-keto sulfones from acid sensitive acetylene precursors

Lei's group developed dioxygen triggered oxidative difunctionalization of terminal alkyne **A** gives *β*-keto sulfones **B** from good to excellent yield (Scheme 15).[53] In this reaction pyridine acts a base and prevents atom transfer radical addition (ATRA) process to afford β-keto sulfone instead of vinyl sulfone.



Scheme 15. Synthesis of *β*-keto sulfones *via* pyridine catalyzed dioxygen triggered oxidative radical process



Scheme 16. Mechanism of synthesis of aryl (β-keto)sulfone *via* pyridine catalyzed dioxygen triggered oxidative radical reaction

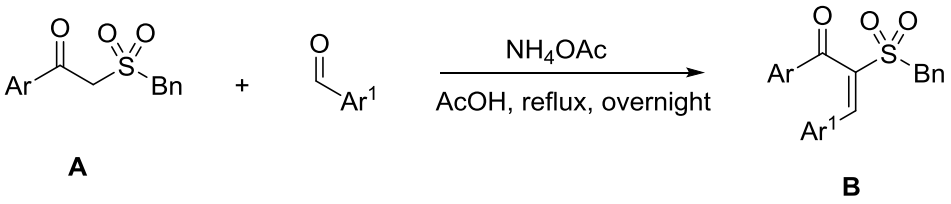
The mechanism of this reaction can be explained as depicted in Scheme 16 which involves generation of the more stable tosyl radical **III** from the interaction of *p*-toluene sulfinic acid with pyridine followed by oxygen molecule. Interaction of the aryl acetylene with *p*-toluene sulfonyl radical followed by molecular oxygen gives peroxy type radical **V**. Single electron transfer and protonation gives β-peroxyvinyl sulfone intermediate **VII**. Subsequent oxygen abstraction and tautomerization afford β-keto sulfone **IX.**

1.4.2. Selected reactions of *β*-keto sulfones

The *β*-keto sulfones have two reactive centers which are active methylene group and carbonyl group. So, the reactivity *β*-keto sulfones can be described by the reactions of each of these two groups separately or the interplay of these reactive groups. As the position of the

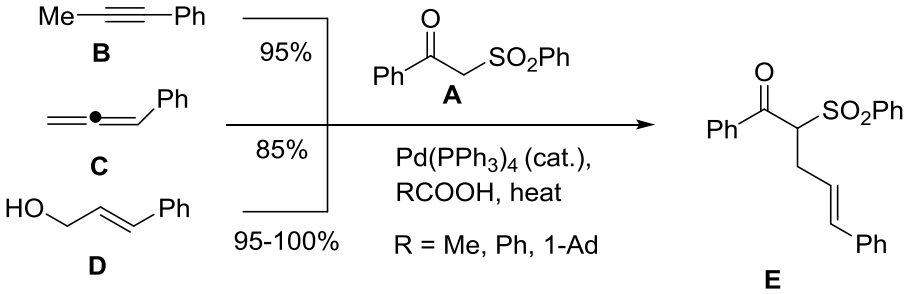
methylene group is in between two electron withdrawing groups which are carbonyl group and sulfonyl moiety, the proton of the methylene group is significantly acidic (pK_a = 9-10).[60]

β-Keto sulfones undergoes a base promoted Knoevenagel type of reaction to afford sulfonyl substituted vinyl ketone derivatives as shown in Scheme 17. Thus treatment of benzyl sulfonyl ethan-2-one **A** with aryl aldehydes gives (*E*)-α-benzylsulfonyl chalcone derivatives **B** which possess antiproliferative activity.[61]



Scheme 17. Knoevengal condensation of *β*-keto sulfones to afford (*E*)-α-benzylsulfonyl chalcone derivatives

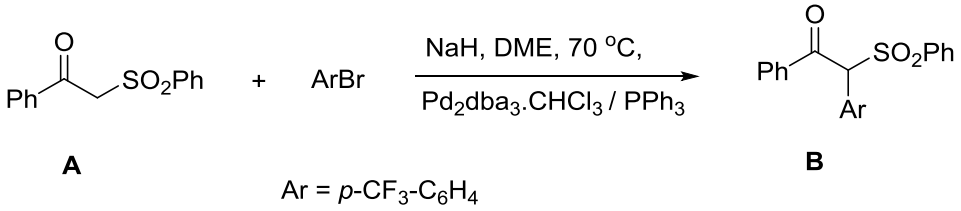
Palladium catalyzed allylic alkylation of phenylsulfonyl acetophenone **A** with either methyl phenyl acetylene **B**,[62] or phenyl allene **C**,[63] or cinnamic alcohol **D**,[64,65] gives corresponding highly *E*-stereoselective α-monoallylated product **E** as depicted in Scheme 18 with excellent yield.



Scheme 18. Pd-catalyzed allylic alkylation of phenylsulfonyl acetophenone for the synthesis of *E*-stereoselective α-monoallylated β-keto sulfone

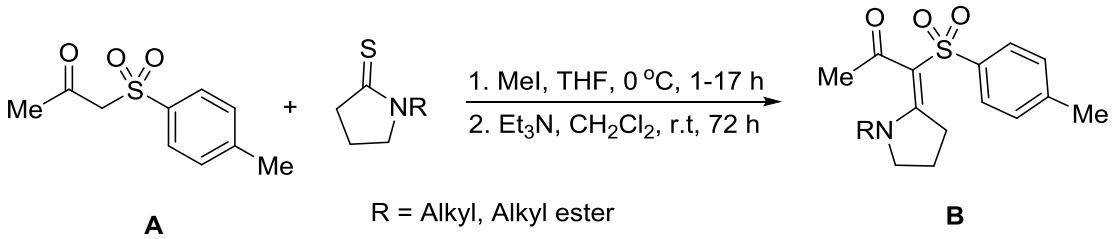
The α-arylated analogue of β-keto sulfone have been synthesized via direct arylation reaction as demonstrated in Scheme 19. Thus, palladium catalyzed reaction of phenylsulfonyl

acetophenone **A** with aryl bromide in the presence of NaH affords α-arylated product **B** with moderate yield.[66]



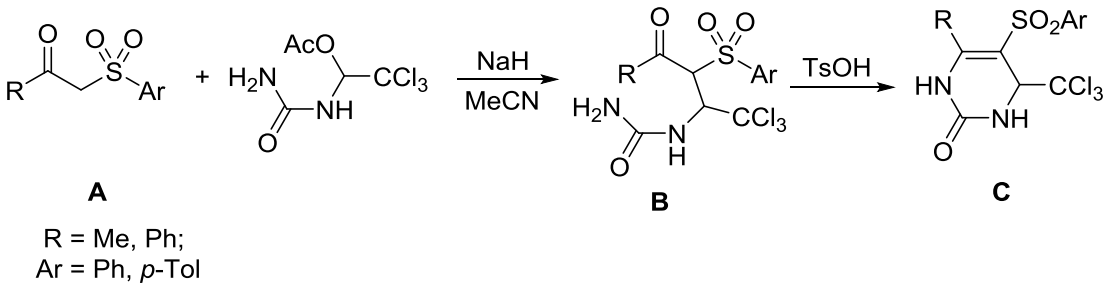
Scheme 19. Pd-catalyzed arylation at *α*-position of phenylsulfonyl acetophenone

Treatment of 1-(4-methylsulfonyl)propane-2-ones **A** with 1-alkylpyrrolidine-2-thione methyl iodide salts in the presence of triethylamine; condensation product *β*-sulfonyl substituted enamines B are obtained as single *E* isomer as depicted in Scheme 20.[67]



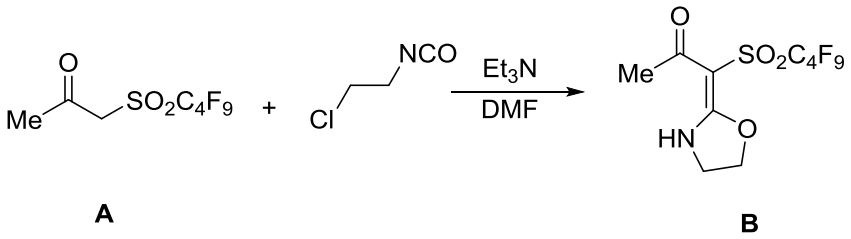
Scheme 20. Alkylation of *β*-keto sulfones with 1-alkylpyrrolidine-2-thione methyl iodide salts for the synthesis of *β*-sulfonyl substituted enamines

When of *β*-keto sulfones **A** are treated with *N*-[1-acetoxy-2,22-trichloro)ethyl] urea in the sodium hydride, trichlorosubstituted oxoalkyl ureas **B** are obtained. Subsequent treatment of these oxoalkyl urea products **B** with para-toluenesulfonic acid give arylsulfonyl-substituted tetrahydropyrimidin-2(1*H*)-ones C as shown in Scheme 21.[68]



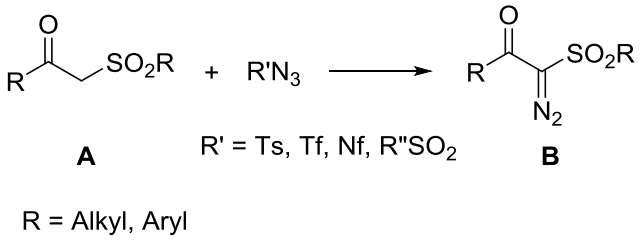
Scheme 21. Alkylation of *β*-keto sulfones with *N*-[1-acetoxy-2,22-trichloro)ethyl] urea to afford arylsulfonyl-substituted tetrahydropyrimidin-2(1*H*)-ones

Treatment of nonaflyl acetone **A** with *β*-chloroethyl isocyanate in the presence of triethyl amine as base affords corresponding 1,3-oxazolidine **B** as shown in Scheme 22. In this reaction triethyl amine deprotonates at the *α*-carbon of **A** and subsequent electrophilic attacks at the isocyanate carbon followed by cyclization and tautomerization gives 1,3-oxazolidine product **B**.[69]



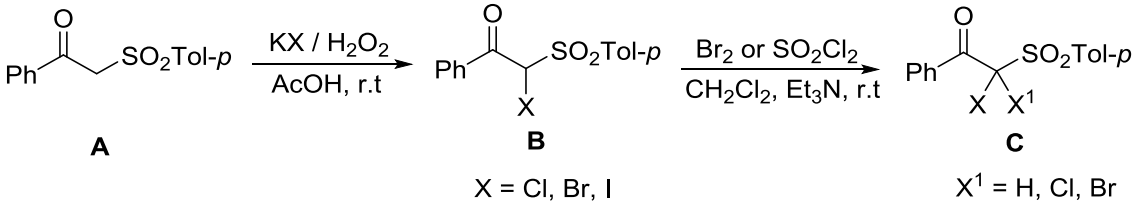
Scheme 22. Synthesis of 1,3-oxazolidine by the reaction of nonaflyl acetone with *β*-chloroethyl isocyanate

α-Diazo *β*-keto sulfones **B** are synthesized by treating correspoding *β*-keto sulfones **A** with different sources of diazo group such asTsN_3,[70] TfN_3,[71] NfN_3,[72] $I(CF_2)_2O(CF_2)_2SO_2N_3$,[73] and ionic-liquid supported sulfonyl azide as depicted in Scheme 23.[74]



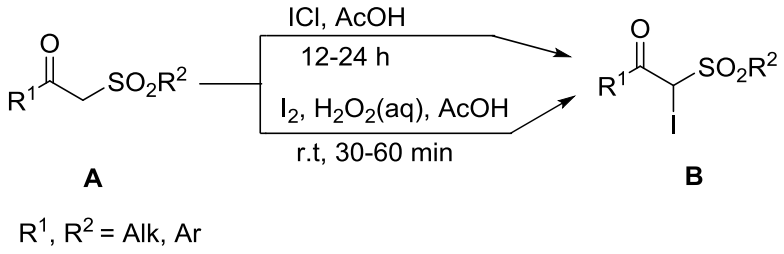
Scheme 23. Synthesis of α-diazo *β*-keto sulfones

Treatment of *p*-toluene sulfonyl acetophenone **A** with potassium halide and hydrogen peroxide in acetic acid gives *α*-monohalogenated product **B** with high yield where potassium halides are the source of halogen atoms. Subsequent treatment of these monohalogenated products **B** with SO_2Cl_2 or Br_2 in the presence of triethylamine give *α,α*-dihalogenated products C as demonstrated in Scheme 24.[75]



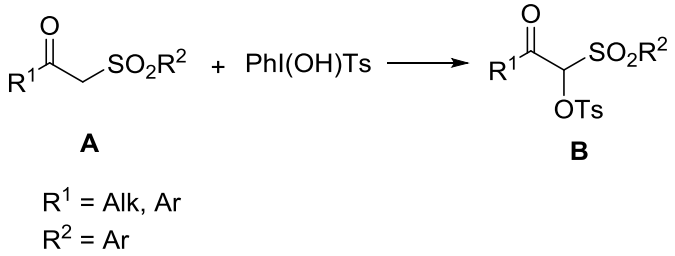
Scheme 24. Chemoselective *α*-mono and di-halogenation at the α-carbon of *β*-keto sulfones

The *α*-iodo *β*-keto sulfone **B** can be prepared efficiently by treating corresponding *β*-keto sulfones A either with iodine monochloride in acetic acid,[76] or molecular iodine in aqueous acidic hydrogen peroxide as shown in Scehme 25.[77]



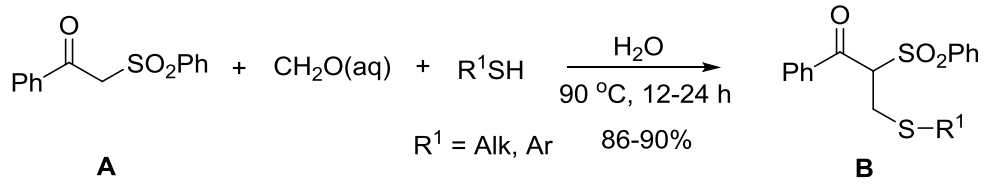
Scheme 25. Synthesis of *α*-Iodo *β*-keto sulfone using iodine monochloride or iodine

Treatment of *β*-keto sulfones **A** with [hydroxyl(tosyloxy)iodo] benzene affords to *α*-tosyloxy substituted *β*-keto sulfones B from very good to excellent yields as depicted in Scheme 26.[78] No solvent is required in this reaction.



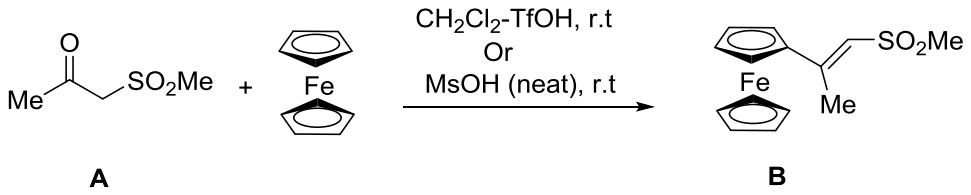
Scheme 26. Synthesis of α-tosyloxy *β*-keto sulfone *via* solvent free reaction

Treatment of *β*-keto sulfone **A** with formalin, and thiol or thiophenol in water gives Mannich type thiesters **B** in good to excellent yield (Scheme 27).[79] In this reaction first *β*-keto sulfones interact with formaldehyde to give *α*-methylene *β*-keto sulfones through a Knoevangal type reaction. Later the intermediate is attacked by thiol and affords the product via a Michael addition reaction.



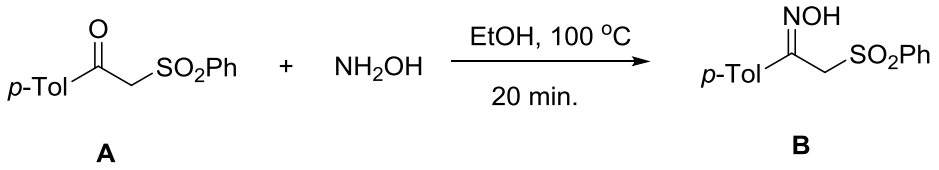
Scheme 27. Incorporation of methylthiol moieties at *α*- position of *β*-keto sulfones by Mannich type reaction

Treatment of methyl sulfonyl A acetone with triflic acid or methanesulfonic acid affords (*E*) β-ferrocenyl-α,β unsaturated sulfone **B** *via* Friedel-Crafts type reaction as depicted in Scheme 28.[80]

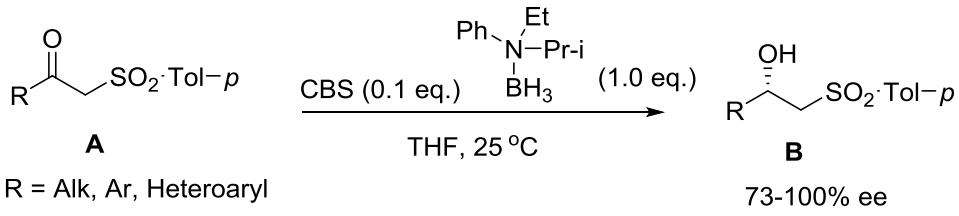


Scheme 28. Synthesis of (*E*) β-ferrocenyl-α,β-unsaturated sulfone *via* Friedel-Crafts type reaction

Treatment β-keto sulfone **A** with hydroxyl amine under reflux in ethanol afford β-hydroxy sulfone **B** as demonstrated in Scheme 29.[81]

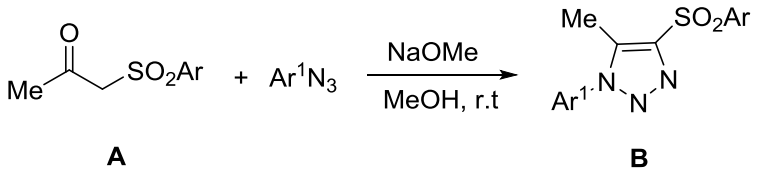


Corey-Bakshi-Shibata (CBS) catalyzed asymmetric reduction of *β*-keto sulfones **A** with N-ethyl-N-isopropylaniline-borane complex give optically active *β*-hydroxy sulfones with excellent enatiometric excess (ee) as shown in Scheme 30.[82]



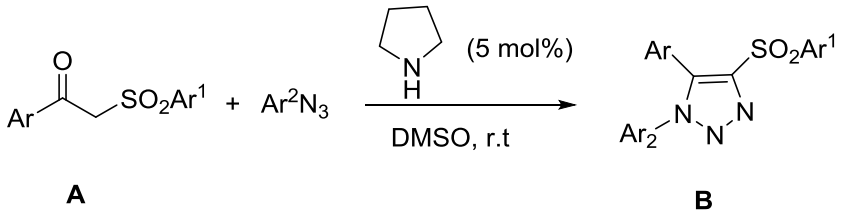
Scheme 29. Asymmetric borane reduction of *β*-keto sulfones for convertion into *β*-hydroxy sulfones

When *β*-keto sulfone **A** is treated with aryl azide in the presence of sodium methoxide, N-aryl substituted 1H-1,2,3-trizoles **B** are afforded from good to excellent yield via base catalyzed Dimroth reaction (Scheme 31).[83]



Scheme 30. Synthesis of *N*-substituted triazoles from *β*-keto sulfones *via* base catalyzed Dimroth reaction

The reaction of *β*-keto sulfones **A** with arylazide in the presence of pyrrolidine as ograno catalyst affords 4-sulfonyl 1,2,3-triazoles **B** from good to excellent yields as depicted in Scheme 32.[84] In this reaction pyrrolidine forms enamine with the *β*-keto moiety which played the key role for the success of this reaction.



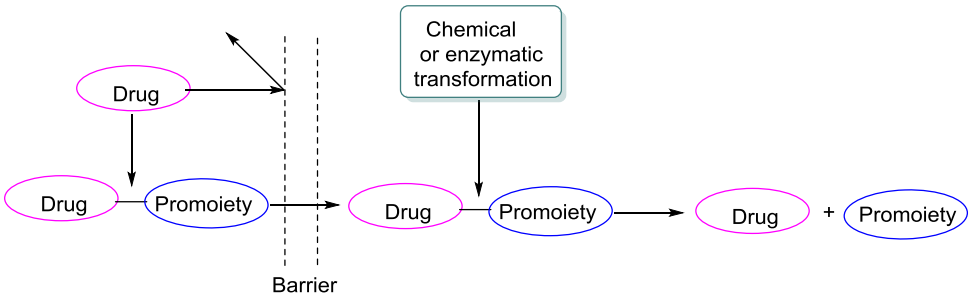
Scheme 31. Synthesis of N-substituted triazoles from *β*-keto sulfones using pyrolidine as organo catalyst

1.4.3. Biological activity

The β-keto sulfone contains biologically important sulfonyl moiety which captivates attention of the pharmaceutical chemist to investigates their pharmacological properties. Research shows that certain types of (β-keto) sulfones are potent and selective inhibitors of 11β-hydroxysteroiddehydrogenase type 1 (11β-HSD1).[85] In addition the (β-keto) sulfones are active against pernicious microorganism[86] and cyclic (β-keto) sulfones also exhibit fungicidal activities.[87]

1.5. Short overview of prodrugs

Prodrugs are the compounds which are metabolized after entering into the cell of the body to act as drugs.[88] The chemical modification of a pharmacologically active agent that must undergo transformation to release the active drug after entering into the cell is a well known and convenient strategy to enhance the physiochemical, biopharmaceutical or pharmacokinetic properties of pharmacologically potent compounds.[89-92] By designing and synthesizing prodrugs different parameters such as poor cell permeability, low aqueous solubility, chemical instability, insuffient oral absorption, rapid pre-systemic metabolism etc. can be overcome.[92,93] In short, prodrugs enhance the absorbtion, distribution, metabolization, and excretion (ADME) properties of drugs.[94] Currently 5-7% of approved drugs can be sorted out as prodrugs and in 2001-02 about 15% of new drugs were approved as prodrugs.[95]



Scheme 32. General representation of prodrug concept[96]

Some common functional groups which are convenient to design prodrugs are carboxylic, hydroxyl, amine, phosphate/phosphonate, and carbonyl group. These groups can be converted into esters, carbonates, carbamates, amides, phosphates, and oximes to obtain the corresponding prodrugs.[96] In addition thiols can be converted into thioethers,[97] or thioesters[98] whereas amine can be converted into imines,[99,100] and N-Mannich bases[101] as the prodrug synthetic approach.

1.5.1. Prodrug of nucleoside analogues

One of the convenient and effective methods used to synthesize nucleoside prodrugs is the incorporation of different liphophilic groups either at the base moieties or at the sugar moieties *via* acylation or an alkylation reaction to improve cell permeability to the drug. Gemcitabine is a well-known anticancer drug which is widely used to treat breast cancer, pancreatic cancer, bladder cancer, lung cancer, and others.[102] However, research shows that significant amount of gemcitabine converts into corresponding uridine analogues in the cell as a result of the deamination by the cytidine deaminase enzyme.[103] 4-*N*-Alkonylation of gemcitabine can significantly reduce the extent of deamination process and exhibit better efficiency.[104] On the basis of the finding 4-*N* acylated produg of gemcitabine LY2334737 has been developed (Figure 4).[105,106]

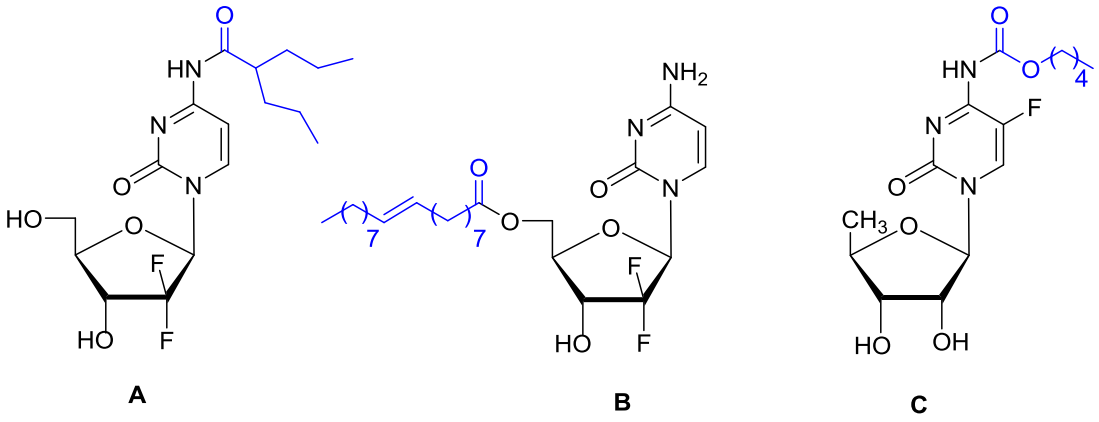


Figure 4. 5'-Acylated CP-4126 (A), 4-*N*-acylated prodrug LY2334737 (B) of Gemcitabine and Capecitabine (C) prodrug of 5-fluorouracil

The 5'-acylated gemcitabine analogues which is also known as CP-4126 is a well-known prodrug (Figure 4).[107] The lipophilic alkyl long chain enhances the cell permeability of the original drug gemcitabine. After entering into the cell the prodrug converts into original drug

gemcitabine by the esterase enzyme.[108] Capecitabine is a prodrug of 5-fluorouracil which is widely used for the treatment of breast cancer, gastric cancer, and colorectal cancer.[109,110]

Mericitabine which is also known as RG7128 is an antiviral prodrug of PSI-6130 and used for the treatment of hepatitis C virus infected patients as a combination therapy with other antiviral drugs (Figure 5).[111] The drug acts by inhibiting NS5B RNA polymerase, a primary transcript RNA.[17]

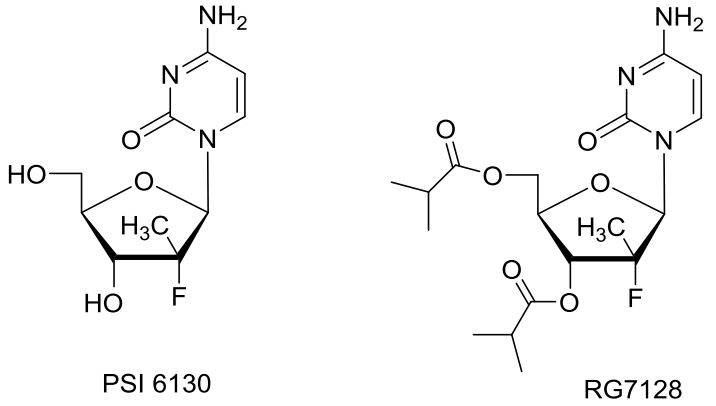


Figure 5. PSI 6130 and its prodrug mericitabine (RG7128**)**

2. RESEARCH OBJECTIVES

My first research objective was the synthesis of 5-(1-halo-2-tosylvinyl)pyrimidine nucleosides (**A**). Recently, (β-halo)vinyl sulfone analogues gained considerable amount of attention because of their reactivity and important biological activities. As (β-halo)vinyl sulfones possess reactive halovinyl sulfonyl moiety along with electronegative halogen atom at the β-position, I thought it would be interesting to synthesize nucleoside analogues modified with (β-halo)vinyl sulfone and exploring their reactivities and biological properties. To get access to 5-(β-chloro/bromo)vinyl sulfones (**A**) I explored transition-metal catalyzed halovinylsulfonylation of 5-alkynyl uracil and cytosine nucleosides with *p*-toluenesulfonyl hydrazine in the presence of *tert*-butyl hydrogen peroxide. The synthesis of 5-(β-iodo)vinyl sulfones (**A**) would be attempted via *N*-iodosuccinamide mediated halosulfonylation reactions of the 5-alkynyl uracil and cytosine nucleosides with sodium salt of sulfinic acid.

The typical reaction of the unsubstituted vinyl sulfone with nucleophiles occurs via the Michael addition reactions. As (β-halo)vinyl sulfone possesses halogen atom at the β-position, it would be fascinating to explore these type of reactions with the (β-halo)vinyl sulfone. So, I was planning to explore reactivity of the novel (β-halo)vinyl sulfones with different nucleophiles i.e., thiolates, amines, amino acid to observe whether it underwent a Michael addition or conjugated addition-elimination reaction to give 5-(1-substituted-2-tosylvinyl)pyrimidine nucleosides (**B**). I was also eager to investigate the feasibility of bioconjugation of these novel halovinyl sulfone analogues by reacting them with biomolecules such as tripeptide L-glutathione. The (β-chloro/bromo/iodo)vinyl sulfones would be prepared to study the mechanism of the conjugated addition-elimination reactions employing kinetic and synthetic approaches. Since (β-halo)vinyl sulfones as well as their

products of the addition-elimination reactions with nucleophiles are trisubstituted alkenes, the stereochemistry at the double bond of these novel pyrimidine-based 5-(β-halo)vinyl sulfones will be established using advanced 2D-NMR techniques and single X-ray crystallographic method. Moreover, since 5-modified pyrimidine nucleosides have been demonstrated to possess antiviral and antiproliferative properties, I would explore the biological properties of these novel (β-halo)vinyl sulfone in various viral (e.g. herplex simplex virus, human cytomegalovirus, parafluenza virus) and cancer (e.g. murine leukemia, human T-lymphocyte, human cervix carcinoma) cells. I was also planning to investigate if changing the polarity of the (β-halo)vinyl sulfone analogues by incorporating different liphophilic alkyl groups (as an carboxylic esters) at the sugar moiety would increase their antproliferative properties.

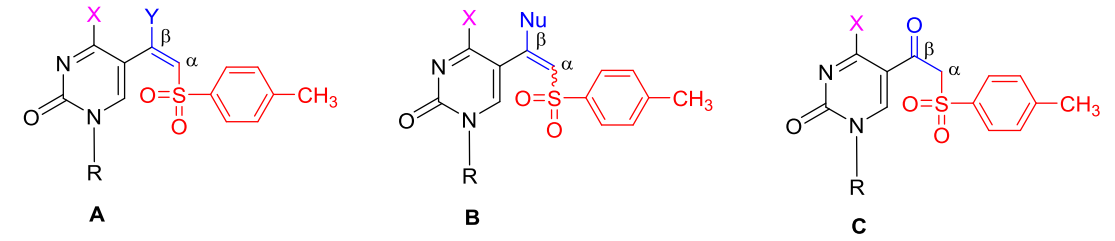


Figure 6. General structure of 5-modified pyrimidine nucleoside analogues

My second research objective was the synthesis of novel 5-(β-keto)sulfone analogues of pyrimidine nucleoside (**C**) and exploring their reactivity at the α-carbon of these sulfones. Synthesis of these novel (β-keto)sulfone analogues would be attempted by di-oxygen triggered oxidative radical reaction of the 5-ethynyl pyrimidine nucleosides with *p*-toluenesulfinic acid in the presence of pyridine and oxygen. Alternatively the (β-halo)vinyl sulfone analogues would be converted into corresponding β-keto sulfone analogues by applying one-pot synthetic protocol which would involve conversion of the (β-halo)vinyl

sulfone into the corresponding (β-amino)vinyl sulfone followed by acid hydrolysis of the latter intermediate into (β-keto)sulfone will be also developed. Since the α-carbon of 5-(β-keto)sulfones **C** possess acidic protons, I would explore further modification of these novel probes. My ultimate goal was to verify the feasibility of the reactivity at the α-carbon of β-keto sulfone analogues with different electrophiles and possibly nucleophiles and finally demonstrate the feasibility of the bioconjugation of the 5-(β-keto)sulfone modified DNA with proteins.

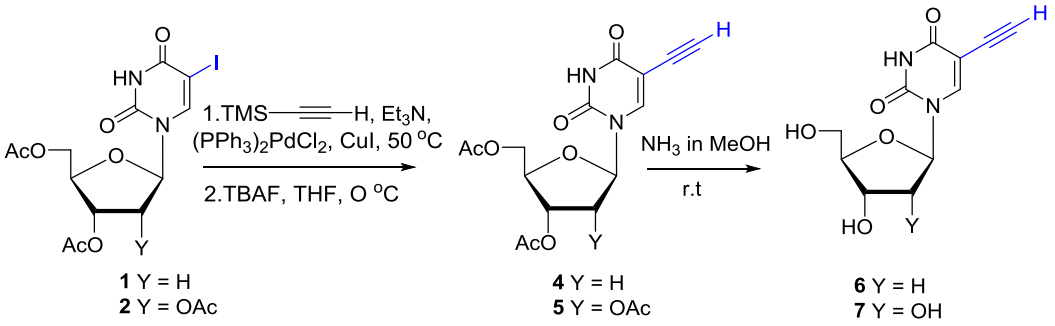
My third and final research objective was to incorporate the novel 5-(β-chloro)vinyl and 5-(β-keto)sulfone-2'-deoxyuridine triphosphatesinto DNA so that study of DNA-proteins interactions under the physiological conditions could be explored. Thus, Yoshikawa phosphorylation of 5-(β-chloro)vinyl or 5-(β-keto) sulfones followed by coupling of the resulting 5'-*O*-monophosphate with ammonium pyrophosphate should afford the corresponding 5'-*O*-triphosphates. These novel 5-modified 2'-deoxyuridine nucleotides will be attempted to be incorporated into DNA fragments by polymerase-catalyzed reaction. If successful, I was planning to investigate bioconjugation of these active probes with tripeptide L-glutathione. My final goal was to explore bioconjugation of such modified DNA with transcription factors such as c-Myc protein which is rich in nucleophilic thiol (cysteine) residues. If successful, further study might uncover critical information of the role of this protein in gene expression and DNA replication.

3. RESULTS AND DISCUSSIONS

3.1. Chemistry of (*E*)-5-(1-halo-2-tosylvinyl)pyrimidine nucleosides

3.1.1. Synthesis of (*E*)-5-(1-chloro/bromo/iodo-2-tosylvinyl)uracil nucleosides

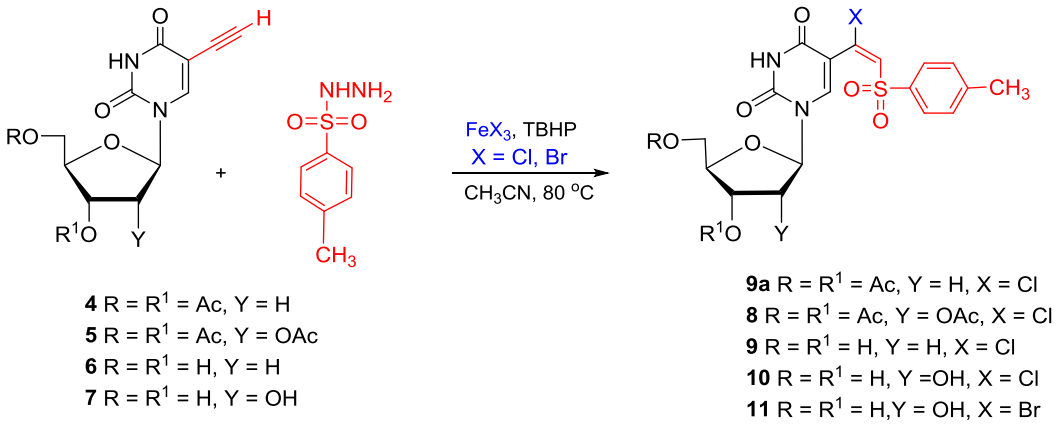
General approaches for the synthesis of targeted 5-modified pyrimidine nucleosides involve investigation of halosulfonylation reactions with the nucleoside-derived terminal alkynes. Thus, in the first step a reactive acetylene group was incorporated at C5-position of the uracil nucleosides which involved highly efficient Sonogashira coupling of 5-iodo uracil nucleoside analogues following Robin's procedure[112] followed by sequential deprotection of the protecting groups (Scheme 34).



Scheme 33. Incorporation of reactive acetylene group at 5 position of uracil nucleosides

Iron (III) chloride mediated and TBHP promoted regio and stereoselective chlorosulfonylation reaction of protected 5-ethynyluridine **5** gave a single *E* isomer of 2',3',5'-tri-*O*-acetyl-5-(1-chloro-2-tosylvinyl)uridine (**8**) with 68% yield. Analogous treatment of protected 2'-deoxyuridine **4** afforded 3',5'-di-*O*-acetyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (**9**) with 76% yield. Treatment of 5-ethynyluridine **7** with *p*-toluenesulfonyl hydrazide and iron(III) chloride in the presence of tertiary butyl hydrogenperoxide gave (*E*)-5-(1-chloro-2-tosylvinyl)uridine **10** with 59% yield. Analogous treatment of **6** in the same

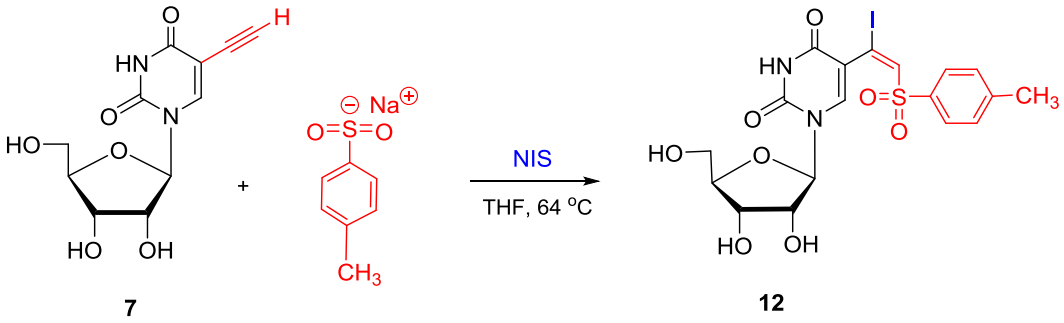
condition gave (*E*)-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (**9**) with 90% yield (Scheme 35).[90]



Scheme 34. Synthesis of (*E*)-5-(1-chloro/bromo-2-tosylvinyl)uracil nucleosides via iron catalyzed halosulfonylation reaction

Treatment of 5-ethynyluridine **7** with *p*-toluenesulfonyl hydrazide and iron(III) bromide in the presence of tertiary butyl hydrogenperoxide gave (*E*)-5-(1-bromo-2-tosylvinyl)uridine **11** with 41% yield. The structure of **8-11** was determined by using NMR techniques and the *E* stereochemistry was established based on literature report and analysis from X-ray crystallography as described in Section 3.1.3.2.

The 5-(1-iodo-2-tosylvinyl)uracil nucleoside analogues were synthesized by NIS promoted halosulfonylation reaction with 5-ethynyl uracil nucleosides and sodium salt of *p*-toluenesulfinic acid as depicted in Scheme 36.



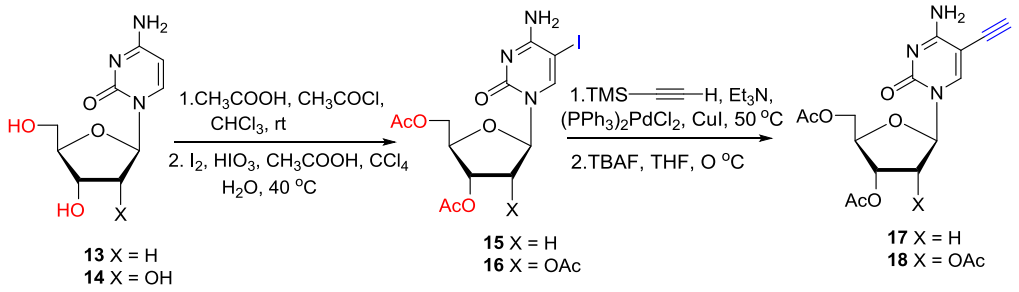
7 12

Scheme 35. Synthesis of (*E*)-5-(1-iodo-2-tosylvinyl)uracil nucleosides via NIS promoted halosulfonylation reaction

Thus treatment of 5-ethynyluridine **7** with sodium salt of *p*-toluenesulfinic acid gave (*E*)-5-(1-iodo-2-tosylvinyl)uridine **12** with 42% yield. The structure of the compound was established by the applying different NMR techniques and mass was determined by using high resolution mass spectrometry. In ^{1}H NMR **12** has vinylic proton peak at 7.72 ppm while the vinylic carbon peaks appeared at 105.5 ppm.s

3.1.2. Synthesis of (*E*)-5-(1-chloro/bromo/iodo-2-tosylvinyl)cytosine nucleosides

The *β*-halo sulfonylation reaction was also extended to cytosine nucleoside. An acetylene functional group at the C5-position offers a convenient way to synthesize 5-(β-chlorovinyl) sulfone of cytidine or 2'-deoxycytidine nucleosides. The acetylene moiety was efficiently incorporated at C5- position of these nucleosides via an acetyl protection at the sugar moiety, iodination at 5 position, Sonogashira coupling, silyl deprotection (Scheme 37).[113]



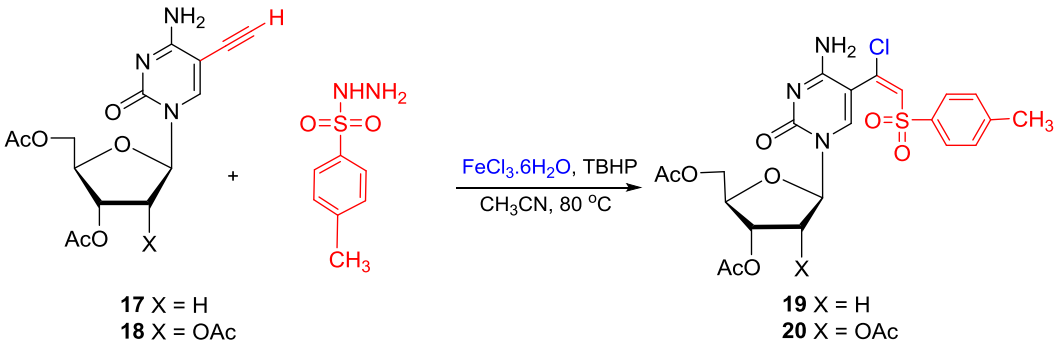
13 X = H
14 X = OH

15 X = H
16 X = OAc

17 X = H
18 X = OAc

Scheme 36. Synthesis of acetyl protected 5-ethynylcytidine and 2'-deoxycytidine analogues

Thus, treatment of 5 ethynyl analogue **17** with tosyl hydrazide in the presence of iron (III) chloride and tertiary butyl hydrogen peroxide gave (*β*-chloro)vinyl sulfone analogue **19** with 68% yield. Analogous treatment of **18** with tosyl hydrazide in the similar reaction condition gave **20** with 60% yield (Scheme 38).



17 X = H
18 X = OAc

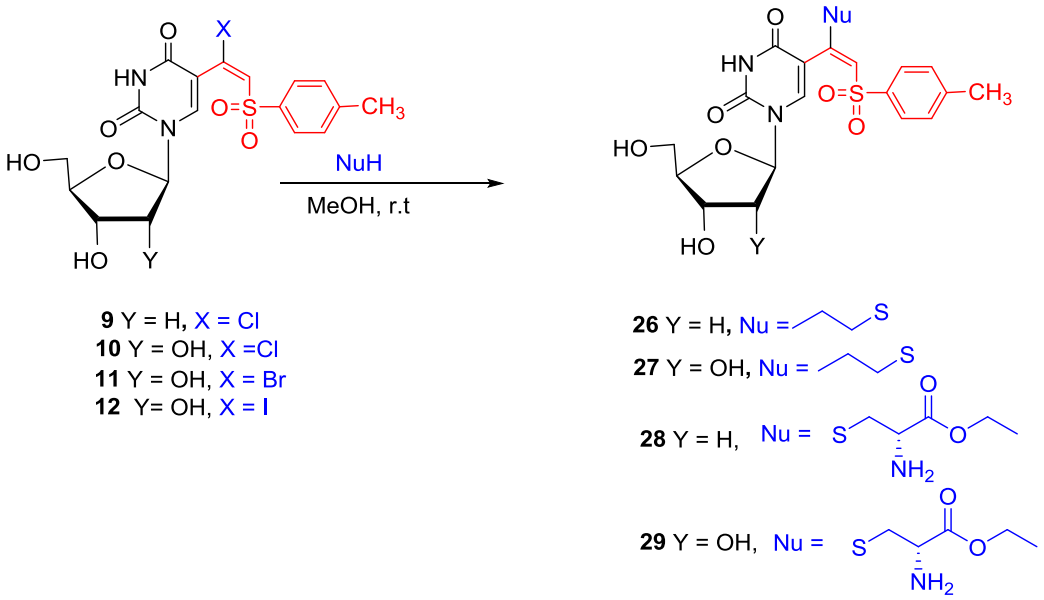
19 X = H
20 X = OAc

Scheme 37. Synthesis of (*E*)-*β*-chlorovinyl sulfone of protected cytidine and 2'-deoxycytidine nucleosides

The structure of the compounds **19** and **20** were established by applying different NMR techniques such as ^{1}H, COSY, HMQC, DEPT-135, and ^{13}C. The exact mass was determined by applying MALDI-TOF mass spectrometry. In proton NMR vinyl peak of **19** and **20** were obtained at 7.67 and 7.61 ppm respectively. For both **19** and **20** methyl peak of tosyl group was observed at 2.40 ppm while the aromatic peaks were observed in between 7.00 to 8.00 ppm. In ^{13}C NMR the vinyl peak of **19** and **20** was observed at 137.0, and 138.0 ppm respectively while the methyl carbon peak of tosyl group were appeared at 21.0, and 21.8 ppm. Other aromatic peaks of the tosyl group were observed from 125 to 145 ppm. In addition a characteristics *β*-carbon peak was observed at 143.0, and 139.5 ppm respectively. In mass spectrum the characteristics isotopic pattern of one chlorine atom (peak intensity 3:1) was observed. Finally, the experimental mass value $[M + H]^{+}$ 526.1048 and 583.1025 confirmed the successful synthesis of **19** and **20**.

3.1.3. Addition-elimination reactions of (*E*)-5-(1-halo-2-tosylvinyl)pyrimidine nucleosides with nucleophiles

The reactivity of the (β-halo)vinyl sulfones of uracil and cytosine nucleosides was studied in details, and it was observed that they react efficiently with different nucleophiles such as amines, thiols, amino acid etc. through addition-elimination process to give the substituted products from moderate to good yield.

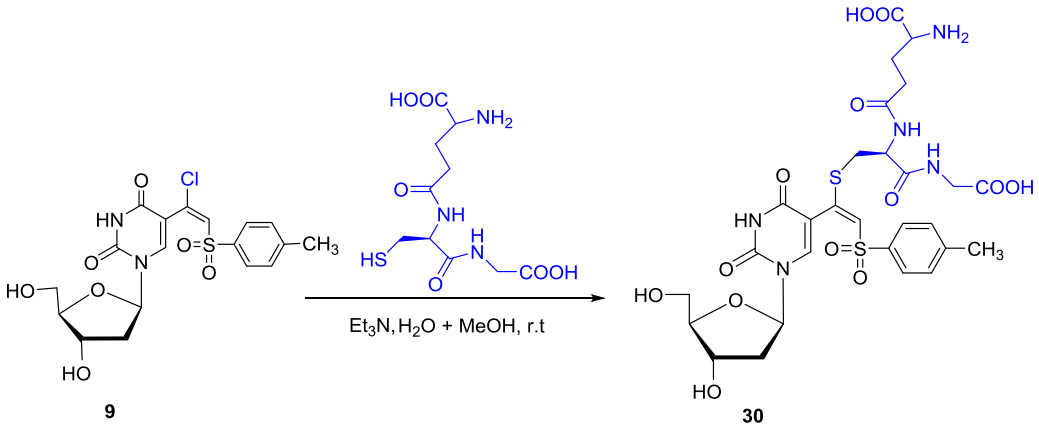


Scheme 38. Displacement of halogen atoms of uracil nucleosides by thiolates and amino acid to afford analogues with retention of configuration

Treatment of **9** with propane thiol in the presence of triethyl amine afforded propanethio substituted analogue **26** with 73% yield. Analogous treatment of **10** with propane thiol in the presence of triethyl amine gave **27** with 75% yield (Scheme 39). Treatment of **9** with important amino acid derivative L-cysteine ethyl ester hydrogen chloride in the presence of triethyl amine afforded **28** as a 85/15 *E/Z* mixture and the yield was 60%. Analogous treatment of **10** with amino acid derivative L-cysteine ethyl ester hydrogen chloride afforded **29** as a 85/15 *E/Z* mixture and the yield was 56% (Scheme 39).

The addition-elimination reaction of chlorovinyl sulfone analogue **9** was extended with well-known biomolecule and antioxidant L-glutathione. It is noteworthy to mention that glutathione is a tripeptide and thus can serve as a model compound to study bioconjugation with protein.

Thus, treatment of chlorovinyl sulfone analogue **9** with L-glutathione in the presence of triethylamine in mixed solvent (water and methanol) afforded glutathione incorporated product **30** with 56% yield (Scheme 40).



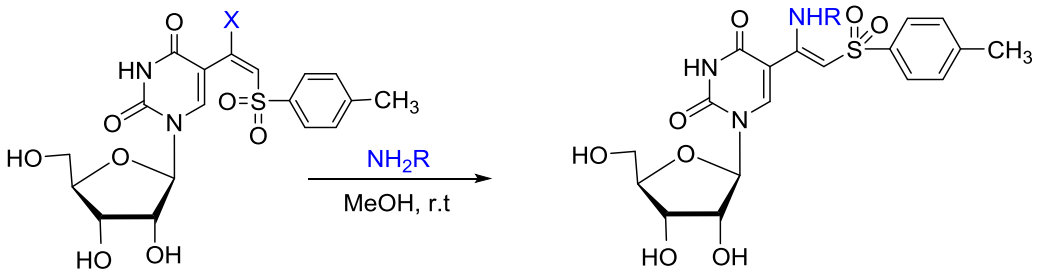
Scheme 39. Conjugation of 5- (*β*-chloro)vinyl sulfone of 2'-deoxyuridine with L-glutathione via displacement reaction

The structure of the compound **30** was determined by applying different NMR techniques such as ^{1}H, COSY, HMQC, DEPT-135, and ^{13}C. The exact mass was confirmed by taking MALDI-TOF mass spectrometry. In proton NMR distinguished peaks of glutathione moiety were observer at the region from 2.0- 5.0 ppm. Each peaks was figured out from their correlation in COSY.

In carbon NMR four peaks of carbonyl carbon of the glutathione moiety were detected at 170.5, 174.0, 175.2, and 176.2 ppm. The remaining six carbon peaks of glutathione were

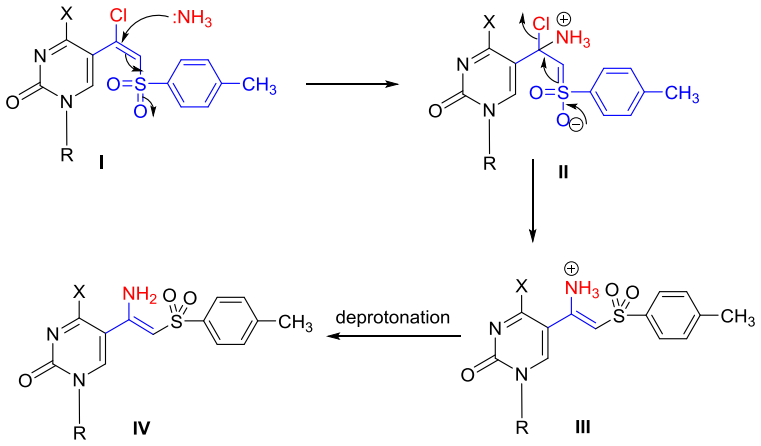
observed at the region from 25.0 to 55.0 ppm. Finally, the experimental mass value of **30** verified the success of this reaction.

The success of the reaction is very encouraging because addition-elimination reaction of nucleophile with chlorovinyl sulfone probe can be performed in physiological condition and therefore can be explored for the bioconjugation of nucleic acid with protein. One of the potential candidate of such protein is c-Myc protein. Note that c-Myc is a DNA transcription regulatory protein which controls about 15% of gene expression[114] and modified DNA- c-Myc protein interaction can significantly modulate the gene expression process and thus might play a crucial role in biological system.



Scheme 40. Substitution of halogen atoms of 5-(β-halovinyl) sulfone of uridine and 2'-deoxyuridine with amino group

Interestingly, treatment of *E* vinyl sulfone **10** with methanolic ammonia for 3 h gave (*Z*)-5-(1-amino-2-tosylvinyl) uridine **32** in 84% yields with a complete inversion of configuration (Scheme 41). The plausible mechanism of the reaction can be explained as depicted in the Scheme 42.



Scheme 41. Plausible mechanism for the substitution of chlorine atom by amino group in (*β*-halo)vinyl sulfones

If the nucleophiles after the addition-elimination type reactions are able to form hydrogen bonding with the oxygen atom of the sulfonyl moiety, then the products possess Z stereochemistry,[89] means inversion of configuration takes place as the halo vinyl substrates have E stereochemistry. When *β*-halo vinyl sulfones were treated with methanolic ammonia, it gave the Z isomer exclusively (Figure 7).

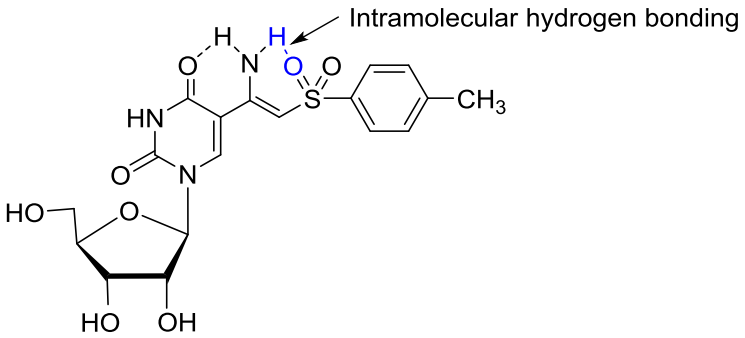


Figure 7. Intramolecular hydrogen bonding of amino group with oxygen atom of uracil ring and sulfone moiety

The *Z* stereochemistry of the vinylic position of (*β*-aminovinyl) sulfone analogue **32** was confirmed by Tsui from X-ray crystallography.[89] However, crystal was not obtained from the

5 modified amino vinyl sulfone analogue **32** as it readily converted into corresponding *β*-keto sulfone analogue during the approach to get crystal in MeOH or CH_3CN solvent.

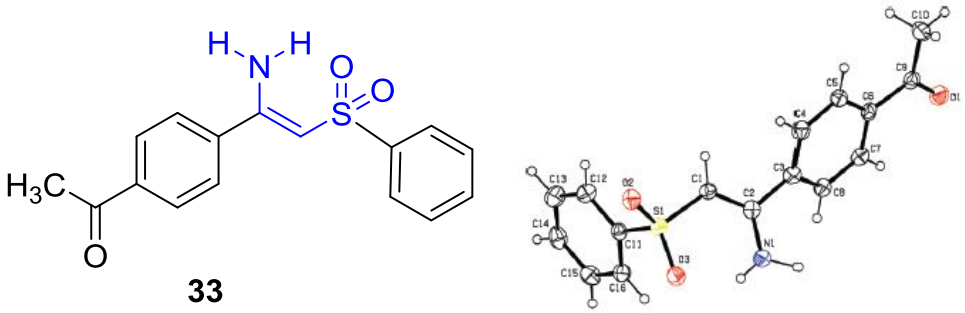


Figure 8. Structure of β-aminovinyl sulfone analogue **33** (left) and its X-ray structure (right).[89]

The *Z*-5-(*β*-amino)vinyl sulfone of uracil derivative **35** was synthesized from the corresponding *E*-5-(*β*-chloro)vinyl sulfone analogue **34**.[90] In the NOESY experiment correlation was observed between the vinylic and H6 proton in **35**. This correlation is only possible when vinyl position possesses *Z* stereochemistry. It is important to mention that in similar NOESY experiment with *E*-5-(*β*-chloro)vinyl sulfone **34** no correlation observed between those two protons (Figure 9).

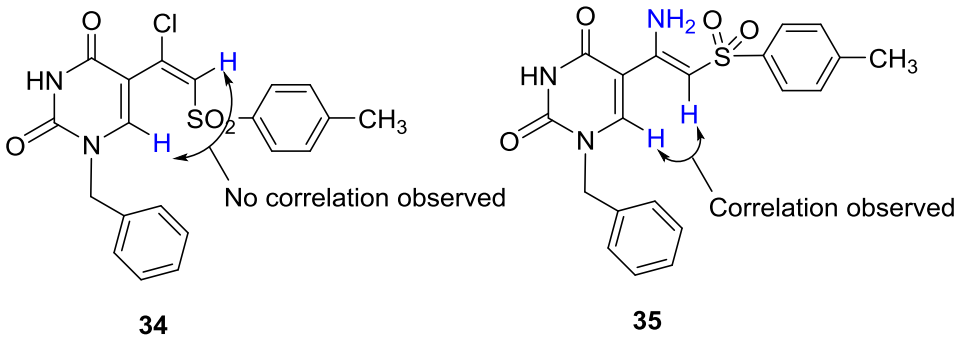


Figure 9. NOESY correlation between the vinylic proton and the H6 proton

Interesting findings from the NOESY experiments and the recent literature report by Tsui leads me to draw the conclusion that the vinylic position of the novel (*β*-amino)vinyl sulfone analogue **32** possesses *Z* stereochemistry.

However, when *β*-halo vinyl sulfones were treated with butyl amine, the product **33** was a mixture of *E*/*Z* isomers. The rationale for this can be explained that amino group has two hydrogen atoms which can form two intramolecular hydrogen bondings as shown in figure 10. The butyl amino group has only one hydrogen atom attached to the nitrogen atom which can form hydrogen bonding either with oxygen atom of the uracil base or oxygen atom of the sulfonyl group. This competition might results the mixtures of *E*/*Z* isomers.

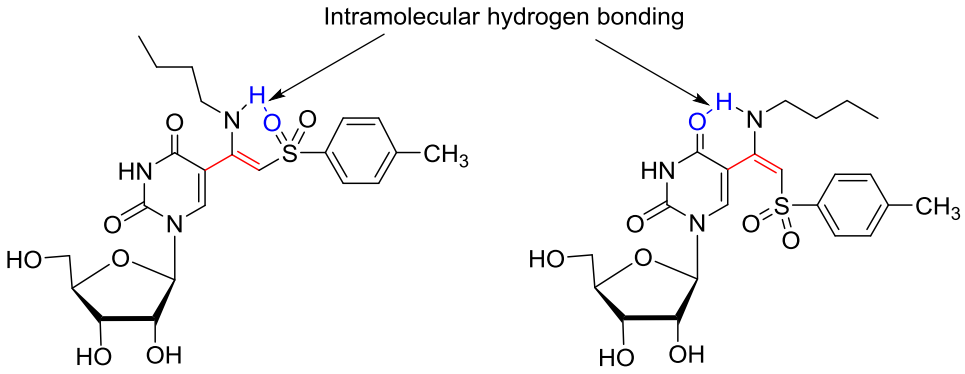
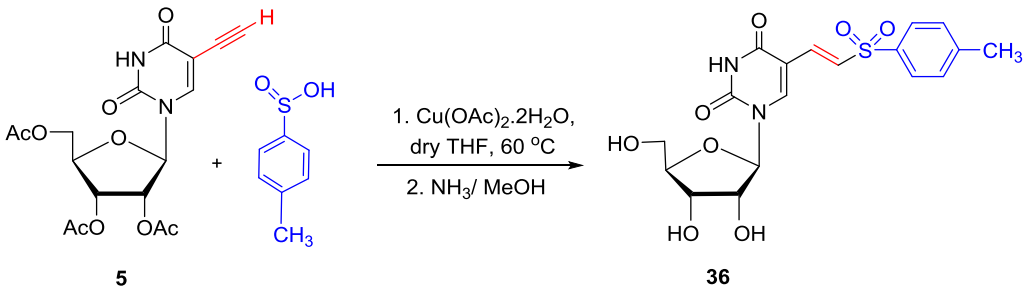


Figure 10. Intramolecular hydrogen bonding in (β-aminobutyl)sulfone **33**

To study the kinetics of the addition-elimination reactions of these halo vinyl sulfone and to compare reactivity of these halovinylsulfones with the unhalogenated vinyl sulfones, the (*E*)-5-(2-tosylvinyl)uridine (**36**) was synthesized.[115] Thus treatment of **5** with *p*-toluenesulfinic acid in the presence of copper(II) acetate catalyst followed by acetyl deprotection gave **36** with 35% yield (Scheme 43).



5 36

Scheme 42. Synthesis of (*E*)-5-(2-tosylvinyl)uridine

The structure of **36** was established by applying different NMR techniques and molecular weight was determined by high resolution mass spectrometry. In ^{1}H NMR, two vinylic proton showed two doublet at 7.19 and 7.62 ppm respectively. The coupling constant value was 15.0 Hz which indicated that the vinylic position possessed *E* stereochemistry. In addition the tosyl peak was observed at 7.39 and 7.73 ppm respectively. The experimental mass of **36** was 447.0802 $[M+Na]^+$ which is very much similar to the theoretical value 447.0833.

3.1.3.1. Kinetics of addition-elimination reaction of (E)-5-(1-halo-2-tosylvinyl)uridine

The kinetics of the addition-elimination reactions was studied by examining reaction profile of *β*-halovinylsulfones **10**, **11**, **12,** or **36** with *n*-propenthiol. Kinetic data for the reactions between *β*-halovinylsulfone **10, 11, 12,** and **36** and *n*-propanethiol in the presence of TEA were acquired by ^{1}H NMR at room temperature using a Bruker 400 MHz spectrometer. For each of the experiment propanethiol (200 µL of the 29.8 mM in DMSO-d_6) was added to NMR tube containing 200 µL of the 29.8 mM of *β*-halovinylsulfone (**10, 11, 12,** and **36)** in DMSO-d_6 and the proper ratio of PrSH to substrates (**10, 11, 12,** and **36)** was confirmed by proton integration. Then TEA (200 µL of the 29.8 mM in DMSO-d_6) was added for the total 9.93 mM concentration of substrates and the reaction mixture was scanned once every 5 minutes over first 30 minutes and later every 10 min. The procedure was repeated in

duplicate. For each of the halovinyl sulfones **10, 11, 12** tested, proton spectra showed formation of the substitution product **27**. However, unsubstituted vinyl sulfone **36** was unreactive in that nucleophilic environment. The profile for the reaction was measured by integrating disappearance of the signal of H6 of substrate **10**, **11 ,** and **12** at 8.25, 8.21, and 8.07 ppm and appearance of H6 signal at 7.96 ppm for the product **27** on ^{1}H NMR spectra. The y-intercept for each plot was set up to 1/[starting *β*-halovinylsulfone concentration]. Plots show data collected within ~40% (**10**), ~55% (**11**) and ~60% (**12**) conversion to the β-(propylthio)vinylsulfone (**27**) product.

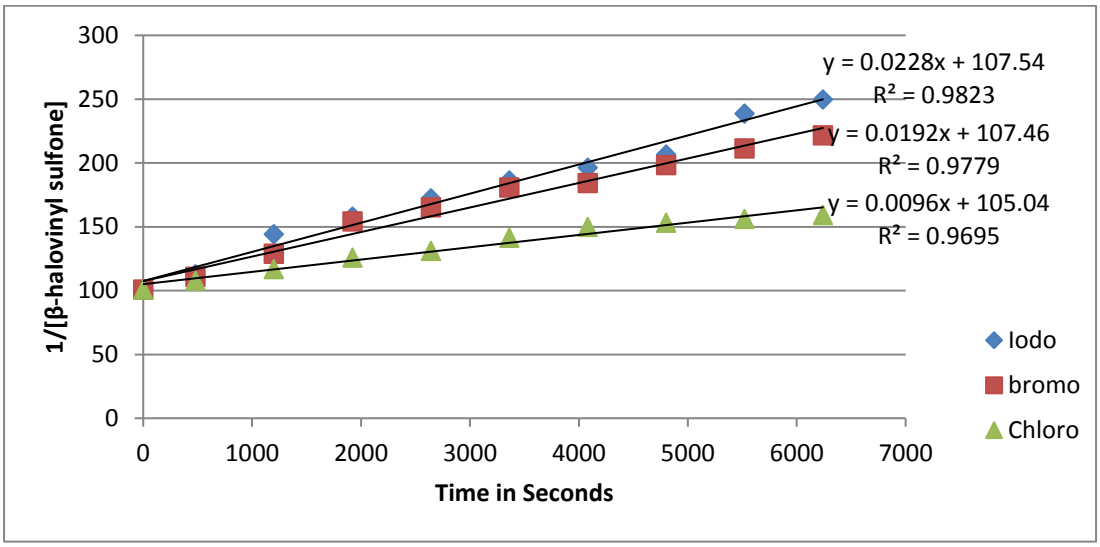
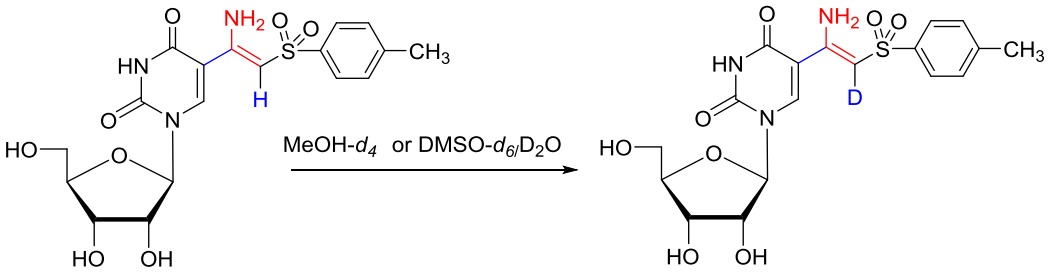


Figure 11. The rate plots for the reactions between **10** (chloro), **11** (bromo), and **12** (iodo) with n-propanethiol in the presence of TEA. The second order rate constant was calculating by plotting 1/[*β*-halo vinyl sulfone] as a function of time

The calculated second order rate constants for the substitution reaction were 0.0096 $M^{-1}s^{-1}$, 0.0192 $M^{-1}s^{-1}$ and 0.0228 $M^{-1}s^{-1}$ for **10** (chloro), **11** (bromo), and **12** (iodo), respectively. Plots depicted in Figure 11 showed that iodo and bromo vinylsufones are more reactive compare to chloro counterpart (I ≥ Br > Cl).

3.1.3.2. Mechanism and stereochemistry

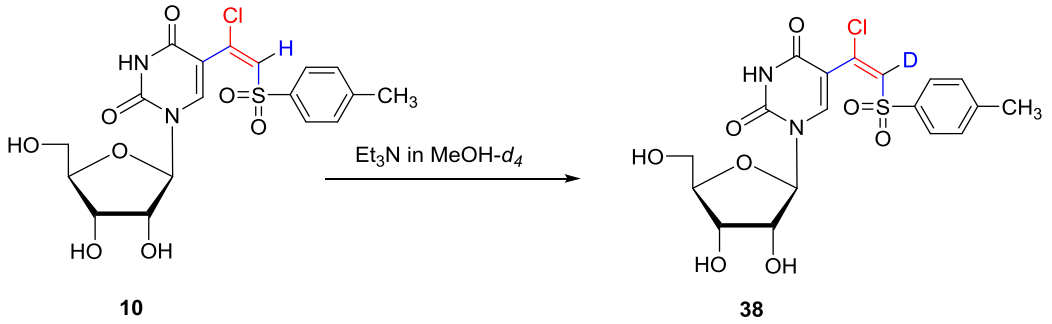
When β-substituted vinylsulfones were dissolved in protic polar deuterated solvent the alpha vinylic proton exhibited interesting behavior. Thus, when β-amino vinyl sulfone **32** was dissolved in D_2O or MeOH-d_4 the alpha-vinylic proton underwent quick exchange with deuteriumto give deuterium labeled compound **37** (Scheme 44)**.**



32 **37**

Scheme 43. Deuterium exchange at vinylic proton of 5- (*β*-aminovinyl) sulfone of uridine to afford α-deuterated analogue

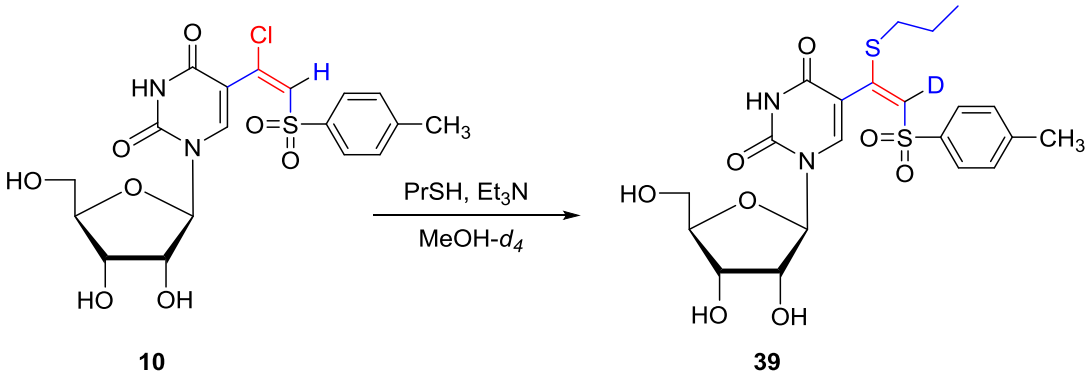
When **10** was dissolved in MeOH-d_4, no deuteration at the vinyl proton was observed. However in the presence of triethyl amine deuteration of the vinylic proton was observed and gave deuterated compound **38** (Scheme 45). However, the rate of deuteration of chlorovinyl analogue **10** was slow compare to the amino analogue **32**.



10 **38**

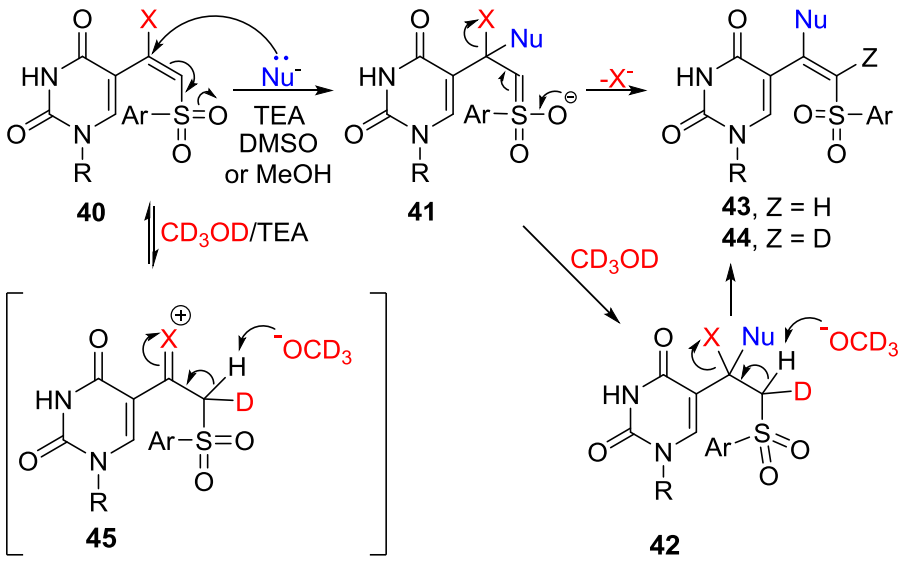
Scheme 44. Deuterium exchange at the vinylic proton of *β*-chloro analogue

In the presence of propanethiol and triethyl amine fast deutaration was observed and afforded the α-deuterated compound **39** as shown in Scheme 46**.**



Scheme 45. Deuterium exchange at vinylic proton of *β*-chloro sulfone to give α-deuterated *β*-propanethio-analogue

From these observations the plausible mechanism of the addition-elimination reaction could be proposed (Scheme 47). The *β*-carbon of the vinyl sulfone is vulnerable to the nucleophilic attack as it is partially positive due to the inductive effect of the halogen atom and neighboring electron withdrawing sulfonyl moiety. So, subsequent attack of the nucleophile and elimination of halogen atom give compound **43**. It is also observed that under nucleophilic condition (PrSH/MeOH-d_4/TEA) the vinylic proton is exchanged by deuterium to give compound **44.** It is entirely possible that enolate like basicity of intermediate **41** is quenched by deuterium and subsequent deprotonation of more acidic proton of intermediate **42** gave deuterium incorporated compound **44**.



Scheme 46. Plausible mechanism for substitution of halogen in *β*-halovinyl sulfones with nucleophiles

Deuterium exchange was observed when *β*-amino vinyl sulfone **31** is treated with DMSO-d_6/D_2O or MeOH-d_4. However, vinyl thiether analogue **27** did not undergo such exchange in MeOH-d_4 in the presence of TEA. Although deuterium exchange of the vinyl proton was observed in the chlorovinyl sulfone analogue **10** in MeOH-d_4 in the presence of TEA, this exchange was slower compare to the aminovinyl analogue **31.** In addition, in the presence of nucleophilic condition (PrSH/ TEA/ MeOH-d_4) deuterium exchange occured faster compare to the absence of propane thiol. From this behavior of chlorovinyl compound **10,** it is believed that faster deuterium acquision occurs through intermediate **42** instead of intermediate **45**.

It is reported in the literature[3-4] that the expected *β*-halo vinyl sulfone should have possessed *E* configuration at the double bond. In order to establish unequivocally the stereaochemistry in the novel nucleoside analogues, crystal of 5-(1-chloro-2-tosylvinyl)uridine (**10**) was obtained by slow evaporation of ACN in H_2O. X-ray analysis of

the single crystal of **10** was accuired in Dr. Raptis's Lab at FIU from the X-ray analysis *E* stereochemistry around double bond was concretely established (Figure 12).[90]

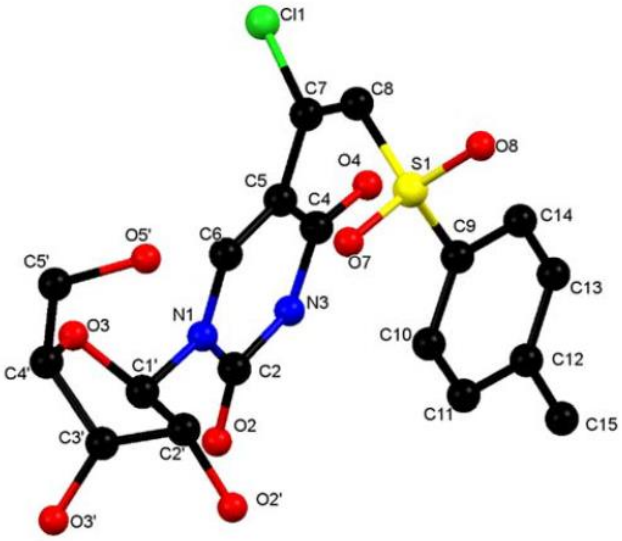


Figure 12. Ball-and-stick representation of 5-(1-chloro-2-tosylvinyl)uridine **10** showing the atom labeling scheme. H-atoms and interstitial H_2O molecules are omitted for clarity.

In 5-(1-chloro-2-tosylvinyl)uridine **5** the glycosyl torsion angle C6−N1−C1′−O4′ is 43.9°, and the furanose pseudorotation angle is 164.9° ($^2T_3$ conformation). The C3′−C4′−C5′−O5′ torsion angle is 53.6° and is in the g+/gg range. Nearly parallel uracil and benzene ring orientation allowed favorable π-π interactions.

After addition-elimination reaction, the stereochemistry of the vinyl position of the products was studied. The crystal of the model compound 1-*N*-Benzyl-5-(1-(propylthio)-2-tosylvinyl)uracil **45** was developed by the slow evaporation of acetonitrile and stereochemistry was confirmed after running X-ray of the crystal.

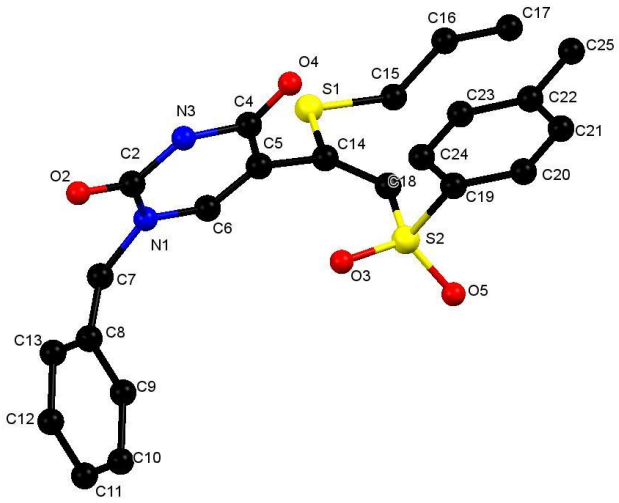


Figure 13. Ball-and-stick representation of 1-*N*-benzyl-5-(1-propanethiovinyl)uracil **45** showing the atom labeling scheme. Disordered parts and H-atoms are not shown for clarity

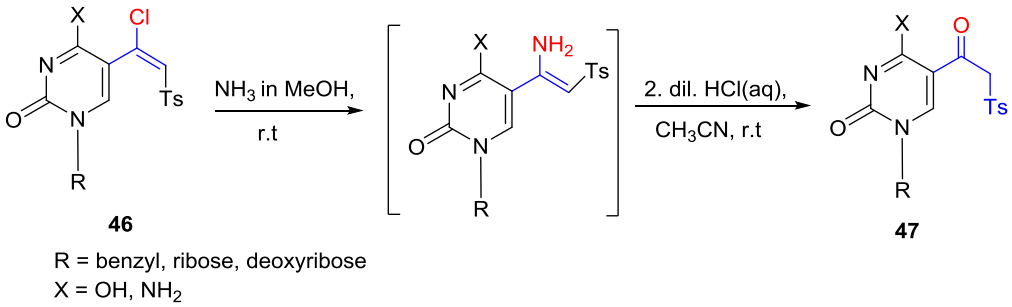
The 1-*N*-benzyl-5-(1-propanethiovinyl)uracil **45** crystallizes in the monoclinic $P2_1/n$ space group with the whole molecule in the asymmetric unit. The molecule contains four intermolecular H-bonds including two from the uracil base: (N1-C13: 2.867(9) Å and N3-O5: 2.920(4) Å). Compare to corresponding β-ketosulfone analogue as discussed in (Section 3.2.1), the pi-pi interactions are weaker.

From the X-ray data it is clear that the propanethio analogue **45** possesses *E* stereochemistry. That means after the addition-elimination reaction the *E* configuration of the double bond remained unchanged for that substituted product. As an amino group can form hydrogen bonding with oxygen atom of the tosyl group and the uracil moiety, it was interesting to explore the stereochemistry of the double bond of the nucleoside analogue 5-(1-amino-2-tosylvinyl) uridine **32**. As crystal of amino compound was not obtained, the stereochemistry of the double bond having amino group was determined by analyzing the 1-*N*-benzyl-5-(1-amino-2-tosylvinyl)uracil (**35**) by NOESY techiques. In a NOESY experiment,

the vinyl proton of chloro compound did not show any correlation with H6 proton while in the amino compound the vinyl proton showed strong correlation with the corresponding H6 proton (Figure 9). This correlation could be only possible if the double bond of the vinilic position possesses *Z* stereochemistry. So, the amino analogue **31** has *Z* stereochemistry at the double bond due to the formation of hydrogen bonding of the amino group with the sulfonyl moiety of the tosyl group. The *Z* stereochemistry at the double bond of analogue **31** is totally consistent with the literature report published by Tsui *et al.*[89]

3.2. Chemistry of 5-(2-tosylacetyl)uracil and cytosine nucleosides

The C-5 position of the pyrimidine nucleoside is an active site where different reactive functional groups such as alkene, alkyne, azide etc can be incorporated to synthesize novel pyrimidine nucleosides.[116] So, acetylene functional group was incorporated at the C-5 position of the pyrimidine nucleosides. The 5-(1-chloro-2-tosylvinyl)pyrimidine nucleosides were synthesized via halovinyl sulfonylation reactions as discussed above in section 3.1.1. These (*β*-chloro)vinyl sulfones **46** were converted into the corresponding *β*-keto sulfones **47** by the treatment of **46** with methanolic ammonia followed by acid hydrolysis of the intermediary (*β*-amino)vinyl sulfone (Scheme 48).



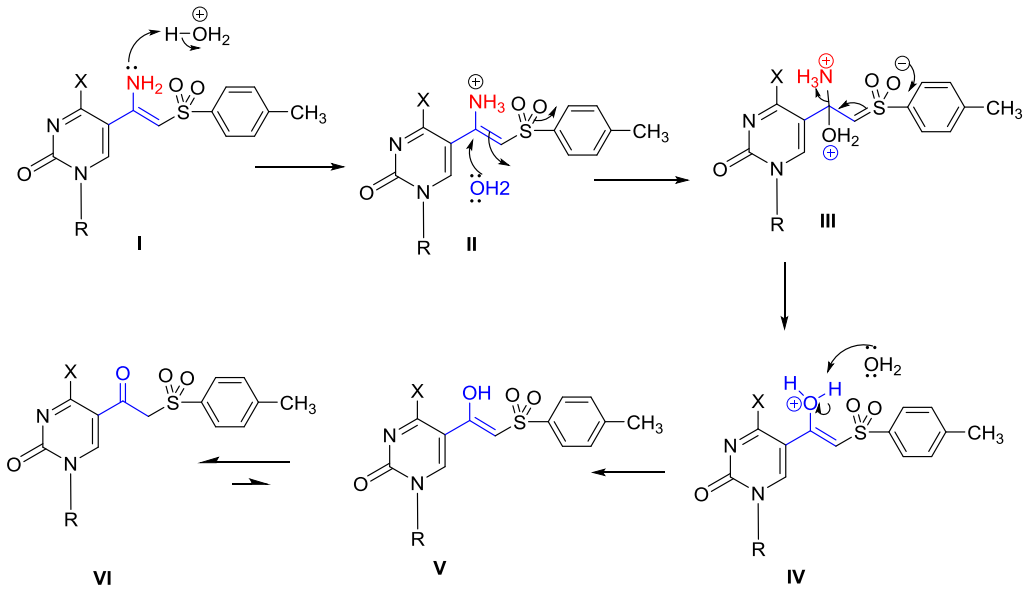
46

R = benzyl, ribose, deoxyribose
X = OH, NH_2

47

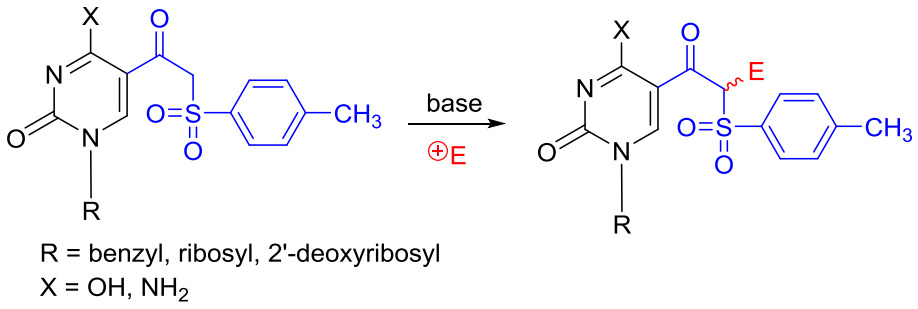
Scheme 47. Synthesis of *β*-keto sulfone from β-chloro vinyl sulfone analogue via substitution by ammonia and acid hydrolysis

The plausible mechanism for the conversion of **46** to **47** can be explained in the following way. Firstly, conversion of (*β*-chloro)vinyl sulfone (e.g. **46**) into (*β*-amino)vinyl sulfone occurs *via* addition-elimination reactions as depicted in Scheme 42. The basic amino group in (*β*-amino)vinyl sulfone **I** accepts proton in acidic media and developed positive charge at the nitrogen atom to give intermediates **II** (Scheme 49). Water attacks at the *β*-carbon of **II** follow by elimination of ammonia pathway afford intermediate **IV**. Subsequent deprotonation and tautomerization generates *β*-keto sulfone **VI**.



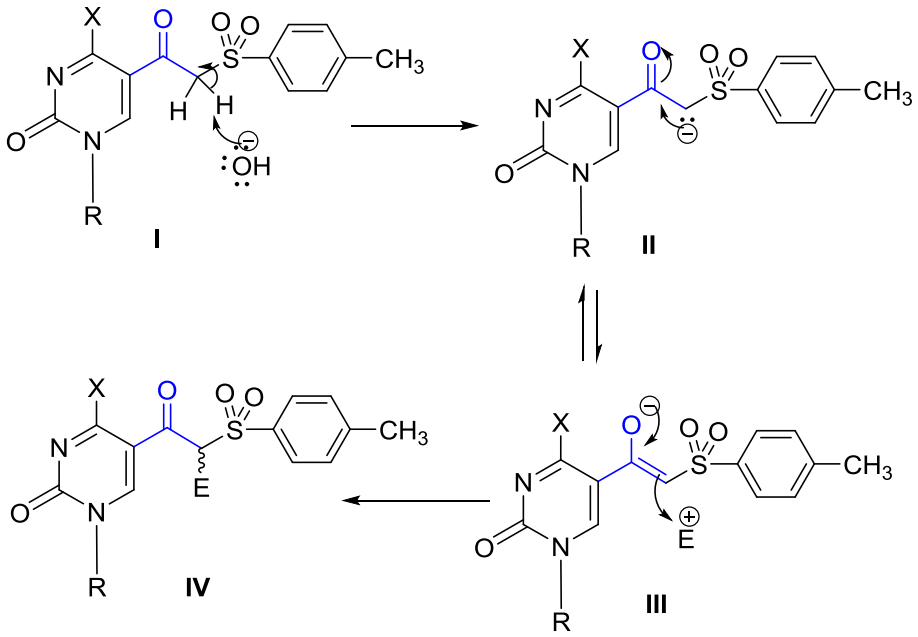
Scheme 48. Plausible mechanism of the conversion of *β*-amino sulfone into *β*-keto sulfone

Since the *α*-proton of *β*-keto sulfone is acidic (pKa = ~9-10) and under sufficient basic conditions the *α*-carbon can trap electrophile. From this assumption, different electrophiles can be inserted at the α-carbon of the β-keto sulfone analogues as illustrates in Scheme 50.



Scheme 49. General scheme for the trapping of eletrophiles at the α-carbon of β-keto sulfone

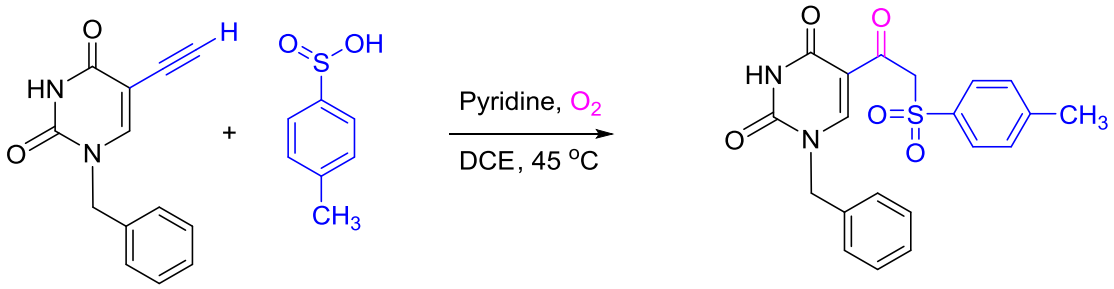
The plausible mechanism of this reaction is very straightforward and is depicted in Scheme 51. First the base picks up an acidic proton from the α-carbon and thus generates carbanion **II** which can be stabilized by forming enolate ion **III**. The π electron of the enolate ion attacks the electrophile and gives product **IV** as mixture of isomers. The rationale of forming products **IV** can be explained from the sp^2 hybridization of α-carbon of **III** which can attack electrophile either from the top face or bottom face.



Scheme 50. Plausible mechanism of electrophile trapping at the α- carbon

3.2.1. Synthesis of 5-(2-tosylacetyl)uracil nucleosides

Initially the synthesis of uracil 5-(β-keto)sulfone were attempeted by pyridine-catalyzed oxidative radical reaction of 5-ethynyluracil analogues. Thus, treatment of 1-*N*-benzyl-5-ethynyluracil **48** with *p*-toluenesulfinic acid and pyridine in the presence of oxygen afforded 5-(β-keto)sulfone derivative **49** with the yield of 44% (Scheme 52).

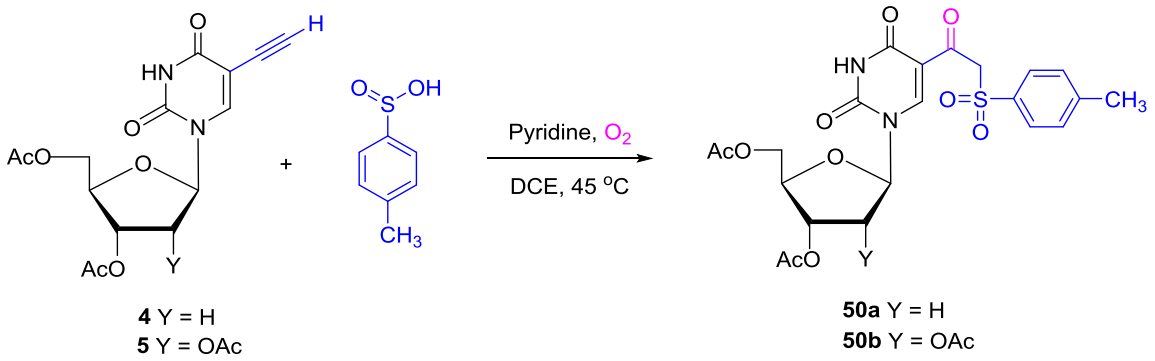


48 **49**

Scheme 51. Synthesis of 5-(β-keto)sufone of 1-*N*-benzyl-5-ethynyluracil

The 5-(β-keto)sulfone **49** was characterized by NMR techniques and mass was confirmed from HRMS-TOF analysis. In ^{1}H NMR the α-methylene peak was observed at 4.98 ppm and three peaks from tosyl group appeared at 2.42, 7.29 and 7.79 ppm. In ^{13}C NMR the methylene carbon peak appeared at 52.9 ppm while β-carbonyl carbon peak was detected at 183.8 ppm.

Treatment of acetyl protected 5-ethynyl-2'-deoxyuridine **4** with *p*-toluenesulfinic acid by pyridine-catalyzed aerobic oxidative reaction afforded protected 5-(β-keto)sulfone analogue **50a** with 16% yield (Scheme 53). Analogous treatment of protected 5-ethynyluridine **5** with sulfinic acid afforded **50b** with 20% yield. The low yield of these reactions could be explained by the acid sensitive β-glycosidic bond which cleaved during the reaction. Both of the products were characterized using NMR techniques.

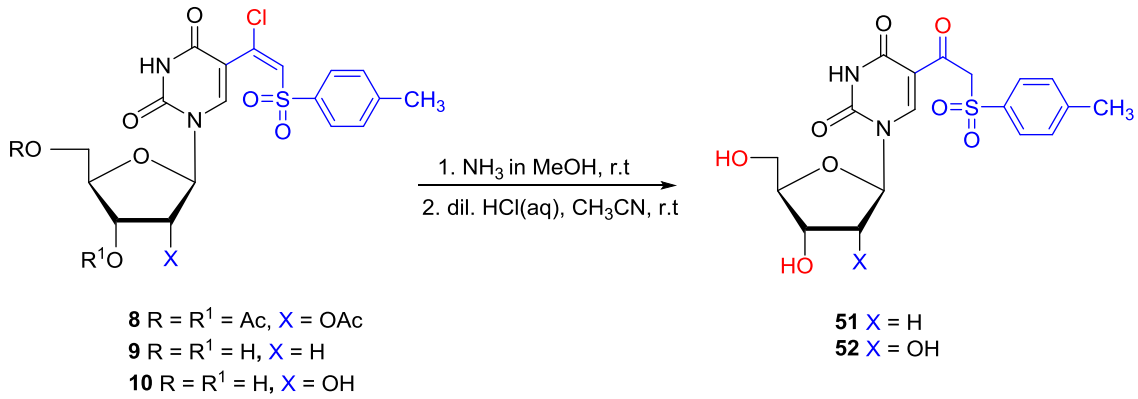


Scheme 52. Synthesis of 5-(β-keto)sufone of protected uridine from the corresponding 5-ethynyl substrates

In **50a** the two peaks of α-methylene proton were observed at 4.95 and 5.05 ppm and the three tosyl peaks appeared at 2.45, 7.35, and 7.85 ppm. In **50b** the two peaks from α-methylene protons appeared at 4.90 and 5.10 ppm. The chemical shift value of H6 for both **50a** and **50b** were appeared in the relatively downfield region compared to the 5-ethynylsubstrates **4** and **5** (8.50 ppm *vs* 7.83 ppm) probably the result of the incorporation of electron withdrawing β-keto sulfonyl moiety at the C5 position.

As a result of the low yield of the oxidative radical process, the 5-(β-keto)sulone of uracil nucleosides were synthesized from their 5-(β-halo)vinylsulofne precursors by applying one pot synthetic protocol as depicted in Scheme 48. Thus, acetyl protected (**8**) or unprotected (**9-10**) *β*-chlorovinyl sulfone analogues of uracil nucleosides were efficiently converted into corresponding *β*-keto sulfones (**51-52**) by a one pot synthetic protocol. Thus, treatment of the vinyl sulfones (**8-10**) with methanolic ammonia gave *β*-amino vinyl sulfones in quantitative yield. Subsequent acid hydrolysis of these *β*-amino vinyl sulfones in acetonitrile gave *β*-keto sulfones in high yield. Analogoulsy, acetyl protected *β*-chlorovinyl sulfones **8** was converted into corresponding *β*-keto sulfone with 60% (**52**) yields and unprotected chlorovinyl sulfone

analogues **9** and **10** into corresponding *β*-keto sulfones afforded 74% and 70% yields respectively (Scheme 54).



8 R = R^1 = Ac, X = OAc
9 R = R^1 = H, X = H
10 R = R^1 = H, X = OH

51 X = H
52 X = OH

Scheme 53. Synthesis of 5-(β-keto)sulfone of uridine and 2'-deoxyuridine from their (β-chlorovinyl) sulfone analogue

The structure of (*β*-keto) sulfone analogues **51** and **52** were established using different NMR (^{1}H, COSY, HMQC, DEPT-135 and ^{13}C) techniques. In **51** and **52**, a higher chemical shift value of H6 proton was observed at 8.68 and 8.80 ppm respectively as compared to the chemical shift value of H6 proton in the corresponding *β*-chlorovinyl sulfone substrates **8-10**. The reason of higher chemical shift value of the keto analogues can be explained by the incorporation of electron withdrawing keto group at C5 position. In compounds **51** and **52**, the peaks of methylene group appeared at 5.15 and 5.14 ppm respectively. In *β*-keto sulfone analogues **51** and **52,** characteristics peaks of carbonyl carbon were observed at 184.0 and 185.0 ppm respectively. In addition, distinct peak of methylene carbon peaks were observed at 64.0 and 64.3 ppm respectively. In agreement with the proton NMR there was also higher chemical shifts value of C6 carbon peaks those were 149.0 and 149.3 ppm respectively. The mass obtained from the high resolution mass spectroscopy (HRMS) was in accord with the calculated value.

The single crystal of 1-*N*-benzyl-5-(2-tosylacetyl)uracil (**49**) for X-crystallography was obtained by diffusion crystallization (ethyl ether into a MeOH solution) of the corresponding aminovinylsulfone **35**. Compound **49** was crystallized in the triclinic *P-1* space group with the whole molecule in the asymmetric unit. Several intermolecular H-bonds stabilize the crystal structure, including a uracil N-O H-bond (N3-O4: 2.900(3) Å) (Figure 14). The (*β*-keto) sulfone analogue **49** is also stabilized by an intermolecular C-H-π-interaction between the tosyl methyl group and the phenyl ring from the benzyl group (C(H)-centroid distance: 3.893 Å). The π-π interaction usually present between the uracil and the tosyl groups is weak or negligible in this analogue possibly due to other H-bonding and C-H-π interactions mentioned above.

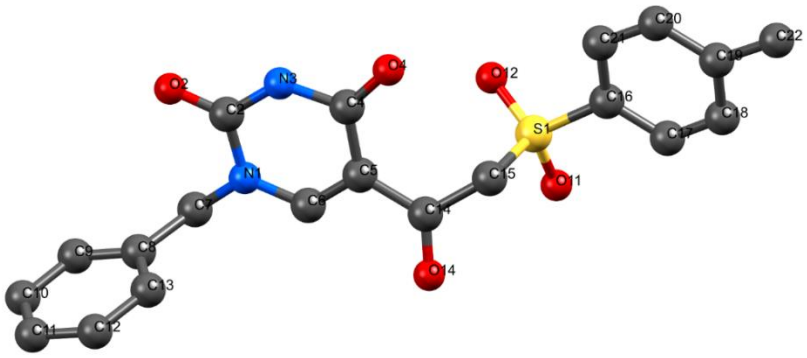
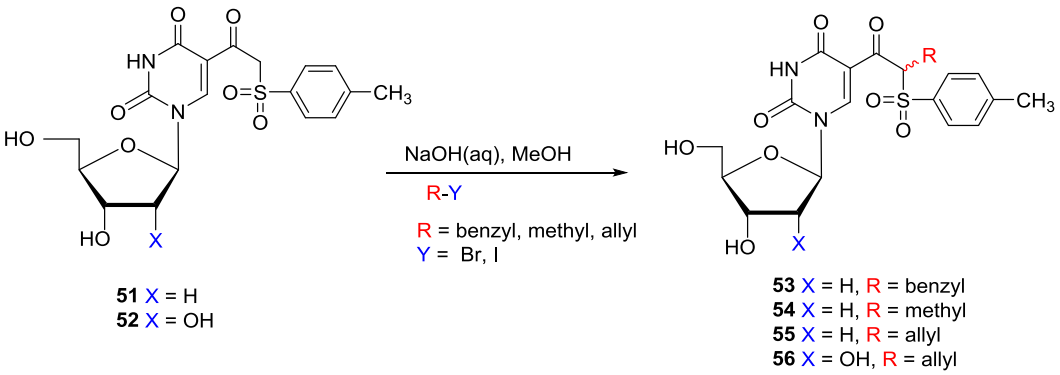


Figure 14. Ball-and-stick representation of 1-*N*-benzyl-5-(2-tosylacetyl)uracil (**49**); H-atoms have been hidden for clarity

3.2.1.1. Electrophile trapping at the *α*- carbon of the corresponding *β*-keto sulfone

Since the *α*-CH_2 proton in *β*-keto sulfones is acidic (*p*Ka = 10-11) the *β*-keto sulfone analogues (**51-52**) when treated with different electrophile sources such as benzyl bromide, methyl iodide, allyl bromide in the presence of dilute NaOH can serve as a convenient substrates for the synthesis of α-alkylated products **53-56** (Scheme 55).



Scheme 54. Incorporation of electrophiles at the *α*-carbon of (*β*-keto) sulfone of uridine and 2'-deoxyuridine

It is interesting to see that during the electrophile trapping, diastereomeric products were obtained and the ratio of the diastereomers varied upon treatment with different electrophiles. When benzyl bromide was used as electrophile source **51** gave (50/50) mixtures of diasteremeric product **53** with 28% yield. The reason of getting low yield was the formation of byproducts. Apart from the expected diastereomeric products, there were two additional compounds were characterized. First byproduct was benzylated product at N3 position and the second one was a consequence of benzylation both at *N3* and α-carbon of the β-keto sulfone. Surprisingly, dibenzylation at α-carbon was not observed. In **53** two peaks of H6 proton at 8.6 and 8.7 ppm with the integration ratio of 1:1 were observed which can be considered as the evidence for a 50/50 diastereomeric product. In ^{1}H NMR triplet at 6.5 ppm was observed

which indicated the C proton. The methylene proton of the benzyl group was detected at 3.1-3.2 ppm as a multiplet. In addition the aromatic peak of the benzyl group was observed in the region from 7.0-7.3 ppm. In ^{13}C NMR the α-carbon peak was observed at 55.0 ppm while the methyl carbon peak of benzyl group was detected at 31.0 ppm. The aromatic peaks of the benzyl group were found in the region from 126.0-131.0 ppm which collapsed with the tosyl peaks. The mass obtained from the high resolution mass spectroscopy (HRMS) of compound **53** was 515.1437 $[M+H]^+$ which is in accord with the calculated value.

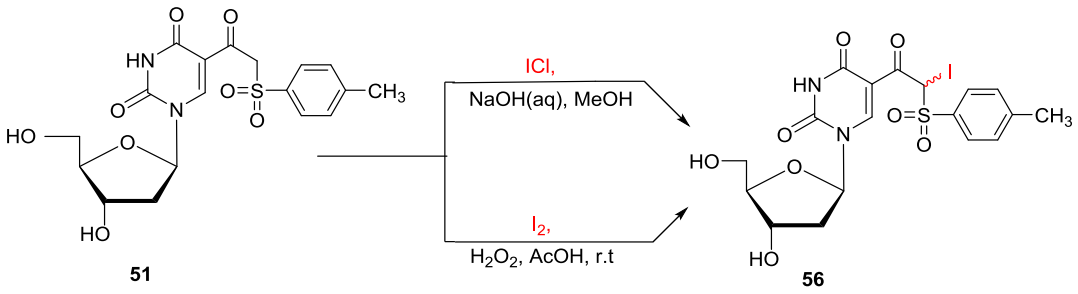
Treatment of **51** with methyl iodide afforded (48/52) mixtures of diasteriomeric product **54** with the yields of 31%. In addition, two other byproducts were also observed. These byproducts were characterized as *N*3 methylated byproduct and both *N*3 and α-carbon methylated byproduct. Like benzylation reaction, dimethylated product at α-carbon was not observed. In this α-methylated analogue **54** two proton peaks of H6 at 8.71 and 8.73 were observed with the integral ratio 96: 104 which meant that the diasteriomeric ratio was 48/52. The α-CH proton peak was observed at 5.96 ppm as a quartet while the methyl peak was observed at 1.34 ppm. In ^{13}C NMR α-C peak was observed at 66.0 ppm and the methyl carbon peak was found at 10.0 ppm.

Analogous treatment of **51** with allyl bromide gave 55/45 mixtures of diastereomeric product **55** afforded 31% yield. In addition, two byproducts were also produced in similar way as discussed before. The 104: 96 integral ratio of H6 proton of the α-substituted analogue **55** at 8.70 ppm and 8.75 ppm indicated the diastereomeric ratio is 52:48. The α-CH peak was detected as a doublet at 6.15 ppm. The allyl sp^3 hybridized CH_2 proton peak was obtained at 2.8 ppm and sp^2 hybridized CH proton peak was detected at 5.6 ppm. The sp^2 hybridized CH_2 proton peak of allyl group was observed at 5.0 ppm. In ^{13}C NMR α-carbon peak was observed

at 68.0 ppm. The three carbon peaks of allyl group were found at 30.0, 118.0 and 133.0 ppm respectively.

Treatment of **52** with allyl bromide afforded allyl substituted product **56** with the as a diastereomeric mixtures of 52/48 with the yields of 32%. Two byproducts were obtained in the similar way as discussed before. No disubstitution observed at the α-carbon. In **56** the integral ratio of H6 proton indicated the diastereomeric product was a 52/48 mixtures. The multiplet at 6.14-6.20 ppm confirmed the presence of α-CH peak. The three vinyl peaks was observed at 2.8, 4.9 and 5.6 ppm respectively. In ^{13}C NMR α-carbon peak was observed at 69.0 ppm. The allyl peaks were observed at 30.0, 127.0 and 133.0 ppm respectively.

The 5-(α-iodo-β-keto)sulfone of 2'-deoxyuridine **56** was synthesized as an alternative probe to incorporate nucleophile at the α-carbon of the corresponding sulfones *via* substitution reaction. Thus, treatment of β-keto sulfone analogue **51** with iodine monochloride afforded 5-(α-iodo-β-keto) sulfone of 2'-deoxyuridine **56** as 50/50 mixtures of diastereomeric mixtures with the yields of 44% yield (Scheme 56). Alternatively **56** was also synthesized using molecular iodine in the presence of hydrogen peroxide and acetic acid and the yield was 55%.



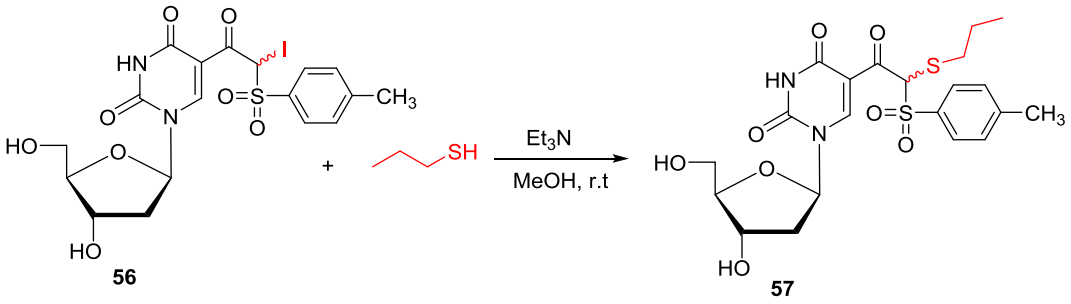
Scheme 55. Synthesis of 5-(α-iodo β-keto sulfone) of 2'-deoxyuridine

The structuce of the novel probe **56** was established by using different NMR techniques. In ^{1}H NMR α-proton of CH group was observed at 7.30 ppm. The higher chemical shift value

of this proton can be explained as the incorporation of the relatively electronegative iodine atom at the α-carbon. In ^{13}C NMR the α-carbon peak was observed at 128.0 ppm

3.2.1.2. Nucleophile trapping at the α-carbon of the β-keto sulfones

Treatment of 5-(α-iodo-β-keto) sulfone **56** with propane thiol in the presence of triethylamine gave propane thio nucleophile incorporated product **60** as a 50/50 mixture of diasteromers and yield was 20% (Scheme 57). Relatively low yield of this reaction could be explained due to the formation of methyl ester as by product which was the major product in this reaction.



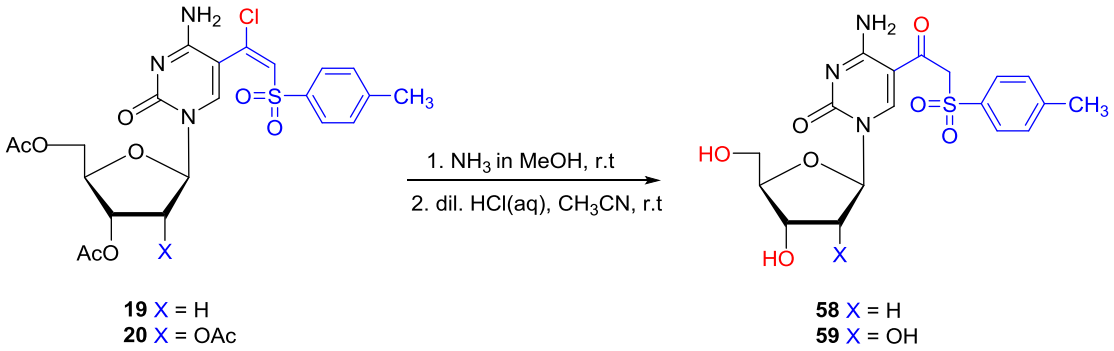
Scheme 56. Synthesis of 5- (*α*-propanethio *β*-keto sulfone) of 2'-deoxyuridine

The structure of the compound **57** was established by NMR techniques. Thus, in ^{1}H NMR three signals of the propanethio group were observed at 2.80-2.85 (multiplet), 1.40-1.60 (multiplet), and 0.85 (triplet) respectively. This ractions implied the feasibility of the incorporation the thiol residue of the important amino acid cysteine at the α-position of the corresponding *β*-keto sulfone analogue **56**.

3.2.2. Synthesis and reactivity of 5-(2-tosylacetyl)cytosine nucleosides

Acetyl protected *β*-chlorovinyl sulfone analogues (**19-20**) of cytosine nucleosides have been efficiently converted into corresponding *β*-keto sulfones (**58-59**). Thus treatment of the (β-chloro)vinyl sulfones (**19-20**) with methanolic ammonia gave *β*-amino vinyl sulfones in

excellent yield. Subsequent acid hydrolysis of these *β*-amino vinyl sulfones in acetonitrile gave *β*-keto sulfones (**61-62**) in good yield (Scheme 58)



19 X = H
20 X = OAc

58 X = H
59 X = OH

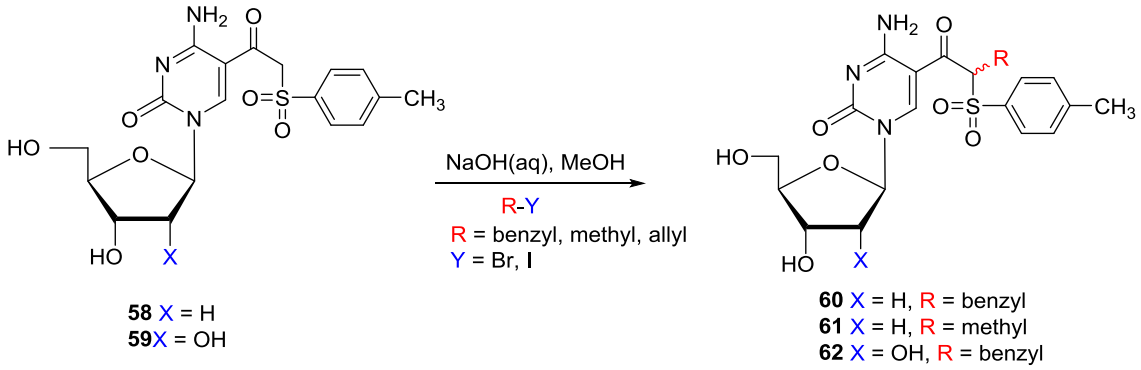
Scheme 57. Synthesis of 5-(*β*-keto)sulfone of cytidine and 2'-deoxycytidine

When *β*-chlorovinyl sulfones were treated with methanolic ammonia, it converted into the corresponding *β*-aminovinyl sulfones with the deprotection of the acetyl group simultaneously. When *β*-chlorovinyl sulfones **19** and **20** were converted into corresponding *β*-keto sulfone the yields were 68% (**58**) and 57% (**59**) respectively.

The structures of these *β*-keto sulfone analogues (**58-59**) were determined by applying different NMR techniques and actual mass was obtained from high resolution mass spectrometry. In *β*-keto sulfone analogues **58** and **59**, chemical shift value of H6 proton was observed at 9.0 and 9.05 ppm respectively which was higher compare to chemical shift value of H6 proton of the corresponding *β*-chlorovinyl sulfone analogues **19** and **20**. The higher chemical shift value of the keto analogues can be explained by the incorporation of electron withdrawing keto group at C5 position. In **58** methylene peak was appeared at 4.80-5.0 ppm as doublet of doublet. In **59**, the same peak was observed at 4.75-4.98 ppm as doublet of doublet. In **58** and **59,** characteristics peaks of *β*-keto carbonyl carbon were observed at 185.0 and 186.0 ppm respectively. In addition characteristic peaks of methylene carbon of these compounds were also observed at 62.4 and 63.0 ppm respectively. In complete accord with

the proton NMR, the higher chemical shift value of C6 carbon was observed at 152.0 and 152.5 ppm respectively. The mass obtained from the high resolution mass spectroscopy (HRMS) of compound **58** was 446.1002 $[M+Na]^+$. The theoretical mass of the compound was also 446.1002 $[M+Na]^+$ means the theoretical mass was in complete accord with the experimental value of the corresponding compound

The *β*-keto sulfone analogues of cytosine nucleosides (**58-59**) were treated with different electrophiles and these electrophiles were efficiently trapped at the α-carbon of the corresponding keto sulfone analogues with high yield. Thus treatment of *β*-keto sulfone analogue **58** with benzyl bromide in the presence of dil. NaOH, the α-benzylated product **60** was obtained as a 52/48 mixture of diastereomers with 68% yield. Interestingly, no dibenzylation at the α-carbon was observed (Scheme 59).



Scheme 58. Synthesis of 5-(*α*-subsitituted *β*-keto sulfone) of cytidine and 2-deoxycytidine

The structure of the benzylated product **60** was established by applying different NMR techniques. In ^{1}H NMR two multiplets of α-proton was observed at 5.45-5.52 and 5.55-5.62 ppm. In addition methylene proton of benzyl group was found at 3.30-3.40 ppm as a multiplet. Aromatic proton of benzyl group was observed at 7.05-7.25 ppm. In ^{13}C NMR α-carbon peak was obtained at 69.0 ppm. The methylene carbon peak of benzyl group was observed at 32.0

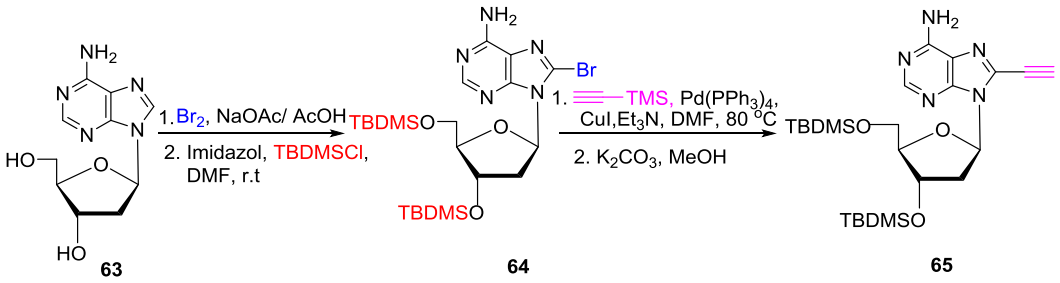
ppm and the aromatic peaks were collapsed with the tosyl peaks in the region between 127.0 ppm to 130.0 ppm.

Analogues treatment of **59** with benzyl bromide in the presence of dil. NaOH afforded α-benzylated product **61** with 67% yield (Scheme 59). Benzyl disubtitution at *α*-carbon was not observed. In ^{1}H NMR of **61**, α-proton peak was appeared at 5.60-5.70 ppm as a multiplet. The methylene peak of benzyl group was observed at 3.18-3.30 ppm as a multiplet. The aromatic peaks were appeared at7.10-7.25 ppm. In ^{13}C NMR the *α*-carbon peak was appeared at 70.0 ppm. The methlene carbon peak of benzyl group was found at 31.0 ppm. Aromatic peak of the benzyl group was observed at the region from 126.0 to 129.0 ppm which collapsed with the tosyl peaks.

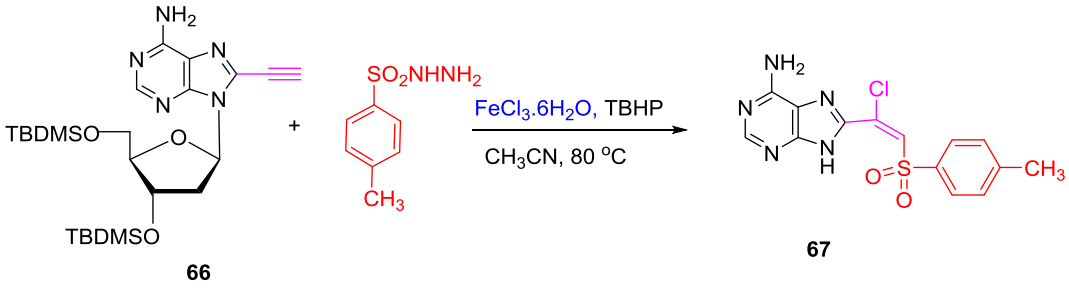
Treatment of compound **58** with methyl iodine in basic medium gave 52/48 mixture of diastereomers **62** with 68% yield (Scheme 59). As usual no dimethylation observed at the α-carbon. Analogue **62** was characterized by applying different NMR techniques. In ^{1}H NMR α-proton peak was observed at 5.20-5.30 ppm. The methyl proton was appeared at 1.32-1.38 ppm. In ^{13}C NMR peaks of α-carbon and methyl group were found at 64.0 ppm and 12.0 ppm respectively.

3.3. Incorporation of (β-halo)vinyl sulfone probes at 8-position of 2'-deoxyadenosine

The *β*-halo sulfonylation reaction was also extended into the purine nucleosides by the reaction of terminal alkyne group *via* halosulfonylation reactions. Thus the acetylene group was incorporated at the 8 position of the 2'-deoxyadenosine analogue following the literature procedure[117,118] which involved TBDS protection at the sugar moiety, bromination at 8 position, Sonogashira coupling, and silyl deprotection as depicted in Scheme 60.

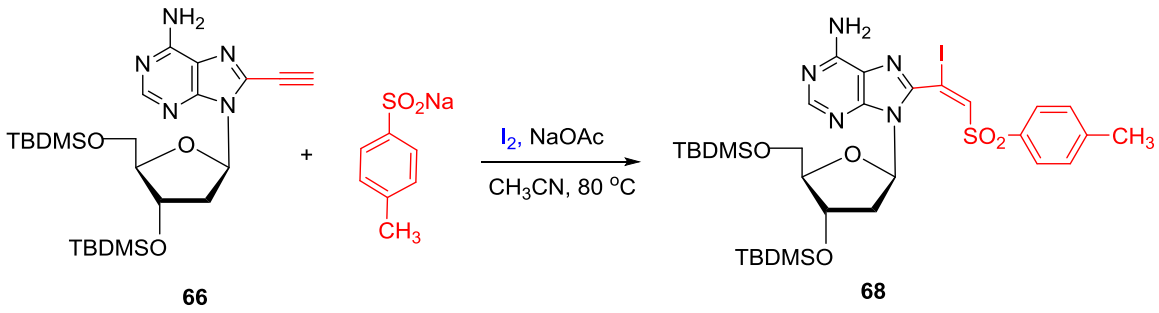


Scheme 59. Synthesis of protected 8-acetylene 2'-deoxyadenosine



Scheme 60. Attempted halosulfonylation of 8-ethynyl -2'-deoxyadenine **23** with tosyl hydrazide. Synthesis of 8-(1-chloro-2-tosylvinyl)adenine

Treatment of 2'-deoxy-3',5'-bis(*O*-tert-butyldimethylsilyl)-8-ethynyl adenosine **66** with tosyl hydrazide gave 8-(1-chloro-2-tosylvinyl)adenine (**67**) with the yields of 44% (Scheme 61). In this halosulfonylation reaction the cleavage of β-glycosidic took place which was confirmed from the proton NMR as there was no peak of the sugar moiety observed. In aditio there was a peak of the NH proton observed at 13.5 ppm. The vinyl peak was observed at 7.90 ppm. The three tosyl peaks were appeared at 2.40, 7.45, and 7.80 ppm. In the carbon NMR of **67,** vinyl peak was detected at 136.4 ppm while the C-Cl peaks appeared at 143.0 ppm. The five tosyl peaks were found at 21.0, 128.0, 130.0, 135.0, and 145.0 ppm.



Scheme 61. Synthesis of 8-(β-iodovinyl)sulfone analogue of 2'-deoxyadenosine

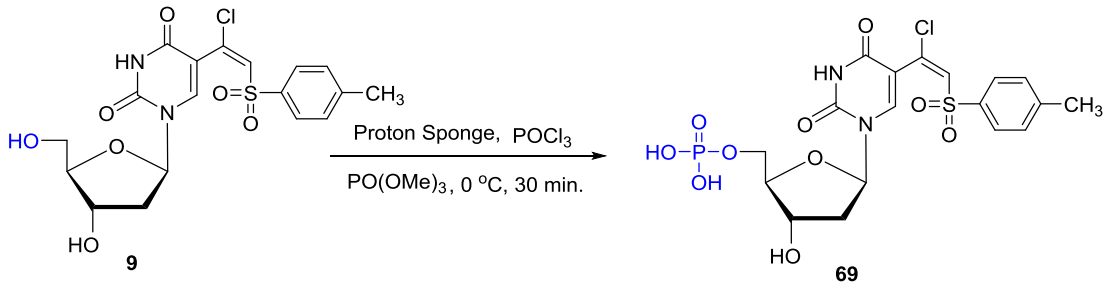
Interestingly when silyl protected 8-ethynyl adenosine analogue **66** is reacted with sodium salt of p-toluenesulfinic acid and iodine, no glycosidic bond cleavage was observed. Thus, treatment of **66** with sodium tosylate and iodine in the presence of sodium acetate afforded 8-(β-iodovinyl)sulfone of silyl protected 2'-deoxyadenosine analogue **68** with 48% yield (Scheme 62). In the proton NMR of **68** the vinyl peak was observed at 7.60 ppm. The three tosyl peaks were appeared at 2.40, 7.25, and 7.70 ppm. In the carbon NMR of the vinyl peak was detected at 136.0 ppm while the C-I peaks appeared at 129.0 ppm. The C_H peaks from tosyl group were converged at 130.0 ppm. The successful conversion of 8-ethylnyl-2'-deoxyadenosine **66** to 8-(β-iodovinyl)sulfone of 2'-deoxyadenosine **68** showed that halovinylsulfonylation can be extended to the purine nucleoside analogues.

3.4. Incorporation of (*E*)-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine nucleotide at DNA

3.4.1. Synthesis of 5'-phosphates

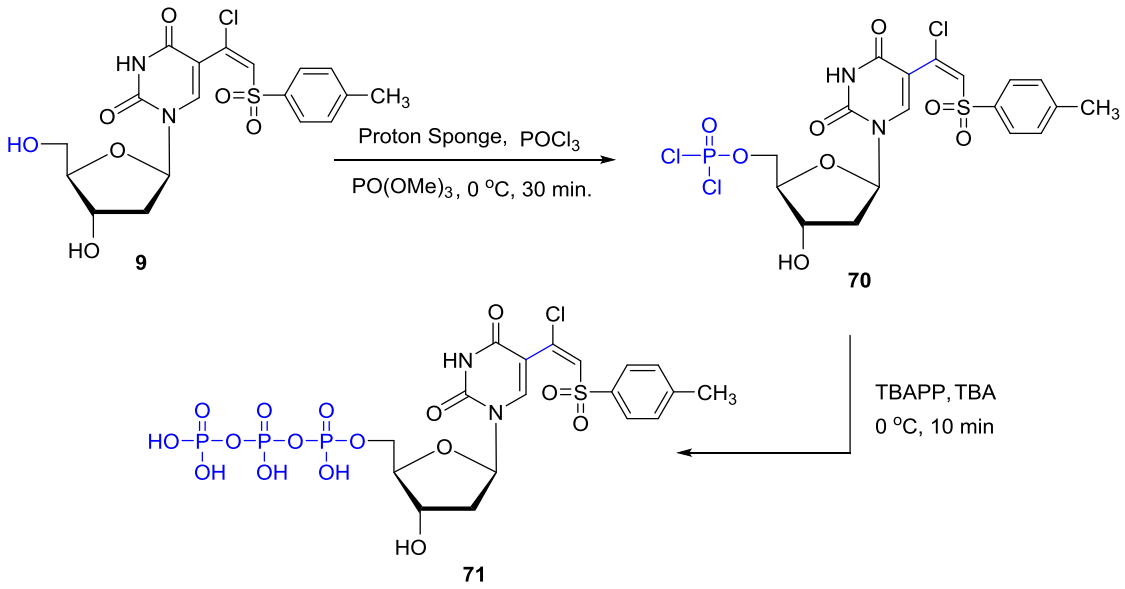
Phosphorylation is an important technique to study important biological properties of the nucleotides in living cell. Phosphorylation at 5' position of the 2'-deoxynecloside analogues are widely useful technique to DNA polymerase mediated incorporation in DNA. Here novel 5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine analogues have been triphosphorylated at 5'-position in order to polymerase mediated incorporation in DNA and to study the DNA-protein

interaction by reacting with protein molecules. Phosphorylation was accomplished by applying modified Yoshikawa method.[119]



Scheme 62. Synthesis of 5'-monophosphate of 5-(*β*-chlorovinyl sulfone) of 2'-deoxyuridine by modified Yoshikawa method

Treatment of *β*-chlorovinyl sulfone analogue **9** with 1.8 equiv. of phosphoryl chloride in the presence of 2.0 equiv. of proton sponge in trimethyl phosphate solvent at 0 °C gave 5-dichlorophosphate intermediate **70** in very good yield (~70%; TLC). Subsequent treatment of intermediate **70** with 4.2 equiv. of tributylammonium pyrophosphate (TBAPP) and 2.7 equiv. of tributylamine (TBA) at rt afforded 5'-triphosphorylated analogue **71** in moderate yield (32%) as depicted in Scheme 64. Alternatively, the intermediate **70** have been efficiently converted into 5'-monophosphate **69** by quenching the reaction with TEAB buffer.



Scheme 63. Synthesis of 5'-triphosphate of 5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine

The 5'-triphosphosphate **71** was characterized by applying different NMR techniques such as ^{31}P, ^{1}H, COSY, HMQC, DEPT-135, ^{13}C; and FT-ICR mass spectrometry. In phosphorous NMR three peaks were observed at δ -23 (β-P), -11.5 (α-P), -10.5 (γ-P). The peaks at -23.0 ppm confirmed that triphophorylation had been taken place as *β*-phosphorous atom of triphosphorylated compound gave peak at around -23.0 ppm. The chlorine atom of *β*-chlorovnyl sulfone analogue was readily substituted by nucleophile through addition-elimination type reaction. So, it was a concern whether the chlrorine survived at that basic condition after triphosphorylation reaction. However, the mass spectrum showed the presence of chloride as it has a classic isotopic pattern at the molecular ion peak and the peak intensity is 3:1. The observed molecular mass was 680.95172 $[M-H]^-$ which was in very good agreement with the theoretical mass of the compound 680.95186 ppm and the mass error was

only 0.2 ppm. All the proton and carbon peaks were assigned by analyzing different NMR spectrum and all the values were in accordance with the expected values.

3.4.2. DNA polymerase catalyzed incorporation into DNA

To study the bioconjugation of the novel (*β*-chloro)vinyl sulfone analogue with proteins *in vivo*, it is indispensable to incorporate this novel probe into DNA. The phosphorylated (*β*-chloro)vinyl sulfone analogue **71** was incorporated into double strand DNA by using DNA polymerase where the extent of incorporation was tested both in DNA leading and lagging strand by using bacterial replication DNA polymerase, the Klenow fragment of DNA polymerase I (pol I) and human repair DNA polymerase, DNA polymerase β (pol β). The concentration of the DNA pol I (pol I) was 5 U in each time, however different concentrations of DNA polymerase β (pol β) such as 0.5 nM, 1 nM, 5 nM, 10 nM, 25 nM, and 50 nM were used. The concentration of the (*β*-chloro)vinyl sulfone analogue **71** was 50 μM. The polymerase mediated DNA incorporation was monitored by applying polyacrylamide gel electrophoresis (PAGE) technique. The details of nucleotide sequence and number, primer, and template are tabulated below:

Table 3. Detailed information of nucleotides used during polymerase mediated incorporation

Nucleotide	nt	Sequence (5'-3')
Upstream primer	31	GCA GTC CTC TAG TCG TAG TAG CAG ATC ATC A
# Downstream primer	39	CAA CCG GCA TTA GGT GTA GTA GCT AGA CTT ACT CAT TGC
Template	71	GCA ATG AGT AAG TCT AGC TAC TAC ACC TAA TGC CGG TTG ATG ATG ATC TGC TAC TAC GAC TAG AGG ACT GC

Downstream primer was used only in one nucleotide gap DNA template

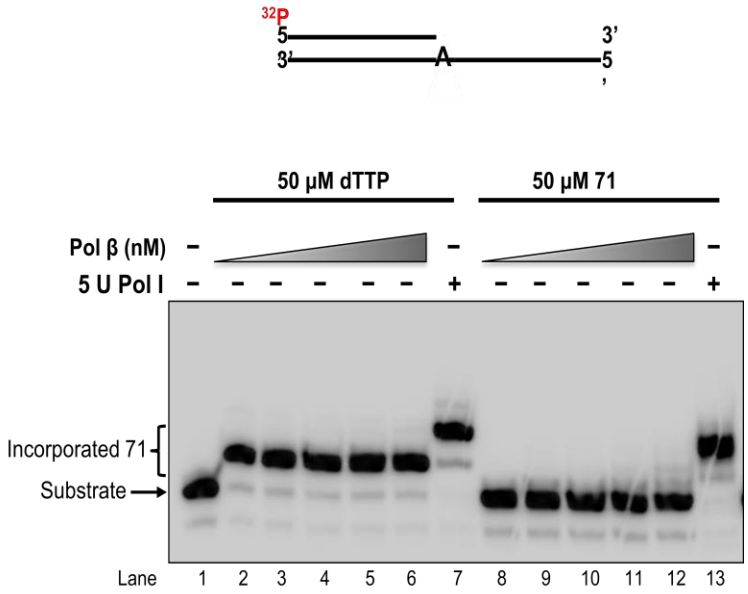


Figure 15. Incorporation of (*β*-chloro)vinyl sulfone analogue into DNA open template by DNA polymerase

During the polymerase mediated incorporation approach of the substrate **71** in DNA open template which leads to DNA synthesis, human repair DNA polymerase (pol β) was unable to incorporate the substrate into DNA at any of the concentration mention above (Fig.15, Lane 8-12). However, bacterial polymerase pol I can efficiently incorporated the substate (Figure 15, Lane 13) at 5 U concentrations like the natural substrate deoxythymidine triphosphate (dTTP). However due to the excessive activities of the pol I on the substrate one more unit of the substrate **71** incorporated and thus mismatch happened which was obvious in PAGE analysis (Figure 15, Lane13).

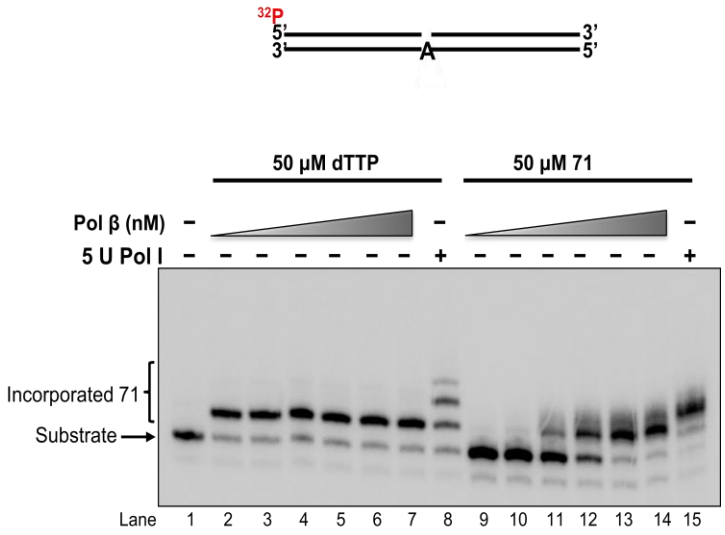
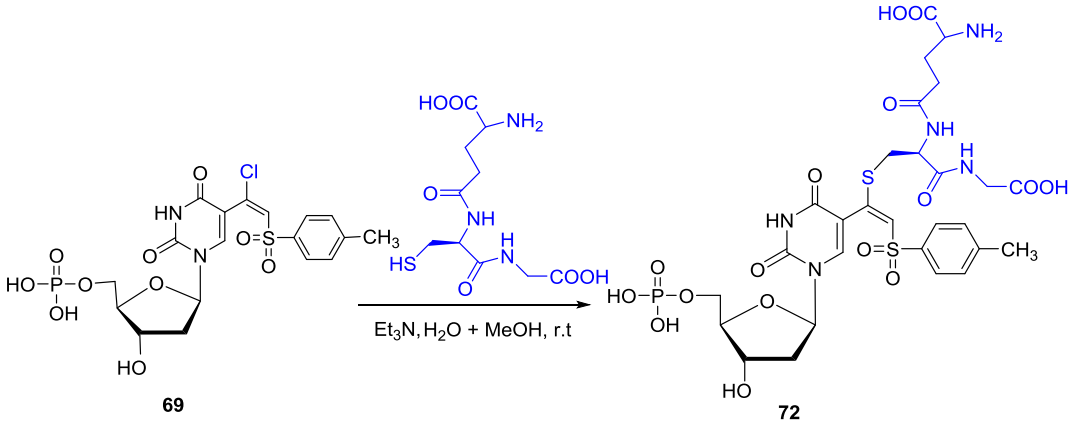


Figure 16. Incorporation of (*β*-chloro)vinyl sulfone analogue into DNA one gap nucleotide template by DNA polymerase

During the polymerase mediated incorporation of the substrate into the one gap nucleotide template of DNA which causes lagging during the DNA synthesis, both human repair DNA polymerase (pol β) and bacterial polymerase (pol I) efficiently incorporated the chlorovinyl substrate **71** (Figure 16, Lane 13-15). However, human repair DNA polymerase (pol β) mediated incorporation of the substrate is dependent on the concentration of the polymerase. From the PAGE analysis it was obvious that 0.1 and 0.5 nM concentration of pol β couldn't incorporate the substrate **58** (Figure 16, Lane 8-9). However, at 1.0 nm concentration of pol β the incorporation was observed (Figure 16, Lane 10), though the extent of incorporation was low at that concentration. With the increase of the concentration of the pol β the extent of incorporation enhanced significantly. When 25.0 nM pol β was used the polymerase incorporated the substrate **71** (Lane 13) as like the natural substrate dTTP (Lane 6). Similar to the open template, 5 U concentration of pol I efficiently incorporated the substrated at the template with the mismatched (one more unit incorporated) (Figure 16, Lane 15).

3.4.3. Conjugation of phosphorylated chlorovinyl sulfone analogue with L-glutathione

Treatment of monophosphorylated (β-chloro)vinyl sulfone **69** with L-glutathione in the presence of triethyl amine in methanol-water mixed solvent system gave the bioconjugated product **72** with 55% yield (Scheme 65).



Scheme 64. Conjugation of 5-(β-chlorovinyl sulfone) of 2'-deoxyuridine-5'-monophosphate with L-glutathione

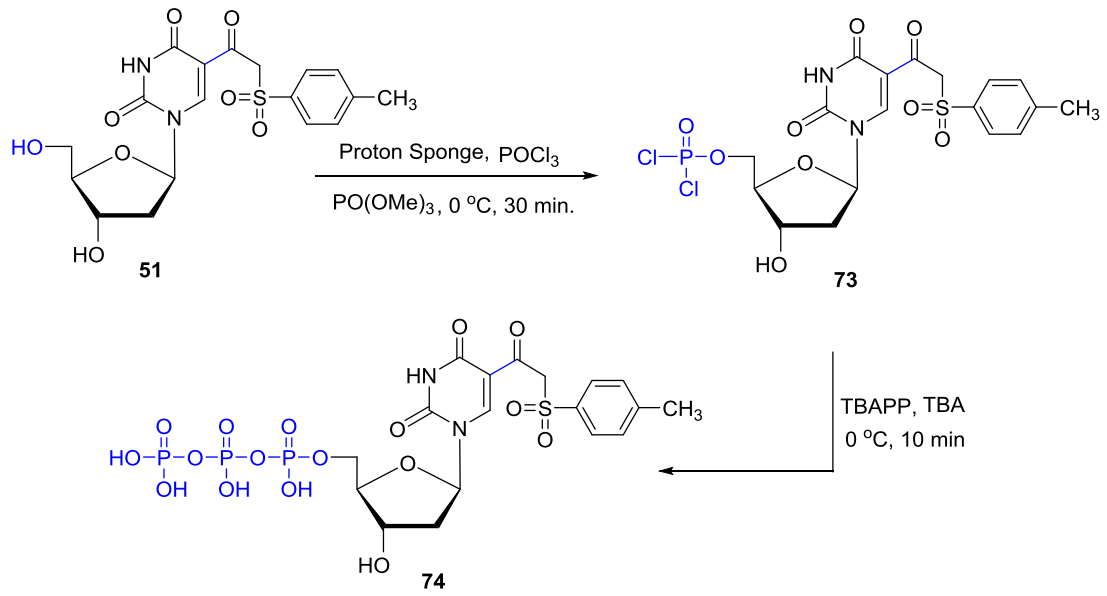
The structure of the bioconjugated analogue **72** was determined by applying different NMR techniques and experimental mass value was obtained from FT-ICR mass spectrometry where the ionization was carried out in the negative mood.

In the proton NMR distinct peaks of glutathione were observed in the region from 2.0 to 5.0 ppm. The three amino acids peak of glutathione which are cysteine, glutamic acid and glycine were appeared in that region. It was difficult to figure out the multiplicity of some of the proton peaks due to the peak overlapping with the sugar moieties of the nucleosides. In carbon NMR the four carbonyl carbon peaks of the amide and carboxylic acid groups were observed at 171.0, 175.0, 177.0, and 177.0 ppm. The other six carbon peaks were appeared from 25.0 to 65.0 ppm. Finally the experimental mass value which was similar with the theoretical value confirmed the presence of **72**.

3.5. Incorporation of 5-(2-tosylacetyl)-2'-deoxyuridine nucleotide at DNA

3.5.1. 5'- Phosphorylation

Novel 5-(2-tosylacetyl)-2'-deoxyuridine analogues have been triphosphorylated at 5'-position in order to polymerase mediated incorporation in DNA and to study the DNA-protein interaction by reacting with protein molecules. Phosphorylation was accomplished by applying modified Yoshikawa method. Treatment of *β*-keto analogue **51** with 1.7 equiv. of phosphoryl chloride in the presence of 2.0 equiv. of proton sponge in trimethyl phosphate solvent gave 5'-dichlorophosphate intermediate compound **73** in very good yield (~70%; TLC). Subsequent treatment of 5'-monophosphorylated intermediate **73** with 4.1 equiv. of tributylammonium pyrophosphate (TBAPP) and 2.5 equiv. of tributyl amine (TBA) gave 5'-triphosphorylated analogue **74** in moderate yield (38%) as depicted in Scheme 66.



Scheme 65. 5'-Phosphorylation of 5-(2-tosylacetyl)-2'-deoxyurindine

The 5'-triphosphate **74** was characterized by applying different NMR techniques such as ^{31}P, ^{1}H, COSY, HMQC, DEPT-135, ^{13}C; and FT-ICR mass spectrometry. In phosphorous

NMR three peaks were observed at -23.5, -11.7, -11.0 ppm. The peaks at -23.5 ppm confirmed that triphophorylation had been taken place as *β*-phosphorous atom of triphosphorylated compound gave peak at around -23.0 ppm. It was a concerned whether the acidic α-methylene proton survived at that basic condition. However, the presence of proton peak at 4.20 ppm and integration value indicated the survival of the α-methylene proton. In addition in DEPT-135 NMR α-CH_2 peak was observed at 67.0 ppm which also ensured the presence of unaffected α-methylene group. Finally the mass of the compound was determined by ultra high resolution mass spectroscopy in negative mood. The experimental value of the compound **72** was 662.98595 $[M-H]^-$ compared to the theoretical value 662.98575 $[M-H]^-$ with a mass error of only 0.3 ppm.

3.5.2. Incorporation into DNA by DNA polymerase

Both human DNA repair polymerase (pol β) and bacterial polymerase (pol I) efficiently incorporated *β*-keto sulfone analogue **74** into DNA open template. At 5 U concentration pol I efficiently incorporated the substrate **74** like the natural substrate dTTP. However, the human DNA repair polymerase β mediated incorporation was dependent on the concentration of the polymerase (Figure 17)

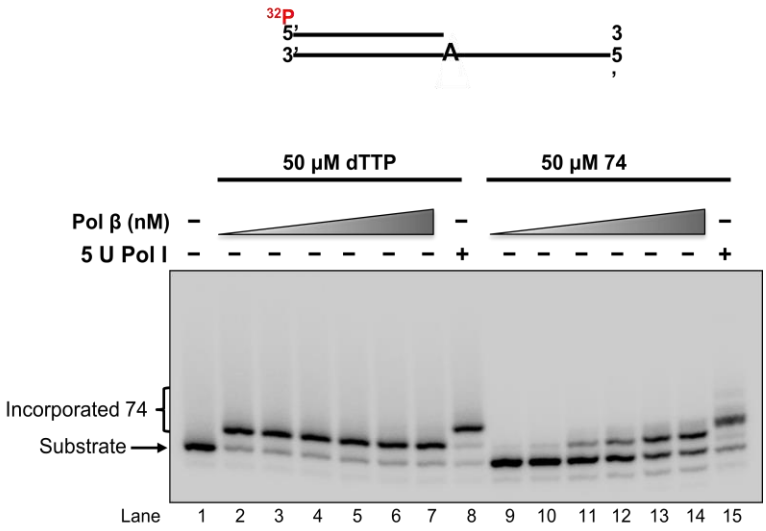


Figure 17. Incorporation of 5 (*β*-keto)sulfone analogue into DNA open template by DNA polymerase

Incorporation started to take place at 1.0 nM polymerase concentration and at 25.0 nM pol β the incorporation was about 50% (Figure 17, Lane 13) which increased to about 70% at 50.0 nM concentration of the polymerase β.

Polymerase mediated incorporation of the (*β*-keto)sulfone analogue **74** into one nucleotide gap template was observed when either of the polymerase was used. 5 U concentration of the bacterial polymerase (pol I) efficaciously incorporated the (*β*-keto)sulfone analogue **74** (Figure 18, Lane 15). In this nucleotide template enhance activities of the human repair DNA polymerase (pol β) was observed. Even at 0.1 nM of this polymerase incorporated about 50% of the (*β*-keto)sulfone analogue **72** (Figure 18, Lane 9) and only 1.0 nM pol β incorporated the substrate **74** (Figure 18, Lane 11) like the natural substrate dTTP (Figure 18, Lane 4).

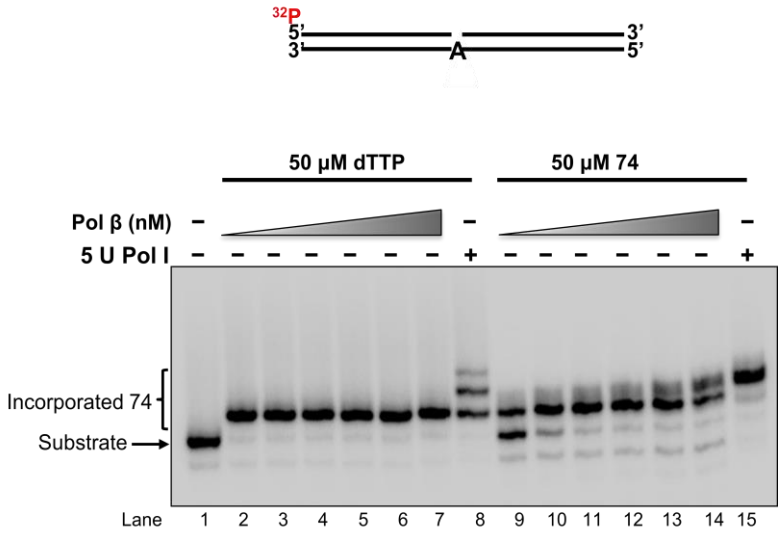


Figure 18. Incorporation of (*β*-keto)sulfone analogue into DNA one nucleotide gap template by DNA polymerase

3.6. Prodrugs of C5 modified uracil nucleosides

A number of C5-modified uracil nucleoside analogues has been synthesized in our lab[90] and their anticancer and antiviral activities were tested at Rega Institute for Medical Iesearch in Belgium. Interestingly, it has been observed that the half maximal inhibitory concentration (IC_{50}) of the acetyl protected chlorovinyl sulofne analogue **9a** against the proliferation of murine leukemia cells (L1210) were much lower as compared to its unprotected chlorovinyl sulfone counterpart **9** (5.6 ± 4.7 µM *vs.* >200 µM). Therefore, based on these findings liphophilic long alkyl chain was incorporated at the 5' and/or 3' position of at 5-modified analogue **9** and a number of 5-arylated analogues *via* esterification reaction.

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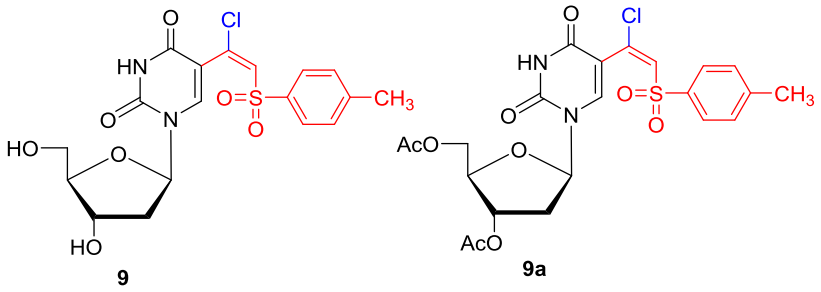


Figure 19. Lipophobic (**9**) and lipophilic (**9a**) 5-modified-2' deoxynucleoside analogues

3.6.1. Synthesis of prodrug of (*E*)-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine

To incorporate more liphophilic long alkyl chain at the sugar moiety of the nucleoside analogues more reactive undecanoic anhydride was prepared from undecanoic acid. Thus treatment of undecanoic acid with thionyl chloride at ambient temperature for 5 h gave undecanoyl chloride with quantitative yield.

Treatment of undecanoyl chloride with undecanoic acid in the presence of triethylamine gave undecanoic anhydride with quantitative yield.

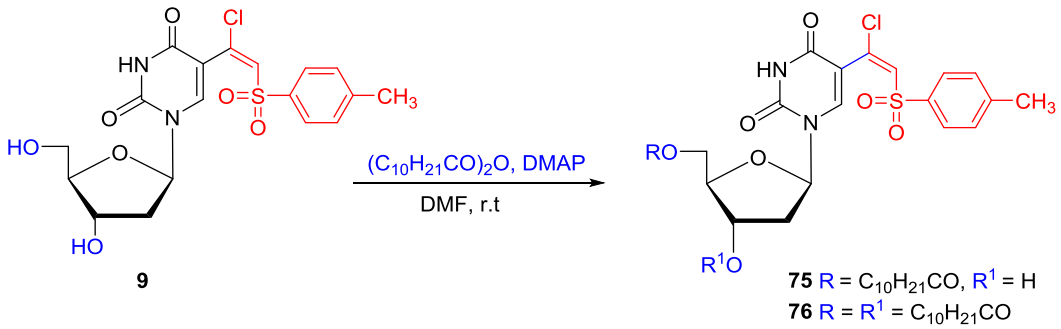
$$C_{10}H_{21}COOH \xrightarrow[CH_2Cl_2,\ r.t]{SOCl_2} C_{10}H_{21}COCl \xrightarrow[Et_3N,\ CH_2Cl_2,\ r.t]{C_{10}H_{21}COOH} (C_{20}H_{42}CO)_2O$$

Scheme 66. Preparation of undecanoic anhydride from undecanoic acid

Further treatment of undecanoyl chloride with undecanoic acid for overnight and evaporation of volatiles under reduced pressure gave undecanoic anhydride as white solid with quantitative yield (Scheme 67).

Treatment of *β*-chloro vinyl sulfone analogue **9** with undecanoic anhydride gave mono and diprotected products from moderate to good yield. When *β*-chloro vinyl sulfone analogue **9** was treated with 1.2 equiv. of undecanoic anhydride for two hours, the major product was (*E*)-5'-*O*-undecanoyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (**75**) with 45% yield.

However, treatment of *β*-chloro vinyl sulfone analogue **9** with 3 equiv.of undecanoic anhydride for 6 h gave (*E*)-3',5'-Di-*O*-undecanoyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (**76**) as major product with 75% yield (Scheme 68). Also small quantities of 3'-*O*-undecanoyl product was isolated (see Experimental Part).



Scheme 67. Synthesis of undecanoate protected 5-(1-chloro-2-tosyl)vinyl-2'-deoxyuridine

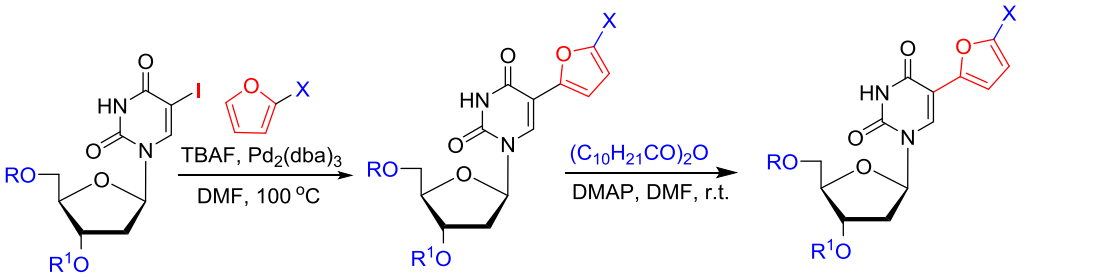
Both mono and di-protected compounds **75** and **76** were characterized by applying NMR techniques i.e ^{1}H, COSY, HMQC, DEPT-135, ^{13}C and mass of the products were determined by high resolution mass spectrometry. The proton NMR peak at 0.88, 1.24-1.39, 1.62, and 2.34 ppm and integration value of number of proton confirmed the presence of one undecanoyl group in the sugar moiety. The higher chemical shift value of H5' proton at 4.40 ppm while almost unchanged chemical shift value of H3' proton which is at 4.17 ppm indicated that esterification took place at 5'-OH group. In addition, the presence of 3'-OH at 3.00 ppm further justified the regioselective esterification of **9**. Eleven carbon peaks at 14.2, 22.0, 25.0 29.2, 29.4, 29.5, 29.7, 29.8, 32.2, 34.4, and 174.0 ppm in compound **75** indicated the presence of one undecanoyl group. Finally, experimental mass value of 633.2013 $[M+Na]^{+}$ confirmed the presence of mono protected analogue **75**.

The integration values of different proton peaks at the region from 0.84 to 2.34 ppm and higher chemical shift values of H3' and H5' at 4.42 ppm and 5.27 ppm indicated the presence

of diprotected compound. In addition the different carbon peaks and experimental mass value 801.3505 $[M+Na]^+$ confirmed the diprotected compound **76**.

3.6.2. Synthesis of prodrug of 5-(fur-2-yl/5-heptylfur-2-yl)-2'-deoxyuridine

The 5-(fur-2-yl)-2'-deoxyuridines **78** and **80** have been synthesized from 5-iodo substrates **2** and **77** following the literature procedure.[91] Analogously, tetrabutylammonium fluoride (TBAF)-mediated direct C-H arylation of 5-iodouracil nucleosides **2** and **77** with 2-heptylfuran provided 5-(heptylfur-2-yl)-2'-deoxyuridine **79** and **81**.[120] Treatment of unprotected derivatives **80** or **81** with undecanoic anhydride gave mono and diacylated products from moderate to good yield. Thus, treatment of 5-(fur-2-yl) **80** or 5-(5-heptylfur-2-yl) **81** analogues with 1.2 equiv. of undecanoic anhydride for two hours afforded 5'-*O*-undecanoyl-5-(fur-2-yl)-2'-deoxyuridine (**82**) or 5'-*O*-undecanoyl-5-(5-heptylfur-2-yl)-2'-deoxyuridine (**84**) with the yield of 52% and 48% respectively. Analogues treatment of **80** or **81** with 3 equiv. of undecanoic anhydride for 6 h afforded 3',5'-di-*O*-undecanoyl-5-(fur-2-yl)-2'-deoxyuridine (**83**) or 3',5'-di-*O*-undecanoyl-5-(5-heptylfur-2-yl)-2'-deoxyuridine (**85**) as major products with the yield of 77% and 80% respectively (Scheme 69). Also small quantities of 3'-*O*-undecanoyl products were isolated (see Experimental Part)



Scheme 68. Synthesis of 5-(fur-2-yl)/5-(heptafur-2-yl)-2'-deoxyuridine by direct arylation and their undecanoate protected analogues

Both mono and diprotected compounds were characterized by applying NMR techniques and mass values were determined by HRMS. Accumulating the number of proton present in the compound **82** or 84, it was confirmed that these are monoprotected compounds. The regio-selectivity of the monoesterified products were confirmed by comparing the chemical shift value of H3' and H5' proton of the products (**82** and **84**) with the starting compound (**63** and **64**) respectively. In the mono protected analogues **82** and **84** the proton peak of H5' is at 4.25 ppm and 4.28 ppm which are significantly higher than the corresponding proton of the unprotected compounds **80** or **81**. Additionally, the H3' proton peaks were almost unchanged (4.36 ppm and 4.33 ppm respectively) in the monoprotected products (**82** and **84**) compared to the unprotected ananogues (**80** and **81**). This could be only possible when acyl protection takes place at 5'OH group while 3'OH group would be unprotected. In addition each of the compound **82** and **84** has one carbon peak at 174.0 ppm which also indicated the presence of mono-esterified products. Finally, the experimental mass value of the products **82** and **84** which are 485.2264 $[M+Na]^+$ and 583.3359 $[M+Na]^+$ respectively confirmed the presence of monoprotected products.

The higher chemical shift values of H3' and H5' proton at 5.27 ppm and 4.36 ppm respectively suggested the presence of diprotected analogue **83**. The integration value of different proton peaks at the region from 0.82 to 2.40 ppm also agreed this claim. Similar finding was observed in diprotected 5-(heptylfur-2-yl) analogue **85**. Two different carbonyl carbon peaks and experimental mass value of 653.3778 $[M+Na]^+$ and 751.4873 $[M+Na]^+$ confirmed the synthesis of diprotected 5-arylated analogues **83** and **85** respectively.

3.6.3. Selected antiviral and anticancer activities

All of the uracil nucleoside analogues (Scheme 68 and 69) were sent to our collaborators at Raga Institute for Medical Research in Belgium to test their antiviral and anticancer activities. The antiproliferative activity of all of the compounds (**9, 9a**, and **75-76**) was tested in murine leukemia (L1210), human leukemia (CEM), and human cervical carcinoma (HeLa) cells. Only acetyl protected (β-halo)vinyl sulfone **9a** inhibited the growth of those cells in lower μM range (Table 4). Interestingly, (β-halo)vinyl sulfone derivatives with a larger acyl protected group **75** and **76** didn't show improved activity. The 5-(fur-2-yl) analogues (**78-85**) were also tested against those carcinogenic cells and the acetylprotected 5-heptylfur-2-yl)-2'-deoxyuridine **79** inhibited the T-lympocyte cells (CEM) in the μM range. Interestingly unprotected 5-(5-heptylfur-2-yl) analogue **81** exhibited improved activity against the same cells. Both acetyl protected analogue **79** and unprotected analogue **81** showed moderate activities against the proliferation of murine leukemia cells (L1210) (Table 4). Unprotected 5-(fur-2-yl) analogue **80** is moderately active against human cervix carcinoma cells (HeLa) proliferation (Table 4).

Table 4. Inhibitory effects on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa).[120]

Compound	IC_{50} (μM)		
	L1210	CEM	HeLa
9a	5.6 ± 4.7	11± 10	23 ± 8
79	65 ± 10	36 ± 3	> 100
80	>100	> 100	32 ± 1
81	48± 6	16 ± 4	> 100
84	> 100	93 ± 4	> 100
85	> 100	78 ± 16	> 100

IC_{50} is 50% inhibitory concentration

The 5-(β-chlorovinyl)sulfone analogues **(9, 9a** and **76-77**) and 5-(fur-2-yl) analogues (**78-85**) were tested against a broad range of DNA and RNA viruses and the human immunodeficiency virus (HIV). The (β-chloro)vinyl sulfone **9a** showed an EC_{50} of 4 μM for the Oka strain (VZV TK^+) and marginal activity against human cytomegalovirus (HCMV) (Table 5). The 3',5'-di-*O*-acetyl -5-(5-heptylfur-2-yl) (**79**) and 5-(5-heptylfur-2-yl) (**81**) derivatives inhibited the replication of HCMV and varicella zoster virus (VZV) bearing a wild-type thymidine kinase (TK^+) with 50% effective concentrations (EC_{50}) in the range of 10-20 μM (Table 5). 5-(Heptylfur-2-yl)-2-deoxyuridine **81** was equally active against TK^+ and TK^- deficient VZV mutant virus. However, neither of the arylated analogues **79** and **81** were able to decrease herpes simplex virus 1 (HSV-1) and 2 (HSV-2) induced cytopathic effect (Table 5).

Table 5. Anti-herpesvirus activity of the tested compounds in HEP (human embryonic lung) fibroblasts

Compd	Cytotoxicity (μM)	EC_{50} (μM)						
	MCC	HSV-1 (KOS)	HSV-2 (G)	HSV-1 TK^-(KOS ACV)	HCMV (AD-169)	HCMV (Davis)	VZV TK^+ (Oka)	VZV TK^- (07-1)
9a	> 100	> 100	> 100	> 100	> 20	20	4	>20
79	100	> 100	> 100	> 100	10 ± 2	12 ±4	20	>20
80	> 100	4.0 ± 0	47 ± 37	> 100	45	20	32	>100
81	100	> 100	> 100	> 100	10	20	13 ±2	12 ±5

EC_{50} concentration required to reduce virus-induced cytopathogenicity by 50%
MCC is the Minimum Cytoxic Concentration required to cause a microscopically detectable alteration of normal cell morphology

In contrast, the 5-(fur-2-yl)uracil nucleoside **80** emerged among the compounds synthesized as the most potent inhibitor the HSV-1 TK^+ strain Kos with an EC_{50} of 4 μM. However, this analogue (**80**) was less active against HCMV, HSV-2, and VZV TK^+ Oka strain strain than against HSV-1 while it lacke activity against TK^- HSV-1 and VZV (Table 5). The 5-(5-heptylfur-2-yl)-2'-deoxyuridine (**81**) displayed antiviral activities against parainfluenza virus (Table 6) while all others compounds were inactive.

Table 6. Activity of 5-(5-heptylfur-2-yl)-2'-deoxyuridine against parainfluenza virus

Compound	Cytotoxicity (μM)	EC_{50} (μM)
	MCC	Parainfluenza-3 virus
81	> 100	14.8 ±8

EC_{50} concentration required to reduce virus-induced cytopathogenicity by 50%
MCC is the Minimum Cytoxic Concentration required to cause a microscopically detectable alteration of normal cell morphology

4. EXPERIMENTAL

4.1. General synthetic procedures

^{1}H NMR spectra at 400 MHz and ^{13}C NMR at 100.6 MHz were recorded in $CDCl_3$ unless otherwise noted. All chemical shift values are reported in parts per million (ppm) and referenced to the residual solvent peaks [$CDCl_3$ (7.26 ppm) or DMSO-d_6 (2.54 ppm)] for ^{1}H NMR and the $CDCl_3$ (77.16 ppm) or DMSO-d_6 (39.52 ppm) for ^{13}C NMR spectra, with coupling constant (*J*) values reported in Hz. HRMS were obtained in TOF (ESI) mode unless otherwise specified. TLC was performed on Merck kieselgel 60-F_{254}, and products were detected with 254 nm light. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. Purity, yields and ratio of the products (crude and/or purified) were established via NMR with calibrated standards. All reagents and solvents were purchased from commercial suppliers and used without further purification. The 5-ethynylpyrimidine nucleosides[112,113] or 8-ethynyl-2'-deoxyadenosine[117] were synthesized following the literature procedure.

4.2. Synthesis

5-(1-Chloro/bromo-2-tosylvinyl)uracl/cytosine nucleosides (9a, 8-11, and 19-20). **Procedure A**. 5-Ethynyluracil/cytosine and tosyl hydrazide were dissolved in the acetonitrile and treated with iron(III) halide in the presence of tertiary butyl hydrogen peroxide at 80 ^{o}C. The volatiles were evaporated and the residue was column chromatographed to afford (**9a, 8-11, and 19-20**).

(*E*)-3',5'-Di-*O*-acetyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (9a). Treatment of 3',5'-di-*O*-acetyl-2'-deoxy-5-ethynyluridine **4** (336 mg, 1 mmol) with *p*-toluenesulfonyl hydrazide (372 mg, 2 mmol) and $FeCl_3 \cdot 6H_2O$ (540 mg, 2 mmol) by procedure A (column

chromatography; hexane/EtOAc, 1:1 → 4:6) gave **9a** (400 mg, 76%): ^{1}H NMR δ 2.12 (s, 3H), 2.16 (s, 3H), 2.27-2.37 (m, 1H), 2.42 (s, 3H), 2.58 (ddd, *J* = 1.7, 5.5, 14.3 Hz, 1H), 4.30-4.35 (m, 2H), 4.46 (td, *J* = 2.3, 5.0 Hz, 1H), 5.27 (d, *J* = 6.4 Hz, 1H), 6.35 (dd, *J* = 5.5, 8.5 Hz, 1H), 6.89 (s, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.88 (s, 1H), 8.83 (s, 1H); ^{13}C NMR δ 21.02, 21.04, 21.8, 28.8, 38.2, 64.0, 74.4, 83.1, 85.7, 109.2, 127.9, 130.2, 134.7, 136.7, 139.9, 141.5, 145.6, 149.3, 158.9, 170.5, 170.6; HRMS calcd for $C_{22}H_{23}{}^{35}ClN_2NaO_9S$ $[M+Na]^+$ 549.0705, found 549.0708.

(*E*)-2',3',5'-Tri-*O*-acetyl-5-(1-chloro-2-tosylvinyl)uridine (8). Treatment of 2,3,5-tri-*O*-acetyl-5-ethynyluridine **5** (79 mg, 0.2 mmol) with *p*-toluenesulfonyl hydrazide (52.2 mg, 0.28 mmol) in the presence of $FeCl_3 \cdot 6H_2O$ (108 mg, 0.4 mmol) by procedure A gave **8** (80 mg, 68%): ^{1}H NMR δ 2.12 (s, 3H), 2.14 (s, 3H), 2.18 (s, 3H), 2.42 (s, 3H), 4.37-4.47 (m, 3H), 5.36-5.41 (m, 1H), 5.44 (t, *J* = 5.5 Hz, 1H), 6.10 (d, *J* = 5.3 Hz, 1H), 6.94 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.82 (s, 1H), 8.63 (s, 1H); ^{13}C NMR δ 20.6, 20.7, 21.0, 21.8, 63.2, 70.4, 73.4, 80.7, 88.0, 109.4, 128.1, 130.2, 135.3, 136.7, 139.6, 141.6, 145.5, 149.2, 159.6, 169.6, 169.7, 170.5; HRMS calcd for $C_{24}H_{25}{}^{35}ClN_2NaO_{11}S$ $[M+Na]^+$ 607.0760, found 607.0755.

(*E*)-5-(1-Chloro-2-tosylvinyl)-2'-deoxyuridine (9). Treatment of 5-ethynyl-2'-deoxyuridine **6** (50.5 mg, 0.2 mmol) with *p*-toluenesulfonyl hydrazide (52.2 mg, 0.28 mmol) in the presence of $FeCl_3 \cdot 6H_2O$ (108 mg, 0.4 mmol) by procedure A (column chromatography; $CHCl_3$/MeOH; 9:1→85:15) gave **9** (82 mg, 90%) as white solid. ^{1}H NMR (MeOD-d_4) δ 2.25-2.34 (m, 1H), 2.38 (ddd, *J* = 13.4, 6.0, 4.1 Hz, 1H), 2.45 (s, 3H), 3.78 (dd, *J* = 12.0, 3.5 Hz, 1H), 3.84 (dd, *J* = 12.0, 2.9 Hz, 1H), 3.99 (dd, *J* = 6.3, 3.0 Hz, 1H), 4.37-4.53(m, 1H), 6.31 (t, *J* = 6.4 Hz, 1H), 7.23 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 2H), 7.69 (d, *J* = 7.8 Hz, 2H), 8.31 (s, 1H);

^{13}C NMR (MeOD-d_4) δ 21.6, 41.8, 62.7, 72.0, 87.0, 89.3, 109.7, 129.1, 131.0, 136.0, 138.3, 142.0, 143.9, 146.8, 151.3, 161.6; HRMS calcd for $C_{18}H_{19}{}^{35}ClN_2NaO_7S$ $[M+Na]^+$ 465.0494, found 465.0493

(*E*)-5-(1-Chloro-2-tosylvinyl)uridine (10). Treatment of 5-ethynyluridine **7** (54 mg, 0.2 mmol) with *p*-toluenesulfonyl hydrazide (80 mg, 0.4 mmol) in the presence of FeCl3·6H_2O (108 mg, 0.4 mmol) by procedure A (column chromatography; $CHCl_3$/MeOH; 95:5 → 90:10) gave **10** (54 mg, 59%): UV (MeOH) λmax 242, 276 nm (ε 14 100, 10 800), λmin 230, 261 nm (ε 13 200, 10 200); ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 3.58 (ddd, *J* = 12.0, 5.0, 3.1 Hz, 1H), 3.67 (ddd, *J* = 11.9, 4.9, 3.1 Hz, 1H), 3.90 (q, *J* = 3.2 Hz, 1H), 4.01 (q, *J* = 4.9 Hz, 1H), 4.07 (q, *J* = 5.0 Hz, 1H), 5.14 (d, *J* = 5.0 Hz, 1H), 5.47 (d, *J* = 5.6 Hz, 1H), 5.80 (d, *J* = 4.8 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.53 (s, 1H), 7.57-7.79 (m, 2H), 8.25 (s, 1H), 11.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.1, 60.5, 69.5, 74.1, 79.1, 85.0, 88.5, 108.8, 127.6, 129.8, 134.0, 136.9, 139.6, 141.5, 144.8, 149.8, 159.4; HRMS calcd for: $C_{18}H_{19}{}^{35}ClN_2O_8S$ [M+H]+ for 459.0623, found 459.0620.

(*E*)-5-(1-Bromo-2-tosylvinyl)uridine (11). Treatment of **7** (54 mg, 0.2 mmol) with *p*-toluenesulfonyl hydrazide (80 mg, 0.4 mmol) in the presence of $FeBr_3$ (36 mg, 0.2 mmol) and column chromatography; $CHCl_3$/MeOH, 95:5 → 90:10) gave **11** (41.3 mg, 41%): UV (MeOH) λmax 239, 277 nm (ε 13 500, 10 200), λmin 232, 264 nm (ε 12 800, 9600); ^{1}H NMR (DMSO-d_6) δ 2.4 (s, 3H), 3.54-3.72 (m, 2H), 3.88-3.93 (m,1H), 3.96-4.1 (m, 2H), 5.1-5.2 (m, 2H), 5.48 (d, *J* = 5.6 Hz, 1H), 5.8 (d, *J* = 4.6 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 2H), 7.65 (S, 1H), 7.68 (d, *J* = 7.5 Hz, 2H), 8.22 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.1, 60.5, 69.6, 74.2, 85.0, 88.5, 110.4, 127.7, 129.3, 129.8, 136.7, 136.9, 140.7, 144.8, 149.7, 159.3; HRMS calcd for $C_{18}H_{19}$ $^{81}BrN_2NaO_8S$ [M+Na]+ 526.9917, found 526.9916.

(*E*)-3',5'-Di-*O*-acetyl-5-(1-chloro-2-tosylvinyl)-2'-deoxycytidine (19). Treatment of 3',5'-di-***O***-acetyl-5-ethynylcytidine **17** (156 mg, 0.465 mmol) with *p*-toluenesulfonyl hydrazide (261 mg, 1.39 mmol) in the presence of $FeCl_3 \cdot 6H_2O$ (252 mg, 0.93 mmol) by procedure A (column chromatography; $CHCl_3$/MeOH; 100:0 → 90:5) gave **19** (168 mg, 68%): ^{1}H NMR (DMSO-d_6) δ 1.97 (s, 3H), 2.08 (s, 3H), 2.28-2.35 (m, 2H), 2.40 (s, 3H), 4.15-4.26 (m, 3H), 5.18 (br, 1H), 6.12 (br, 1H), 7.40 (d, J = 6.8 Hz, 2H), 7.65 (d, J = 7.1 Hz, 2H), 7.70 (s, 1H), 7.80 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 20.3, 20.7, 21.0, 38.5, 63.0, 74.0, 75.0, 81.5, 86.0, 101.0, 127.5, 130.0, 136.0, 136.3, 141.2, 145.0, 147.0, 159.5, 169.5, 170.0. HRMS calcd for $C_{22}H_{25}{}^{35}ClN_3O_8S$ $[M+H]^+$ for 526.1051, found 526.1046

(*E*)-2',3',5'-Tri-*O*-acetyl-5-(1-chloro-2-tosylvinyl)cytidine (20). Treatment of 3',5'-di-*O*-acetyl-5-ethynylcytidine **18** (66 mg, 0.167 mmol) with *p*-toluenesulfonyl hydrazide (90 mg, 0.5 mmol) in the presence of $FeCl_3 \cdot 6H_2O$ (91 mg, 0.33 mmol) by procedure A (column chromatography; $CHCl_3$/MeOH; 100:0 → 90:5) gave **20** (48 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H), 2.14 (s, 3H), 2.16 (s, 3H), 2.40 (s, 3H), 4.38-4.48 (m, 3H), 5.38 (dd, J = 10.1, 5.5 Hz, 1H), 5.49 (t, J = 5.2 Hz, 1H), 6.06 (d, J = 5.3 Hz, 1H), 7.10 (s, 1H), 7.32 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.3 Hz, 1H), 7.88 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.8, 21.8, 63.0, 70.0, 74.0, 80.0, 89.5, 101.0, 128.0, 131.0, 136.0, 137.5, 139.5, 144.0, 146.0, 153.0, 160.5, 169.5, 169.7, 170.5

(*E*)-5-(1-Iodo-2-tosylvinyl)uridine (12). 5-Ethynyluridine **7** (26.8 mg, 0.1 mmol) was dissolved in 5 mL THF.Then sodium *p*-toluenesulfinate (35.6 mg, 0.2 mmol) and NIS (23 mg, 0.1 mmol) were sequentially added into the solution and the resulting mixtures were stirred at 64 ^{o}C for 4 h. Volatiles were evaporated and column chromatography; $CHCl_3$/MeOH; 95:5 → 90:10) gave **12** (21.5 mg, 42%): UV (MeOH) λmax 235, 262, 291 (sh) nm (ε 14 150, 12 650),

λmin 220, 252 nm (ε 13 100, 12 400); 1H NMR (DMSO *d*6) δ 2.4 (s, 3H), 3.54-3.70 (m, 2H), 3.87-3.92 (m, 1H), 3.97-4.09 (m, 2H), 5.8 (d, *J* = 4.9 Hz, 1H), 7.4 (d, *J* = 8.1 Hz, 2H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.72 (s, 1H), 8.07 (s, 1H), 11.6 (s, 1H); 13C NMR (DMSO-*d*6) δ 21.1, 60.7, 69.7, 74.1, 85.1, 88.4, 99.5, 111.4, 127.6, 129.8, 136.7, 138.3, 142.6, 144.6, 149.8, 159.2; HRMS calculated for $C_{18}H_{19}IN_2NaO_8S$ [M+Na]+ 572.9799, found 572.9798.

β-Substituted analogues after displacement of halogen via addition-elimination reaction (26-29, 32-33). Procedure B. Chlorovinyl sulfone analogues were dissolved in methanol. Then methanolic ammonia, or thiol nucleophilic sources with triethyl amine was added into the solution and the resulting mixtures were stirred at ambient temperature. The volatiles were evaporated and the residue was column chromatographed to afford **(26-29, 32-33).**

(*E*)-5-(1-(propylthio)-2-tosylvinyl)-2'-deoxyuridine (26). Treatment of **9** (30 mg, 0.07 mmol) with *n*-Propanethiol (7.4 µL, 6.2 mg, 0.08 mmol) in the presence of Et_3N (11.3 µL, 8.2 mg, 0.08 mmol) for 2 h following the procedure B (column chromatography; $CHCl_3$: MeOH = 95:5 → 90:10) gave ***E*-26** (22 mg, 68%). UV (MeOH) λ_{max} = 275 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.93 (t, *J* = 7.3 Hz, 3H) 1.47-1.64 (m, 2H), 2.00-2.11 (m, 1H), 2.14-2.19 (m, 1H), 2.39 (s, 3H), 2.80 (t, *J* = 7.2 Hz, 2H), 3.55 (dd, *J* = 3.5, 7.9 Hz, 2H), 3.82 (dd, *J* = 3.04, 6.04 Hz, 1H), 4.22-4.26 (m, 1H), 5.00 (t, *J* = 4.9 Hz, 1H), 5.27 (d, *J* = 4.2 Hz, 1H), 6.17 (t, *J* = 6.7 Hz, 1H), 6.46 (s, 1H), 7.38 (d, *J* = 8.1 Hz, 2H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.89 (s, 1H), 11.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.2, 20.6, 21.1, 33.6, 61.2, 70.4, 84.6, 87.7, 122.3, 127.0, 129.5, 138.8, 143.7, 149.8, 160.1; HRMS calcd for $C_{21}H_{26}N_2NaO_7S_2$ $[M + Na]^+$ 505.1074, found 505.1077.

(*E*)-5-(1-Propylthio-2-tosylvinyl)uridine (27). Treatment of **10** (48 mg, 0.1 mmol) with *n*-propanethiol (11 μL, 9 mg, 0.12 mmol) in the presence of Et_3N (17 μL, 12 mg, 0.12 mmol) for 2 h following the procedure B and column chromatography ($CHCl_3$/MeOH, 95:5 → 9:1) gave **27** (38 mg, 75%) as white solid: 1H NMR (DMSO-*d*6) δ 0.95 (t, *J* = 7.8 Hz, 3H), 1.55 (m, 2H), 2.40 (s, 3H), 2.80 (t, *J* = 7.8 Hz, 2H), 3.52-3.68 (m, 2H), 3.90 (m, 1H), 3.95-4.07 (m, 2H), 5.04 (brs, 1H) 5.15 (brs, 1H), 5.45 (d, *J* = 5.8 Hz, 1H), 5.80 (d, *J* = 5.1 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 8.2 Hz, 2H), 7.96 (s, 1H), 8.32 (s, 1H), 11.45 (s, 1H); 13C NMR (DMSO-*d*6) δ 13.2, 20.6, 21.1, 33.6, 60.7, 69.8, 74.0, 85.0, 88.2, 109.2, 122.1, 127.1, 128.4, 129.5, 138.2, 138.8, 143.6, 150.1, 160.1; HRMS calculated for $C_{21}H_{26}N_2NaO_8S_2$ [M+Na]+ 521.1023, found 521.1019.

***S*-[(*E*)-1-(2'-Deoxyuridin-5-yl)-2-tosylvinyl]-L-cysteine ethyl ester (28).** Treatment of **9** (30 mg, 0.07 mmol) with L-cysteine ethyl ester hydrogen chloride (36 mg, 0.02 mmol, 3 eq.) in the presence of Et_3N (11.3 μL, 8.2 mg, 0.08 mmol, 1.2 eq.) for 2 h following the procedure B (column chromatography; $CHCl_3$: MeOH = 95:5 → 90 : 10) gave **28** (27 mg, 70 %, *E/Z*, 80:20) as a mixture of region isomers and further purification gave **28** (19 mg, 50 %) as single isomer. ^{1}H NMR (400 MHz, MeOD-d_4) δ 1.32 (t, *J* = 7.2 Hz, 3H), 2.14 (ddd, *J* = 5.9, 7.3, 13.5 Hz, 1H), 2.30 (ddd, *J* = 3.1, 6.2, 13.6 Hz, 1H), 2.43 (s, 3H), 3.02 (dd, *J* = 7.8, 10.5 Hz, 1H), 3.12 (dd, *J* = 5.6, 10.5 Hz, 1H), 3.76 (dd, *J* = 3.7, 11.9 Hz, 1H), 3.83 (dd, *J* = 3.6, 11.9 Hz, 1H), 3.99 (d, *J* = 14.9 Hz, 1H), 4.03 (q, *J* = 3.4 Hz, 1H), 4.08 (dd, *J* = 5.6, 7.8 Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 4.38 (dt, *J* = 3.0, 6.0 Hz, 1H), 4.75 (d, *J* = 14.9 Hz, 1H), 6.24 (t, *J* = 6.7 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.99 (s, 1H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 14.5, 21.7, 37.1, 41.7, 62.8, 63.3, 63.6, 67.1, 72.7, 74.5, 87.0, 89.4, 116.4, 130.0, 130.9, 137.1, 138.1, 146.5, 151.6, 163.6, 172.8; ^{13}C NMR (101 MHz, MeOD) δ 172.77,

163.62, 151.64, 146.47, 138.11, 137.10, 130.89, 129.98, 116.37, 89.40, 87.01, 74.51, 72.73, 67.13, 63.64, 63.30, 62.79, 41.73, 37.08, 21.66, 14.47; HRMS calcd for $C_{23}H_{29}N_3NaO_9S_2$ $[M+Na]^+$ for 578.1237, found 578.1239

***S*-[(*E*)-1-(Uridin-5-yl)-2-tosylvinyl]-L-cysteine ethyl ester (29).** Treatment of **10** (19 mg, 0.04 mmol) with L-cysteine ethyl ester hydrogen chloride (22.8 mg, 0.12 mmol) in the presence of Et_3N (45.7 μL, 33.2 mg, 0.32 mmol) for 2 h following the procedure B and column chromatography; $CHCl_3$/MeOH, 95:5 → 9:1) gave **29** (*E/Z*, 85:15; 13 mg, 56%) as white solid. The *E*-isomer had: 1H NMR (DMSO-*d*6) δ1.20 (t, *J* = 7.2 Hz, 3H), 2.40 (s, 3H), 2.95 (dd, *J* = 10.5, 7.8 Hz, 1H), 3.09 (dd, *J* = 10.5, 5.6 Hz, 1H), 3.53-3.58 (m, 2H), 3.61-3.63 (m, 1H), 3.87-3.90 (m, 1H), 3.95–4.00 (m, 2H), 4.04–4.12 (m, 4H), 5.00-5.20 (m, 2H), 5.45 (d, *J* = 5.7 Hz, 1H), 5.80 (d, *J* = 5.1 Hz, 1H), 6.58 (s, 1H), 7.48 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.96 (s, 1H), 11.50 (s, 1H); 13C NMR (DMSO-*d*6) δ 14.0, 21.0, 37.0, 53.5, 61.5, 61.6, 69.5, 74.0, 85.0, 88.5, 96.0, 116.0, 122.5, 127.5, 128.5, 129.5, 138.5, 143.5, 150.5, 160.0, 173.0; HRMS calculated for $C_{23}H_{29}N_3NaO_{10}S_2$ [M+Na]+ 594.1187, found 594.1173.

***S*-[(*E*)-1-(2'-Deoxyuridin-5-yl)-2-tosylvinyl]-L-glutathione (30).** The 5-(β-chloro)vinyl sulfone analogue **9** (25 mg, 0.056 mmol) was dissolved in 1 mL methanol-water (80:20) cosolvent system. Then, L-glutathione (25.8 mg, 0.084 mmol) and Et_3N (59.0 μL, 42.5 mg, 0.42 mmol) were sequentially added and the resulting mixtures were stirred at ambient temperature for 4 h. The volatile were evaporated and reverse phase HPLC separation gave **30** (22 mg, 55%) as white solid. 1H NMR (D_2O) δ 2.15 (q, *J* = 7.2 Hz, 2H), 2.27-2.39 (m, 2H) 2.45 (s, 3H), 2.50 (t, *J* = 7.5 Hz, 2H), 3.24-3.45 (m, 2H), 3.70-3.80 (m, 3H), 3.85-4.02 (m, 2H), 4.08 (br, 1H), 4.50 (br, 1H), 4.60-4.70 (m, 2H), 6.25 (t, *J* = 6.7 Hz, 1H), 6.85 (s, 1H), 7.42 (d, *J* = 8.1 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.70 (s, 1H); ^{13}C NMR (D_2O) δ 21.0,

26.4, 31.0, 33.0, 39.5, 43.8, 52.0, 54.0, 61.0, 70.0, 85.5, 94.0, 108.0, 126.0, 130.0, 135.0, 136.0, 142.0, 145.8, 151.0, 159.8, 170.7, 174.0, 175.0, 176.2; HRMS calculated for $C_{28}H_{35}N_5O_{16}PS_2$ $[M-H]^-$ 792.12633, found 792.121640

(*Z*)-5-(1-Amino-2-tosylvinyl)uridine (32). Treatment (2 h) of **10** (25 mg, 0.054 mmol) with NH_3/MeOH (3 mL) by the procedure B and column chromatography; $CHCl_3$/MeOH, 9:1) gave **32** (20 mg, 84%): 1H NMR (DMSO-*d*6) δ 2.37 (s, 3H), 3.59 (ddd, *J* = 2.8, 5.3, 12.3 Hz, 1H), 3.68 (ddd, *J* = 3.0, 5.0, 12.3 Hz, 1H), 3.86-3.8 (m, 1H), 4.00 (q, *J* = 5.19 Hz, 1H), 4.10-4.14 (m, 2H), 5.11 (d, *J* = 5.6 Hz, 1H), 5.21 (s, 1H) ,5.29 (t, *J* = 5.1 Hz, 1H), 5.44 (d, *J* = 5.4 Hz, 1H), 5.72 (d, *J* = 4.2 Hz, 1H), 6.99 (s, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.76 (d, *J* = 8.2 Hz, 2H), 8.33 (s, 1H), 11.66 (s, 1H); 13C NMR (DMSO-d_6) δ 20.9, 60.2, 69.1, 73.9, 84.8, 88.2, 89.1, 107.2, 125.5, 129.4, 141.5, 142.2, 142.3, 149.4, 150.7, 161.6; HRMS calculated for $C_{18}H_{22}N_3O_8S$ $[M+H]+$ 440.1122, found 440.1100.

5-[1-(n-Aminobutyl)-2-tosylvinyl]uridine (33). Treatment of β-chlorovinyl analogue **10** (25 mg, 0.054 mmol) with n-butyl amine 27 μL, 20 mg, 0.27mmol) for 5 h following the procedure B and column chromatography; $CHCl_3$/MeOH, 95:5 → 90:10) gave **33** (19 mg, 71%) as *E*/*Z* mixtures (85:15). The major isomer had: 1H NMR (MeOH-d_4) δ 0.85 (t, *J* = 7.5 Hz, 3H), 1.40 (sex, *J* = 7.2 Hz, 2H), 1.58 (quin, *J* = 7.5 Hz, 2H), 2.40 (s, 3H), 3.00 (t, *J* = 7.0 Hz, 2H), 3.75 (dd, *J* = 10.3, 6.0 Hz, 1H), 3.82 (dd, *J* = 10.2, 5.8 Hz, 1H), 4.0-4.05 (m, 1H), 4.20 (ddd, *J* = 9.4, 6.4, 2.3 Hz, 1H), 4.25 (dd, *J* = 9.7, 4.6 Hz, 1H), 5.00 (s, 1H), 5.9 (d, *J* = 4.5 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.95(s, 1H).

(*E*)-5-(2-Tosylvinyl)uridine (36). *Step a*. *p*-Toluenesulfonic acid (62 mg, 0.4 mmol) was added to a solution of 2',3',5'-tri-*O*-acetyl-5-ethynyluridine **5** (68 mg, 0.17 mmol) in dry THF

(3 mL) containing Cu(OAc)2•2H2O (3 mg, 0.015 mmol) under nitrogen at ambient temperature. The resulting solution was stirred at 60 °C (oil bath) for 7 h. After cooling to ambient temperature, the volatiles were evaporated under the reduced pressure and the residue was column chromatographed (hexane/EtOAc, 1:1→4:6) to give (*E*)-2',3',5'-tri-*O*-acetyl-5-(2-tosylvinyl)uridine (31.5 mg, 35%): ^{1}H NMR δ 2.10 (s, 3H), 2.12 (s, 2H), 2.21 (s, 3H), 2.42 (s, 3H), 4.30 – 4.51 (m, 3H), 5.31 (t, *J* = 5.3 Hz, 1H), 5.36 (t, *J* = 5.2 Hz, 1H), 6.00 (d, *J* = 4.8 Hz, 1H), 7.19 (d, *J* = 15.0 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 15.0 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 2H), 7.79 (s, 1H), 8.70 (s, 1H). *Step b.* Methanolic ammonia (1.5 mL) was added to the solution of the material (25.5 mg, 0.045 mmol) in MeOH (1.5 mL) at 0 °C and the resulting solution was stirred for overnight. Volatiles were evaporated and the residue was column chromatographed (CHCl3/MeOH, 9:1) to give **36** (12 mg, 63%): 1H NMR (MeOD-*d*4) δ 2.42 (s, 3H), 3.77 (dd, *J* = 12.4, 2.6 Hz, 1H), 3.93 (dd, *J* = 12.4, 2.6 Hz, 1H), 4.02 (dt, *J* = 5.2, 2.6 Hz, 1H), 4.12 – 4.21 (m, 2H), 5.87 (d, *J* = 3.1 Hz, 1H), 7.30 (d, *J* = 15.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 15.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 8.61 (s, 1H); 13C NMR (MeOD-*d*4) δ 21.5, 61.5, 70.4, 76.2, 86.2, 91.3, 108.7, 128.2, 128.5, 131.1, 136.2, 139.6, 145.8, 146.6, 151.1, 163.3. HRMS calculated for $C_{18}H_{20}N_2NaO_8S$ [M+Na]+ 447.0833, found 447.0802.

Synthesis of protected 5-(β-keto sulfone) of uracil nucleoside (49, 50a and 50b). General Procedure C. Protected 5-ethylnyluracil nucleosides were taken in a round bottom flask and dissolved in 1, 2 dichloroethane. Then freshly prepared *p*-toluenesulfinic acid and pyridine were sequentially added into the solution. A balloon filled with oxygen was connected with the flask and the resulting mixtures were placed in an oil bath and stirred at 45

^{o}C for 4 h. The reaction mixtures were washed with dil. HCl followed by $NaHCO_3$ solution. The organic layer was column chromatographed to afford (**35, 50a,** and **50b**).

1-*N*-benzyl-5-(2-tosylacetyl)uracil (49). Treatment of 1-*N*-benzyl-5-ethynyluracil **48** (45.2 mg, 0.2 mmol) with *p*-toluenesulfinic acid (156 mg, 1.0 mmol) and pyridine (66 μL, 64.9 mg, 0.82 mmol) in the presence of oxygen (balloon) by procedure C and column chromatography (Hexane : EtOAc = 1:1) gave **49** (35 mg, 44%) as white solid. 1H NMR δ 2.42 (s, 3H), 4.98 (s, 4H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.34-7.31 (m, 2H), 7.45-7.37 (m, 3H), 7.79 (d, *J* = 8.3 Hz, 2H), 8.23 (s, 1H), 8.46 (s, 1H); 13C NMR δ 21.8, 52.9, 65.4, 112.0, 128.58, 128.61, 129.4, 129.6, 129.9, 133.9, 137.0, 145.3, 149.6, 151.7, 160.5, 183.8; HRMS calcd for $C_{20}H_{19}N_2O_5S$ [M + H]+ 399.1009, found 399.1017.

3',5'-Di-*O*-acetyl-5-(2-tosylacetyl)-2'-deoxyuridine (50a) Treatment of protected 5-ethynyl-2'-deoxyuridne **4** (25 mg, 0.074 mmol) with *p*-toluenesufinic acid (58 mg, 0.372mmol) and pyridine (14.5 μL, 14.2 mg, 0.372 mmol) in the presence of oxygen (balloon) by procedure C and column chromatography (Hexane/EA ; 60:40 → 40:60) gave **50a** (6 mg, 16%) as white solid: ^{1}H NMR ($CDCl_3$) δ 2.13(s, 3H), 2.16 (s, 3H), 2.45 (s, 3H), 2.58-2.66 (m, 1H), 2.74-2.78 (m, 1H), 4.35 (dd, *J* = 6.9, 3.8 Hz, 1H), 4.36-4.40 (m, 2H), 4.93 (d, *J* = 13.0 Hz, 1H), 5.04 (d, *J* = 13.1 Hz, 1H), 5.24-5.30 (m, 1H), 6.35 (dd, *J* = 11.9, 6.4 Hz, 1H), 7.35 (d, *J* = 7.8 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 8.40 (s, 1H), 8.60 (s, 1H).

2',3',5'-Tri-*O*-acetyl-5-(2-tosylacetyl)uridine (50b) Treatment of protected 5-ethynyluridine **5** (36 mg, 0.09 mmol) with *p*-toluenesufinic acid (78 mg, 0.5 mmol) and pyridine (40 μL, 39.2 mg, 0.5 mmol) in the presence of air (balloon) and column chromatography (Hexane/EA ; 60:40 → 40:60) gave **50b** (9 mg, 20%) as white solid: ^{1}H NMR ($CDCl_3$) δ 2.11(s, 3H), 2.14 (s, 3H), 2.19 (s, 3H), 2.45 (s, 3H), 4.35 (ddd, *J* = 12.1, 6.9,

3.8 Hz, 2H), 4.40-4.44 (m, 1H), 4.93 (d, *J* = 13.3 Hz, 1H), 5.06 (d, *J* = 13.3 Hz, 1H), 5.33-5.38 (m, 1H), 6.35 (d, *J* = 4.5 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 2H), 8.50 (s, 1H), 8.65 (s, 1H)

5-(2-Tosylacetyl)uracil/cytosine nucleosides (51-52 and 58-59). **Procedure D.** Acetyl protected (**8** and **19-20**) or unprotected (**9-10**) 5-(1-chloro-2-tosylvinyl)pyrimidine nucleosides were dissolved in methanolic ammonia and the resulting mixtures were stirred at 0 ^{o}C → r.t; The volatiles were evaporated and the residue was dissolved in 4 mL CH_3CN. The solution was acidified (pH ~ 4) by adding dil. HCl (aq) and stirred for 2h. After neutralizing the solution with dil. NaOH(aq), the volatiles were evaporated and the residue was column chromatographed to give the products (**51-52** and **58-59**).

5-(2-Tosylacetyl)-2'-deoxyuridine (51). Treatment of **9** (96 mg, 0.21 mmol) with methanolic ammonia (3 mL) for 2 h and subsequent acid hydrolysis as described in procedure D followed by column chromatography ($CHCl_3$/MeOH ; 100:0 → 95:5) gave **51** (68 mg, 72%) as white solid: ^{1}H NMR (DMSO-d_6) δ 2.10-2.27 (m, 2H, H2',2''), 2.40 (s, 3, Me), 3.51-3.63 (m, 2H, H5',5''), 3.87 (q, *J* = 3.1 Hz, 1H, H4'), 4.23 (q, *J* = 3.4 Hz, 1H, H3'), 5.10 (t, *J* = 4.5 Hz, 1H, 5'OH), 5.15 (s, 2H, α-CH_2), 5.30 (d, *J* = 4.1 Hz, 1H, 3'OH), 6.06 (t, *J* = 6.3 Hz, 1H, H1'), 7.42 (d, *J* = 8.2 Hz, 2H, Ar), 7.74 (d, *J* = 8.1 Hz, 2H, Ar), 8.68 (s, 1H, H6), 11.80 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.0, 41.0, 60.5, 64.0, 70.0, 86.0, 88.0, 110.5, 128.0, 130.0, 137.0, 144.0, 148.0, 149.0, 161.0, 184.0; HRMS calcd for $C_{18}H_{20}N_2NaO_8S$ $[M+Na]^+$ for 447.0833, found 447.0874

5-(2-Tosylacetyl)uridine (52). Treatment of **10** (96 mg, 0.21 mmol) with methanolic ammonia (3 mL) for 2 h and subsequent acid hydrolysis as described in procedure D followed by column chromatography ($CHCl_3$/MeOH ; 100:0 → 95:5) gave **52** (65 mg, 70%) as white

solid: ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, Me), 3.55-3.75 (m, , 1H, H5'5''), 3.93 (q, *J* = 3.2 Hz, 1H, H4'), 3.95 (t, *J* = 4.9 Hz, 1H, H3'), 4.08 (t, *J* = 5.0 Hz, 1H, H2'), 5.12 (t, *J* = 4.5 Hz, 1H, 5'OH), 5.14 (s, 2H, α-CH_2), 5.21 (d, *J* = 4.1 Hz, 1H, 3'OH), 5.51 (d, *J* = 4.9 Hz, 1H, 2'OH), 5.75 (d, *J* = 2.2 Hz, 1H, H1'), 7.42 (d, *J* = 7.8 Hz, 2H, Ar), 7.74 (d, *J* = 7.8 Hz, 2H, Ar), 8.80 (s, 1H, H6), 11.85 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.0, 60.0, 64.2, 69.5, 75.0, 85.0, 89.5, 111.0, 128.0, 130.0, 137.0, 144.5, 148.0, 149.5, 161.0, 185.0

5-(2-Tosylacetyl)-2'-deoxycytidine (58). Treatment of acetyl protected (β-chloro) vinyl sulfone **19** (150 mg, 0.28 mmol) with methanolic ammonia (5 mL) for 12 h and subsequent acid hydrolysis as described in procedure D followed by column chromatography ($CHCl_3$/MeOH ; 100:0 → 95:10) gave **58** (70 mg, 59%) as white solid: ^{1}H NMR (DMSO-d_6) δ 2.05-2.10 (m, 1H, H2'), 2.30 (ddd, *J* = 13.2, 8.2, 2.1 Hz, 1H, H2''), 2.40 (s, 3H, Me), 3.62-3.68 (m, 1H, H5'), 3.70-3.76 (m, 1H, H5''), 3.92 (q, *J* = 3.4 Hz, 1H, H4'), 4.30 (quint, *J* = 4.6 Hz, 1H, H3'), 4.86 (d, *J* = 14.1 Hz, 1H, α-CH), 4.95 (d, *J* = 14.1 Hz, 1H, α-CH), 5.29 (d, *J* = 4.4 Hz, 1H, 3'OH), 5.34 (t, *J* = 5.3 Hz, 1H, 5'OH), 6.09 (t, *J* = 5.9 Hz, 1H, H1'), 7.45 (d, *J* = 8.3 Hz, 2H, Ar), 7.75 (d, *J* = 7.8 Hz, 2H, Ar), 8.10 (d, *J* = 5.4 Hz, 2H, NH_2), 9.02 (s, 1H, H6); ^{13}C NMR (DMSO-d_6) δ 21.0, 41.5, 61.0, 62.5, 69.5, 86.0, 88.0, 103.0, 128.0, 130.0, 136.0, 144.5, 152.0, 152.5, 163.0, 185.5; HRMS calcd for $C_{18}H_{22}N_3O_7S$ $[M+H]^+$ 424.1178, found 424.1176

5-(2-Tosylacetyl)cytidine (59). Treatment of acetyl protected (β-chloro) vinyl sulfone **20** (35 mg, 0.06 mmol) with methanolic ammonia (4 mL) for 12 h and subsequent acid hydrolysis as described in procedure D followed by column chromatography ($CHCl_3$/MeOH ; 100:0 → 95:10) gave **59** (17 mg, 64%) as white solid: ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H, Me), 3.68 (ddd, *J* = 12.5, 5.2, 3.0 Hz, 1H, H5'), 3.85 (ddd, *J* = 12.1, 4.5, 2.3 Hz, 1H, H5''),

3.95 (q, *J* = 3.4 Hz, 1H, H4'), 3.98-4.02 (m, 1H, H3') 4.03-4.09 (m, 1H, H2'), 4.80 (d, *J* = 14.1 Hz, 1H, α-CH), 4.95 (d, *J* = 14.1 Hz, 1H, α-CH), 5.05 (d, *J* = 6.5 Hz, 1H, 3'OH), 5.45 (t, *J* = 5.0 Hz, 1H, 5'OH), 5.58 (d, *J* = 4.8 Hz, 1H, 2'OH), 5.71 (d, *J* = 1.9 Hz, 1H, H1'), 7.43 (d, *J* = 8.3 Hz, 2H, Ar), 7.74 (d, *J* = 8.2 Hz, 2H, Ar), 8.06 (d, *J* = 3.2 Hz, 1H, NH), 8.13 (d, *J* = 3.2 Hz, 1H, NH), 9.05 (s, 1H, H6); ^{13}C NMR (DMSO-d_6) δ 21.0, 60.0, 62.5, 68.3, 74.0, 84.0, 91.0, 103.5, 128.0, 130.0, 136.0, 145.0, 152.0, 152.5, 162.5, 185.5

Incorporation of electrophiles at the α-carbon of β-keto sulfone moiety of pyridmidine nucleoside analogues (53-56 and 60-62). Procedure E. 5-(β-keto)sulfones of uracil/cytosine nucleoside were dissolved in 1 mL MeOH. Then 5M NaOH/H_2O solution (2 equiv.) were added and the solution was stirred for 20 min. Finally, 1.2 equiv. of the electrophile source (R-X) was added and the resulting solution was stirred at ambient temperature. The solution was neutralized by adding dil. HCl and volatiles were evaporated. The residue was column chromatographed to give the desired products (**53-56** and **60-62**).

5-(2-Benzyl-2-tosylacetyl)-2'-deoxyuridine (53). Treatment of **51** (18 mg, 0.042 mmol) with NaOH(aq) (16 µL, 3.36 mg, 0.084 mmol), and benzyl bromide (10 µL, 14.4 mg, 0.05 mmol) for 3 h as described in procedure E followed by column chromatography ($CHCl_3$/MeOH; 100:0 → 95:5) gave **53** as a (50/50) mixture of diastereomers (6 mg, 28%) along with byproducts. ^{1}H NMR (DMSO-d_6) δ 2.05-2.20 (m, 1.0, H2'), 2.22-2.32 (m, 1.0, H2''), 2.40 (s, 1.5, Me), 2.41 (s, 1.5, Me), 3.25-3.30 (m, 1, Bn), 3.10-3.20 (m, 1.0, Bn), 3.54-3.68 (m, 2.0, H5',5''), 3.88 (q, *J* = 3.2 Hz, 1.0, H4'), 4.20 (dd, *J* = 9.2, 4.2 Hz, 0.5, H3'), 4.25 (dd, *J* = 9.2, 4.2 Hz, 0.5, H3'), 5.12-5.18 (m, 1.0, 5'OH), 5.28 (d, *J* = 4.3 Hz, 0.5, 3'OH), 5.33 (d, *J* = 4.3 Hz, 0.5, 3'OH), 5.97 (t, *J* = 6.2 Hz, 0.5, H1'), 6.06 (t, *J* = 6.3 Hz, 0.5, H1'), 6.52 (t, *J* = 3.4 Hz, 0.5, α-CH), 6.56 (t, *J* = 3.4 Hz, 0.5, α-CH), 7.05 (d, *J* = 7.1 Hz, 2.0, Ar) 7.13 (t, *J*

= 6.5 Hz, 1.0, Ar), 7.18 (t, *J* = 6.9 Hz, 2.0, Bn) 7.42 (d, *J* = 7.9 Hz, 1.0, Ar), 7.44 (d, *J* = 7.9 Hz, 1.0, Ar), 7.67 (d, *J* = 8.1 Hz, 1.0, Ar), 7.69 (d, *J* = 8.1 Hz, 1.0, Ar), 8.63 (s, 0.5, H6), 8.70 (s, 0.5, H6), 11.50 (s, 1.0, NH); ^{13}C NMR (DMSO-d_6) δ 21.0, 31.3, 31.6, 40.5, 40.9, 54.8, 60.7, 60.8, 69.9, 86.0, 86.5, 88.0, 88.2, 111.0, 111.4, 126.6, 128.4, 128.5, 128.6, 128.8, 128.9, 129.6, 129.8, 134.5, 136.1, 136.2, 145.0, 145.1, 148.0, 148.1, 149.1, 149.2, 161.4, 161.5, 186.1, 186.2; HRMS calcd for $C_{25}H_{27}N_2O_8S$ $[M+H]^+$ 515.1488, found 515.1437

5-(2-Methyl-2-tosylacetyl)-2'-deoxyuridine (54). Treatment of **51** (16 mg, 0.037 mmol) with NaOH(aq) (16 µL, 2.96 mg, 0.074 mmol), and methyl iodide (4.60 µL, 10.50 mg, 0.074 mmol) for 4 h as described in procedure E followed by column chromatography ($CHCl_3$/MeOH; 100:0 → 95:5) gave **54** as a (50/50) mixture of diastereomers (5 mg, 31%) and two byproducts. ^{1}H NMR (DMSO-d_6) δ 1.32 (t, *J* = 7.4 Hz, 3.0), 2.10-2.18 (m, 1.0, H2'), 2.20-2.30 (m, 1.0, H2''), 2.39 (s, 1.5, Me), 2.40 (s, 1.5, Me), 3.53-3.64 (m, 2.0, H5',5''), 3.87-3.92 (m, 1H, H4'), 4.21-4.29 (m, 1.0, H3'), 5.11-5.16 (m, 1.0, 5'OH), 5.30 (d, *J* = 4.3 Hz, 0.5, 3'OH), 5.33 (d, *J* = 4.3 Hz, 0.5, 3'OH), 5.97 (q, *J* = 6.5 Hz, 1.0, α-CH), 6.05 (t, *J* = 6.3 Hz, 0.5, H1'), 6.11 (t, *J* = 6.4 Hz, 0.5, H1'), 7.42 (t, *J* = 7.6 Hz, 2.0, Ar), 7.65 (d, *J* = 8.1 Hz, 1.0, Ar), 7.68 (d, *J* = 8.1 Hz, 1.0, Ar), 8.71 (s, 0.5, H6), 8.73 (s, 0.5, H6), 11.80 (s, 1.0, NH); ^{13}C NMR (DMSO-d_6) δ 11.0, 11.2, 21.1, 40.8, 41.0, 60.8, 60.9, 65.5, 70.0, 86.0, 86.5, 88.0, 88.1, 111.0, 111.2, 128.8, 128.9, 129.6, 129.8, 145.0, 145.1, 148.0, 148.1, 149.1, 149.2, 161.3, 161.4, 186.1, 187.8

5-(2-Allyl-2-tosylacetyl)-2'-deoxyuridine (55). Treatment of **51** (18 mg, 0.042 mmol) with NaOH(aq) (16 µL, 3.36 mg, 0.084 mmol), and Allyl bromide (6 µL, 8.38 mg, 0.069 mmol) for 4 h as described in procedure E followed by column chromatography ($CHCl_3$/ MeOH; 100:0 → 95:5) gave **55** as a (52/48) mixture of diastereomers (6 mg, 31%) and two

byproducts: ^{1}H NMR (DMSO-d_6) δ 2.05-2.20 (m, 1.10, H2'), 2.22-2.35 (m, 0.90, H2''), 2.39 (s, 1.65, Me), 2.40 (s, 1.35, Me), 2.66-2.78 (m, 2.00, CH_2), 3.56-3.53-3.68 (m, 2.00, H5',5''), 3.89 (q, *J* = 3.3 Hz, 1.00, H4'), 4.21-4.29 (m, 1.00, H3'), 4.93-5.01 (m, 1.65, allyl), 5.11-5.17 (m, 1.00, 5'OH), 5.30 (d, *J* = 4.3 Hz, 0.55, 3'OH), 5.35 (d, *J* = 4.3 Hz, 0.45, 3'OH), 5.55-5.66 (m, 1.00, allyl), 6.05 (t, *J* = 6.2 Hz, 0.45, H1'), 6.10 (t, *J* = 6.2 Hz, 0.55, H1'), 6.18 (d, *J* = 2.6 Hz, 0.55, α-CH), 6.21 (d, *J* = 2.6 Hz, 0.45, α-CH), 7.40 (d, *J* = 8.2 Hz, 1.10, Ar), 7.44 (d, *J* = 8.2 Hz, 0.90, Ar), 7.65 (d, *J* = 7.5 Hz, 2.00, Ar), 8.71 (s, 0.45, H6), 8.73 (s, 0.55, H6), 11.80 (s, 1.00, NH); ^{13}C NMR (DMSO-d_6) δ 21.1, 30.0, 30.3, 40.5, 41.0, 60.8, 60.9, 68.9, 70.0, 70.1, 86.0, 86.5, 88.0, 88.1, 110.8, 111.2, 118.0, 129.0, 129.6, 129.9, 133.0, 134.5, 134.6, 148.0, 148.1, 149.0, 149.2, 161.5, 161.6, 186.5, 186.7

5-(2-Allyl-2-tosylacetyl)uridine (56). Treatment of **51** (18 mg, 0.04 mmol) with NaOH(aq) (16 µL, 3.36 mg, 0.084 mmol), and allyl bromide (6 µL, 8.38 mg, 0.069 mmol) for 4 h as described in procedure E followed by column chromatography ($CHCl_3$/ MeOH; 100:0 → 95:5) gave **56** as a (52/48) mixture of diastereomers (6 mg, 32%) and two byproducts: ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 0.48, Me), 2.40 (s, 0.52, Me), 2.66-2.78 (m, 2.00, allyl), 3.57-3.63 (m, 0.96, H5'), 3.69-3.77 (m, 1.04, H5''), 3.4 (q, *J* = 3.3 Hz, 1.00, H4'), 3.95-4.02 (m, 1.00, H3'), 4.03-4.09 (m, 1.00, H2'), 4.93-5.01 (m, 1.04, allyl), 4.93-5.10 (m, 0.96, allyl), 5.13 (d, *J* = 5.3 Hz, 1.04, 3'OH), 5.20 (d, *J* = 5.4 Hz, 0.96, 3'OH), 5.23-5.28 (m, 1.00, 5'OH), 5.48 (d, *J* = 5.3 Hz, 0.48, 3'OH), 5.58 (d, *J* = 5.2 Hz, 0.52, 3'OH), 5.59-5.67 (m, 1.00, allyl), 5.71 (d, *J* = 3.7 Hz, 0.48, H1'), 5.78 (d, *J* = 4.0 Hz, 0.52, H1'), 6.13-6.21 (m, 1.00, α-CH), 7.42 (d, *J* = 8.2 Hz, 0.96, Ar), 7.46 (d, *J* = 8.2 Hz, 1.04, Ar), 7.65 (d, *J* = 7.1 Hz, 2.00, Ar), 8.81 (s, 0.48, H6), 8.90 (s, 0.52, H6), 11.80 (s, 1.00, NH); ^{13}C NMR (DMSO-d_6) δ 21.1, 30.0, 30.1, 60.0, 60.2, 68.8, 69.0, 69.2, 69.6, 74.4, 74.6, 85.0, 85.3, 89.2, 89.6, 111.2, 111.4, 117.6,

128.8, 129.0, 129.8, 130.0, 133.0, 135.4, 145.0, 148.5, 148.8, 149.4, 149.6, 161.4, 161.5, 186.7, 186.8

5-(2-Benzyl-2-tosylacetyl)-2'-deoxycytidine (60). Treatment of **58** (18 mg, 0.043 mmol) with NaOH(aq) (11 µL, 2.2 mg, 0.055 mmol), and benzyl bromide (6.5 µL, 9.4 mg, 0.05 mmol) for 8 h as described in procedure E followed by column chromatography ($CHCl_3$/ MeOH; 100: 0 → 95:5) gave **60** as a (52/48) mixture of diastereomers (15 mg, 68%). ^{1}H NMR (DMSO-d_6) δ 1.93-2.03 (m, 1.04, H2'), 2.22-2.30 (m, 0.96, H2''), 2.38 (s, 1.42, Me), 2.41 (s, 1.58, Me), 3.20-3.30 (m, 2.00, Bn), 3.54-3.68 (m, 2.00, H5',5''), 3.89 (q, *J* = 3.5 Hz, 0.52, H4'), 4.00 (q, *J* = 3.5 Hz, 0.48, H4'), 4.20 (quint, *J* = 4.9 Hz, 0.52, H3'), 4.38 (quint, *J* = 4.7 Hz, 0.48, H3'), 5.28 (d, 0.52, *J* = 4.6 Hz, 3'OH), 5.35 (d, *J* = 4.3 Hz, 0.48, 3'OH), 5.48 (dd, *J* = 11.3, 3.6 Hz, 0.48, α-CH), 5.58 (dd, *J* = 10.7, 4.4 Hz, 0.48, α-CH), ,5.68 (t, *J* = 4.6 Hz, 0.48, 5'OH), 5.75 (t, *J* = 4.9 Hz, 0.52, 5'OH), 5.94-6.20 (m, 1.00, H1'), 7.08-7.23 (m, 5.00, Bn), 7.42 (d, *J* = 8.2 Hz, 1.04, Ar), 7.46 (d, *J* = 8.2 Hz, 0.96, Ar), 7.67 (d, *J* = 9.7 Hz, 0.52, Ar) 7.71 (d, *J* = 8.8 Hz, 0.48, Ar), 8.03 (d, *J* = 3.6 Hz, 0.52, NH_2), 8.14 (d, *J* = 3.5 Hz, 0.48, NH_2), 8.92 (s, 0.48, H6), 9.06 (s, 0.52, H6); ^{13}C NMR (DMSO-d_6) δ 21.0, 31.8, 32.2, 41.4, 41.6, 60.5, 60.8, 68.5, 69.0, 69.4, 69.6, 69.8, 86.0, 86.8, 87.6, 88.0, 104.0,104.8, 126.7, 126.9, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.8, 129.9, 133.7, 133.8, 135.6, 135.8, 145.1, 145.3, 151.0, 151.2, 152.0, 162.0, 162.4, 187.4, 188.0

5-(2-Methyl-2-tosylacetyl)-2'-deoxycytidine (61). Treatment of **58** (18 mg, 0.043 mmol) with NaOH(aq) (20 µL, 4.0 mg, 0.1 mmol), and benzyl bromide (7.5 µL, 9.4 mg, 0.086 mmol) for 24 h as described in procedure E followed by column chromatography ($CHCl_3$/ MeOH; 100: 0 → 95:5) gave **61** as a (52/48) mixture of diastereomers (15 mg, 68%).^{1}H NMR (DMSO-d_6) δ 1.33 (d, *J* = 6.5 Hz, 1.44, CH_3), 1.37 (d, *J* = 6.5 Hz, 1.56, CH_3), 2.03-2.18 (m,

0.96, H2'), 2.28-2.35 (m, 1.04, H2''), 2.40 (s, 3.00, Me), 3.60-3.95 (m, 2.00, H4' & H5'5''), 4.20 (quint, 0.52, H3'), 4.39 (q, *J* = 3.5 Hz, 0.52, H4'), 4.00 (q, *J* = 3.5 Hz, 0.48, H4'), 4.20 (quint, *J* = 4.8 Hz, 0.52, H3'), 4.39 (quint, *J* = 4.8 Hz, 0.48, H3'), 5.16-5.28 (m, 1.00, α-CH), 5.30-5.45 (m, 2.00, 3' & 5'OH), 6.00 (t, *J* = 4.8 Hz, 0.52, H1'), 6.15 (t, *J* = 5.5 Hz, 0.48, H1'), 7.42 (d, *J* = 7.8 Hz, 1.04, Ar), 7.46 (d, *J* = 7.8 Hz, 0.96, Ar), 7.62 (d, *J* = 8.2 Hz, 1.04, Ar), 7.66 (d, *J* = 8.2 Hz, 0.96, Ar), 8.05-8.12 (m, 0.52, NH_2), 8.22 (d, *J* = 3.5 Hz, 0.48, NH_2), 9.08 (s, 0.48, H6), 9.16 (s, 0.52, H6); ^{13}C NMR (DMSO-d_6) δ 12.0, 21.0, 41.2, 41.4, 60.2, 60.5, 63.3, 64.1, 68.8, 69.0, , 86.0, 86.8, 87.6, 88.1, 104.0,104.5, 129.0, 129.2, 130.0, 130.5, 133.4, 133.7, 145.1, 145.3, 151.8, 152.0, 152.4, 162.8, 163.0, 189.0, 189.2

5-(2-Benzyl-2-tosylacetyl)cytidine (62). Treatment of **59** (15 mg, 0.034 mmol) with NaOH(aq) (13.6 µL, 2.7 mg, 0.068 mmol), and benzyl bromide (8.5 µL, 11.6 mg, 0.068 mmol) for 8h as described in procedure E followed by column chromatography ($CHCl_3$/ MeOH; 100: 0 → 95:5) gave **62** as a (54/46) mixture of diastereomers (12 mg, 67%). ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 1.62, Me), 2.40 (s, 1.38, Me), 3.15-3.30 (m, 2.00, Bn), 3.72-3.90 (m, 2.00, H5',5''), 3.91-3.97 (m, , 1.00, H4'), 3.98-4.03 (m, 1.00, H3'), 4.04-4.20 (m, 1.00, H2'), 5.28 (d, *J* = 4.6 Hz, 0.54, 3'OH), 5.05 (d, *J* = 6.3 Hz, 0.46, 3'OH), 5.20 (d, *J* = 3.6 Hz, 0.54, 3'OH), 5.45-5.52 (m, 1.00, 2'OH), 5.53-5.58 (m, 1.00, 1'), 5.59-5.63 (m, 1.00, α-CH), 5.72-5.88 (m, 1.00, 5'OH), 7.09-7.23 (m, 5.00, Bn), 7.42 (d, *J* = 8.3 Hz, 1.08, Ar), 7.46 (d, *J* = 8.2 Hz, 0.92, Ar), 7.66 (d, *J* = 8.2 Hz, 1.08, Ar), 7.69 (d, *J* = 8.2 Hz, 0.92, Ar), 7.98-8.08 (m, 1.08, NH_2), 8.15 (d, *J* = 3.6 Hz, 0.92, NH_2), 8.90 (s, 0.54, H6), 9.06 (s, 0.46, H6); ^{13}C NMR (DMSO-d_6) δ 21.0, 31.8, 32.0, 59.4, 60.0, 67.5, 68.2, 68.3, 68.5, 69.2, 74.8, 75.0, 79.2, 83.8, 84.2, 90.6, 91.4, 104.1,104.7, 126.6, 126.9, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.8,

130.1, 133.7, 134.1, 135.6, 135.7, 145.1, 145.2, 151.0, 151.5, 152.0, 162.4, 162.6, 187.4, 188.0

5-(2-Iodo-2-tosylacetyl)-2'-deoxyuridine (56). Method A. (β-Keto)sulfone analogue **51** (18 mg, 0.042 mmol) was dissolved in 1 mL MeOH. Then, NaOH(aq) (8.4 µL, 1.68 mg, 0.042 mmol) was added into the solution and was stirred for 30 min. Later, iodine monochloride (42 µL, 6.18 mg, 0.042 mmol) was added into solution and the resulting mixtures were stirred for 3 h . The solution was neutralized by adding dil. HCl and volatiles were evaporated. The residue was column chromatographed ($CHCl_3$/ MeOH; 100:0 → 95:5) to afford **56** as a (50/50) mixture of diastereomers (8 mg, 35%). ^{1}H NMR (DMSO-d_6) δ 2.08-2.30 (m, 2H, H2'2"), 2.40 (s, 3H, Me), 3.55-3.75 (m, 2H, H5',5"), 3.85 (q, *J* = 3.4 Hz, 1H, H4'), 4.22 (m, 1H, H3'), 5.13 (t, *J* = 4.6 Hz, 0.5H, 5'OH), 5.17 (t, *J* = 4.5 Hz, 0.5H, 5'OH), 5.28 (d, *J* = 4.4 Hz, 0.5H, 3'OH), 5.31 (d, *J* = 4.6 Hz, 0.5H, 3'OH), 5.58-6.10 (m, 1H, H1'), 7.30 (s, 1H, α-CH), 7.45 (d, *J* = 8.1 Hz, 2H, Ar), 7.75 (d, *J* = 8.1 Hz, 2H, Ar), 8.80 (s, 0.5H, H6), 8.82 (s, 0.5H, H6), 11.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.1, , 40.5, 41.0, 60.8, 60.9, 68.9, 69.9, 70.0, 86.3, 86.5, 88.0, 88.1, 107.7, 107.9, 128.5, 129.5, 129.6, 129.7, 129.8, 133.3, 133.4, 134.5, 134.6, 145.0, 145.2, 150.0, 150.2, 161.0, 161.1, 186.5

Method B. (β-Keto)sulfone **51** (40 mg, 0.094 mmol) was taken in a round bottom flask containing 0.3 mL of acetic acid. Then iodine (15 mg, 0.056 mmol) and 50% H_2O_2(aq) (60 µL, 0.94 mmol) were sequentially added into the flask. The resulting mixtures were stirred for 1 h at ambient temperature. The volatiles were evaporated and column chromatography ($CHCl_3$/ MeOH; 100:0 → 95:5) afforded 5-(α-iodo-β-keto)sulfone **56** (21 mg, 41%).

5-(2-Propanethio-2-tosylacetyl)-2'-deoxyuridine (57). The 5-(α-iodo-β-keto)sulfone **56** (10 mg, 0.018 mmol) was dissolved in 1 mL of MeOH. Then 1:1 mixture of propanethiol and

TEA(8.5 µL, 6.8 mg, 0.076 mmol) was added into a stirring solution of **56** at ambient temperature for 24 h. The volatiles were evaporated and column chromatography ($CHCl_3$/ MeOH; 100:0 → 95:5) afforded **57** as a (50/50) mixture of diastereomers (2 mg, 22%). ^{1}H NMR (DMSO-d_6) δ 2.08-2.30 (m, 2H, H2'2''), 2.40 (s, 3H, Me), 3.55-3.75 (m, 2H, H5',5''), 3.85 (q, *J* = 3.4 Hz, 1H, H4'), 4.22 (m, 1H, H3'), 5.13 (t, *J* = 4.6 Hz, 0.5H, 5'OH), 5.17 (t, *J* = 4.5 Hz, 0.5H, 5'OH), 5.28 (d, *J* = 4.4 Hz, 0.5H, 3'OH), 5.31 (d, *J* = 4.6 Hz, 0.5H, 3'OH), 5.58-6.10 (m, 1H, H1'), 7.30 (s, 1H, α-CH), 7.45 (d, *J* = 8.1 Hz, 2H, Ar), 7.75 (d, *J* = 8.1 Hz, 2H, Ar), 8.80 (s, 0.5H, H6), 8.82 (s, 0.5H, H6), 11.90 (s, 1H, NH)

8-(1-Chloro-2-tosylvinyl)adenine (67). Treatment of 8-ethynyl adenosine analogue **66** (100 mg, 0.198 mmol) with *p*-toluenesulfonyl hydrazide (110.6 mg, 0.594 mmol) in the presence of $FeCl_3 \cdot 6H_2O$ (108 mg, 0.396 mmol) by procedure A (column chromatography; $CHCl_3$/MeOH; 100:0 → 95:5) gave **67** (30 mg, 44%):) ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.49 (bs, 2H), 7.81(d, *J* = 8.2 Hz, 2H), 7.88 (s, 1H), 8.18 (s, 1H), 13.6 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 21.2, 119.4, 128.1, 129.8, 135.2, 136.4, 139.6, 145.0, 149.0, 150.7, 153.0, 156.2.

3',5'-Bis-(*O*-tert-butyldimetylsilyl)-8-(1-iodo-2-tosylvinyl)-2'-deoxyadenosine (68). TBDMS protected 8 ethynyl 2'-deoxyadenosine **66** (200 mg, 0.4 mmol) was dissolved in CH_3CN. Then, sodium salt of *p*-toluenesulfinic acid (205.6 mg, 1.2 mmol) , iodine (152.0 mg, 0.60mmol), and sodium acetate (49.2 mg, 0.6 mmol) were sequentially added into the solution. The resulting mixtures were stirred at 80^{o}C for 5 h. The volatiles was evaporated and column chromatography (Hexane/EA; 70:30 → 50:50) gave **68** (148 mg, 48%) as pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6H), 0.15 (s, 6H), 0.80 (s, 9H), 0.90 (s, 9H), 2.30-2.35 (m, 1H), 2.40 (s, 3H), 3.45-3.55 (m, 1H), 3.80 (bs, 1H), 4.00- 4.10 (m, 2H), 4.85 (bs, 1H), 5.85 (s,

2H), 6.20 (dd, *J* = 13.5, 6.7 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 2H), 7.58 (s,1H), 7.68 (bs, 2H), 8.32 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -0.6, -0.5,-0.4, -0.3, 18.0, 18.5, 22.0, 26.0, 38.0, 63.0, 73.0, 86.0, 88.0, 120, 128.3, 130.0, 136.0, 146.0, 147.5, 150.0, 153.0, 156.0.

***S*-[(*E*)-1-(2'-Deoxyuridin-5-yl)-2-tosylvinyl]-L-glutathione monophosphate (72).** The 5'-monophosphate **69** (12 mg, 0.022 mmol) was added in 1 mL of methanol-water (50:50) co-solvent system. Then, L-glutathione (8.9 mg, 0.029 mmol) and triethylamine (25.0 μL, 17.8 mg, 0.176 mmol) were sequentially added into the solution. The resulting mixtures were stirred at ambient temperature for 4 h. The volatiles were evaporated and sephadex column separation (TEAB buffer 0.1-0.3M) gave **72** (10 mg, 57%) as white solid. 1H NMR (D_2O) δ 2.15 (q, *J* = 7.4 Hz, 2H), 2.28-2.41 (m, 2H), 2.44 (s, 3H), 2.52 (dt, *J* = 15.5, 7.5 Hz, 2H), 3.32-3.38 (m, 1H), 3.70-3.90 (m, 5H), 4.05 (q, *J* = 3.9 Hz, 1H), 4.45(br, 1H), 4.65-4.72 (m, 2H), 6.30 (t, *J* = 6.4 Hz, 1H), 6.95 (s, 1H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.95 (s, 1H); ^{13}C NMR (D_2O) δ 20.8, 26.2, 31.5, 33.5, 39.5, 42.5, 52.0, 54.0, 59.0, 64.0, 85.0, 86.0, 108.7, 127.0, 130.0, 133.0, 136.0, 142.0, 146.3, 150.6, 161.8, 170.7, 174.0, 175.0, 176.2; ^{31}P NMR (D_2O) δ 3.75; HRMS calculated for $C_{28}H_{35}N_5NaO_{13}S_2$ $[M+Na]^+$ 736.1570, found 736.1513

General procedure for the phosphorylation of 5-(β-chloro)vinyl and 5-(β-keto)sulfone of 2'-deoxyuridne, synthesis of 5'-triphosphates (71 and 74). Procedure F. The 5-(β-Chloro)vinyl sulfone **9** or 5-(β-keto)sulfone **51** and proton sponge were placed in a flame dry round bottom flask. 1 mL trimethyl phosphate was added into the flask and the mixtures were allowed to cool at 0 ^{o}C for 5 min. $POCl_3$ was added and the mixtures were stirred for 30 min. at 0 ^{o}C. Then TBAPP 0.5M solution in DMF and tributylamine were added sequentially and the resulting mixtures were stirred for 10 min at 0 ^{o}C. The reactions were

quenched by dropwise adding 2M TEAB buffer. The reaction mixtures were extracted with ethylacetate two times and the aqueous layer was collected. The volatiles were evaporated and the residue was column chromatographed using Sephadex as adsorbent and TEAB buffer as eluting solovent to afford product **71** or **74**.

(*E*)-5-(1-Chloro-2-tosylvinyl)-2'-deoxyuridine triphosphate (71). Treatment of chlorovinyl sulfone **28** (30 mg, 0.067 mmol) solution in 0.5 mL $PO(OMe)_3$ with $POCl_3$ (16 μL, 26.2 mg, 0.17 mmol) in the presence of proton sponge (37 mg, 0.172 mmol) and subsequent treatment of the mixtures with 0.5 M TBAPP solution (700 μL, 192 mg, 0.35 mmol) and TBA (50 μL, 39 mg, 0.21 mmol) by procedure F and column chromatography (TEAB buffer; 0.1M → 0.5M) afforded 5'-triphosphate **71** (15 mg, 32%). ^{1}H NMR (D_2O) δ 2.28-2.40 (m, 2H, H2',2''), 2.45 (s, 3H, CH_3), 4.14-4.20 (m, 2H, H5',5''), 4.15-4.28 (m, 1H, H4'), 4.62 (dd, *J* = 5.7, 2.9 Hz, H3'), 6.28 (t, *J* = 6.7 Hz, 1H, H1'), 7.44 (d, *J* = 8.0 Hz, 2H, Ar), 7.45 (s, 1H, vinyl), 7.65 (d, *J* = 8.2 Hz, 2H, Ar), 8.20 (s, 1H, H6); ^{13}C NMR (D_2O) δ 21.0, 39.0, 66.0, 71.0, 85.0, 86.0, 108.5, 128.0, 130.5, 135.0, 136.0, 141.0, 142.5, 146.0, 150.0, 161.0; ^{31}P NMR (D_2O) δ -23.00 (β-P), -11.50 (α-P), -10.50 (γ-P); HRMS calcd for $C_{18}H_{21}ClN_2O_6P_3S$ $[M-H]^-$680.9513, found 680.9517

Note: Treatment of the crude product of the reaction between **9** and $POCl_3$ with 2M TEAB buffer and column chromatography using Sephadex as adsorbent and TEAB buffer as eluting system (0.1M → 0.25 M) gave 5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine phosphate **69**.

5-(2-Tosylacetyl)-2'-deoxyuridine triphosphate (74). Treatmento of *β*-keto sulfone analogue **51** (20 mg, 0.047 mmol) solution in 0.5 mL $PO(OMe)_3$ with $POCl_3$ (10 μL, 16.4 mg, 0.107 mmol) in the presence of proton sponge (21 mg, 0.097 mmol) and subsequent treatment of the mixtures with 0.M TBAPP solution (400 μL, 110 mg, 0.2 mmol) and TBA

(28 μL, 22 mg, 0.118 mmol) by procedure F and column chromatography (TEAB buffer; 0.1M → 0.5M) afforded desired 5'-triphosphate **74** (12 mg, 38%). ^{1}H NMR (D_2O) δ 2.35-2.48 (m, 2H, H2',2''), 2.45 (s, 3H, CH_3), 3.58-3.68 (m, 2H, H5',5''), 4.21 (dd, *J* = 5.6, 4.0 Hz, 2H, α-CH_2), 4.28 (br, 1H, H4'), 4.62 (dd, *J* = 6.0, 2.8 Hz, H3'), 6.13 (t, *J* = 6.4 Hz, 1H, H1'), 7.47 (d, *J* = 8.0 Hz, 2H, Ar), 7.65 (d, *J* = 8.0 Hz, 2H, Ar), 8.46 (s, 1H, H6); ^{13}C NMR (D_2O) δ 21.0, 39.0, 59.0, 65.0, 71.0, 87.0, 88.0, 112.0, 128.0, 130.5, 134.0, 148.0, 149.0, 150.0, 161.0, 185.0; ^{31}P NMR (D_2O) δ -23.50 (β-P), -11.70 (α-P), -11.00 (γ-P); HRMS calcd for $C_{18}H_{22}N_2O_{17}P_3S$ $[M-H]^-$ 662.9852, found 680.9859

General procedure for esterification of 5-(β-chloro)vinyl sulfone and 5-heteroarlyated compounds. Synthesis of liphophilic analogues (75-76 and 82-85). Procedure G. 5-(β-Chloro)vinyl sulfone or 5-arlyated compound was dissolved in 1 mL DMF. Undecanoic anhydride and DMAP were sequentially added into the solution and stirred at ambient temperature. The volatiles were evaporated and the residue was partitioned between CH_2Cl_2 and 0.1 M HCl/H_2O. The organic layer was washed with $NaHCO_3$/H_2O and brine and was column chromatographed to afford (**75-76** and **82-85).**

(*E*)-5'-*O*-Undecanoyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (75). Treatment of 5-(β-chloro)vinyl sulfone **9** (25 mg, 0.056 mmol) with undecanoic anhydride (24 mg, 0.067 mmol) and DMAP (6.0 mg, 0.05 mmol) in DMF at ambient temperature for 2 h by Procedure G and column chromatography (hexane/EtOAc, 90:10 → 60:40) afforded **75** (15 mg, 45%): ^{1}H NMR δ 0.88 (t, *J* = 6.6 Hz, 3H, CH_3), 1.24-1.39 (m, 14H, 7 x CH_2), 1.62 ("quin", *J* = 7.4 Hz, 2H, CH_2), 2.10-2.18 (m, 1H, H2'), 2.34 (t, *J* = 7.7 Hz, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.49 (ddd, *J* =13.8, 6.3, 4.0 Hz, 1H, H2''), 3.00 (br s, 1H, 3'-OH), 4.17-4.40 (m, 4H, H3',4',5',5''), 6.26 (t, *J* = 6.5 Hz, 1H, H1'), 6.85 (s, 1H, CH), 7.35 (d, *J* = 8.1 Hz, 2H, Ar), 7.74 (d, *J* = 8.1

Hz, 2H, Ar), 8.33 (s, 1H, H6), 8.44 (s, 1H, NH); ^{13}C NMR δ 14.2, 21.9 (CH_3), 22.0, 25.0, 29.2, 29.4, 29.5, 29.7, 29.8, 32.2, 34.4 (CH_2), 40.8 (C2'), 63.7 (C5'), 71.6 (C3'), 84.7 (C4'), 85.8 (C1'), 108.2 (C5), 128.0 (Ar), 130.3 (Ar), 134.0 (Ar), 136.0 (=CH), 140.8 (Ar), 143.0 (=CCl), 146.0 (C6), 149.3 (C2), 159.0 (C4), 174.0 (C=O); HRMS calcd for $C_{29}H_{39}{}^{35}ClN_2NaO_8S$ $[M+Na]^+$ 633.2013, found 633.2018.

Note: The (*E*)-3'-*O*-undecanoyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (7 mg, 20%) were also isolated: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H, CH_3), 1.22 -1.37 (m, 14H, 7 x CH_2), 1.58 -1.68 (m, 2H, CH_2), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2), 2.38 -2.42 (m, 1H, H2'), 2.44 (s, 3H, CH_3-tosyl), 2.5 -2.58 (m, 1H, H2''), 3.89 -3.94 (dd, 1H, H5'), 4.01 -4.06 (dd, 1H, H5''), 4.15 -4.19 (m, 1H, H4'), 5.38 -5.42 (dd, 1H, H3'), 6.34 -6.38 (dd, 1H, H1'), 6.87 (s, 1H, vinyl), 7.34 (d, *J* = 8.1 Hz, 2H-tosyl), 7.70 (d, *J* = 8.1 Hz, 2H-tosyl), 8.34 (s, 1H, H6), 8.44 (s,1H, NH).

(*E*)-3',5'-Di-*O*-undecanoyl-5-(1-chloro-2-tosylvinyl)-2'-deoxyuridine (76). Treatment of (25 mg, 0.056 mmol) with undecanoic anhydride (62 mg, 0.17 mmol) and DMAP (6.0 mg, 0.05 mmol) in DMF for 6 h by Procedure G and column chromatography (hexane/EtOAc, 100:0 → 80:20) gave **76** (32 mg, 75%): ^{1}H NMR δ 0.84-0.90 (m, 6H, 2 x CH_3), 1.20-1.37 (m, 28H, 14 x CH_2), 1.60-1.68 (m, 4H, 2 x CH_2), 2.35 ("t", *J* = 8.2 Hz, 4H, 2 x CH_2), 2.38-2.42 (m, 1H, H2'), 2.46 (s, 3H, CH_3), 2.57 (ddd, *J* = 12.8, 5.4, 2.1 Hz, 1H, H2''), 4.29-4.32 (m, 1H, H4'), 4.42 (dd, *J* = 12.1, 2.4 Hz, 1H, H5'), 4.50 (dd, *J* = 12.1, 2.3 Hz, 1H, H5''), 5.23-5.27 (m, 1H, H3'), 6.35(dd, *J* = 8.5, 5.5 Hz, 1H, H1'), 6.88 (s, 1H, vinyl), 7.34 (d, *J* = 8.1 Hz, 2H, Ar), 7.70 (d, *J* = 8.1 Hz, 2H, Ar), 7.90 (s, 1H, H6), 8.34 (s, 1H, NH); ^{13}C NMR δ 14.2, 21.8 (CH_3), 22.8, 24.9, 25.0, 29.3, 29.37, 29.43, 29.5, 29.58, 29.63, 29.69, 29.72, 32.0, 34.2, 34.3 (CH_2), 38.2 (C2'), 63.8 (C5'), 74.2 (C3'), 83.3 (C4'), 85.8 (C1'), 109.1 (C5), 128.0 (Ar), 130.2 (Ar),

134.9 (=CH), 136.9 (Ar), 139.9 (Ar), 141.6 (C6), 145.6 (=CCl), 149.1 (C2), 158.6 (C4), 173.3, 173.4 (C=O); HRMS calcd for $C_{40}H_{59}{}^{35}ClN_2NaO_9S$ $[M+Na]^+$ 801.3527, found 801.3505.

3',5'-Di-*O*-acetyl-5-(5-heptylfur-2-yl)-2'-deoxyuridine (79). 2[121] (150 mg, 0.34 mmol) was dissolved in 2 mL of DMF. 2-Heptylfuran (0.6 mL, 565 mg, 3.4 mmol), TBAF (2.3 mL, 2.38 mmol), and $Pd_2(dba)_3$ (12 mg, 0.021 mmol) were sequentially added into the solution. The resulting mixtures were stirred at 100 ^oC for 1 h. The volátiles were evapórated and column chromatography; hexane/EtOAc, 80:20 → 60:40 gave **79** (86 mg, 60%): ^1H NMR δ 0.87 (t, *J* = 7.1 Hz, 3H, CH_3), 1.22-1.37 (m, 8H, 4 x CH_2), 1.61 (q, *J* = 7.4 Hz, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.25 ("ddd", *J* = 16.6, 8.7, 2.2 Hz, 1H, H2'), 2.50-2.57 (m, 1H, H2''), 2.59 (t, *J* = 7.5 Hz, 2H, CH_2), 4.30-4.34 (m, 1H, H4'), 4.38-4.42 (m, 2H, H5',5''), 5.28 ("dt", *J* = 6.4, 1.6 Hz, 1H, H3'), 6.05 (d, *J* = 3.3 Hz, 1H, furan), 6.40 (dd, *J* = 8.6, 5.5 Hz, 1H, H1'), 6.98 (d, *J* = 3.3 Hz, 1H, furan), 7.85 (s, 1H, H6), 9.30 (s, 1H, NH); ^{13}C NMR δ 14.2 (CH_3), 20.9, 21.1 (Ac), 22.8, 28.1, 28.2, 29.2, 29.3, 31.9 (CH_2), 38.1(C2'), 61.2 (C5'), 74.7 (C3'), 82.7 (C4'), 85.7 (C1'), 107.4 (furan), 108.2 (C5), 111.0 (furan), 131.3 (C6), 143.8, 149.4 (furan), 156.2 (C2), 159.9 (C4), 170.3, 170.5 (Ac); HRMS calcd for $C_{24}H_{32}N_2NaO_8$ $[M+Na]^+$ 499.2056, found 499.2078.

5-(5-Heptylfur-2-yl)-2'-deoxyuridine (81). 5-Iodo-2'-deoxyuridine **77** (53 mg, 0.15 mmol) was dissolved in 2 mL DMF. Then, 2-heptylfuran (0.29 mL, 249 mg, 1.5 mmol), TBAF (1 mL, 1.05 mmol), $Pd_2(dba)_3$ (10 mg, 0.017 mmol) were sequentially added into the solution. The mixtures were stirred at 100 ^oC for 1 h. The volatiles were evaporated and column chromatography (hexane/EtOAc, 20:80) gave **81** (35 mg, 61%): UV (MeOH) λ_{max} 256, 326 nm (ε 14 250, 11 300), λ_{min} 287 nm (ε 4000); ^1H NMR (DMSO-d_6) δ 0.86 (t, *J* =6.7

Hz, 3H, CH_3), 1.24-1.31 (m, 8H, 4 x CH_2), 1.60 (quin, *J* = 6.7 Hz, 2H, heptyl), 2.17 ("dd", *J* = 6.6, 4.9 Hz, 2H, H2',2"), 2.60 (t, *J* = 7.4 Hz, 2H, CH_2), 3.60-3.62 (m, 2H, H5',5"), 3.83 (q, *J* = 3.3 Hz, 1H, H4'), 4.29 (quin, *J* = 4.2, 1H, H3'), 5.05 (t, *J* = 5.0 Hz, 1H, 5'-OH), 5.28 (d, *J* = 4.1 Hz, 1H, 3'-OH), 6.11 (d, *J* = 3.1 Hz, 1H, furan), 6.21 (t, *J* = 6.6 Hz, 1H, H1'), 6.72 (d, *J* = 3.1 Hz, 1H, furan), 8.27 (s, 1H, H6), 11.58 (s, 1H, NH); ^{13}C NMR δ 14.2, 22.1, 27.0, 28.6, 31.1, 39.4, 60.8, 70.3, 84.5, 87.4, 105.7, 106.8, 108.6, 133.6, 144.5, 149.6, 154.8, 160.0; HRMS calcd for $C_{20}H_{29}N_2O_6$ $[M+H]^+$ 393.2020, found 393.2023

5'-*O*-Undecanoyl-5-(fur-2-yl)-2'-deoxyuridine (82). Treatment of **80**[122] (25 mg, 0.08 mmol) with undecanoic anhydride (34 mg, 0.096 mmol) in the presence of DMAP (6.0 mg, 0.05 mmol) in DMF for 2 h by Procedure G and column chromatography; hexane/EtOAc, 100:0 → 70:30) gave **82** (18 mg, 52%) and **83** (5 mg, 10%) in addition to unchanged **80** (~15%; TLC). Compound **82** had: ^{1}H NMR δ 0.88 (t, *J* = 7.1 Hz, 3H, CH_3), 1.22-1.40 (m, 14H, 7 x CH_2), 1.60 ("quin", *J* = 7.3 Hz, 2H, CH_2), 2.10-2.17 (m, 1H, H2'), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2), 2.50 (ddd, *J* =13.7, 6.3, 3.9 Hz, 1H, H2"), 3.05 (br s, 1H, 3'-OH), 4.15 (q, *J* = 3.9, 1H, H4'), 4.25 (dd, *J* = 12.1, 3.3 Hz, 1H, H5'), 4.36-4.42 (m, 2H, H3',5"), 6.26 (t, *J* = 6.3 Hz, 1H, H1'), 6.60 (dd, *J* = 3.3, 1.8 Hz, 1H, furan), 7.05 (d, *J* = 3.5 Hz, 1H, furan), 7.38 (d, *J* = 1.2 Hz, 1H, furan), 8.25 (s, 1H, H6), 8.38 (s, 1H, NH); HRMS calcd for $C_{24}H_{34}N_2NaO_7$ $[M+Na]^+$ 485.2264; found 485.2271.

Note: 3'-*O*-Undecanoyl-5-(fur-2-yl)-2'-deoxyuridine (6.0 mg, 15%) was also isolated: ^{1}H NMR ($CDCl_3$) δ 0.85-0.93 (m, 6H, 2 x CH_3), 1.22 -1.37 (m, 24H, 12 x CH_2), 1.58 -1.68 (m, 4H, 2 x CH_2), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2- undecanoyl), 2.45 (dt, 1H, H2'), 2.55 (dt, 1H, H2"), 2.64 (t, 2H, *J* = 7.6 Hz, CH_2- heptyl), 3.90 - 4.00 (dd, 1H, H5'5"), 4.13 -4.18 (m, 1H,

H4'), 5.38 -5.42 (dd, 1H, H3'), 6.05 (d, , *J* = 3.2 Hz, 1H-furan), 6.29 -6.35 (dd, 1H, H1'), 6.90 (d, *J* = 3.2 Hz, 1H-furan), 8.10 (s, 1H, H6), 8.44 (s,1H, NH).

3',5'-Di-*O*-undecanoyl-5-(fur-2-yl)-2'-deoxyuridine (83). Treatment of **80**[122] (25 mg, 0.08 mmol) with undecanoic anhydride (84 mg, 0.24 mmol) in the presence of DMAP (6.0 mg, 0.05 mmol) by Procedure G (6 h) gave **83** (38.5 mg, 77%): ^{1}H NMR δ 0.82-0.91 (m, 6H, 2 x CH_3), 1.20-1.40 (m, 28H, 14 x CH_2), 1.60-1.68 (m, 4H, 2 x CH_2), 2.24 (ddd, *J* = 14.6, 8.6, 6.5 Hz, 1H, H2'), 2.33-2.40 (m, 4H, 2 x CH_2), 2.54 (ddd, *J* = 14.1, 5.6, 1.3 Hz, 1H, H2''), 4.30 ("q", *J* = 2.7 Hz, 1H, H4'), 4.36 (dd, *J* = 12.2, 2.8 Hz, 1H, H5'), 4.45 (dd, *J* =11.8, 3.5 Hz, 1H, H5''), 5.27 ("dt", *J* = 6.4, 1.6 Hz, 1H, H3'), 6.40 (dd, *J* = 8.8, 6.1 Hz, 1H, H1'), 6.47 (dd, *J* = 3.3, 1.8 Hz, 1H, furan), 7.05 (d, *J* = 3.4 Hz, 1H, furan), 7.33 (d, *J* = 1.6 Hz, 1H, furan), 8.00 (s, 1H, H6), 8.95 (s, 1H, NH); ^{13}C NMR δ 14.5, 23.0, 25.1, 25.2, 29.3, 29.4, 29.6, 29.7, 29.9, 32.3, 34.1, 34.3, 34.5, 39.0 (C2'), 64.0 (C5'), 74.6 (C3'), 83.2 (C4'), 86.0 (C1'), 108.0 (C5), 110.0 (furan), 112.5 (furan), 133.0 (C6), 142.0 (furan), 146.0 (furan), 149.5 (C2), 160.2 (C4), 173.2, 173.4 (C=O); HRMS calcd for $C_{35}H_{54}N_2NaO_8$ $[M+Na]^+$ 653.3778, found 653.3778.

5'-*O*-Undecanoyl-5-(5-heptylfur-2-yl)-2'-deoxyuridine (84). Treatment of **81** (25 mg, 0.064 mmol) with undecanoic anhydride (27.2 mg, 0.0768 mmol) in the presence of DMAP (6.0 mg, 0.05 mmol) for 2 h and column chromatography (hexane/EtOAc, 100:0 → 80:20) gave **84**(17 mg, 48%). ^{1}H NMR δ 0.85-0.90 (m, 6H, 2 x CH_3), 1.28-1.31 (m, 22H, 11 x CH_2), 1.54-1.64 (m, 4H, 2 x CH_2), 2.13-2.16 (m, 1H, H2'), 2.27-2.32 (m, 2H, CH_2), 2.46 (ddd, *J* =13.9, 6.4, 4.3 Hz, 1H, H2''), 2.56 (t, *J* = 7.4 Hz, 2H, CH_2), 2.98 (s, 1H, 3'-OH), 4.19 (q, *J* = 3.5, 1H, H4'), 4.28 (dd, *J* = 12.3, 3.4 Hz, 1H, H5'), 4.33-4.41 (m, 2H, H3',5''), 6.05 (d, *J* = 3.2 Hz, 1H-furan), 6.28 (t, *J* = 6.4 Hz, 1H, H1'), 6.90 (d, *J* = 3.2 Hz, 1H-furan), 8.10 (s, 1H, H6), 8.44 (s,1H, NH); ^{13}C NMR δ 14.2, 22.9 (CH_3), 24.9, 28.1, 28.2, 29.2, 29.2, 29.3, 29.4, 29.5,

29.6, 29.7, 31.9, 32.1, 34.2, 34.3, 34.4 (CH_2), 40.7 (C2'), 63.6 (C5'), 71.8 (C3'), 84.4 (C4'), 85.5 (C1'), 108.2 (C5), 110.6 (furan), 113.0 (furan), 129.0 (furan), 133.0 (C6), 149 (C2), 156.0 (furan), 159.6 (C4), 174.0 (C=O); HRMS calcd for $C_{31}H_{48}N_2NaO_7$ $[M+Na]^+$ 583.3359, found 583.3375.

Note: 3'-*O*-undecanoyl-5-(heptylfur-2-yl)-2'-deoxyuridine (6.1 mg, 17%) was also isolated: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 7.1 Hz, 3H, CH_3), 1.22 -1.40 (m, 14H, 7 x CH_2), 1.60 -1.65 (m, 2H, CH_2), 2.35 (t, *J* = 7.6 Hz, 2H, CH_2), 2.44 -2.58 (m, 2H, H2'), 4.0 (dd, 2H, H5'), 4.15(m, 1H, H4'), 5.40 (dd, 1H, H3'), 6.35 (dd, 1H, H1'), 6.6 (dd, 1H, furan), 7.05 (d, *J* = 3.5 Hz, 1H, furan), 7.38 (d, *J* = 1.2 Hz, 1H, furan), 7.34 (d, *J* = 8.1 Hz, 2H-tosyl), 8.25 (s, 1H, H6), 8.38 (s, 1H, NH).

3',5'-Di-*O*-undecanoyl-5-(5-heptylfur-2-yl)-2'-deoxyuridine (85). Treatment of **81** (25 mg, 0.064 mmol) with undecanoic anhydride (65 mg, 0.19 mmol) in the presence of DMAP (6.0 mg, 0.05 mmol) for 6 h and column chromatography (hexane/EtOAc, 100:0 → 80:20) gave **85** (36 mg, 80%). ^{1}H NMR δ 0.83-0.92 (m, 9H, 3 x CH_3), 1.22-1.38 (m, 36H, 18 x CH_2), 1.55-1.70 (m, 6H, 3 x CH_2), 2.25 (ddd, *J* = 14.7, 8.5, 6.6 Hz, 1H, H2'), 2.30-2.40 (m, 4H, 2 x CH_2), 2.53-2.57 (m, 1H, H2''), 2.60 (t, *J* = 7.6 Hz, 2H, CH_2), 4.31 (q, *J* = 3.0 1H, H4'), 4.38 (dd, *J* = 12.2, 2.8 Hz, 1H, H5'), 4.42 (dd, *J* =11.3, 3.8 Hz, 1H, H5''), 5.26 ("dt", *J* = 6.8, 1.6 Hz, 1H, H3'), 6.05 (d, *J* = 3.2 Hz, 1H, furan), 6.37 (dd, *J* = 8.8, 6.1 Hz, 1H, H1'), 6.95 (d, *J* = 3.3 Hz, 1H, furan), 7.88 (s, 1H, H6), 9.00 (s, 1H, NH); HRMS calcd for $C_{42}H_{68}N_2NaO_8$ $[M+Na]^+$ 751.4873, found 751.4851.

4.3. Incorporation of 5-modified nucleotides into DNA

Materials of enzymatic reactions

All DNA oligonucleotides were synthesized following the Integrated DNA Technologies (Coralville, IA). The radionucleotides γ-^{32}P] ATP (6000 mCi/mmol) was purchased from MP biomedicals Inc. (Santa Ana, CA) while T4 polynucleotide kinase and deoxynucleoside 5'-triphosphates (dNTPs) were purchased from Thermo Scientific (Pittsburgh, PA). Micro Bio-Spin TM 6 Columns were purchased from Bio-Rad (Hercules, CA). All other chemicals were purchased from Thermo Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Purified DNA polymerase β (pol β) were purified following the procedures described previously.[123,124] Klenow fragment was bought from New England Biolabs (Ipswitch, MA).

Oligonucleotide substrates

Substrates with an upstream primer annealed to the template strand were designated as open template substrates. The substrates were made by annealing upstream primer (31nt) with the template strand (71 nt) at a molar ratio of 1:3. The substrate containing one-nucleotide gap were made by annealing an upstream primer and downstream primer with the template strand at the molar ratio of 1:3:3. The open template and one-nucleotide gap substrates were employed to mimic the intermediates formed during DNA replication.

Enzymatic activity assay

Nucleotides incorporation by DNA polymerases were performed by incubating different concentrations of pol β or Klenow fragment with 25 nM ^{32}P labeled substrates at 37°C for 15 min. The enzymatic reactions were assembled in the presence of 5-(β-chlorovinyl)sulfone of dU (50 μM) or 5-(β-keto)sulfone of dU (50 μM) alone or in the presence of 50 μM 5-(β-chlorovinyl)sulfone of dU or 50 μM 5-(β-keto)sulfone of dU along with 50 μM dATP, 50 μM

dCTP and 50 μM dGTP. This allows to examine if 5-(β-chlorovinyl)sulfone of dU or 5-(β-keto)sulfone of dU residue can be directly incorporated into a double-strand DNA during DNA leading and lagging strand maturation. DNA synthesis was separated in a 15% urea denaturing polyacrylamide gel were detected by Pharos FX Plus PhosphorImager (Bio-Rad Laboratory, CA).

4.4. Cytotoxic evaluation

Proliferation Assays

Human cervical carcinoma (HeLa) cells were seeded in 96-well plates at 15,000 cells/well in the presence of 5-fold dilutions of the compounds. After 4 days of incubation, the cells were trypsinized and counted by means of a Coulter counter (Analis, Belgium). Suspension cells (Mouse leukemia L1210 and human lymphoid CEM cells) were seeded in 96-well plates at 60,000 cells/well in the presence of the compounds. L1210 and CEM cells were allowed to proliferate for 48 h or 96 h, respectively and then counted. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce cell proliferation by 50%.

Antiviral Assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strain G, varicella-zoster virus (VZV) strain Oka, TK^- VZV strain 07-1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Sindbis, reovirus-1, Punta Toro, human immunodeficiency virus

type 1 strain IIIB and human immunodeficiency virus type 2 strain ROD. The antiviral, other than anti-HIV, assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 CCID50 of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 or 100 plaque forming units (PFU) (VZV or HCMV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%. Cytotoxicity of the test compounds was expressed as the minimum cytotoxic concentration (MCC) or the compound concentration that caused a microscopically detectable alteration of cell morphology

5. CONCLUSION

I have successfully synthesized 5-(1-chloro/bromo-2-tosylvinyl)pyrimidine nucleosides from their corresponding 5-ethynyl analogues via transition metal catalyzed halosulfonylation reaction with moderate to excellent yield (45-90%). The 5-(1-iodo-2-tosylvinyl)pyrimidine nucleosides analogues were obtained via halosulfonylation reaction by reaction of 5-ethynyl pyrimidine nucleoside analogue with sodium salt of *p*-toluenesulfinic acid and *N*-iodosuccinamide in moderate yield (42-50%). The physical, chemical, and biological properties of these novel 5-(1-halo-2-tosylvinyl)pyrimidine nucleosides analogues were also explored. It was discovered that unlike the unsubsituted vinyl sulfone, the (β-halo)vinyl sulfones underwent addition-elimination reaction efficiently with varieties of nucleophiles such as amines, thiols etc. Although the (β-halo)vinyl sulfones substrates possesses exclusively *E* stereochemistry at the vinylic position, their addition-elimination products possess either *E* or *Z* stereochemistry which was found to be dependent on the type of nucleophiles substitute the halogen atom at the β-position. If the halogen atom is substituted by the thiolate nucleophile, the addition-elimination reaction occurs with retention of configuration to give exclusively *E* stereochemistry. If the same halogen atom of the vinyl sulfone analogue is replaced by amino nucleophile in the analogous reaction the stereochemistry of resulting aminovinylsulfones is *Z* which means that reaction occurs with the inversion of configuration. From the kinetics data it was determined that the rate of reaction follows the descending order as I ≥ Br > Cl. That means the rate of addition-elimination reaction of novel (β-halo)vinyl sulfone analogues is dependent on the halogen

atoms attached at the β-position. The vinylic proton exchange with deuterium during the addition-elimination reaction was investigated by carrying the reaction in different deuterated solvents such as DMSO-d_6, D_2O, or MeOH-d_4. The cysteine and tripeptide L-glutathione were incorporated at the β-position of the uracil nucleoside analogues via conjugated addition-elimination reaction. Antiproliferative and antivirul properties of the (β-halo)vinyl sulfone analogues were examined and it was found that acetyl protected (β-chloro)vinyl sulfone analogue inhibited the growth of the L1210, CEM, and HeLa cancer cells in the lower μM range. The lipophilic undecanoyl groups were incorporated at the sugar moiety via esterification reaction to enhance the cell permeability of these analogues but unfortunately they did not show improved cytostatic activity.

Novel (β-keto)sulfone analogues of 5-modified pyrimidine nucleoside have been synthesized and their reactivity at the acidic α-carbon were explored. So, 5-(β-chloro)vinylsulfones were efficiently converted into the corresponding 5-(β-keto)sulfone analogues *via* a one pot synthetic procedure. Thus, (*E*)-(β-chloro)vinylsulfone analogues were converted into (*Z*)-(β-amino)vinylsulfone by treatment with methanolic ammonia. Subsequent acid hydrolysis efficiently converted the intermediary amino sulfone into β-keto sulfone analogues with good yield (60-70%). Since the proton at α-carbon of β-keto sulfone is acidic, novel chemistry at the α-carbon of β-keto sulfones were investigated. The reactivity at the α-carbon of β-keto sulfone analogues with different electrophiles and nucleophiles were elaborately explored to find a suitable way to incorporate amino acid residues at the α-carbon which would verify the feasibility of the bioconjugation of the 5-(β-keto)sulfone-modified DNA with proteins. Different electrophiles derived from benzyl, methyl, or allyl halides were efficiently incorporated at the α-carbon of β-keto sulfone analogues. In addition, it was

demonstrated that in two step protocols the α-carbon of β-keto sulfones can be also substituted by nucleophile. Thus, treatment of 5-(β-keto)sulfone-modified 2'-deoxyuridine with iodinemonochloride afforded the product with the α-carbon substituted by iodo group. Subsequently, this iodo group served as a good leaving group and was displaced by the thiolate in the presence of triethyl amine. Such approach might be extended to incorporate amino acid possessing active nucleophilic groups at the α-carbon of the β-keto sulfones.

The silyl protected 8-(1-iodo-2-tosylvinyl)-2'-deoxyadenosine was also efficiently synthesized by the iodine mediated halosulfonylation of the corresponding 8-alkynyl substrates with the yield of 48%. The success of this reaction was encouraging to extend this halosulfonylation reaction in future into other purine nucleoside systems.

The 5-(β-chloro)vinylsulfone- or 5-(β-keto)sulfone modified 2'-deoxyuridine were efficiently converted to the 5'-triphosphates by applying Yoshikawa method followed by treatment with ammonium pyrophosphate in the presence of tributylamine. These nucleotides were successfully inserted into the DNA by polymerase-catalyzed reactions with either human DNA repaired polymerase (pol β) or bacterial polymerase (pol I). The (β-chloro)vinyl sulone analogue was efficiently incorporated both into the open template that leads the DNA synthesis and one nucleotide gap template that lags the DNA synthesis via bacterial polymerase (Pol I) catalyzed reaction. However, the human DNA polymerase (pol β) catalyzed process was successful to incorporate the chlorovinyl analogue into the one nucleotide gap template only. The (β-keto) sulfone analogue was efficiently inserted into DNA open template and one nucleotide gap template either by human DNA repaired polymerase (pol β) or bacterial polymerase (pol I).

The 5-(β-chloro)vinyl and/or 5-(β-keto) sulfones might be bioconjugated with the proteins which are involved in DNA replication process. Specifically it would be interested to examine whether the (β-chloro)vinyl sulfone modified DNA might bioconjugate with thiol residues of transcription factors such as c-Myc protein and thus uncover the mystery of critical biological functions related to DNA-c-Myc protein interactions.

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PUBLICATIONS AND PRESENTATIONS

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