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Design and Synthesis of Novel Nucleoside Analogues: Oxidative and Reductive Approaches toward Synthesis of 2'-Fluoro Pyrimidine Nucleosides

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DESIGN AND SYNTHESIS OF NOVEL NUCLEOSIDE ANALOGUES: OXIDATIVE
AND REDUCTIVE APPROACHES TOWARD SYNTHESIS OF 2'-FLUORO
PYRIMIDINE NUCLEOSIDES

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Ramanjaneyulu Rayala

2015

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

This dissertation, written by Ramanjaneyulu Rayala, and entitled Design and Synthesis of Novel Nucleoside Analogues: Oxidative and Reductive Approaches toward Synthesis of 2'-Fluoro Pyrimidine Nucleosides, 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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DEDICATION

This Ph.D. is dedicated to:

Padma Rayala, Nagaiah Rayala, Aneela Rayala, Yashitha Rayala, Radhika Mandapalli,
Muralidhar Mandapalli, Rishitha Chowdary Mandapalli, and Srikarprasad Chowdary
Mandapalli.

You have been a great inspiration.

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ABSTRACT OF THE DISSERTATION

DESIGN AND SYNTHESIS OF NOVEL NUCLEOSIDE ANALOGUES: OXIDATIVE
AND REDUCTIVE APPROACHES TOWARD SYNTHESIS OF 2'-FLUORO
PYRIMIDINE NUCLEOSIDES

by

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Florida International University, 2015

Miami, Florida

Professor Stanislaw F. Wnuk, Major Professor

Fluorinated nucleosides, especially the analogues with fluorine atom(s) in the ribose ring, have been known to exert potent biological activities. The first part of this dissertation was aimed at developing oxidative desulfurization-fluorination and reductive desulfonylation-fluorination methodologies toward the synthesis of 2'-mono and/or 2',2'-difluoro pyrimidine nucleosides from the corresponding 2'-arylthiopyrimidine precursors. Novel oxidative desulfurization-difluorination methodology was developed for the synthesis of α,α -difluorinated esters from the corresponding α -arylthio esters, wherein the arylthio group is present on a secondary internal carbon. For the reductive desulfonylation studies, cyclic voltammetry was utilized to measure the reduction potentials at which the sulfone moiety of substrates can be cleaved.

The 5-bromo pyrimidine nucleosides and 8-bromo purine nucleosides act as crucial intermediates in various synthetic transformations. The second part of the present dissertation was designed to develop a novel bromination methodology using 1,3-dibromo-5,5-dimethylhydantoin (DBH). Various protected and deprotected pyrimidine

and purine nucleosides were converted to their respective C5 and C8 brominated counterparts using DBH. The effect of Lewis acids, solvents, and temperature on the efficiency of bromination was studied. Also, *N*-bromosuccinimide (NBS) or DBH offered a convenient access to 8-bromotoyocamycin and 8-bromosangivamycin.

Third part of this research work focuses on the design and synthesis of 6-*N*-benzylated derivatives of 7-deazapurine nucleoside antibiotics, such as tubercidin, sangivamycin and toyocamycin. Target molecules were synthesized by two methods. First method involves treatment of 7-deazapurine substrates with benzylbromide followed by dimethylamine-promoted Dimroth rearrangement. The second method employs fluoro-diazotization followed by S_NAr displacement of the 6-fluoro group by a benzylamine. The 6-*N*-benzylated 7-deazapurine nucleosides showed type-specific inhibition of cancer cell proliferation at micromolar concentrations and weak inhibition of human equilibrative nucleoside transport protein (hENT1).

In the fourth part of this dissertation, syntheses of C7 or C8 modified 7-deazapurine nucleosides, which might exhibit fluorescent properties, were undertaken. 8-Azidotoyocamycin was synthesized by treatment of 8-bromotoyocamycin with sodium azide. Strain promoted click chemistry of 8-azidotoyocamycin with cyclooctynes gave the corresponding 8-triazolyl derivatives. Alternatively, 7-benzotriazolyl tubercidin was synthesized by iodine catalyzed CH arylation of tubercidin with benzotriazole.

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LIST OF ABBREVIATIONS

Ac	acetyl
Ar	aromatic (NMR)
β	beta
Bn	benzyl
br	broad (NMR)
<i>t</i> -But	<i>tert</i> -butyl
calcd	calculated (MS or HRMS)
CDA	cytidine deaminase
° C	degrees Celsius
δ	delta
d	doublet (NMR) or deuterium (in DMSO- <i>d</i> ₆)
DAST	diethylaminosulfur trifluoride
dCK	deoxycytidine kinase
DCM	dichloromethane
DCTD	deoxycytidylate deaminase
dFdC	2',2'-difluoro-2'-deoxycytidine
dFdCMP	2',2'-difluoro-2'-deoxycytidine monophosphate
dFdCDP	2',2'-difluoro-2'-deoxycytidine diphosphate
dFdCTP	2',2'-difluoro-2'-deoxycytidine triphosphate
DMAP	4-(<i>N,N</i> -dimethylamino)pyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide

DNA	deoxyribonucleic acid
dUMP	deoxyuridine monophosphate
ϵ	epsilon
ESI	electrospray ionization
Et	ethyl
FdC	2'-fluoro-2'-deoxycytidine
g	gram(s)
h or hr or hrs	hour(s)
Hz	hertz
hCNT	human concentrative nucleoside transporter
hENT	human equilibrative nucleoside transporter
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
IC ₅₀	half maximal inhibitory concentration
<i>J</i>	coupling constant in Hz (NMR)
λ	lambda
L	liter(s)
LDA	lithium diisopropylamide
m	milli; multiplet (NMR)
μ	micro
M	moles per liter
MeFdC	2'-deoxy-2'-fluoro-2'-C-methylcytidine
min	minute(s)

mol	mole(s)
MS	mass spectrometry
m/z	mass to charge ratio (MS)
NMR	nuclear magnetic resonance
nM	nano Molar
<i>p</i>	para
PET	Positron Emission Tomography
pyr	pyridine
%	percentage
q	quartet (NMR)
quin	quintet (NMR)
RNA	ribonucleic acid
RNR	Ribonucleotide reductase
rt	room temperature
RP	reverse phase (HPLC)
s	second(s); singlet (NMR)
SET	single electron transfer
t	triplet (NMR)
TBDMS	<i>tert</i> -butyldimethylsilyl
TBDPS	<i>tert</i> -butyldiphenylsilyl
TBAF	tetra- <i>n</i> -butylammonium fluoride
TBHP	<i>tert</i> -butyl hydroperoxide
TEA	triethylamine

THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
t_R	retention time (HPLC)
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
VIS	visible
<i>vs</i>	versus

1. INTRODUCTION

In 1909, the term “nucleoside” was proposed by Levene and Jacobs to describe carbohydrate derivatives of purines and pyrimidines.¹ Nucleosides, held together by phosphodiester linkages, are the core components of the biopolymers DNA and RNA. Nucleosides are composed of a furanose sugar moiety, linked to either a purine or a pyrimidine heterocyclic base via a beta-glycosidic bond. The four nucleosides prevailing in RNA are adenosine, guanosine, cytosine and uridine. Whereas, 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine and thymidine form the core part in DNA (see Figure 1 for structures of RNA and DNA nucleosides). Development of drug therapy progressed rapidly after the discovery of DNA as the primary genetic material in 1944² and subsequent elucidation of its physical structure in 1953.³ Since then, DNA and RNA have been regarded as potential targets for drug design, as they play vital role in many biological processes. Since nucleosides form the major part of DNA and RNA, nucleosides have also been considered prospective targets.

Figure 1. Structures of RNA and DNA nucleosides³

1.1. Notable nucleoside analogues with anticancer and antiviral activity

Pyrimidine nucleoside analogues such as 5-fluoro-2'-deoxyuridine (Floxuridine), 1-(β -D-arabinofuranosyl)cytosine (araC) were among the initial nucleoside analogues approved by U.S. Food and Drug Administration (FDA) for cancer treatment (Table 1). Remarkable biological activity resulting from slight structural modifications to the normal nucleosides prompted various scientists all over the world to develop modified nucleoside analogues as anticancer agents. Currently, there are 11 nucleoside based drugs approved by FDA for the treatment of cancer.

Table 1. FDA approved anticancer purine and pyrimidine nucleoside analogues.⁴

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

Parallel to the anticancer drugs, researchers also developed various nucleoside analogues with antiviral properties. The sounding success of prominent antiviral drugs such as Acyclovir (anti-HSV), and Zidovudine (anti-HIV) revealed the potential value of

modified nucleoside analogues as antiviral agents (Table 2). There are currently 13 FDA approved nucleoside based drugs used in the treatment of various viruses. Most recent of them being Sofosbuvir, an anti-HCV drug, which is considered to be a huge step forward in HCV treatment.^{5,6}

Table 2. Antiviral properties of purine and pyrimidine nucleoside analogues.⁴

Drug	Category	Year
2'-deoxy-2'-fluoro-2'-methyluridine-5'-phosphate (Sofosbuvir)	Uridine	2013
Tenofovir disoproxil fumarate, mixture of purine analogues (Tenofovir)	Purine	2008
1-2-deoxy- β -L-erythro-pentofuranosylthymine (Telbivudine)	Thymidine	2006
5-fluoro-1-(2 <i>R</i> ,5 <i>S</i>)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (Emtricitabine)	Cytidine	2003
2',3'-didehydro-2',3'-dideoxythymidine (Stavudine)	Thymidine	2001
2',3'-dideoxy-3'-thiacytidine (Lamivudine)	Cytidine	1998
2',3'-didehydro-2',3'-dideoxythymidine (Stavudine)	Thymidine	1994
3'-azido-3'-deoxythymidine (Zidovudine)	Thymidine	1987
2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6 <i>H</i> -purin-6-one (Acyclovir)	Guanosine	1982
(<i>E</i>)-5-(2-bromovinyl)-2'-deoxyuridine (Brivudine)	Uridine	1980
9- β -D-arabinofuranosyladenine (Vidarabine)	Adenosine	1976
5-ethyl-2'-deoxyuridine (Edoxudine)	Thymidine	1969
5-iodo-2'-deoxyuridine (Idoxuridine)	Uridine	1962

1.2. Importance of fluorine substitution

Fluoro organic compounds have been known to possess a wide variety of biological activity and several of them are used as drugs for the treatment of various types of diseases.^{7,8} Introduction of fluorine atom(s) into the organic molecules dramatically changes their electronic and steric properties, which often leads to the molecules rendering potent biological activities.^{9,10} Though a fluorine atom is close in size to a hydrogen atom, presence of fluorine causes various electronic changes because of its electronegativity. Moreover, fluorine atoms can also act as hydrogen bond

acceptors. Also, a fluorine atom can serve as a replacement for hydrogen, since the Van der Waals radius of fluorine (1.35 Å) is so close to that of a hydrogen atom (1.1 Å). The C-F bond strength (484 kJ/mol) is also greater than that of a C-H bond (411 kJ/mol), offering a measure of increased biological and chemical stability to the structure.

1.3. Prominent nucleoside analogues with fluorine atom in sugar moiety

Of the variety of nucleoside analogues with biological activity, the class of fluoro nucleosides is known to possess a wide range of medicinal and biological activity.¹¹ Prominent examples of fluorinated nucleoside analogues include: 2'-deoxy-2',2'-difluorocytidine (**1**, Gemcitabine)^{12,13} the phosphoramidate analogue of 2'-deoxy-2'-fluoro-2'-*C*-methyluridine (**2**, Sofosbuvir) and 2-chloro-1-(2'-deoxy-2'-fluoroarabinofuranosyl)uracil (**3**, Clofarabine)^{14,15} (Figure 2). Gemcitabine (**1**) and Clofarabine (**3**) are potent anti-cancer drugs that are commonly used in the treatment of various types of cancers.^{13,16} Sofosbuvir (**2**) acts as potent inhibitor of Hepatitis C virus replication.^{5,6}

Figure 2. Examples of the prominent nucleoside drugs with fluorine modifications in sugar moieties.

1.4. Gemcitabine, a critical anticancer drug

Gemcitabine is a nucleoside analog that has been used as a chemotherapeutic agent for last two decades. Gemcitabine is approved in the treatment of various types of cancers including pancreatic, breast, ovarian, and non-small cell lung cancer.^{13,16-20}

1.4.1. Existing methods for the synthesis of gemcitabine

Gemcitabine was first synthesized by Hertel and co-workers in 1988.¹² Their method used ethyl bromodifluoro acetate and (*R*)-2,3-*O*-isopropylidene glyceraldehyde as the precursors for the construction of ribose sugar with two fluorine atoms at the 2-position (Figure 3). Coupling of the latter to the heterocyclic base and subsequent deprotection gives **1**. As shown in Figure 2, their approach suffers from two stereoselective setbacks: one being the Reformatsky reaction (the desired product is generated in 65% and required separation from the *threo* byproduct) and the other being the coupling of the sugar derivative to pyrimidine base (the desired β anomer is generated in 10%).

Figure 3. Original synthesis of gemcitabine developed by Hertel.¹²

Over the last 25 years, several research groups have published improved methods for the synthesis of gemcitabine. Some of these synthetic approaches followed Hertel's original approach and differ mainly in employing either different protecting groups on the ribose or different coupling conditions to improve stereoselectivity/isomer-separation. Additionally gemcitabine was also synthesized from various furanose and pyranose derivatives. The synthesis of gemcitabine has been subject to an excellent review published recently.²¹

Even though the synthesis of gemcitabine is widely studied from the stand point of sugar-base coupling, much less attention has been paid toward the synthesis from parent nucleosides. To our knowledge, only three literature examples have described the synthesis of gemcitabine *via* direct carbohydrate fluorination of the parent nucleoside derivatives.

A 1996 patent from Eli Lilly reported the synthesis of 2'-deoxy-2',2'-difluorocytidine derivative **6** by treating the corresponding 2'-keto cytidine derivative **5** with DAST and Pyr.HF reagent combination (Figure 4). Whereas a more recent Chinese patent (2013) described the similar transformation with the new deoxyfluorinating agents Xtalfluor-M and Xtalfluor-E.

Figure 4. Synthesis of 2'-difluorocytidine derivatives from 2'-keto derivatives by deoxyfluorination

Synthesis of difluorouridine derivative **10** by desulfurization-difluorination of the corresponding dithiouridine derivative **9** with DBH/Py.9HF reagent combination was reported in a 2007 patent (Figure 5). However, the crucial uridine-2'-dithioacetal precursor **9**, was prepared by coupling the uracil base with the pre-constructed dithioacetal ribose derivative **8**, which in turn was prepared from the open chain ribonolactone derivative **7**.²²

Figure 5. Synthesis of 2'-deoxy-2',2'-difluorouridine derivative by desulfurization-difluorination of the corresponding 2'-dithioacetal derivative

1.4.2. Mechanism of action of gemcitabine

The mechanism of action of gemcitabine has been widely studied^{13,16-20} and is only discussed briefly here. Gemcitabine (dFdC) is a prodrug and must be metabolized to the active triphosphate form 2',2'-difluoro-2'-deoxycytidine triphosphate (dFdCTP) to exert biological activity (Figure 6). Human nucleoside transporters (hNTs), an important class of membrane proteins, mediate the cellular uptake of gemcitabine. Gemcitabine is converted into its monophosphate (dFdCMP) by deoxycytidine kinase (dCK); to its diphosphate (dFdCDP) by pyrimidine nucleoside monophosphate kinase (UMP-CMP kinase); and subsequently to the active metabolite triphosphate by nucleoside diphosphate

kinase. Gemcitabine may become inactivated through deamination by cytidine deaminase (CDA) and, when in the monophosphate form by deoxycytidylate deaminase (dCTD). The product of gemcitabine deamination by CDA is 2',2'-difluoro-2'-deoxyuridine (dFdU), which is cytotoxic itself. Also, dFdU monophosphate (dFdUMP) could inhibit the activity of thymidylate synthase, directly effecting the deoxynucleotide triphosphate (dNTP) pool. Gemcitabine can also become inactivated by dephosphorylation of the monophosphate form by 5'-nucleotidases (5'-NTs), converting nucleotides back to nucleosides. These enzymes play a critical role in the balance of dNTP pools and, therefore, in gemcitabine metabolism. In this way, the rate-limiting step of phosphorylation by dCK may be also be affected, compromising the overall beneficial cytotoxicity of gemcitabine.

Figure 6. Gemcitabine cellular metabolism.¹⁸

hNT: human nucleoside transporter; dFdCMP: gemcitabine monophosphate; dFdCDP: gemcitabine diphosphate; dFdCTP: gemcitabine triphosphate; dFdU: 2',2'-difluoro-2'-deoxyuridine, dFdUMP: 2',2'-difluoro-2'-deoxyuridine monophosphate.

The most important mechanism of action of gemcitabine is inhibition of DNA synthesis. When dFdCTP is incorporated into DNA, a single deoxynucleotide is incorporated afterwards, and stops chain elongation. The non-terminal position of gemcitabine makes DNA polymerases unable to proceed, as well as inhibits removal of gemcitabine by exonucleases.

An additional mechanism of action of gemcitabine is inhibition of deoxycytidylate deaminase (dCTD), by dFdCTP and indirectly by dFdCDP.^{23,24} By covalently binding to the active site, dFdCDP inhibits Ribonucleotide reductase (RNR), which catalyzes the reduction of ribonucleotides to deoxyribonucleotides.²⁵⁻³¹ The covalent inhibition of RNR causes decrease in the dNTP pool and consequently reduces dCTD activity.^{32,33} In addition, decrease in the dNTP pool stimulates dFdC phosphorylation, thereby increasing the level of dFdCTP, making dFdCTP more likely to be incorporated into DNA competing with the NTPs.^{25,27} In addition, another important mechanism of gemcitabine is the induction of apoptosis through caspase signaling. In response to cellular stress, Gemcitabine activates p38 mitogen-activated protein kinase (MAPK) to trigger apoptosis in tumour cells, but not in normal cells.

The widely accepted mechanism of covalent inhibition of RNR by dFCDP in the presence and absence of reductant is shown in Figure-7.³¹ Crucial steps in RNR mechanism include generation of tyrosine (Y122) radical from R2 subunit of RNR, abstraction of a hydrogen radical from Cysteine (C439) in R1 subunit of RNR by Y122

radical to produce **A**, subsequent abstraction of H3' by C439 radical to produce the crucial C3'-centered radical **B** (first two steps are not shown in figure). The C3'-centered radical then loses a molecule of HF to generate C2'-centered radical **C**, which is resonance stabilized by oxygen centered radical intermediate **D**, which eventually leads to covalent inhibition of RNR.

Figure 7. Mechanism for the covalent inhibition of RNR by dFdCDP in the absence and in the presence of reductant, proposed by Prof. Stubbe, et al.³¹

1.5. Short survey on desulfurization-fluorination reactions

Although the preparation of the organofluorine compounds remains a difficult task, in the last 40 years numerous methods of incorporating fluorine into organic molecules have been developed, as summarized in many excellent reviews.³⁴⁻³⁸ Oxidative desulfurization-(di)fluorination is one of the most important fluorination protocols for the

preparation of numerous fluoro/*gem*-difluoro organic compounds.^{39,40} Various reagents or reagent-combinations have been developed for *oxidative* desulfurization-fluorination reactions over the years. Of which, desulfurization-difluorination of aldehyde and ketone dithiolanes with oxidants such as *N*-halosuccinimides (NBS, NIS) or 1,3-dibromo-5,5-dimethylhydantoin (DBH) and pyridinium poly(hydrogen fluoride) (PPHF)⁴¹ and desulfurization-difluorination of alkyl aryl thioethers with DBH and Olah's reagent (Py.9HF)^{42,43} share similar mechanistic traits (Scheme 1).

Katzenellenbogen's protocol:

Haufe's protocol:

Scheme 1. *Oxidative* desulfurization-difluorination of aldehyde, ketone dithiolanes; and alkyl aryl thioethers.

Hara's research group has developed three reagents or reagent combinations for *oxidative* desulfurization-fluorination reactions. First among them was Iodine pentafluoride (IF₅),⁴⁴⁻⁴⁷ which mainly causes the desulfurization-difluorination of benzyl sulfides (Scheme 2). Later, an air- and moisture-stable IF₅-pyridine-HF reagent^{48,49} was developed for the desulfurization-difluorination of benzyl sulfides, thioacetals, and dithianes. Recently, an air-stable fluorinating agent BrF₃-KHF₂⁵⁰ was also developed for

desulfurization-difluorination of benzyl sulfides, dithioacetals, and (phenylthio)glycosides.

Scheme 2. Hara's *oxidative* desulfurization-difluorination of benzylic sulfides.

Interesting point to note is that none of the above mentioned methods provide access to *gem*-difluoro products from alkyl aryl thioethers where in the thioether is present on an internal secondary carbon atom (See Figure 13 in Section 2).

1.6. 5-Bromopyrimidines and 8-bromopurines: Syntheses and importance

The C-5 halogenated pyrimidine and C-8 halogenated purine nucleosides are widely used as substrates in reactions involving direct displacement of halo groups with nucleophiles.^{51,52} Especially, 5-bromopyrimidine and 8-bromopurine nucleoside derivatives have been shown to possess interesting synthetic and biological properties.^{51,52} Also, the halogenated pyrimidine and purine nucleoside derivatives are often used in transition metal catalyzed cross-coupling reactions⁵³ resulting in the syntheses of a variety of nucleoside analogues with biological activity and/or fluorescent probes.⁵⁴ For example, a number of 5-substituted uracil derivatives, especially arabinofuranosyl- and 2'-deoxyuridines, have been investigated extensively for the clinical treatment of viral diseases.⁵⁵ The 5-bromo and 5-iodo uridine derivatives are used as substrates for high yield coupling with terminal alkynes to generate 5-alkynyluracil nucleosides with antiviral activity^{56,57} and the 5-alkynyl products can also be transformed into bifurane derivatives which possess potent and selective inhibition of *Varicella-Zoster*

virus.^{56,58} Moreover, radiolabeled 5-bromo- and 5-iodouracil nucleosides have applications in cellular biochemistry.⁵⁹

Halogenated pyrimidine⁵² and purine⁵¹ nucleosides have been prepared by direct treatment with halogens or halonium ion (X^+) sources but most of these methods utilize harsh reaction conditions. Various C5-bromouridine derivatives have been synthesized using $Br_2/Ac_2O/AcOH$,⁵² Br_2/H_2O ,⁶⁰ *N*-bromosuccinimide (NBS) in DMF⁶¹ or ionic liquids,⁶² combination of 3-chloroperoxybenzoic acid/HBr in aprotic solvents,⁶³ ceric ammonium nitrate (CAN)/LiBr in protic or aprotic solvents,⁶⁴ or KBr/Oxone.⁶⁵ The C5-bromination of cytidine nucleosides has been accomplished with $Br_2/CCl_4/h\nu$ ⁶⁶ or NBS in DMF⁶¹ or ionic liquids.⁶² On the other hand, the synthesis of C8-bromo purine nucleosides has been typically achieved with $Br_2/AcOH/AcONa$ ⁶⁷ or NBS/DMF.⁶¹ Hence, a universal brominating reagent for C5-bromination of pyrimidines and C8-bromination of purines and also development of a bench-friendly bromination methodology is warranted.

1.6.1. 1,3-dibromo-5,5-dimethylhydantoin, an important brominating reagent

The 1,3-dibromo-5,5-dimethylhydantoin (DBDMH or DBH, Figure 8) is a useful reagent for various organic transformations⁶⁸⁻⁷⁰ including aromatic bromination.⁷¹⁻⁷⁵ Enhanced efficiency of DBH towards aromatic bromination in the presence of acids has been known.⁷²⁻⁷⁴ Moreover, Lewis acid-catalyzed benzylic bromination with DBH has also been reported.⁷⁶ DBH also efficiently oxidizes thiols to disulfides with DBH.^{77,78} In addition, α -bromination of aliphatic ketones is achieved with DBH in combination with *p*-toluenesulfonic acid (TsOH).⁷⁹ Interestingly, compared to NBS, DBH can donate two bromines and can be doubly efficient.

Figure 8. Structures of DBH and NBS

1.7. Selected aspects of the chemistry of 7-deazapurine nucleosides

The 7-deazapurine nucleosides, namely, tubercidin, sangivamycin and toyocamycin (**19a-c**, Figure 9) are a group of natural products isolated from the species *Streptomyces*. Analogues and derivatives of these 7-deazapurine nucleoside antibiotics have been synthesized and subjected to extensive biological testing.⁸⁰⁻⁸⁴ Chemical modifications of the parent 7-deazapurine antibiotics as well as sugar-base coupling has provided access to a sizeable number of biologically active 7-deazapurine compounds.⁸⁵⁻

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Figure 9. Structures of 7-deazapurine nucleosides

Noteworthy examples of such molecules include: (a) 5-iodotubercidin **20**,⁹⁷ an up-field activator of the p53 pathway; (b) sangivamycin analogues such as 6-hydrazinosangivamycin **22**⁹⁸ and 4-Amino-6-bromo-7-(β -L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (xylocidine, **23**),⁹⁹ *in vitro* down-field inhibitors of PKC and CDK in cancer cell lines; (c) a methyl-substituted tubercidin **21**,¹⁰⁰ which acts against the

replication of polio and dengue viruses; (d) the anti-HSV agent xylobutercidin;¹⁰¹ (e) substituted toyocamycin analogues **24**¹⁰² and 2'- β -C-methyl derivative of toyocamycin,¹⁰³ sangivamycin,¹⁰³ and tubercidin^{104,105} that have activity against HCV; (f) 2'-deoxy-2'-fluoroarabinotubercidin¹⁰⁶ and 2-amino-2'-deoxy-2'-fluoroarabinotubercidin,^{107,108} which exhibit antiviral activity; (g) 4*N*,5-diaryltubercidin derivatives **25**, which act as adenosine kinase inhibitors;^{96,109,110} and (h) tubercidin derivatives such as 4-(het)aryl,¹¹¹ 5-(het)aryl¹¹² and some 4-substituted-5-(het)aryl¹¹³ compounds **26** with nanomolar cytostatic activity against several cancer cell lines. Important base-modified analogues are shown in Figure 10.

Figure 10. Important base-modified 7-deazapurine nucleoside derivatives.

Direct alkylation of exocyclic amino groups on 7-deazapurine nucleosides has not been noted. However, 4-*N*-methyltubercidin (6-*N*-methyl-7-deazaadenosine) **28** was reported to be synthesized from the reaction of tubercidin with methyl iodide followed by 1*N* sodium hydroxide (Scheme 3).⁸⁶ Thus, treatment of tubercidin with CH₃I (rt, 24 h,

DMA) gave the corresponding N1-methyltubercidin hydroiodide **27** after recrystallization from methanol. Base treatment of this cationic intermediate (1N NaOH 100 °C, 1.25 h) produced 4-*N*-methyltubercidin **28** via Dimroth rearrangement.⁸⁶

Scheme 3. Synthesis of 4-*N*-methyltubercidin by Dimroth-rearrangement approach

Even though direct alkylation or Dimroth type rearrangement has been scarce in 7-deazapurine chemistry, the 4-*N*-substituted-7-deazapurine derivatives have been widely prepared by aromatic nucleophilic substitution ($\text{S}_{\text{N}}\text{Ar}$). For example, $\text{S}_{\text{N}}\text{Ar}$ displacement of chloro group from 4-chloro analogues^{86,113} or triazolyl group from 4-*N*-(1,2,4-triazol-4-yl) intermediates¹¹⁴ gave access to various other 4-*N*-modified tubercidin derivatives. Also, 4-*N*-substituted toyocamycin derivatives were prepared from the $\text{S}_{\text{N}}\text{Ar}$ displacement of chloro group from the corresponding 4-chloro compounds.¹⁰² Likewise, the 7-*N*-benzylformycin was prepared via 7-chloro derivative of the *C*-nucleoside antibiotic formycin (3- β -D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine).¹¹⁵

1.8. Nucleoside transport inhibitors

As discussed in previous sections (Chapter 1, Sections 1.1-1.3), nucleoside based drugs have been in use for the treatment of various infections. Since the hydrophilic nature of nucleosides limits their cell permeability, nucleoside-specific membrane transport carriers (NT) facilitate and regulate the cellular uptake and therapeutic actions

of many nucleoside based drugs.^{116,117} The nucleoside transporter proteins are classified into two categories: equilibrative NT (ENT) and concentrative NT (CNT). Human equilibrative NTs (hENTs) are found in the outer plasma membrane and some intracellular membranes of most, if not all, human cells.¹¹⁸

Nitrobenzylmercaptapurine ribonucleoside (NBMPR, **29a**) and structurally related hENT1 probes such as 6-*N*-(4-nitrobenzyl)adenosine (**29b**)¹¹⁹ and 5'-S-{2-(6-[3-(fluorescein-5-yl)thioureido-1-yl]hexanamido)}ethyl-6-*N*-(4-nitrobenzyl)-5'-thioadenosine (FITC-SAHENTA)¹²⁰ inhibit hENT1 with nM affinity (Figure 11). Discovery and development of such nucleoside transport inhibitors has greatly aided investigations on the structure, function, and cell surface abundances of hENT1 proteins.^{116,120,121}

Figure 11. Structures of Important nucleoside transport inhibitors.

2. RESEARCH OBJECTIVES

(i) The first research objective of this dissertation was to develop novel fluorination methodologies for the synthesis of mono-fluoro and *geminal*-difluoro uridine analogues from their parent nucleoside precursors. These strategies were envisioned to eliminate the current need to couple fluorinated sugar precursors with nucleosides bases, a process which has severe regiochemical and stereochemical limitations. On the basis of literature

precedence,¹²² I would explore the possibility of insertion of the fluorine or *geminal*-difluoro unit into the 2'-position of ribose moiety of nucleosides using *oxidative* desulfurization-fluorination of substrates of type **30** and **31** with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH or DBH) and Olah's reagent (Py.9HF) (Figure 12).

Figure 12. Different classes of 2'-arylnucleosides for oxidative desulfurization-fluorination and reductive desulfonylation-fluorination procedures.

A complementary approach was designed based on *reductive*-desulfonylation/fluorination processes of 2'-arylsulfonyl-2'-deoxyuridine **32** or 2'-arylsulfonyl-2'-deoxy-2'-fluorouridine **33** with *electrophilic* fluorine reagents (Figure 12). Treatment of sulfone **32** or α -fluorosulfone **33** with organic electron donors such as tetrakis(dimethylamino)ethylene (TDAE), Murphy's reagent, would be expected to lead to the reductive cleavage of sulfone moiety and generation of C2'-centered carbanion. The intermediate carbanion would then be quenched with fluoronium ion (F⁺) to give mono- or di- fluorinated uridine derivatives. Alternatively, treatment of 2'-arylsulfonyl-2'-deoxyuridine **32** with a base would lead to abstraction of the most acidic proton H2' and then the intermediary carbanion would be quenched with electrophilic fluorine (F⁺) to give products of type **33**.

(ii) Since nucleosides are complex molecules and also because of the lack of literature precedence for the desulfurization-difluorination of aryl-alkyl thioethers wherein the arylthio unit is positioned on a secondary internal carbon atom, I planned to perform model studies on simpler aryl-alkyl thioether substrates of type **34-37** (Figure 13). The proposed substrates would have different functional groups such as ester (**34**), keto (**35**), alkane (**36**) and lactone (**37**), and mimic the C2' of ribose unit in the pyrimidine nucleoside precursor (**30**, Figure 12), which also has a carbon at the carbonyl oxidation state (as an aminated group) on the α -carbon atom.

Figure 13. Various aryl-alkyl thioether substrates for model oxidative desulfurization-difluorination reactions

(iii) Since DBH is a source of electrophilic bromine (Br^+) and as bromonium ions are known to brominate pyrimidines at C-5 position and purines C-8 position, I explored the bromination at C-5 of pyrimidines and C-8 of purines with DBH. The goal of this part of my dissertation was to develop an efficient bromination methodology, which could be applicable for the bromination of all DNA and RNA nucleosides, and which would also be compatible with commonly used protection groups in nucleoside chemistry.

(iv) The next goal of my dissertation was to investigate the behavior of gemcitabine and its 2'-modified analogues such as 2'-deoxy-2'-fluorocytidine (**38**) and 2'-deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130, **39**) in one electron oxidation systems and evaluate if

stereo electronic differences at C2'-position among these substrates would allow us to detect C2' and/or C3' sugar radicals using electron spin resonance (ESR) spectroscopy studies (Figure 14). This part of my dissertation was planned in collaboration with Dr. Michael D. Sevilla's research group of Oakland University.

Figure 14. Gemcitabine and its analogues for one electron oxidation studies

(v) Since 6-*N*-(4-nitrobenzyl) derivatives of adenosine and other adenine nucleosides possess potent nucleoside transport inhibition activity, in the next goal, I proposed to synthesize novel 6-*N*-benzyl-7-deazapurine nucleoside derivatives (**40a-d**) of 7-deazaadenosine antibiotics (**19a-c**) in order to test their biological activity (Figure 15). I envisioned two methods for achieving the synthesis of the target molecules (**40a-d**). One involved alkylation of the 7-deazaadenosine antibiotics (**19a-c**) at N1 with a benzyl bromide followed by a base-promoted Dimroth rearrangement. The second route employed diazotization–fluorodediazoniation followed by S_NAr displacement of fluoride with a benzyl amine.

Figure 15. Structures of 7-deazapurine antibiotics and the proposed 6-N-benzyl analogues.

(vi) I also intended to synthesize 7-deazapurine nucleoside derivatives with various novel modifications at C-8 and C-7 position (Figure 16). For example, 8-bromo-7-deazapurine derivatives (**41**, Y = Br) are envisioned as substrates for the synthesis of novel 8-azido-7-deazapurine analogues (**41**, Y = N₃). These 8-azido-7-deazapurine nucleosides might act as substrates for Copper-catalyzed and copper-free click reactions with alkynes and cycloalkynes respectively. The resultant novel 8-triazolyl-7-deazapurine derivatives (**41**, Y = triazolyl) might possess fluorescent properties because of extended conjugation.

Figure 16. Structures of various 8-modified-7-deazapurine derivatives

Also, the 8-bromo-7-deazapurine substrates (**41**, Y = Br) can be converted to the corresponding 8-alkynyl-7-deazapurine counterpart **42**. The crucial 8-alkynyl intermediates **42** might give access to α -halovinyl (**43**, Z = Cl, Br), β -ketosulfone (**43**, Z =

OH), and α -vinylazide (**44**) derivatives, analogous to recent findings on similar modifications at C5-position of pyrimidines (unpublished results from our research group).

3. RESULTS AND DISCUSSION

3.1. Bromination of nucleobases with 1,3-dibromo-5,5-dimethylhydantoin

Since DBH is known to be a good source of bromonium ion (Br^+), I envisioned a novel bromination protocol toward C5-bromination of pyrimidines and C8-bromination of purines.

3.1.1 Bromination at C5 position of pyrimidine nucleosides with DBH

To test my hypothesis, I initially treated 2',3',5'-tri-*O*-acetyluridine **45a** with 1.1 equivalents of DBH in CH_2Cl_2 at ambient temperature (Scheme 4; Table 3, entry 1). After 28 h of reaction time TLC showed conversion of most of the substrate to a new spot, which was less polar than the substrate spot. The less polar spot was isolated by aqueous workup (95%) and ^1H -NMR of the crude material was very clean and implied a single compound. Characteristic doublet for H5 at 5.73 ppm was missing in ^1H -NMR and the doublet for H6 (7.33 ppm) collapsed to a singlet (7.82 ppm), indicating the formation of 2',3',5'-tri-*O*-acetyl-5-bromouridine (**46a**; 95%). Furthermore, structure of the product was confirmed by comparing NMR data with literature NMR data for 2',3',5'-tri-*O*-acetyl-5-bromouridine.⁶⁴ To optimize the amount of DBH required for the bromination, I treated **45a** with 0.55 equiv. of DBH. But the TLC showed only 60% conversion to **46a** even after 48 h of reaction time. Although, two bromonium ions (Br^+) can be delivered by one equivalent of DBH, it seemed that 1.1 equiv. of DBH is required for complete conversion of **45a** to **46a** at room temperature.

Role of Lewis acids

Since it was reported that Lewis acids enhance the efficiency of bromination by DBH,⁷⁶ I decided to compare the rate of bromination in presence of Lewis acids. Remarkably, treatment of **45a** with 0.55 equiv. of DBH in presence of 0.55 equiv. of trimethylsilyl trifluoromethanesulfonate (TMSOTf) significantly enhanced the rate of bromination yielding **46a** after only 6 h (94% yield) (entry 2). The efficiency of bromination did not differ much when 1.1 equiv. of TMSOTf was used (entry 3). Also, replacing TMSOTf with other organic acids such as *p*-toluenesulfonic acid (TsOH, 0.75 equiv.) required 0.75 equiv. of DBH for complete conversion of **45a** to **46a** (entry 4). Next, I studied the effect of temperature and role of solvents on bromination in order to establish optimal reaction conditions.

Scheme 4. Bromination of uracil-derived nucleosides **1** with 1,3-dibromo-5,5-dimethylhydantoin (DBH). See Table 3 and 4 for specific reaction parameters.

Effect of Temperature

Treatment of **45a** with equimolar quantities of DBH and TMSOTf (0.55 equiv.) at 40 °C in CH₂Cl₂ afforded **46a** quantitatively in only 2 h (entry 5). Whereas, analogous

treatment of **45a** with DBH/TMSOTf at 0 °C gave **46a** in only 65% yield after 8 h (TLC) and required 1.1 equiv. of DBH for complete conversion (entry 6).

Role of Solvents

Treatment of **45a** with 0.55 equiv. of DBH at room temperature in CH₃CN gave **46a** after 11 h (entry 7). Analogous treatment of **45a** with DBH in presence of TMSOTf (0.55 equiv.) yielded **46a** after only 2.5 h (entry 8). Moreover, the DBH/DMF system was much more effective than CH₃CN or CH₂Cl₂ affording **46a** in much shorter time (entries 9-10). In addition, bromination of **45a** with DBH in the presence or absence of TMSOTf was less efficient in polar protic solvent MeOH, producing **46a** in low yields. Hence, it can be concluded that bromination of **45a** with DBH and/or TMSOTf was very efficient in polar aprotic solvents such as CH₃CN or DMF. However, it is noteworthy that prior removal of these polar solvents from crude reaction mixture was required before proceeding to aqueous workup, which is not the case with CH₂Cl₂.

Table 3. Effect of various reaction parameters on C5-bromination of 2',3',5'-tri-O-acetyluridine **45a** with DBH^a

Entry	Solvent	Temp. (°C)	DBH (equiv.)	TMSOTf (equiv.)	Time (h)	Yield ^{b,c} 46a (%)
1	CH ₂ Cl ₂	25	1.1	-	28	95
2	CH ₂ Cl ₂	25	0.55	0.55 ^d	6	94
3	CH ₂ Cl ₂	25	0.55	1.10	6	91
4	CH ₂ Cl ₂	25	0.75	0.75 ^e	8	94
5	CH ₂ Cl ₂	40	0.55	0.55	2	98

6	CH ₂ Cl ₂	0	1.1 ^f	0.55	3	98
7	CH ₃ CN	25	0.55	-	11	86 ^g
8	CH ₃ CN	25	0.55	0.55	2.5	90
9	DMF	25	0.55	-	0.6	95
10	DMF	25	0.55	0.55	0.3	98

^a Bromination was performed on 0.1 mmol scale of **45a**. ^b Isolated yield after aqueous work-up. ^c Purity of the product **46a** was determined by TLC and ¹H NMR and was higher than 97% unless otherwise noted. ^d Reaction without TMSOTf showed 60% conversion to **46a** (TLC) after 48 h and complete conversion after 68 h with purity over 90% (¹H NMR). ^e TsOH was used instead of TMSOTf. ^f Reaction with 0.55 eq. of DBH was complete in 65% after 8 h. ^g With purity over 90%.

Extension to other protected uridine nucleosides

I also wanted to test the applicability of the optimized bromination procedure for the 5-bromination of the other uracil nucleosides. Thus, treatment of 1-(2,3,5-tri-*O*-acetyl-β,D-arabinofuranosyl)uracil **45b** with DBH and TMSOTf yielded the corresponding C5-brominated analogue **46b** in 91% yield after 10 h (Scheme 4; Table 4, entry 2). Replacing CH₂Cl₂ with polar aprotic solvent such as CH₃CN generated **46b** in 98% yield after only 2 h (entry 3). In addition, treatment of 3',5'-di-*O*-acetyl-2'-deoxyuridine **45c** with DBH produced the 5-bromo counterpart **46c** in 72% isolated yield after 18 h (entry 4). **46c** was also obtained after only 2.5 h in the presence of TMSOTf (entry 5). Moreover, the rate of bromination increased five-fold when the reaction was carried out at 40 °C (entry 6).

C5-Bromination of unprotected uridine nucleosides with DBH

On the basis of the successful C5-bromination of protected uridine nucleosides, I envisioned the extension of DBH bromination protocol for the C5-bromination of unprotected uridine nucleosides. Thus, bromination of uridine **45d** with DBH in DMF yielded 5-bromouridine **46d** in 75% crystallized yield after only 20 minutes (Scheme 4; Table 4, entry 7). Analogously, treatment of 1-(β ,D-arabinofuranosyl)uracil **45e** and the acid sensitive 2'-deoxyuridine **45f** with DBH at ambient temperature produced the corresponding 5-brominated products in good yields (entries 8 and 9).

Extension to cytidine nucleosides

With the successful application of C5-bromination of uridine nucleosides with DBH, I imagined the extension of the DBH-bromination methodology toward the synthesis of C5-brominated cytidine analogues. Thus, treatment of cytidine **47a** with 0.55 equiv. of DBH at room temperature in DMF produced 5-bromocytidine **47b** after only 30 minutes (72% yield, Figure 17; Table 4, entry 10).

Table 4. Bromination at C5 position of the pyrimidine based nucleosides **45a-f** and **57-59^a**

Entry	Substrate	Product	Solvent	Temp. (°C)	DBH (equiv.)	TMSOTf (equiv.)	Time (h)	Yield ^b (%)
1	45a	46a	CH ₂ Cl ₂	25	0.55	0.55	6	94 ^c
2	45b	46b	CH ₂ Cl ₂	25	0.55	0.55	10	91 ^c
3	45b	46b	CH ₃ CN	25	0.55	0.55	2	98 ^c
4	45c	46c	CH ₂ Cl ₂	25	1.10	-	18	72 ^d
5	45c	46c	CH ₂ Cl ₂	25	0.55	0.55	2.5	90 ^c

6	45c	46c	CH ₂ Cl ₂	40	0.55	0.55	0.5	93 ^c
7	45d	46d	DMF	25	0.55	-	0.33	75 ^e
8	45e	46e	DMF	25	0.55	-	1	65 ^d
9	45f	46f	DMF	25	0.55	-	0.75	80 ^d
10	47a	47b	DMF	25	0.55	-	0.5	72 ^d
11	48a	48b	DMF	25	0.55	-	0.5	74 ^{d,f}
12	49a	49b	DMF	25	0.55	-	0.5	98 ^c
13	45d	46d	MeOH	25	0.55	-	1	70 ^e
14	47a	47b	MeOH	25	0.55	-	1	68 ^d

^a Bromination was performed on 0.25-2.0 mmol scale. ^b Isolated yield. ^c After aqueous work-up with purity higher than 97% (¹H NMR). ^d After column chromatography. ^e After crystallization. ^f Direct crystallization of the crude reaction mixture from MeOH gave **48b** in 46% yield.

Analogously, bromination of 4-*N*-benzoylcytidine **48a** proceeded smoothly generating **48b** (entry 11), which implies that the presence of an electron withdrawing group on the cytosine ring (*N*4-benzoyl) does not affect the efficiency of bromination.

Protection group compatibility

I also found that DBH bromination methodology was compatible with common protection groups used in nucleoside chemistry. Thus, treatment of 5'-*O*-(*tert*-butyldimethylsilyl)-2',3'-*O*-isopropylideneuridine **49a** with 0.55 equiv. of DBH in DMF afforded the corresponding 5-bromo product **49b** in quantitative yield (Figure 17; Table 4, entry 12). It was already shown that base-labile acetyl groups were stable during DBH-bromination (e.g., Table 3). Therefore, it can be concluded that acid

labile as well as base labile protection groups are stable toward DBH-bromination protocol.

Figure 17. Selected pyrimidine nucleoside precursors (series a) and their brominated products (series b).

C5-Bromination of unprotected pyrimidine nucleosides with DBH in MeOH

Since DMF has to be removed from the crude reaction mixture before purification of the brominated products, I used a low-boiling polar solvent such as MeOH as a replacement for DMF. To my surprise, treatment of uridine **45d** with DBH (0.55 equiv.) in MeOH at room temperature for 1 h yielded 5-bromouridine **46d** in 70% yield (crystallization) (Scheme 4; Table 4, entry 13). Analogously, cytidine **47a** was also brominated using DBH in MeOH generating 5-bromocytidine **47b** (Figure 17; Table 4, entry 14) after only 1 h in 68% yield (column chromatography). Therefore, it can be concluded that DBH/MeOH system offers a convenient access to the unprotected 5-bromopyrimidine nucleosides.

3.1.2 Bromination at C8 position of purine nucleosides with DBH

Next, I planned the extension of the DBH and DBH/TMSOTf combination toward the synthesis of 8-bromo purine nucleosides. Thus, treatment of adenosine **50a** with DBH (1.75 equiv.) in DMF at room temperature gave 8-bromoadenosine **50b** (48% yield) after 5 h at ambient temperature (Figure 18, Table 5, entry 1). Analogous treatment of 2'-

deoxyadenosine **51a** with DBH (1.5 equiv.) afforded 8-bromo-2'-deoxyadenosine **51b** in 68% yield in 3.5 h (entry 2).

Figure 18. Selected purine nucleoside precursors (series **a**) and their brominated products (series **b**).

The 2',3',5'-tri-*O*-acetylguanosine **52a** was converted to the 8-bromo derivative **52b** upon treatment with 0.55 equiv. of DBH in DMF at room temperature for 2.5 h (entry 3). Replacement of DMF with CH₃CN decreased the rate of bromination yielding **52b** after 4 h at room temperature (entry 4). On the other hand, treatment of guanosine with 0.75 equiv. of DBH in DMF at ambient temperature produced 8-bromoguanosine in 48% yield after recrystallization from water (entry 5). Use of TMSOTf did not improve the yield of 8-bromoguanosine **53b**, though less equiv. of DBH was required for complete conversion (entry 6). Treatment of inosine or acetyl protected inosine with DBH or DBH/TMSOTf in DMF failed to afford 8-bromoinosine, which was in accordance with the reported failed bromination attempts of inosine with NBS in DMF.¹²³

Table 5. Bromination of selected purine nucleosides at ambient temperature (see Figure 11 for structures)^a

Entry	Substrate	Product	Solvent	DBH (equiv.)	TMSOTf (equiv.)	Time (h)	Yield ^b (%)
1	50a	50b	DMF	1.75	-	5	48 ^{c,d}
2	51a	51b	DMF	1.50	-	3.5	68 ^{c,d}
3	52a	52b	DMF	0.55	-	2.5	83 ^c
4	52a	52b	CH ₃ CN	0.55	-	4	98 ^e
5	53a	53b	DMF	0.75 ^f	-	2.5	51 ^g
6	53a	53b	DMF	0.60	0.55	0.5	48 ^g
7	50a	50b	MeOH	1.75	-	16	47 ^{c,j}
8	51a	51b	MeOH	1.50	-	3.5	70 ^j
9	53a	53b	MeOH	0.55	-	1	50 ^g
10	54a	54b	MeOH	0.55	-	0.5	72 ⁱ

^aBromination was performed on 0.5-1 mmol scale. ^b Isolated yield. ^c After column chromatography. ^d Reaction showed formation of the product in approximately 80% yield (TLC). ^e Isolated yield after aqueous work-up. ^f Reaction with 0.55 equiv. of DBH was completed in 24 h. ^g After crystallization from water. Bromination was quantitative as judged by TLC. ⁱ After filtration from reaction. ^j Reaction showed formation of the product in approximately 70% yield (TLC).

In conclusion, DBH and/or DBH/TMSOTf also effected bromination of purine nucleosides at the C8-position, although reactions usually required higher equivalency of DBH, longer reaction times, and produced the corresponding brominated products in lower yields when compared to the pyrimidine analogues.

C8-Bromination of unprotected purine nucleosides with DBH in MeOH

Next, I designed the extension of the DBH/MeOH system toward the synthesis of 8-bromo purine nucleosides. Thus, treatment of adenosine **50a** with DBH (1.75 equiv.) in MeOH at room temperature gave 8-bromoadenosine **50b** (47% yield) after 16 h at ambient temperature (Figure 18, Table 5, entry 7). Analogous treatment of 2'-deoxyadenosine **51a** with DBH (1.5 equiv.) showed only ~70% conversion to 8-bromo-2'-deoxyadenosine **51b** (entry 8, TLC scale reaction). Interestingly, treatment of guanosine **53a** with DBH (0.55 equiv.) in MeOH at room temperature yielded 8-bromoguanosine **53b** in 50% yield after recrystallization of the crude reaction mixture from water (entry 9). In contrast, treatment of 2'-deoxyguanosine **54a** with DBH in MeOH at room temperature for 30 minutes led to the solidification of product **54b** from the reaction. The resultant solid was collected by vacuum filtration and was >95% pure on ¹H-NMR (entry 10). In conclusion, DBH/MeOH system works very efficiently with guanine nucleosides and moderately efficient with adenine nucleosides.

3.1.3. Bromination at C8 position of 7-deazapurine nucleosides with NBS and DBH

Encouraged by my findings of bromination of purine nucleosides at C8 position with DBH, I further explored possibility of bromination of 7-deazapurine antibiotics (**19b-c**) with DBH as well as with NBS (Scheme 5). Previously, 8-bromotoyocamycin (**55**) was prepared either from coupling 8-bromo-7-deazapurine derivative to the ribose sugar derivative¹²⁴ or by treating **19c** with Br₂/H₂O¹²⁵. I achieved the synthesis of compound **55** by two procedures, both proceeding by electrophilic aromatic substitution. One involved electrophilic aromatic substitution using *N*-bromosuccinimide (NBS) in anhydrous dimethylformaldehyde (DMF). The second route employed substitution using

1,3-dibromo-5,5-dimethylhydantoin (DBH/DBDMH) in MeOH. Thus, treatment of **19c** with NBS in DMF at ambient temperature for 2.5 h yielded **55** in 45% after column chromatography. Reaction took 4 h to complete when DMF was replaced with MeOH to give **55** in 64% after recrystallization. However, bromination of **19c** using DBH in MeOH proceeded efficiently in 30 min by which time product solidified out of solution, was collected by vacuum filtration and the mother liquor was recrystallized to give **55** in 85% overall yield. The DBH/MeOH method was the most efficient of the three for synthesizing **55** in terms of yield, reaction time, product isolation.

Scheme 5. Synthesis of 8-bromo-7-deazanucleosides. *Reagents and conditions:* d) NBS (1.1-1.5 eq), DMF, rt, 1-2.5 h; e) DBH (0.75-1.1 eq), MeOH, rt, 30-45 min.

Over the years, the synthesis of 8-bromosangivamycin **56** has mainly been achieved by conversion of **55** to **56** using concentrated ammonium hydroxide and 50% hydrogen peroxide solution.¹²⁴ Owing to the successful preparation of **55**, we envisioned the synthesis of **56** using NBS/DMF and DBH/MeOH systems. Thus, treatment of sangivamycin (**19b**) with NBS in DMF at ambient temperature for 1.5 h gave 8-bromosangivamycin (**56**) in 63% yield after column chromatography. Even though the reaction proceeded faster in DBH/MeOH system (30 min) the yield was low (40%), which can be attributed to the presence of some unidentified byproducts. Fortunately, there is literature precedence for convenient synthesis of **56** from **55**.¹²⁴ Thus, treatment

of **55** with $\text{NH}_4\text{OH}/50\%\text{H}_2\text{O}_2$ system gave **56** in 86% yield after vacuum filtration from the reaction mixture. In summary, an efficient bromination methodology for the synthesis of 8-bromotoyocamycin and 8-bromosangivamycin was developed using NBS/DMF and DBH/MeOH systems.

3.1.4. Plausible mechanism of bromination of nucleobases with DBH

Bromination of nucleosides using DBH is expected to proceed via aromatic electrophilic bromination pathway with bromonium ion (Br^+) as the bromine source from DBH.

Plausible mechanism for C-5 bromination of pyrimidines by DBH

Carbocation intermediate (type **A**) is generated upon reaction of pyrimidine base **57** with DBH (Scheme 6). The intermediate **A** is highly unlikely to exist in resonance with the intermediate **B**, because of the presence of electropositive carbonyl group adjacent to the N1 centered cation. Abstraction of the proton from C5 by the anion **C** regenerates the aromaticity, thus providing the 5-bromopyrimidine product **58**.

Scheme 6. Plausible mechanism of C-5 bromination of pyrimidine nucleosides by DBH.

Plausible mechanism for C-8 bromination of purines by DBH

I hypothesize that the N₇-C₈ double bond of purine **59** abstracts a bromonium ion (Br⁺) from DBH to give nitrogen centered cation **A**, which is stabilized by three resonance structures **B-D** (Scheme 7). Then aromaticity is regenerated by abstraction of proton at C8 position in **A'**, probably by hydantoin intermediate **E**, to give the 8-bromopurine product **60**.

Scheme 7. Plausible mechanism of C-8 bromination of purine nucleosides by DBH.

Plausible mechanism for TMSOTf activation of DBH.¹²⁶

Upon reaction with TMSOTf (**61**), DBH generates a silylenol intermediate **A** along with a reactive bromonium triflate (TfOBr) intermediate **B**. Intermediate **B** acts as the actual bromonium ion (Br⁺) source for the bromination of the pyrimidine/purine substrate **62** to generate the corresponding brominated product **63** along with triflic acid (TfOH, **C**) (Scheme 8). Triflic acid **C** reacts with the silylenol intermediate **A** to give another silylenol intermediate **D** and one more equivalent of TfOBr (**E**). Second equivalent of substrate **62** gets brominated with **E** to release TfOH (**F**), which then reacts with the silylenol intermediate **D** to give 5,5-dimethylhydantoin (DMH, **G**) and TMSOTf.

Scheme 8. Plausible mechanism of TMSOTf enhanced DBH bromination.

3.2. Attempted synthesis of 2'-deoxy-2',2''-difluoropyrimidine nucleosides

Because of the ease of preparation of 2'-*S*-aryl-2'-deoxy-2'-thiouridine (**64**, Figure 19) and 2'-deoxy-2'- α -fluorosulfone derivative (**65**), I explored the possibility of introduction of mono and/or difluoro unit into the C2' position using *oxidative* desulfurization-(di)fluorination and *reductive* desulfonylation-fluorination methods. The oxidative fluorination processes were envisioned to proceed via (i) desulfurization-difluorination of the uridine-2'-thioethers (substrate **64**, X = H) or (ii) desulfurization-fluorination of uridine α -fluorothioethers (substrate **64**, X = F) by using halonium ion (Br^+ , I^+) reagents as oxidants and a nucleophilic fluorine source (F^-) for quenching the intermediary C2'-carbocation. Whereas, the reductive fluorination processes were proposed to proceed via (i) abstraction of the C2'-proton (from substrate **65**, X = H) using a base and quenching the intermediary C2'-carbanion with electrophiles such as F^+ , H^+ , CH_3^+ , etc.; or (ii) cleavage of the sulfonyl moiety (from substrate **65**, X = H or F) with single electron transfer reagents and quenching the resultant C2'-carbanion with electrophiles such as F^+ , H^+ , CH_3^+ , etc.

Figure 19. General scheme for the proposed C2'-quarternization of 2'-arylthiouridine derivatives

3.2.1. Oxidative desulfurization-fluorination approaches

The *oxidative* fluorination approach is modeled on Haufe's desulfurization-difluorination of aryl-alkyl thioethers using DBH and Olah's reagent (Py.9HF) for the synthesis of *gem*-difluoroalkanes (Scheme 9).^{42,43}

Scheme 9. General scheme for Haufe's desulfurization-difluorination of aryl-alkyl thioethers.

3.2.1.1. Model desulfurization-fluorination studies

To apply Haufe's desulfurization-difluorination methodology to nucleoside substrates, it is necessary to test the general applicability of the method on model compounds first. As the C2'-position, where the thioether functionality is present, is in α -position to an aminor carbon, substrates with an aminor or electron withdrawing group on the α -position should be used in model studies. Another reason for conducting model reactions on such substrates which would mimic C2' of ribose unit in the pyrimidine nucleoside substrates is because all previous desulfurization-difluorination protocols were successful only for the compounds where the sulfenyl/phenylthio group is present on the

terminal and primary carbon. Therefore, I developed a desulfurization-difluorination protocol of α -thioesters, where in the sulfenyl group is present in the middle of the chain on a secondary carbon.

Initially, open chain aryl-alkyl thioethers (of type **72-75**, Scheme 10) with an ester group on the α -position (prepared conveniently from commercially available ethyl 2-bromoalkanoate **71a** or **71b**) were tested for desulfurization-difluorination protocol. Thus, treatment of ethyl 2-(phenylthio)octanoate **72** with DBH (3 eq.) and Olah's reagent (6 eq.) in CH_2Cl_2 at ambient temperature for 2 h gave ethyl 2,2-difluorooctanoate **77** in 80% yield after only aqueous workup (Table 6, entry 1). However, analogous treatment of **72** with DBH/Py.9HF for 4 h gave **77** in 90% yield after aqueous workup (entry 2). To study the effect of temperature, phenylthioether **72** was treated with DBH (3 eq.) and Olah's reagent (6 eq.) in CH_2Cl_2 at 35 °C and the difluoro product **77** was obtained in 85% yield after only 1 h (entry 3). But **77** was obtained in 95% yield after stirring the reaction for 2 h at 35 °C (entry 4).

Scheme 10. Desulfurization-difluorination of α -thioesters.

To test the influence of the substituent on the para position of aromatic ring of the thioether substrates, 4-chlorophenyl thioether (**73-74**) and 4-methoxyphenyl thioether (**75**) were selected. Thus, treatment of ethyl 2-((4-chlorophenyl)thio)hexanoate **73** with

DBH (3 eq.) and Olah's reagent (6 eq.) in CH₂Cl₂ at ambient temperature for 1 h gave ethyl 2,2-difluorohexanoate **76** in 80% yield after only aqueous workup (Table 6, entry 5). However, difluoro ester **76** was obtained in 90% yield when the reaction was stirred for 2 h (entry 6). In addition, treatment of 4-chlorophenyl thioether **73** with DBH (3 eq.) and Olah's reagent (6 eq.) in CH₂Cl₂ at 35 °C generated the difluoro product **76** in 95% yield after only 1 h (entry 7). Interestingly, no *geminal* bromo-fluoro by product was observed in crude NMR and GC-MS. When less equivalents of DBH (3 equiv. *vs* 2 equiv.) and Olah's reagent (6 equiv. *vs* 4 equiv.) were used, decreased yield of the difluoro product **76** (80%) was observed (entry 8). Thus, 3 equiv. of DBH and 6 equiv. of Olah's reagent were found to be optimal for the desulfurization-difluorination reactions.

DBH *vs* NBS *vs* NIS

Treatment of ethyl 2-((4-chlorophenyl)thio)octanoate **74** with *N*-bromosuccinimide (NBS; 3 eq.) and Olah's reagent (6 eq.) in CH₂Cl₂ at 35 °C for 2 h followed by aqueous workup showed complex ¹H-NMR spectrum and the difluoroocatnoate **77** was only found to be 25% according to the ¹⁹F-NMR of the crude. There was no increase in % of difluoro product **77** even after stirring for 4 h at 35 °C (entry 9). Analogous treatment of **74** with *N*-iodosuccinimide (NIS; 3 eq.) and Olah's reagent (6 eq.) in CH₂Cl₂ at 35 °C for 24 h gave very complex ¹H-NMR and ¹⁹F-NMR, with the difluoroproduct **77** was found to be 5% (entry 10). Thus, DBH was proven to be more effective than other halogen oxidants such as NBS or NIS, as observed by Haufe, et al.⁴³

Table 6. Desulfurization-difluorination studies of α-thioester derivatives with DBH/Py.9HF^a

Entry	Substrate	X	Oxidant (eq)	Py.9HF (eq)	Temp. (°C)	Time (h)	Yield ^b
1	72	H	DBH (3)	6	25	2	80%
2	72	H	DBH (3)	6	25	4	90%
3	72	H	DBH (3)	6	35	1	85%
4	72	H	DBH (3)	6	35	2	95%
5	73	Cl	DBH (3)	6	25	1	85%
6	73	Cl	DBH (3)	6	25	2	90%
7	73	Cl	DBH (3)	6	35	1	95%
8	73	Cl	DBH (2)	4	35	1	80%
9	74	Cl	NBS (3)	6	35	4	25%
10	74	Cl	NIS (3)	6	35	24	5%
11	75	OCH ₃	DBH (3)	6	35	16	70%

^a Reactions were typically carried out in 0.5 mmol scale in CH₂Cl₂

^b Yields were determined based on conversion of substrate to product as determined by ¹H, ¹³C, ¹⁹F NMR and/or GC-MS.

Continuing with my optimization studies, I then treated ethyl 2-((4-methoxyphenyl)thio)hexanoate **75** under the established conditions of DBH/Py.9HF (3equiv./6equiv.) in CH₂Cl₂ at 35 °C. However, the reaction was very sluggish in nature and gave ethyl 2,2-difluorohexanoate **76** in 70% NMR purity after 16 h of stirring (entry 11). Thus, it was concluded that 4-chlorophenyl thioethers reacted faster than the corresponding phenyl and 4-methoxyphenyl thioethers, as noted by Haufe, et al.⁴³

The variations in reactivity with change in the para substituent of the aromatic group of the thioether can be explained by the plausible mechanism proposed by Haufe, et al., for the desulfurization-difluorination reactions.⁴³

Scheme 11. Proposed mechanism for the oxidative desulfurization-difluorination of alkyl-aryl thioethers by DBH/Py.9HF

The electron-donating groups (such as OMe) on the phenyl ring are thought to stabilize the cationic charge on the resonance stabilized carbenium-sulfonium ion intermediate (type C, Scheme 11) and thus promote the first fluorination step; while electron-withdrawing substituents (such as Cl) are thought to ease the elimination of arylsulfenyl bromide (from type E) in the last step and therefore promote the second fluorination step. The proposed mechanism was supported by the observation that 4-chlorophenyl thioether took half the time than the regular phenyl thioether (Table 6, entries 1-4 vs 5-7); whereas 4-methoxyphenyl thioether reaction never went to completion under analogous reaction conditions (entry 11).

Ester vs aldehyde vs ketone vs alkane

Next, I attempted to extend the desulfurization-difluorination of 2° alkyl-aryl thioethers to substrates with other functional groups on the α -position of the thioether moiety. Thus, the 2-((4-chlorophenyl)thio)-3-phenylpropanal (**78**)¹²⁷ and 2-((4-

chlorophenylthio)-1-phenylpropan-1-one (**79**),¹²⁸ which have a carbonyl functional group on the α -carbon similar to an ester; and α -thioalkane (**80**), which does not have a carbonyl group on the α -carbon; were prepared from their corresponding α -bromo counterparts (Figure 20).

Figure 20. α -Thioaldehyde, α -thioketone and α -thioalkane substrates for attempted desulfurization-(di)fluorination reactions.

However, treatment of α -thioaldehyde **78** with DBH (3 equiv.) and Py.9HF (6 equiv.) in CH_2Cl_2 at 35 °C for 1 h, followed by aqueous workup showed complex ^1H -NMR spectrum and no signals in the ^{19}F -NMR. And similar results were obtained when α -thioketone **79** and α -thioalkane **80** were treated with DBH/Py.9HF under analogous reaction conditions. No fluorination was observed with either of the three substrates even after using excess amounts of DBH and Py.9HF, at higher temperatures and for prolonged reaction times. Thus it was implied that my protocol for the desulfurization-difluorination of secondary/internal thioethers works for compounds with only ester functional groups on the α -carbon.

As a result of functional group similarities between esters and lactones, I then tried to extend the application of the desulfurization-defluorination methodology towards the synthesis of *gem*-difluorinated lactone derivatives. Thus, treatment of 2-phenylthio-5-pentanolide **81**¹²⁹ with DBH (4 equiv.) and Py.9HF (8 equiv.) in CH_2Cl_2 at 35 °C for 2 h, followed by aqueous workup gave yellow oil, which showed complex ^1H -NMR

(Scheme 12). The ^{19}F -NMR of the crude material showed triplets in the range of -100 ppm ($J = 15.8$ Hz), in agreement with the literature ^{19}F -NMR peaks for *gem*-difluorolactones,¹³⁰ thus indicating the presence of the *gem*-difluorinated product **82**. Increase in duration of the reaction gave even more complex ^{19}F -NMR spectrum. Therefore, considering the volatile and unstable nature of the difluorolactones, I chose to pursue less volatile 2,3-dideoxy-2-thioribonolactone precursors of type **83**.

Scheme 12. Attempted synthesis of α,α -difluoro- δ -valerolactone **82**.

Thus, treatment of (*S*)-(+)-2-phenylthio-4-benzyloxymethyl-4-butanolide **83**¹³¹ with DBH (4 equiv.) and Py.9HF (8 equiv.) in CH_2Cl_2 at 35 °C for 16 h, followed by aqueous workup showed complex TLC and multiple products on ^1H -NMR (Scheme 13). The ^{19}F -NMR of the crude material showed multiplets in the region of -140 ppm and -150 ppm. And HRMS showed m/z corresponding to α -fluorosulfide **84** and α -fluorosulfoxide **85**. This indicates that monofluorination occurred, albeit in very low yield.

Scheme 13. Attempted synthesis of α,α -difluoro- γ -butyrolactone **86**.

3.2.1.2. Oxidative desulfurization-fluorination approaches with uridine derivatives

Although my attempts in *oxidative* desulfurization-difluorination of 2,3-dideoxy-2-(phenylthio)-2'-S-ribonolactone precursor (**83**) were partially encouraging, I next attempted analogous fluorination on 2'-S-aryl-2'-thiouridine analogues **89/90** and 2'-S-aryl-2'-deoxy-2'-fluoro-2'-thiouridine analogue **93**, since the substrates were readily available for us (Scheme 14). The precursors 2'-S-(4-chlorophenyl)-2'-thiouridine **87** and 2'-S-(4-methoxyphenyl)-2'-thiouridine **88** were synthesized in three steps from uridine following the well-established procedure of preparing 2,2'-anhydroarabinouridine¹³² from uridine followed by ring opening with thiolates.^{133,134} The precursor 2'-S-(4-chlorophenyl)-2'-thiouridine **87** was prepared by a slight modification of the published procedure.¹²¹ The 4-chlorothiophenol reagent was stirred with PPh₃ in DMF for 30 min at room temperature before refluxing with 2,2'-anhydroarabinouridine, to reduce all the possible disulfide to the free thiol. Subsequent protections of 3',5'-hydroxyl groups and/or N3 with either acetyl or benzyl protection groups gave the desired 2'-S-aryl-2'-thiouridine precursors **89/90**. The *second class* of substrates required for desulfurization-fluorination reactions is α -fluoro thioether derivatives **93**, which can be synthesized from thioether **90** by oxidation to the corresponding sulfoxides and subsequent fluorination of sulfoxides **92**^{135,136} with DAST/SbCl₃, as reported.¹³⁴ In addition, sulfone precursors for *reductive* desulfonylation-fluorination reactions were also readily prepared, following literature protocols.¹³⁴ Thus, oxidation of the 2'-S-aryl-2'-thiouridine precursors **89/90** with *meta*-chloroperoxybenzoic acid (mCPBA) at ambient temperature gave the corresponding 2'-deoxy-2'-arylsulfonyluridine derivatives **91/92**. Analogously, 2'-deoxy-2'-fluoro-2'-(4-methoxyphenyl)sulfonyluridine derivative **95** was obtained by treatment of 2'-deoxy-2'-

fluoro-2'-(4-methoxyphenyl)sulfonyluridine derivative **94** with mCPBA at ambient temperature.

Scheme 14. Synthesis of 2'-arylthio substrates for the *desulfurization-fluorination* reactions.

Treatment of 3',5'-di-*O*-acetyl-2'-*S*-(4-chlorophenyl)-2'-thiouridine **89a** with DBH/Py.9HF at -78 °C, under Argon atmosphere for 2 h showed three new spots on TLC (Scheme 15). Aqueous workup followed by column chromatography yielded three different products in 41%, 18% and 30% respectively. The first product was established to be 5-bromo-3',5'-di-*O*-acetyl-2'-*S*-(4-chlorophenyl)-2'-thiouridine (**96a**, 41%). Signature peak (d or dd) for H5 at 5.67 ppm in ¹H-NMR was missing and the corresponding doublet for H6 (7.17 ppm) was collapsed to a singlet (6.98 ppm), implying a C5-brominated product, which probably was caused by DBH. The remaining two products were C5-brominated sulfoxide diastereoisomers [**97** (*R/S*-*S*)]. Doublet of doublets for H2' was found at 3.92 ppm for the faster moving sulfoxide and at 3.72 for

the slower moving isomer. In comparison, peak for H2' for the C5-brominated product **96** was at 3.47 ppm, and for the substrate **89a** was at 3.78 ppm.

Fluorination attempts of **89a** employing Py.9HF/NBS also gave the corresponding 5-brominated derivative **96a** as major product (conditions not shown in scheme), while no desired fluorination was observed (^{19}F -NMR; data not shown). Based on these findings, a novel and efficient protocol for C5-bromination of pyrimidine nucleosides and C8-bromination of purine nucleosides with DBH and/or Lewis acids has been developed.¹³⁷ Analogous treatment of **89a** with the recently developed NIS/DAST system^{138,139} at rt as well as at 35 °C gave the corresponding 5-iodinated analogue **96b** in 73% and 60% isolated yields respectively, with no indication of the fluorinated products.

Scheme 15. Oxidative desulfurization-difluorination of 2'-S-aryl-2'-thiouridine analogue **89a**. Conditions *A*: DBH (1.1 eq), Py.9HF (excess), CH_2Cl_2 , -78 °C, 2 h; Conditions *B*: NIS (3 eq), DAST (6 eq), CH_2Cl_2 , 0 °C (6 h), then rt (32 h); Conditions *C*: NIS (3 eq), DAST (6 eq), CH_2Cl_2 , 35 °C, 24 h.

Treatment of **89a** with DBH (3 eq.)/Py.9HF (6 eq.)/ CH_2Cl_2 /35 °C/24 h gave a complex reaction mixture showing formation of the 5-bromo-2'- α -fluorosulfoxide **99a** (<10% according to ^1H , ^{19}F NMR of the crude; data not shown; Figure 21). Changing the acetyl protection group for a benzyl group did not improve the outcome of the reaction. Treatment of the fully benzylated **89b** with DBH (5 eq.)/Py.9HF (10 eq.)/ CH_2Cl_2 /35

$^{\circ}\text{C}$ /overnight gave a complex mixture indicating the formation of 5-bromo-2'- α -fluorosulfide **98** or 5-bromo-2'- α -fluorosulfoxide **99b** (^1H , ^{19}F -NMR of the crude).

Figure 21. Products for attempted fluorination of 2'-*S*-(4-chlorophenyl)-2'-thiouridine derivatives **89a** and **89b**

Similarly, treatment of fully benzylated 2'-*S*-(4-methoxyphenyl)-2'-thiouridine **90b** with NIS (2.1 eq.)/Py.9HF (3 eq.) gave the corresponding 5-iodocounterpart (**100**, 29%) and the α -fluorothioether (**94b**, 12%) (Scheme 16). Analogous treatment of **90b** with the recently developed NIS/DAST system^{138,139} yielded the α -fluorothioether **94b** in 44% isolated yield when 2.1 equiv. of NIS and 6 equiv. of DAST were used. Interestingly, the 5-iodo derivative **100** was not observed with NIS/DAST system. However, both the reactions failed to generate the desired *geminal* difluoro uridine product. On the other hand, treatment of **90b** with DBH (3 eq.)/Py.9HF (6 eq.)/35 $^{\circ}\text{C}$ generated a complex reaction mixture. Thus, it is noteworthy to conclude that NIS/DAST system provided clean conversion of sulfide **90b** to the mono-fluorinated analogue **94b** in respectable yield.

Scheme 16. Attempted oxidative desulfurization-fluorination of 2'-*S*-(4-methoxyphenyl)-2'-thiouridine analogue **90b**. Conditions *A*: NIS (2.1 eq), Py.9HF (3 eq); Conditions *B*: NIS (2.1 eq), DAST (6 eq).

Next, the α -fluorothioether **94b**, was also directly explored as substrate for the second fluorination step leading to the expected *gem*-difluorouridine derivative. Unfortunately, treatment of **94b** with DBH/Py.9HF at -78 °C gave the corresponding 5-brominated derivative as major product (**101**, 50%), with no indication of the *geminal* difluoro product (Scheme 17). The introduction of the desired second fluorine atom was unsuccessful even when excess reagents, higher temperatures, and longer reaction times were employed.

Scheme 17. Attempted oxidative desulfurization-fluorination of 2'-fluoro-2'-*S*-(4-methoxyphenyl)-2'-thiouridine analogue **94b** with DBH/Py.9HF.

3.2.2. Reductive desulfonylation-fluorination approaches

Since the oxidative desulfurization-difluorination approaches on 2'-arylthiouridine derivatives were overshadowed by the formation of 5-halogenated byproducts and were not very promising for the introduction of the geminal difluoro unit, I turned my attention to reductive fluorination approaches of 2'-arylsulfonyluridine substrates. Reductive desulfonylation-(di)fluorination protocols are to the best of my knowledge, unknown processes, while chemical and electrochemical-induced reductive desulfonylation reactions have been known for years.¹⁴⁰⁻¹⁵⁰ Chemical and electrochemical redox

strategies have rarely been reported in nucleoside series, except some reductive dehalogenation or desulfonylation protocols *en route* to 2',3'-dideoxy-2',3'-didehydro nucleosides with anti-HIV and anti-HBV properties.¹⁵¹⁻¹⁶⁰

This part of the dissertation was completed in collaboration with Dr. Maurice Medebielle of Université Claude Bernard Lyon 1, France; Dr. Gamal Giuglio and Dr. Julie Broggi of Aix-Marseille Université, France.

3.2.2.1. Cyclic voltammetry studies of 2'-arylsulfonyl uridine derivatives

Initially, our collaborators performed cyclic voltammetry studies with various 2'-deoxy-2'-*S*-(arylsulfonyl)-2'-thiouridine and 2'-deoxy-2'-fluoro-2'-*S*-(arylsulfonyl)-2'-thiouridine substrates to find out the reduction potential at which the sulfone moiety could be cleaved. Sulfone derivatives **92a** and **92b** (see Scheme 14 for structures), were found to be reduced in two irreversible reduction steps in DMF containing 0.1 M *n*-Bu₄NPF₆ on a glassy carbon electrode, at almost identical reduction potentials [- 2.73 V and - 2.95 V for **92a**; - 2.73 V and - 2.92 V for **92b** (peak potentials at 0.2 V/s, Figure 22). For both substrates, the first irreversible reduction step (E_{pc1}) corresponded to the cleavage of the C2'-S bond with the expulsion of the *p*-methoxybenzenesulfinate,¹⁶¹ that can be irreversibly oxidized at + 0.02 V (E_{pa2}). The irreversibility of the first reduction step (E_{pc1}) is an indication that the first electron transfer is followed by a chemical reaction producing a new product that is in turn reduced at a more negative potential corresponding to the second reduction step (E_{pc2}). Additional irreversible oxidation steps, only observed after scanning first to reduction potentials and then switching to anodic values, located at + 0.57 and + 0.81 V (**92a**, E_{pa3} and E_{pa4}) and + 0.51 V (**92b**, E_{pa3}) (peak potentials at 0.2 V/s) were also noticed.

Figure 22. Cyclic voltammetry of α -sulfones **92a** ($C = 2.25$ mM, blue curve) and **92b** ($C = 2.75$ mM, pink curve); in DMF + $n\text{-Bu}_4\text{NPF}_6$ 0.1M; $v = 0.2$ V/s.

Cyclic voltammetry of 2'- α -fluorosulfone precursors **95a-b** (see Scheme 14 for structures) was also investigated in DMF in order to estimate their reduction potentials. Both substrates are reduced irreversibly at potentials close to -2.67 V (vs Ag/Ag^+) meaning that the reduction takes place at relatively high reduction potential not far from uracil moiety reduction (Figure 23). For both substrates an irreversible oxidation step is located at $+0.025$ V only observed when scanning in the cathodic direction. None of the nucleosides are oxidized up to $+1.5$ V.

Figure 23. Cyclic voltammetry of α -fluorosulfones **95a** (2.64 mM) and **95b** (2.91 mM) in DMF + NBu₄PF₆ 0.1 M; glassy carbon electrode.

The data obtained by cyclic voltammetry in DMF indicated that the cleavage of the C2'-S bond, although possible will require (i) quite negative potential under electrochemical activation and (ii) strong chemical reducing agents. Also glycosidic bond cleavage may operate as well at such negative potentials as it is apparently the case for substrates **92a-b**. However, from cyclic voltammetry data, it is not obvious to conclude what the possible reduction products resulting after the expulsion of the *p*-methoxybenzenesulfinate are, at least on the time scale of the cyclic voltammetry. Tentative isolation and characterization of the reductive products, either with the use of chemical reducing agents or using electrochemical activation, was therefore necessary.

3.2.2.2. Reductive desulfonylation-fluorination studies of 2'-deoxy-2'-arylsulfonyl uridine and 2'-deoxy-2'-fluoro-2'-arylsulfonyl uridine substrates

During preparation of sulfone substrates for the reductive fluorination approaches, I observed an interesting result. Purification of 3',5'-di-*O*-acetyl-2'-deoxy-2'-[(4-chlorophenyl)sulfonyl]uridine (**91a**) on silica gel column resulted in the overall elimination of acetic acid to generate 2',3'-didehydro-2',3'-dideoxysulfone **102** as a major product, which has same R_f value as starting material and thus inseparable (1.5:1 ratio of **102**:**91a**; $^1\text{H-NMR}$) (Scheme 18). Structure of the product was confirmed by $^1\text{H-NMR}$ (doublet at 7.01 ppm with coupling constant of 1.9 Hz for H3') and also by comparing spectral data with similar 2'-phenylselenonyl derivatives prepared by Chattopadhyaya, et al.¹⁶² Generation of the elimination product **102** was indicative of the increased acidity of C2'-H in C2'-sulfones **91a**.

Scheme 18. Stability studies of sulfones **91a-b** on silica gel chromatography or with treatment of base.

To avoid elimination of the acetate group from C3', a stable benzyl protection group was employed (pK_a of AcOH = 5 vs pK_a of BnOH = 15). As expected, *N*3-benzyl-3',5'-di-*O*-benzyl-2'-deoxy-2'-[(4-chlorophenyl)sulfonyl]uridine (**91b**) with benzyl protecting group (poor leaving group) installed on 3',5' and *N*3 positions was stable

during silica gel column purification. Since the C2'-H in C2'-sulfones **91a-b** is quite acidic, I envisioned abstraction of C2'-proton with base followed by entrapment of the resultant of C2'-carbanion with fluoronium ion (F^+) source. However, treatment of sulfone **91b** with KH and Selectfluor in THF/DMF (-78 °C) led to the elimination of 3-*N*-benzyluracil **103** (63%) *via* the glycosidic bond cleavage (Scheme 18). I also isolated 3,5-di-*O*-benzyl-1,2-dideoxy-1,2-didehydro-2-[(4-chlorophenyl)sulfonyl]ribose (**104**; 71%) from the reaction mixture, whose structure was established using spectroscopic data. The singlet from the olefinic proton H1 at 7.4 ppm was diagnostic for the vinyl sulfone sugar **104**. It was also found that treatment of sulfone **91b** with base only, without Selectfluor, also affected glycosidic bond cleavage to give **103** and **104**, consistent with base/Selectfluor system.

Since the base/ F^+ system proved to be too harsh for the sulfone substrates, I imagined an alternative reduction pathway implying the selective cleavage of the sulfone moiety using organic electron donors. Organic electron donors (OED) are powerful reducing agents able to selectively cleave C-X (e.g., X = halogen) bonds by stepwise transfer of one or two electron(s) under mild reaction conditions.¹⁶³⁻¹⁷⁰ Previous literature studies report the efficient reduction of aryl iodides and bromides to aryl anions, as well as the reductive cleavage of phenylalkylsulfones in excellent yields.¹⁷¹ With redox potentials spanning from - 0.62 V to - 1.24 V *vs* SCE in DMF (~ - 0.93 V to - 1.55 V *vs* Ag/Ag⁺ 0.01 M) (Figure 24), OED could allow us to reductively cleave the aryl-alkyl sulfone in the 2'-sulfone precursors **92a-b** and 2'- α -fluorosulfone precursors **95a-b**.¹⁷² Indeed, in view of the high reduction potential of the arylsulfone moiety compared to OED, transferring one electron would theoretically appear difficult. Nevertheless, as

pointed out by Murphy et al.,³⁸ effective redox potentials of organic reducers in solution are much higher than their thermodynamic redox potential determined by electrochemical methods. It is explained by the formation of intimate charge-transfer complexes and ion pairing that eases the electron transfer. Additional π - π stacking between the SED and nucleoside may help to form intimate complex and facilitate the reduction. Hopefully, the generated carbanion intermediate could then be used in electrophile trapping reactions.

Figure 24. Structures of organic electron donors used for the reductive desulfonation.

Thus, treatment of sulfone **92a** was treated with tetrakis(dimethylamino)ethylene (TDAE) in DMF (-78 °C to rt) for 1.25 h followed by aqueous workup and silica gel column chromatography gave furan derivative **105**, the enol type (open chain sugar) product **106**, and the 3'-acetoxy elimination product **107** as major products (Scheme 19). The anticipated reduction of sulfone moiety was never observed, indicating that the TDAE initially acted as a base and then probably as a reducing agent. It is noteworthy to mention that when **92a** was treated with 4-dimethylamino pyridine (DMAP) in DMF at room temperature, almost quantitative formation of **105** was produced, a result supporting the basic pathway (not shown in scheme; data not given).

Analogous treatment of **92a** with one equivalent of the more powerful Murphy's Super Electron Donor (*N,N,N',N'*-Tetramethyl-7,8-dihydro-6H-dipyrido[1,2-a;2',1'-c][1,4]diazepine-2,12-diamine; SED)¹⁷¹ in DMF at ambient temperature for 15 min yielded furan derivative **105** (95%). The nucleoside base uracil **105a** was also identified (but not isolated) by ¹H-NMR in the aqueous phase along with other impurities.

Scheme 19. Reduction studies of acetyl protected sulfone substrates **91a** with TDAE and Murphy's SED.

Since the acetyl protection group in **92a** is base labile and is prone to elimination from the intermediate C2'-carbanion, I decided to use benzylated sulfone substrates. However, treatment of *N*3-benzyl-3',5'-di-*O*-benzyl-2'-deoxy-2'-[(4-chlorophenyl)sulfonyl]uridine (**92b**) with Murphy's reagent in DMF for 15 min at ambient temperature resulted in a similar, rapid glycosidic bond cleavage producing 3-*N*-benzyluracil **103** (69%) and 3,5-di-*O*-benzyl-1,2-dideoxy-1,2-didehydro-2'-[(4-methoxyphenyl)sulfonyl]ribose (**108**; 70%) respectively (Scheme 20). This result is in accordance with the result obtained when **92b** was treated with base (Scheme 18) and indicated that SED (in stoichiometric amount) was probably acting exclusively as base

and not as reducing agent. Also, analogous treatment of **92b** with SED for 30 min at room temperature generated uracil derivative **103** (in 86%), furan derivative **109** as a mixture with vinyl sulfone **108**.

Scheme 20. Reduction studies of benzyl protected sulfone substrate **91b** with Murphy's SED.

Since the 2'-sulfone substrates were too labile under the desired reaction conditions, the corresponding α -fluorosulfone substrates (**95a-b**, see Scheme 14 for structures) were chosen for the further reductive-desulfonylation reactions. Initially, stability of the acetyl protected 2'-(4-methoxyphenylsulfonyl)-2'-deoxy-2'-fluorouridine **95a** towards tetrakis(dimethylamino)ethylene (TDAE) was evaluated. Thus, treatment of **95a** (2'*R/S*, ~1:4.5) with 2 equiv. or 4 equiv. of TDAE in DMF at -20 °C for 1 h or at room temperature for 3 h resulted in recovery of unchanged starting material **95a** along with 3'-deacetylated byproduct. Longer reaction time (12 h) and higher temperature (60 °C) produced only a mixture of mono- and di-deacetylated byproducts but no further degradation or glycosidic bond cleavage were observed. Replacement of DMF with MeCN gave similar results. Addition of TDAE at 0 °C or above did not cause the reduction of the sulfonyl moiety, indicating that TDAE should be added at low temperatures. Also, replacement of TDAE with other reducing agents such as samarium iodide did not affect the reduction of sulfonyl unit.

On the other hand, treatment of α -fluorosulfone derivative **95a** with 1 equiv. of Murphy's Super Electron Donor (SED) in DMF at rt or 120 °C, overnight gave the 3'-deacetylated product (**110**) along with unchanged SM (Scheme 21). Similar results were obtained with increased equiv. of SED (3 eq.) at rt. However, previously known fluorovinyl compound **111**¹⁷³ was obtained in 46% yield when **95a** was treated with 3 eq. of SED (DMF, 120 °C, overnight) after acidic workup (10% HCl). The structure of **111** was established by ¹⁹F-NMR (δ -133.8 ppm, t, J = 4.6 Hz) and LC-MS [ESI⁺ m/z 271 (MH)⁺]. This result indicated that reductive cleavage of sulfone took place with excess SED at high temperature. Noticeably, when **95a** was treated with 4-dimethylamino pyridine (DMAP) in DMF at room temperature, was recovered intact indicating that base-induced pathway is not operating with such α -fluorosulfone.

Scheme 21. Stability studies of acetyl protected α -fluorosulfone **95a** with Murphy's SED.

To avoid the base-promoted elimination of the 3'-acetyl protection group, benzyl protected α -fluorosulfone derivative **95b** was employed to study the reduction of sulfone with SED. However, treatment of *N*3-benzyl,3',5'-di-*O*-benzyl protected sulfone **95b** under analogous reaction conditions (3 eq. SED, DMF, 120 °C, 3 h) produced a complex

reaction mixture (Scheme 22). Careful silica gel column chromatography allowed for separation of two major products, which are found to be glycosidic bond cleavage products furan **112** and 3-*N*-benzyluracil **103**. Trace amounts of sulfone cleavage product **113** was also isolated from the column. Intriguingly, in the furan derivative **112** sulfone moiety was intact and elimination of fluorine was observed. Formation of **112** could have been happened from either the reduction of glycosidic bond followed by base induced fluoride elimination or *vice versa*.

Scheme 22. Stability studies of benzyl protected α -fluorosulfones **95b** with Murphy's SED

Analogous treatment of **95b** with 3 equiv. of SED in DMF at room temperature gave only trace amounts of **112** and **103** along with unchanged SM. Addition of degazed water at 80°C to the crude, in order to favour the reduction product **113** (to enhance H-abstraction), did not change the outcome of the reaction. The elimination product **112** and 3-*N*-benzyluracil **103** were obtained in 80% and 60% isolated yields respectively.

Next, in order to prepare gem-difluorouridine analogues, I attempted to insert a second fluorine atom at C2'-position of 2'-arylsulfonyl-2'-deoxy-2'-fluorouridine derivative **95a** using reductive-desulfonylation/fluorination approach. If successful this method could be expanded toward the synthesis of other 2'-alkyl-2'-deoxy-2'-fluoro uridine nucleosides. Especially an intriguing target in that class of nucleosides would be

2'-C-methyl-2'-deoxy-2'-fluorouridine because of the potent anti-HCV activity possessed by the corresponding cytidine analogue (PSI 6130).⁵

Scheme 23. Attempted synthesis of 2'-2''-difluorouridine by reductive desulfonylation-fluorination of 2'-arylsulfonyl-2'-deoxy-2'-fluorouridine **95a** with TDAE/Selectrfluor

Since **95a** was barely reactive towards TDAE at low temperatures with no indication of the generation of putative C2' anion, I tried a combination of TDAE and UV light activation to enhance the electron transfer. Thus, treatment of **95a** with 5 equiv. of TDAE at -60 °C and Selectrfluor under uv light irradiation produced a mixture of 2',3'-dideoxy-2',3'-didehydro-2'-fluorouridine **111** and 1-(2-deoxy-2-fluoro-β,D-arabinofuranosyl)uracil **114** in addition to the acetyl deprotected byproducts (Scheme 23). Interestingly, no significant by-products resulting from the glycoside bond cleavage was observed. It is noteworthy that when the TDAE was added at 0 °C or room temperature, neither **111** nor **114** were formed, which means the TDAE did not act as a reductant when added at higher temperatures. Furthermore, no formation of the target 2'-deoxy-2',2''-difluorouridine derivative was observed, while the reduction of the employed electrophilic fluorine reagents was detected.

Scheme 24. Reductive-desulfonylation/methylation of 2'-arylsulfonyl-2'-deoxy-2'-fluorouridine **95a** with TDAE/MeI

Since fluoronium ion (F^+) is the most difficult cation to work with, I chose to test our hypothesis using CH_3^+ . However, treatment of **95a** with TDAE/MeI under analogous reaction conditions yielded acetyl deprotected, *N*3-methylated analogue **115** as the major product (Scheme 24).

3.3. One electron oxidation of gemcitabine and analogues

Our laboratory has a long history of studying the mechanism of ribonucleotide reductase (RNR) enzyme via chemical modeling studies.^{174,175} In collaboration with Prof. Stubbe of MIT, we have also been involved in studying mechanism-based inhibitors of RNR by 2'-azido-2'-deoxynucleostides^{176,177} and probing the mechanism of RNR catalyzed reaction with labeled natural substrates.¹⁷⁸ Recently, in collaboration with Dr. Sevilla's group from Oakland University, we began investigating the generation of radicals upon $1e^-$ oxidation of various nucleoside substrates.¹⁷⁹

Building on this interest, I wanted to explore the possibility of formation of sugar-based radicals in the one-electron ($1e^-$) oxidation of 2'-fluorocytidine analogues such as 2'-fluoro-2'-deoxycytidine (FdC, **38**), 2'-deoxy-2',2''-difluorocytidine (dFdC, gemcitabine, **1**), and 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine (MeFdC, PSI 6130, **39**) (Figure 25). Each of the three substrates has different stereo and electronic properties which should interact

with the resultant radical differently. Comparing to 2'-fluoro-2'-deoxycytidine, gemcitabine has an extra fluorine atom, which causes H3' to be more acidic because of its negative inductive effect (-I). Thus I was also hoping that we might capture and analyze the elusive C3' radical, postulated by Stubbe, et al.³¹ as a critical intermediate in the reduction of RNA monomer to DNA monomer. The presence of a positive inductive effect (+I) causing methyl group makes PSI 6130 a versatile candidate and it would be interesting to study the influence of different substituents at 2'-position (H vs F vs CH₃) on radical generation. Electron spin resonance (ESR) spectroscopy was employed to study the results generated during 1e⁻ oxidation of the selected substrates.

Figure 25. Selected substrates for 1e⁻ oxidation studies

3.3.1. Detection of C2' and C3' sugar radicals

The ESR spectrum after γ -irradiation of 2'-deoxy-2'-fluorouridine **38** (7.5 M LiCl/D₂O sample of 2'-dC (2 mg/ml) at pH ca. 10) showed a central anisotropic doublet (ca. 16 G) assigned to α proton at C5 in the 2'-F-dC pi-cation radical (C^{•+}) **116** and is in keeping with the coupling reported for the C5-H alpha proton coupling in the dC cation radical in single crystals (Scheme 25).¹⁸⁰

Scheme 25. One electron oxidation of 2'-deoxy-2'-fluorocytidine

One-electron oxidation of gemcitabine **1** results in the formation of metastable pi-cation radical **1a**, which is unstable even at ca. 155 K (Scheme 26). The metastable $\text{C}^{\bullet+}$ **1a** quickly deprotonates at C3' in the sugar moiety producing $\text{C3}'^{\bullet}$ **117** via a proton-coupled electron-transfer (PCET) mechanism. The presence of 2nd fluorine atom at 2' position may have increased the acidity of $\text{C3}'\text{H}$. Since the presence of one additional fluorine led to such a drastic behavior, I was very interested to study one electron oxidation of 2'-deoxy-2'-fluoro-2'-*C*-methylcytidine (PSI-6130; **39**), which is very active against HCV.¹⁸¹ PSI 6130 has a methyl group (+I) instead of a fluorine atom (-I).

Scheme 26. One electron oxidation of gemcitabine

The anti-HCV analogue PSI-6130 (**39**) also generated $\text{C3}'$ -radical **39a** via pi-cation radical **118**, upon γ -irradiation. But $\text{C3}'$ -radical in PSI-6130 was unstable and immediately converted to $\text{C2}'$ -radical **119** via loss of hydrogen fluoride (Scheme 27). All the results were in accordance with theoretical model studies and ESR spectroscopy.¹⁸²

This is the first non-enzymatic example of C3'-radical studies on gemcitabine and PSI-6130 and support the mechanism of inhibition of ribonucleotide reductase by these compounds.

Scheme 27. One electron oxidation of PSI-6130

3.4. Synthesis and biological activity of 6-*N*-benzyl-7-deazapurine nucleoside derivatives

3.4.1. Synthesis of 6-*N*-benzyl-7-deazapurine nucleosides

I wanted to prepare 6-*N*-benzylated 7-deazaadenosine analogues (**122a-d**) using the Dimroth rearrangement, which is widely studied on various adenine nucleosides and other heterocycles.^{115,183} Initial alkylation takes place at N1 when adenine nucleosides are treated with alkyl/aryl halides to form an N6-centered cationic intermediate (**120a**; Scheme 28). Treatment of the N1-alkylated intermediate **120a** with base leads to ring-opening, C-C bond rotation, and subsequent ring-closing to give the N6-alkylated adenosine product **121**. Interesting thing to note in the Dimroth rearrangement sequence is that N1 of substrate becomes N6 in the product, which was established based on isotope labeling studies.^{184,185}

Scheme 28. General mechanism of Dimroth rearrangement on Adenosine^{184,185}

Thus, treatment of tubercidin (**19a**) with benzyl bromide in DMF (48 h at 40 °C) showed a baseline spot on TLC, which suggests ionic intermediate(s) (Scheme 29). Evaporation of solvent; treatment of crude with acetone and ether; and vacuum filtration gave a hygroscopic white precipitate. The ionic intermediate was immediately dissolved in methanol and refluxed with dimethylamine (2 M solution in THF) (20 h at 65 °C). TLC showed conversion of majority of the base line spot to a less polar product. Aqueous workup followed by column chromatography gave 4-*N*-benzyltubericin **122a** (67%). Structure of the product was established by NMR and HRMS. For comparison, I synthesized the previously known 6-*N*-benzyladenosine (**121**)¹¹⁵ from adenosine (**120**) by following same reaction conditions (BnBr/DMF-Me₂NH/MeOH). I noticed that the TLC during the course of both the reactions was very similar. Therefore it is reasonable to assume that initial alkylation occurred at N3 (same as ring nitrogen N1 in adenosine) in

tubercidin, and treatment of the 3-*N*-benzyl intermediate with Me₂NH/THF in MeOH resulted in Dimroth-type rearrangement to give 4-*N*-benzyltubercidin (**122a**).

Scheme 29. Synthesis of 4-*N*-benzylated tubercidin and sangivamycin by Dimroth rearrangement approach.

It is well-established that the 6-*N*-(4-nitrobenzyl) derivatives of adenosine and other adenine nucleosides act as potent nucleoside transport inhibitors.¹¹⁹ To evaluate the nucleoside transport inhibition properties in a related system, I endeavored to synthesize 4-*N*-(4-nitrobenzyl) derivatives of the 7-deaza antibiotics. Thus, following the protocol for the synthesis of **122a**, reaction of tubercidin **19a** with 4-nitrobenzyl bromide (24 h at 80 °C) followed by treatment of the corresponding N-3 alkylated cationic intermediate with Me₂NH/MeOH produced 4-*N*-(4-nitrobenzyl)tubercidin (**122b**, 56%, Scheme 29). An analogous base line spot was observed when sangivamycin (**19b**) was treated with 4-nitrobenzyl bromide (48 h at 40 °C). Isolation of this ionic intermediate immediately followed by treatment with Me₂NH/MeOH yielded 4-*N*-(4-nitrobenzyl)sangivamycin (**122c**, 45%). Structures of products **122b-c** were established by NMR and elemental analysis.

Since tubercidin and sangivamycin underwent Dimroth-type rearrangement when treated with BnBr followed by Me₂NH, I envisioned the synthesis of 4-*N*-(4-

nitrobenzyl)toyocamycin (**122d**) *via* similar synthetic pathway. However, upon treatment of toyocamycin (**19c**) with 4-nitrobenzyl bromide (63 h at 40 °C), a major less polar spot than substrate was observed on TLC, as opposed to a more polar spot in case of tubercidin and sangivamycin (Scheme 30). Isolation of the new spot by column chromatography and careful spectroscopic evaluation showed the compound as 4-*N*-(4-nitrobenzyl)toyocamycin (**122d**; 46%). Since there was no N3-benzylated cationic intermediate, there was no need for Me₂NH/MeOH treatment. Therefore, a direct alkylation of the exocyclic amine (N4) of toyocamycin was proposed for the formation of **122d**.

Scheme 30. Synthesis of 4-*N*-(4-nitrobenzyl)toyocamycin by direct alkylation.

The direct alkylation of the exocyclic amino group of toyocamycin has not been previously noted. However, direct alkylation on the exocyclic amino group of adenine and guanine with quinone methides is known.¹⁸⁶ The exocyclic amine nitrogen N4 may have become more nucleophilic than the endocyclic nitrogen N3, as a result of the electron withdrawing pull of the cyano group at C5. Another possibility could be that the cylindrical cyano group might exist in synperiplanar orientation with the exocyclic amine group, thereby diminishing the lone-pair donation on N4 toward N3 of the amidine, which is part of the planar hetrocyclic system.¹⁸⁷

Alternatively, the preparation of 6-*N*-(4-nitrobenzyl)tubercidin derivative (**122b**) was envisioned based on synthesis of 6-fluorotubercidin analogue by diazotization-fluorodediazonation^{120,188,189} followed by S_NAr displacement of 6-fluoro group with 4-nitrobenzylamine.^{120,190} Thus, using standard protocol, tubercidin (**19a**) was treated with acetic anhydride in pyridine to give 2',3',5'-tri-*O*-acetyltubercidin (**123**, 86%; Scheme 31). The acetylated tubercidin (**123**) was then treated with sodium nitrite (NaNO₂) in freshly prepared ~55% HF-pyridine at -10 °C^{191,192} for 15 min to yield the protected 4-fluorotubercidin **124** (82%), probably via diazotive-fluorodeamination. The mechanism of this reaction is expected to be similar to 'Balz-Schiemann' type reaction for the preparation of arylfluorides from arylamines using NaNO₂ and 70% HF-pyridine.¹⁹¹ NaNO₂ in the presence of acid (H⁺) generates nitrosonium ion (NO⁺) in situ and aryl-NH₂ attacks the resultant NO⁺ to form diazonium ion **123a** subsequently. Loss of N₂ followed by quenching of intermediate arylcation **123b** with fluoride ion (F⁻) gives the product arylfluoride **124**. I observed that the concentration of HF was crucial in the preparation of **124**. When commercial 70% HF-pyridine reagent was used, the reaction did not yield the corresponding 4-fluoro product **124**. This observation indicated that pH of the reaction, which in turn depends on the percent of HF used, was critical for such diazotive-fluorodeamination reactions.¹⁹²

Scheme 31. Synthesis of 2',3',5'-tri-*O*-acetyl-4-fluorotubercidin (**124**) by diazotative-fluorodeamination.

Treatment of 4-fluorotubercidin derivative **124** with 4-nitrobenzylamine hydrochloride in presence of trimethylamine (Et_3N) in MeOH gave 2',3',5'-tri-*O*-acetyl-4-*N*-(4-nitrobenzyl)tubercidin (**125**, 47%; Scheme 32). Trimethylamine converts 4-nitrobenzylamine hydrochloride to 4-nitrobenzylamine in situ, and the free amine subsequently displaces the fluoride ion, probably, by aromatic nucleophilic substitution ($\text{S}_{\text{N}}\text{Ar}$).¹²⁰ Acetyl deprotection of compound **125** using methanolic ammonia (NH_3/MeOH) at ambient temperature gave **122b** (85%; overall 32% yield). Out of the two methods for the preparation of **122b**, Dimroth alkylation pathway (56% yield) was better in terms of number of steps and overall yield.

Scheme 32. Synthesis of 4-*N*-(4-nitrobenzyl)tubercidin (**122b**) from 2',3',5'-tri-*O*-acetyl-4-fluorotubercidin (**124**).

3.4.2. Biological activity of 6-*N*-benzyl-7-deazapurine nucleoside derivatives

Nucleoside transport inhibition activities were measured in Dr. James D. Young's laboratory of University of Alberta. While antiviral and anti-proliferation activities were studied in Dr. Jan Balzarini's laboratory of Rega Institute for Medical Research.

3.4.2.1. Nucleoside transport inhibition activity

Inhibition of the initial rate of ^3H -uridine (20 μM) uptake by recombinant hENT1 produced in the *Xenopus* oocyte heterologous expression system was determined as described previously.¹¹⁸ The 4-*N*-(4-nitrobenzyl)tubercidin (**122b**) and 4-*N*-(4-nitrobenzyl)sangivamycin (**122c**) fully inhibited labelled uridine uptake, whereas 4-*N*-benzyltubercidin (**122a**) and 4-*N*-(4-nitrobenzyl)toyocamycin (**122d**) analogues exhibited weak inhibition (<45%). Dose-response evaluations indicated that sangivamycin analogue **122c** ($\text{IC}_{50} = 123 \pm 31$ nM) was a better inhibitor of uridine transport by hENT1 than the tubercidin analogue **122b** ($\text{IC}_{50} > 1000$ nM). However, the sangivamycin analogue **122c** was a weaker inhibitor of hENT1-mediated transport than 6-*N*-(4-nitrobenzyl)adenosine, NBMPR, and other derivatives¹¹⁹ containing a nitrogen atom at the 7-position in the adenine ring.

3.4.2.2. Antiviral activity

Table 7. Antiviral activity in human embryonic lung (HEL) cell cultures-part 1

Compd	EC ₅₀ (μM)*				
	HSV-1 (KOS)	HSV-2 (G)	Vaccinia virus	Vesicular stomatitis virus	HSV-1 TK - KOS ACV
122a	>100	>100	>100	>100	>100
122b	>100	>100	34	>100	>100
122c	>100	>100	34	>100	>100
122d	>100	>100	100	>100	>100

*Effective concentration required to reduce virus plaque formation by 50%.
Virus input was 100 plaque forming units (PFU).

The 4-*N*-benzyl-7-deazapurine derivatives (**122a-d**) weakly inhibited Herpes Simplex Virus (HSV; 1 & 2) and Vesicular stomatitis virus (EC₅₀: >100 μM) (Table 7). But *Vaccinia* virus was reasonably inhibited by 4-*N*-(4-nitrobenzyl)tubercidin (**122b**) and 4-*N*-(4-nitrobenzyl)sangivamycin (**122c**) (EC₅₀: 34 μM) and to a weak extent by 4-*N*-benzyltubercidin (**122a**) and 4-*N*-(4-nitrobenzyl)toyocmycin (**122d**) (EC₅₀: >100 μM).

Cytomegalovirus (CMV) and *Varicella Zoster* Virus (VZV) were inhibited at ~20 μM concentrations by 4-*N*-benzyltubercidin (**122a**), 4-*N*-(4-nitrobenzyl)sangivamycin (**122c**) and 4-*N*-(4-nitrobenzyl)sangivamycin (**122d**) analogues (Table 8). But 4-*N*-(4-nitrobenzyl)tubercidin (**122b**) was not very potent (EC₅₀: >100 μM).

Table 8. Antiviral activity human embryonic lung (HEL) cell cultures-part 2

Compd	EC ₅₀ (μM)*			
	CMV (AD - 169 strain)	CMV (Davis strain)	VZV (TK + VZV strain)	VZV (TK - VZV strain)
122a	>20	>20	>20	>20
122b	>100	>100	>100	>100
122c	>20	>20	>20	>20
122d	>20	>20	>20	>20

*Effective concentration required to reduce virus plaque formation by 50%.
Virus input was 100 plaque forming units (PFU).

3.4.2.3. Inhibition of Cell Proliferation

Inhibition of the proliferation of murine leukemia (L1210) cells and human T-lymphocyte (CEM), cervix carcinoma (HeLa), prostate cancer (PC-3), and kidney carcinoma (Caki-1) cells by the 7-deazaadenine nucleoside analogues (**122a-d**) was evaluated (Table 9). The 4-*N*-benzyltubercidin (**122a**) showed marked inhibition of the proliferation of PC-3 cells (IC₅₀: 0.92 μM) and HeLa cells (IC₅₀: 7.4 μM) but no significant activity was found with its nitrobenzyl analogue **122b** (IC₅₀: >50 μM). The 4-*N*-(4-nitrobenzyl)sangivamycin (**122c**) and 4-*N*-(4-nitrobenzyl)toyocamycin (**122d**) analogues inhibited proliferation of L1210, HeLa, and PC-3 cells at ~0.9–9.4 μM with the sangivamycin analogue **122c** showing more potent effects (0.92–3.4 μM). It is intriguing that cytostatic activity of the 6-*N*-benzyl-7-deazapurine analogues was highly dependent on the nature of the tumor cell lines. Proliferation of HeLa and PC-3 tumor cells was highly inhibited by **122a**, **122c**, and **122d** whereas proliferation of L1210 cells

was sensitive only to **122c** and **122d**. The CEM and Caki-1 cells were weakly sensitive to the antiproliferative effects of any of the 6-*N*-benzyl-7-deazapurine compounds **122a**–**122d**.

Table 9. Inhibitory effects on the proliferation of cells in culture

Compd	IC ₅₀ (μM)*				
	L1210	CEM	HeLa	PC-3	Caki-1
122a	172 ± 47	182 ± 20	7.4 ± 2.2	0.92 ± 0.67	116 ± 23
122b	125 ± 28	≥250	143 ± 4	76 ± 10	132 ± 30
122c	0.92 ± 0.04	115 ± 28	1.8 ± 0.2	3.4 ± 0.9	116 ± 23
122d	5.5 ± 1.5	109 ± 16	9.4 ± 3.0	5.6 ± 3.3	98 ± 10

* Half-maximal inhibitory concentration (IC₅₀); data represent the mean_ SD of at least n=2–3 independent experiments; Cell lines: murine leukemia (L1210); human CD4+ T-lymphocytes (CEM); human cervix carcinoma (HeLa); human prostate adenocarcinoma (PC-3); human kidney carcinoma (Caki-1).

3.5. C7- and C8-modified-7-deazapurine nucleoside derivatives

Building upon my interest in working with 7-deazaadenosine nucleosides, I envisioned the synthesis of various 7-substituted or 8-substituted 7-deazaadenosine analogues, which might have fluorescent properties.

3.5.1. Strain promoted click reactions of 8-azido-7-deazanucleosides with cyclooctynes

Initially, I imagined the conversion of 8-halo-7-deazapurines to the corresponding 8-azido-7-deazapurines, which could serve as substrates for Strain-promoted Azide-Alkyne Cycloaddition reactions (SPAAC, Cu-free click chemistry) with

cyclooctynes.^{193,194} I expect that the resulting triazolyl products might have fluorescent properties due to increased conjugation in the 7-deazapurine base part. The Cu-free click chemistry is a rapidly growing field, with various applications for *in vivo* imaging emerging continuously over the past few years.^{193,195-199}

Thus, 8-Bromotubercidin (**126**) was prepared by treatment of tubercidin **19a** with *N*-bromosuccinimide in presence of potassium acetate (KOAc) in DMF at ambient temperature (30 min), as reported in literature.²⁰⁰ I envisioned the preparation of 8-azido-7-deazaadenosine derivatives (**127-129**, Scheme 33) by nucleophilic aromatic substitution of 8-bromo-7-deazaadenosine derivatives (**55**, **56** and **126**) with sodium azide. However, Attempts to synthesize 8-azidotubercidin via nucleophilic aromatic substitution of **126** with sodium azide were unsuccessful, even after using harsh conditions (NaN₃ (1-5 eq), DMF, rt-153 °C, N₂, 24 h; NaN₃ (3 eq), TsOH (3 eq), EtOH, reflux, 24h). Lack of reaction is possibly a consequence of the low electron deficiency of the pyrrole ring in tubercidin **19a** when compared to that of other 7-deazanucleosides **19b-c**. To explain in other words, C8-bromination probably proceeds via electrophilic aromatic substitution, whereas C8-azidation is expected to proceed via nucleophilic aromatic substitution. I envisioned the presence of electron withdrawing groups in the pyrrole ring on 8-bromo-7-deaza nucleosides will enhance the electron deficiency, thereby promoting nucleophilic displacement of bromide with azide. So I chose to pursue 8-bromotoyocamycin and 8-bromosangivamycin substrates, both of which have an electron withdrawing substituent on the 7-position of the pyrrole ring.

Scheme 33. Synthesis of 8-bromo-7-deazaadenosine and 8-azidotoyocamycin derivatives.

Treatment of 8-bromosangivamycin **56** with NaN₃ in DMF at 70 °C for 16 hrs gave a slightly more polar product, which was thought to be 8-azidosangivamycin. UV spectra of the reactions always showed at least 12 nm bathochromic shift. However, ions corresponding to neither product (**128**) nor substrate (**56**) were detected in the high-resolution mass spectroscopy (HRMS). Instead, peaks at *m/z* 258 and 317 were observed, which were unable to be identified with a list of possible products. Similar results were obtained when DMSO or MeOH were used as the reaction solvents.

Next, I synthesized 8-azidotoyocamycin **129** by treating 8-bromotoyocamycin **55** with NaN₃ in DMF at room temperature for 16 h. This transformation was light and, possibly, heat sensitive requiring the reaction, purification, and characterization to be carried out in the dark with limited heating to give 8-azidotoyocamycin (**129**; 46%). The light sensitivity of the product also limited us in recording the analytical data for the compound. Moreover, this azidation (**55** to **129**) was unsuccessful in MeOH or water, indicating that polar aprotic solvents are probably the best solvents for these types of aromatic nucleophilic substitution reactions.

Scheme 34. Click reaction between 8-azidotoyocamycin and cyclooctynes **130** and **131**. *Reagents and conditions:* g) Cyclooctyne **130** or **131** (1 eq), ACN:H₂O (3:1), rt, 4 h.

Having 8-azidotoyocamycin (**129**) in hand, my next goal was to see if the SPAAC reactions would take place between **129** and cyclooctynes. Thus, treatment of **129** with symmetrically fused cyclopropyl cyclooctyne **130** in an aqueous solution of acetonitrile (ACN) at ambient temperature (4 h) gave the corresponding triazolyl product **132** (59%, Scheme 34). Analogous reaction of **129** with a strain modulated dibenzylcyclooctyne **131** produced triazole **133** (47%). These preliminary results indicated that the SPAAC reactions were taking place as expected. With the extended conjugation in the deazapurine part, I expect the triazole products to have fluorescence properties and that could lead to various interesting biological applications.

3.5.1. Attempted synthesis of 8-alkynyl-7-deazanucleosides *via* Sonagashira reaction

Since the attempted synthesis of 8-azido analogues of tubercidin and sangivamycin were unsuccessful, I envisioned the synthesis of 8-vinylazido derivatives (**136**) from the corresponding 8-alkynyl-7-deazapurine derivatives (**135**), which in turn

could be prepared in two steps via Sonagashira coupling of 8-halo-7-deazapurines (**55**, **56** and **126**) with trimethylsilylacetylene (Scheme 35). The vinylazides **136** could be used as substrates for strain-promoted click reactions to give the corresponding triazolyl products (**137**). Also, the crucial 8-alkynyl derivative **135** could also be converted to the corresponding halovinyl sulfone and β -keto sulfones (not shown in scheme). This part of the dissertation is envisioned based on recent literature relating to transformations of alkynes to the corresponding (i) halovinyl sulfones;²⁰¹⁻²⁰³ (ii) β -keto sulfones;²⁰⁴ and (iii) vinylazides.²⁰⁵

Scheme 35. Proposed scheme for the synthesis and subsequent transformations of 8-alkynyl-7-deazapurine nucleoside analogues **135**.

Initially, treatment of 8-bromotubercidin (**126**) with trimethylsilylacetylene (TMSA) in the presence of trimethylamine (Et_3N) and catalytic amounts of bis(triphenylphosphine)palladium dichloride $[(\text{PPh}_3)_2\text{PdCl}_2]$ and copper iodide (CuI)

(anhydrous DMF, 110 °C, sealed flask) showed unchanged starting material as major product (Scheme 35; Table 10, entry 1). Doubling the amounts of CuI and Et₃N did not change the outcome of the reaction (entry 2).

Table 10. Reaction conditions for the attempted Sonagashira cross-coupling reactions on 8-bromo tubercidin and toyocamycin^a

Entry	Substrate	(PPh ₃) ₂ PdCl ₂ (mol %)	CuI (mol %)	Et ₃ N	TMSA	Temp.	Time
1	126	3%	9%	Excess	3 eq.	110 °C	48 h
2	126	3%	15%	Excess	6 eq.	110 °C	48 h
3	126	10%	20%	4 eq.	3 eq.	50 °C	16 h
4	126	15%	30%	11 eq.	10 eq.	50 °C	5 h
5	55	1%	2%	7 eq.	1.2 eq.	80 °C	48 h
6	55	10%	20%	5 eq.	3 eq.	50 °C	24 h
7	55	20%	10%	5 eq.	3 eq.	50 °C	24 h
8	55	15%	30%	14 eq.	10 eq.	50 °C	22 h

^aReactions were typically performed on 0.1 mmol scale in anhydrous DMF. Reaction progress was monitored by TLC.

Treatment of **126** with 0.1 equiv. of Pd and 0.2 equiv. Cu catalysts under analogous reaction conditions showed a minor less polar spot on TLC (entry 3). Encouraged by the observation, I treated 8-bromotubercidin **126** with 0.15 equiv. of Pd catalyst, 0.3 equiv. of Cu catalyst, 11 equiv. of Et₃N, and 10 equiv. of TMSA in anhydrous DMF for 5 hrs at 50 °C. TLC of the crude reaction mixture was complex, but there was a major less polar spot (~50% in comparison with unchanged substrate) (entry 4). ¹H-NMR of the isolated material showed ~55:45 ratio of substrate to product (**126**:**134a**). The mixture of products was then treated with tetrabutylammonium fluoride (TBAF) and the major product was isolated by column chromatography. But ¹H-NMR of

the isolated spot showed absence of the characteristic acetylenic proton peak and was very similar to the ^1H -NMR of 8-bromotubercidin **126**, and no signals for the 8-alkynyl product **134a** were observed. Thus, it can be concluded that Sonagashira cross-coupling of 8-bromotubercidin with trimethylsilylacetylene is a low-yielding reaction.

Because of the ease of preparation and availability of precursors, I used 8-halotoyocamycin derivatives for initial Sonagashira reactions before proceeding to 8-halosonagashira substrates. However, analogous treatment of 8-bromotoyocamycin (**55**) with trimethylsilylacetylene $[(\text{PPh}_3)_2\text{PdCl}_2/\text{CuI}/\text{Et}_3\text{N}/\text{DMF}/40\text{ }^\circ\text{C}]$ also gave unchanged starting material as major product, even after varying the reaction conditions (Scheme 35; Table 10, entries 5-8).

To simplify the purification process, I decided to use protected 8-halo-7-deazapurine derivatives as substrates. Thus, 2',3',5'-tri-*O*-acetyltoyocamycin (**138**) was prepared in 97% isolated yield by treating toyocamycin (**19c**) with $\text{Ac}_2\text{O}/\text{Py}$ (Scheme 36). Treatment of 2',3',5'-tri-*O*-acetyltoyocamycin (**138**) with DBH (1.1 eq., CH_2Cl_2 , 18 h) gave the corresponding 8-bromo-2',3',5'-tri-*O*-acetyltoyocamycin (**139**) in 79% isolated yield. However, treatment of **139** (107 mg) with 0.02 equiv. of Pd catalyst, 0.1 equiv. of Cu catalyst, 0.1 equiv. of Et_3N , and 4 equiv. of TMSA in anhydrous DMF for 19 h at $40\text{ }^\circ\text{C}$ gave a complex reaction mixture. Silica gel column chromatography gave three major fractions. The NMR analysis showed the first two products to be unchanged starting material **139** (27 mg, 25%), and debrominated product **138** (7 mg, 8%). The third fraction (4 mg) was more polar than the substrate on TLC, was blue on TLC plate under 365 nm lamp. But ^1H -NMR of this material did not have characteristic product peaks and structure of this blue spot could not be determined.

Scheme 36. Attempted synthesis of 8-alkynyltoyocamycin derivative **140** via sonagashira coupling.

As iodo is a better substrate than bromo in Sonagashira coupling, I envisioned the use of 8-iodo-7-deazapurine analogues as substrates. Thus, toyocamycin **19c** was converted to the corresponding tri-O-(*tert*-butyldimethylsilyl) protected counterpart **141** using standard procedures ($\text{TBDMSCl}/\text{Imidazole}/\text{DMF}/35\text{ }^\circ\text{C}$; 58%) (Scheme 37). The reaction was sluggish and required longer hours of stirring and more equivalents of reagents than usual. Treatment of silylated toyocamycin **141** with Lithium diisopropylamide (LDA, 5 equiv.) and iodine (I_2 , 1.5 equiv.) in dry THF [$-78\text{ }^\circ\text{C}$ (2 h) to ambient (48 h)] gave the corresponding 8-iodotoyocamycin analogue **142** in 16% isolated yield. Unchanged substrate **141** was also isolated from the column (58%). However, treatment of 8-iodo analogue **142** with trimethylsilylacetylene, $(\text{PPh}_3)_2\text{PdCl}_2$ (10% mol), CuI (20% mol), Et_3N (excess), TMSA (4 equiv.) in DMF at $40\text{ }^\circ\text{C}$ for 48 h gave unchanged starting material **142** as major product even after for 2 days. Strangely, no trace of deiodinated product **141** or acetylenic product **143** was observed on TLC.

Scheme 37. Attempted synthesis of 8-alkynyloxytocamycin derivative **143** via sonagashira coupling.

Considering the cost of 7-deazapurine substrates, I stopped proceeding further in this direction.

3.5.2. Iodine catalyzed direct C-H activation of 7-deazapurine nucleosides

Since I was unable to synthesize the 8-azido or the corresponding vinylazide analogues of tubercidin and sangivamycin, I explored other possibilities for incorporation of an aromatic moiety (e.g. triazolyl) based on direct on C-H activation.²⁰⁶ Incidentally, a recent research paper by Dr. Yotphan, *et al.* described iodine-catalyzed direct activation of indoles with azoles mediated by *tert*-butylhydroperoxide (TBHP) (Scheme 38).²⁰⁷

Scheme 38. Iodine catalyzed oxidative cross-coupling of Indoles and Azoles.²⁰⁷

Because tubercidin **19a** is structurally similar to indole, I imagined oxidative cross-coupling of tubercidin with triazoles to generate the corresponding C7 and/or C8 triazolyl substituted analogues. Thus, initial treatment of tubercidin **19a** with benzotriazole (2 equiv.), I₂ (0.2 equiv.), and TBHP (5-6 M solution in decane, 1 equiv.) in

anhydrous DMF at ambient temperature after 7 h showed unchanged tubercidin as the major product on TLC (Scheme 39). No product formation was observed even after stirring the reaction mixture at 40 °C for 7 hrs. Additional I₂ (0.2 equiv.), and TBHP (1 equiv.) were added and the reaction mixture was stirred at 40 °C for 48 h, by which time the TLC showed ~15% conversion to a more polar product. Analogous treatment of tubercidin **19a** with benzotriazole (2 equiv.), I₂ (0.4 equiv.), and TBHP (2 equiv.) in anhydrous DMF at 35 °C for 72 h also showed ~10% conversion to a less polar product. Additional TBHP (1 equiv.) was added and the reaction mixture was stirred at 35 °C for 24 h (total: 96 h), by which time the TLC showed ~30% conversion to a more polar product. The new spot was isolated by silica gel chromatography in 18% yield and was single product on ¹H, ¹³C NMR. Comparison of ¹H-NMR data with substrate revealed the following: (i) singlet for H2 was still present (shifted to 8.22 ppm from 8.05 ppm); (ii) Doublet for H8 (7.35 ppm) collapsed to a singlet (7.04 ppm); and (ii) doublet for H7 (at 7.6 ppm) is absent. This confirms the structure of the product to be 7-(benzotriazolyl)tubercidin **144**.

Scheme 39. Iodine catalyzed oxidative cross-coupling of tubercidin and benzotriazole.

Fluorescence analysis of the triazolyl compound **144** showed emission at 420 nm with the best quantum yield being ~0.002 units (in MeOH). The fluorescence is stronger in basic pH between 7 and 12 and at pH ~2, it is non-fluorescent. The average lifetime is

about 3.8 ns with two well-defined lifetimes of 1 ns (10%) and 4.1 ns (90%). However, the compound seems to be highly sensitive to UV light, since increase in fluorescence upon irradiation with 280 nm light was observed.

Conversely, treatment of acetyl-protected tubercidin (**123**) with I₂ (0.2 eq.), TBHP (1eq.), in DMF at room temperature for 12 h gave a mixture of three products (Scheme 40). TLC of the reaction mixture showed ~30% conversion to a less polar spot. Isolation of the new spot by column chromatography followed by ¹H-NMR analysis showed the spot as a mixture of three different products (19% yield, 60:25:15 mixture). It is suspected that the mixture of products are 7-substituted analogue **145**, 8-substituted counterpart **146** and probably 7,8-dibenzotriazolyltubercidin (not shown in scheme).

Scheme 40. Iodine catalyzed oxidative cross-coupling of acetylated tubercidin and benzotriazole.

In addition, treatment of sangivamycin (**19b**) with benzotriazole (2 eq.), I₂ (0.4 eq.), TBHP (2 eq.), in anhydrous DMF at 120 °C for 24 h showed unchanged starting material on TLC (Scheme 41). On the other hand, treatment of toyocamycin (**19c**) with benzotriazole (2 eq.), I₂ (0.4 eq.), TBHP (2eq.), in anhydrous DMF at 80 °C for 48 hrs resulted in 30% conversion to new spot on TLC. The isolated spot was not the expected product **147c** and exact structure could not be established by spectroscopic data. These

results reiterate the literature reports that indoles with electron deficient pyrrole rings react sluggishly with azoles.²⁰⁷

Scheme 41. Attempted synthesis of iodine catalyzed oxidative cross-coupling of sangivamycin or toyocamycin and benzotriazole.

4. EXPERIMENTAL SECTION

The ¹H (400 MHz), ¹³C (100.6 MHz), and ¹⁹F (376 MHz) NMR spectra were recorded at ambient temperature in solutions of CDCl₃ or DMSO-*d*₆. Reaction progress was monitored by TLC on Merck Kieselgel 60-F₂₅₄ sheets with product detection by 254-nm light. Products were purified by column chromatography using Merck Kieselgel 60 (230-400 mesh) or by automated flash chromatography using a CombiFlash system. UV spectra were recorded with a Varian Cary 100 Bio UV-visible spectrophotometer. Reagent grade chemicals were used and solvents were dried by reflux and distillation from CaH₂ under N₂ unless otherwise specified, and an atmosphere of N₂ was used for reactions. Purity of the synthesized compounds was determined to be ≥95% by elemental analysis (C, H, N) and/or HPLC on Phenomenex Gemini RP-C18 column with CH₃CN/H₂O solvent system as mobile phase. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) technique or electro-spray ionization

(ESI) techniques. High-resolution mass spectra were obtained in ESI-TOF mode unless otherwise noted.

Chemical shifts (δ) are reported in parts per million (ppm) referenced to the residual solvent peak, and coupling constants (J) are given in Hertz (Hz). Multiplicity is reported using standard abbreviations: singlet (s); doublet (d); triplet (t); multiplet (m); broad (br); inverted commas indicate observed multiplicity. UV spectra were recorded with a Varian Cary 100 Bio UV-visible spectrophotometer.

Some of the data were reprinted with permission from the following papers:

- (i) Adhikary, Amitava; Kumar, Anil; Rayala, Ramanjaneyulu; Hindi, Ragda M.; Adhikary, Ananya; Wnuk, Stanislaw F.; and Sevilla, Michael D., “One-electron oxidation of Gemcitabine and analogs: Mechanism of formation of C3' and C2' sugar radicals”, *J. Am. Chem. Soc.* **2014**, *136* (44), 15646–15653. Copyright © 2014, American Chemical Society.
- (ii) Rayala, Ramanjaneyulu; Theard, Patricia; Ortiz, Heysell; Yao, Sylvia; Young, James D.; Balzarini, Jan; Robins, Morris J., Wnuk, Stanislaw F., “Synthesis of Purine and 7-Deazapurine Nucleoside Analogues of 6-*N*-(4-Nitrobenzyl)adenosine; Inhibition of Nucleoside Transport and Proliferation of Cancer Cells”, *ChemMedChem* **2014**, *9*, 2186-2192. Copyright © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
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Cytostatic activity assays

All assays were performed in 96-well microtiter plates. To each well of 200 μ L were added $(5-7.5) \times 10^4$ tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210), 72 h (human lymphocytic CEM), 96 h (human cervix carcinoma HeLa), 144 h (human prostate cancer PC-3), or 168 h (human kidney Caki-1) at 37 °C in a humidified CO₂-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC₅₀ (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

General procedure for the bromination of protected nucleosides. DBH and/or TMSOTf were added to a stirred solution of substrate in CH₂Cl₂. The resulting brownish-orange solution was stirred at room temperature for time given in the corresponding table (Tables 3, 4, and 5; Section 3.1.) or until TLC showed absence of starting material and formation of less polar product. The reaction mixture was diluted with CHCl₃ and was washed with saturated NaHCO₃/H₂O and brine. The organic layer was dried (MgSO₄) and concentrated *in vacuo* to yield the corresponding brominated product as a colorless foam with purity over 98% (¹H NMR).

General procedure for the bromination of unprotected nucleosides.

Procedure A. DBH was added to a stirred solution of substrate in DMF. The resulting pale-yellow solution was stirred at room temperature for time given in the corresponding table (Tables 3, 4, and 5; Section 3.1.) or until TLC showed absence of starting material and formation of less polar product. Volatiles were evaporated and the residue was co-evaporated with MeCN. The resulting pale solid was crystallized or purified by column chromatography to give the corresponding brominated product as colorless crystals.

Procedure B. DBH was added to a stirred suspension/solution of substrate in MeOH. The resulting pale solution was stirred at room temperature for time given in the corresponding table (Tables 3, 4, and 5; Section 3.1.) or until TLC showed absence of starting material and formation of less polar product. Products were isolated by (a) filtration, if the products precipitated from the reaction mixture; (b) if no precipitate was observed, then volatiles were evaporated and the residue was crystallized or column chromatographed to give the corresponding brominated product as colorless crystals. The isolated brominated products were usually >98% pure by ^1H -NMR.

5-Bromo-2',3',5'-tri-*O*-acetyluridine (46a)⁶⁴. UV (MeOH) λ_{max} 275 nm, λ_{min} 240 nm; ^1H NMR (CDCl_3) δ 9.75 (br s, 1H, NH), 7.82 (s, 1H, H6), 6.06 (d, $J = 4.7$ Hz, 1H, H1'), 5.34-5.29 (m, 2H, H2', H3'), 4.38-4.30 (m, 3H, H4', H5', H5''), 2.18 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.08 (s, 3H, Ac).

5-Bromo-1-(2,3,5-tri-*O*-acetyl- β -D-arabinofuranosyl)uracil (46b)²⁰⁸ UV (MeOH) λ_{max} 276 nm, λ_{min} 239 nm; ^1H NMR (CDCl_3) δ 9.33 (br s, 1H, NH), 7.77 (s, 1H, H6), 6.22 (d, $J = 4.1$ Hz, 1H, H1'), 5.35 (dd, $J = 3.7, 4.1$ Hz, 1H, H2'), 5.04 ("q", $J = 1.9$ Hz, 1H, H3'), 4.38 (dd, $J = 5.8, 12.1$ Hz, 1H, H5'), 4.32 (dd, $J = 3.9, 12.1$ Hz, 1H, H5''), 4.16-4.12 (m, 1H, H4'), 2.09 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.98 (s, 3H, Ac); ^{13}C NMR (CDCl_3): δ 170.5, 169.6, 168.6 (3 x Ac), 158.6 (C4), 149.2 (C2), 139.8 (C6), 96.3 (C5), 84.4 (C1'), 80.7 (C4'), 76.1 (C3'), 74.4 (C2'), 62.5 (C5'), 20.8, 20.6, 20.4 (3 x Ac); MS (ESI) m/z 447 (100, [^{79}Br], MH $^+$), 449 (98, [^{81}Br], MH $^+$).

5-Bromo-3',5'-di-*O*-acetyl-2'-deoxy-uridine (46c)⁶⁴. UV (MeOH) λ_{max} 277 nm, λ_{min} 241 nm; ^1H NMR (CDCl_3) δ 9.59 (br s, 1H, NH), 7.89 (s, 1H, H6), 6.29 ("dd", $J = 5.8, 8.0$ Hz, 1H, H1'), 5.24-5.21 (m, 1H, H3'), 4.40 (dd, $J = 3.1, 12.2$ Hz, 1H, H5'), 4.34-4.28 (m,

2H, H4', H5''), 2.54 (ddd, $J = 2.3, 5.8, 14.3$ Hz, 1H, H2'), 2.23-2.15 (m, 1H, H2''), 2.17 (s, 3H, Ac), 2.11 (s, 3H, Ac).

5-Bromouridine (46d)⁶⁴. UV (MeOH) $\lambda_{\max}$ 279 nm, $\lambda_{\min}$ 241 nm; ^1H NMR (DMSO- d_6) δ 11.81 (br s, 1H, NH), 8.48 (s, 1H, H6), 5.73 (d, $J = 4.5$ Hz, 1H, H1'), 5.42 (d, $J = 5.3$ Hz, 1H, 2'OH), 5.27 (t, $J = 4.7$ Hz, 1H, 5'OH), 5.07 (d, $J = 5.4$ Hz, 1H, 3'OH), 4.04 (q, $J = 4.9$ Hz, 1H, H2'), 3.98 (q, $J = 5.0$ Hz, 1H, H3'), 3.88-3.85 (m, 1H, H4'), 3.71-3.66 (m, 1H, H5'), 3.60-3.55 (m, 1H, H5'').

5-Bromo-1-(β -D-arabinofuranosyl)uracil (46e)²⁰⁹. UV (MeOH) $\lambda_{\max}$ 280 nm, $\lambda_{\min}$ 242 nm; ^1H NMR (DMSO- d_6) δ 11.82 (br s, 1H, NH), 8.07 (s, 1H, H6), 5.96 (d, $J = 4.6$ Hz, 1H, H1'), 5.63 (d, $J = 5.3$ Hz, 1H, 2'OH), 5.48 (d, $J = 4.5$ Hz, 1H, 3'OH), 5.17 (t, $J = 5.4$ Hz, 1H, 5'OH), 4.03 ("q", $J = 4.5$ Hz, 1H, H2'), 3.91 (q, $J = 4.2$ Hz, 1H, H3'), 3.74 ("q", $J = 4.5$ Hz, 1H, H4'), 3.66-3.55 (m, 1H, H5', H5'').

5-Bromo-2'-deoxyuridine (46f)⁶⁴. UV (MeOH) $\lambda_{\max}$ 279 nm, $\lambda_{\min}$ 241 nm; ^1H NMR (DMSO- d_6) δ 11.79 (br s, 1H, NH), 8.39 (s, 1H, H6), 6.10 (t, $J = 6.5$ Hz, 1H, H1'), 5.25 (d, $J = 4.3$ Hz, 1H, 3'OH), 5.17 (t, $J = 4.8$ Hz, 1H, 5'OH), 4.26-4.22 (m, 1H, H3'), 3.79 (q, $J = 3.2$ Hz, 1H, H4'), 3.66-3.54 (m, 2H, H5', H5''), 2.18-2.08 (m, 2H, H2', H2'').

5-Bromocytidine (47b)⁶². UV (MeOH) $\lambda_{\max}$ 289 nm, $\lambda_{\min}$ 265 nm; ^1H NMR (DMSO- d_6) δ 8.39 (s, 1H, H6), 7.83 (br s, 1H, NH), 6.99 (br s, 1H, NH), 5.71 (d, $J = 3.2$ Hz, 1H, H1'), 5.37 (d, $J = 5.0$ Hz, 1H, 2'OH), 5.23 (t, $J = 4.8$ Hz, 1H, 5'OH), 5.00 (d, $J = 5.4$ Hz, 1H, 3'OH), 3.98-3.92 (m, 1H, H2'), 3.85-3.83 (m, 1H, H3'), 3.70 (ddd, $J = 2.7, 4.8, 12.1$ Hz, 1H, H5'), 3.56 (ddd, $J = 2.5, 4.9, 12.1$ Hz, 1H, H5'').

4-N-Benzoyl-5-bromocytidine (48b). mp 193-195 °C; UV (MeOH) $\lambda_{\max}$ 252, 335 nm (ϵ 8900, 13 900), $\lambda_{\min}$ 228, 292 nm (ϵ 7300, 4200); ^1H NMR (DMSO- d_6) δ 12.81 (br s, 1H,

NH), 8.79 (s, 1H, H6), 8.10-8.24 (br s, 2H, Bz), 7.62 (t, $J = 7.3$ Hz, 1H, Bz), 7.53 (t, $J = 7.6$ Hz, 2H, Bz), 5.70 (d, $J = 3.6$ Hz, 1H, H1'), 5.57 (d, $J = 3.9$ Hz, 1H, 2'OH), 5.41 (t, $J = 4.6$ Hz, 1H, 5'OH), 5.10 (d, $J = 5.9$ Hz, 1H, 3'OH), 4.07-4.13 (m, 1H, H2'), 4.04 ("q", $J = 5.9$ Hz, 1H, H3'), 3.90-3.96 (m, 1H, H4'), 3.74-3.82 (m, 1H, H5'), 3.63 (ddd, $J = 2.1, 4.4, 12.2$ Hz, 1H, H5"); ^{13}C NMR (DMSO- d_6) δ 177.8 (Bz), 154.5 (C2), 147.2 (C4), 142.1 (C6), 136.1 (Bz), 132.8, 129.4, 128.4, 95.0 (C5), 89.7 (C1'), 84.5 (C4'), 74.2 (C2'), 68.6 (C3'), 59.5 (C5'); MS (ESI) m/z 426 (100, [^{79}Br], MH^+), 428 (98, [^{81}Br], MH^+). Anal. Calcd for $\text{C}_{16}\text{H}_{16}\text{BrN}_3\text{O}_6 \cdot 0.5 \text{ MeOH}$ (442.24): C, 44.81; H, 4.10; N, 9.50. Found: C, 44.62; H, 3.71; N, 9.13.

5'-O-(*Tert*-butyldimethylsilyl)-2',3'-O-isopropylideneuridine (49b)²¹⁰. UV (MeOH) λ_{max} 276 nm, λ_{min} 241 nm; ^1H NMR (CDCl_3) δ 9.23 (br s, 1H, NH), 7.91 (s, 1H, H6), 5.91 (d, $J = 3.1$ Hz, 1H, H1'), 4.73 (dd, $J = 2.3, 6.2$ Hz, 1H, H2'), 4.68 (dd, $J = 3.1, 6.1$ Hz, 1H, H3'), 4.39 ("q", $J = 2.4$ Hz, 1H, H4'), 3.93 (dd, $J = 2.1, 11.7$ Hz, 1H, H5'), 3.80 (dd, $J = 2.8, 11.7$ Hz, 1H, H5'), 1.58 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 0.90 (s, 9H, 3 x CH_3), 0.11 (s, 6H, 2 x CH_3).

8-Bromoadenosine (50b)²¹¹. UV (MeOH) λ_{max} 264 nm, λ_{min} 232 nm; ^1H NMR (DMSO- d_6) δ 8.12 (s, 1H, H2), 7.58 (br s, 2H, NH_2), 5.83 (d, $J = 6.8$ Hz, 1H, H1'), 5.52 (dd, $J = 3.9, 8.6$ Hz, 1H, 5'OH), 5.48 (d, $J = 6.3$ Hz, 1H, 2'OH), 5.25 (d, $J = 4.4$ Hz, 1H, 3'OH), 5.09 (q, $J = 6.1$ Hz, 1H, H2'), 4.20-4.17 (m, 1H, H3'), 3.99-3.96 (m, 1H, H4'), 3.68 (dt, $J = 3.9, 12.1$ Hz, 1H, H5'), 3.55-3.49 (m, 1H, H5').

8-Bromo-2'-deoxyadenosine (51b)⁵⁴. ^1H NMR (DMSO- d_6) δ 8.11 (s, 1H, H2), 7.51 (br s, 2H, NH_2), 6.29 ("dd", $J = 6.7, 7.9$ Hz, 1H, H1'), 5.34 (d, $J = 4.2$ Hz, 1H, 3'OH), 5.29 ("dd", $J = 4.4, 7.7$ Hz, 1H, 5'OH), 4.50-4.46 (m, 1H, H3'), 3.90-3.87 (m, 1H, H4'), 3.65

("dt", $J = 4.5, 11.9$ Hz, 1H, H5'), 3.51-3.45 (m, 1H, H5''), 3.28-3.21 (m, 1H, H2'), 2.19 (ddd, $J = 2.7, 6.5, 13.2$ Hz, 1H, H2'').

8-Bromo-2',3',5'-tri-*O*-acetylguanosine (52b)²¹². UV (MeOH) λ_{max} 263 nm, λ_{min} 220 nm; ¹H NMR (DMSO-*d*₆) δ 10.95 (br s, 1H, NH), 6.63 (br s, 2H, NH₂), 6.01 ("dd", $J = 4.5, 6.3$ Hz, 1H, H1'), 5.88 ("d", $J = 4.4$ Hz, 1H, H2'), 5.65 (t, $J = 6.3$ Hz, 1H, H3'), 4.42 (dd, $J = 3.7, 11.9$ Hz, 1H, H5'), 4.34-4.30 (m, 1H, H4'), 4.21 (dd, $J = 6.3, 11.9$ Hz, 1H, H5''), 2.11 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.99 (s, 3H, Ac).

8-Bromoguanosine (53b)²¹³. UV (MeOH) λ_{max} 261 nm, λ_{min} 223 nm; ¹H NMR (DMSO-*d*₆) δ 10.87 (br s, 1H, NH), 6.51 (br s, 2H, NH₂), 5.68 ("d", $J = 6.3$ Hz, 1H, H1'), 5.44 ("d", $J = 6.2$ Hz, 1H, H2'), 5.09 (d, $J = 5.0$ Hz, 1H, 2'OH), 5.01 (q, $J = 6.0$ Hz, 1H, 5'OH), 4.95 (t, $J = 5.9$ Hz, 1H, 3'OH), 4.15-4.12 (m, 1H, H3'), 3.87-3.84 (m, 1H, H4'), 3.68-3.62 (m, 1H, H5'), 3.54-3.48 (m, 1H, H5'').

8-Bromo-2'-deoxyguanosine (54b)^{214,215}. UV (MeOH) λ_{max} 261 nm, λ_{min} 221 nm; ¹H NMR (DMSO-*d*₆) δ 10.80 (br s, 1H, NH), 6.49 (br s, 2H, NH₂), 6.15 ("t", $J = 7.3$ Hz, 1H, H1'), 5.25 (d, $J = 4.3$ Hz, 1H, 3'OH), 4.86 (t, $J = 5.9$ Hz, 1H, 5'OH), 4.41-4.37 (m, 1H, H3'), 3.81-3.78 (m, 1H, H4'), 3.65-3.59 (m, 1H, H5'), 3.52-3.46 (m, 1H, H5''), 3.19-3.12 (m, 1H, H2'), 2.13-2.07 (m, 1H, H2'').

8-bromotoyocamycin (55).

Method A. NBS (195.8 mg, 1.1 mmol) was added to a stirred solution of **19c** (291.3 mg, 1 mmol) in anhydrous DMF (8 mL) in a flame dried flask and the resulting pale-yellow solution was stirred at room temperature for 2.5 h, by which time TLC showed >90% conversion to a less polar product. Volatiles were removed from the clear, brown solution using high vacuum rotary evaporator on a hot water bath (<80 °C). The resulting brown

gummy solid was purified using column chromatography (10%, MeOH/EtOAc) and recrystallized from MeOH to give **55** as a colorless solid (166.6 mg, 45%): mp: 200-202 °C; UV (MeOH): λ_{max} 286 nm (ϵ 15 000), λ_{min} 250 nm (ϵ 2200); ^1H NMR (DMSO- d_6): δ 8.20 (s, 1H, H2), 7.09 (br s, 2H, NH₂), 5.94 (d, J = 6.6 Hz, 1H, H1'), 5.46 (d, J = 6.2 Hz, 1H, 2'-OH), 5.30-5.33 ("q", J = 4.1 Hz, 1H, 5'-OH), 5.25 (d, J = 4.8 Hz, 1H, 3'-OH), 5.08 (q, J = 6.0 Hz, 1H, H2'), 4.21 (m, 1H, H3'), 3.96 (q, J = 3.8 Hz, 1H, H4'), 3.69 (dt, J = 4.20, 12.0 Hz, 1H, H5'), 3.50-3.57 (m, 1H, H5"); ^{13}C NMR: δ = 156.1, 153.2, 149.9, 121.7, 114.1 (CN), 102.1, 91.0 (C1'), 87.4, 86.5 (C4'), 71.1 (C2'), 70.6 (C3'), 62.0 (C5'). R_f (EtOAc:*i*PrOH:H₂O, 4:1:2) 0.69 (R_f **19c** 0.68); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₂H₁₃BrN₅O₄⁺: 370.0145 and 372.0125; found 370.0093 and 371.9937.

Method B. DBH (552 mg, 1.93 mmol) was added to a suspension of **19c** (750 mg, 2.57 mmol) in MeOH (120 mL) in a flame dried flask and the resulting yellow suspension was stirred at room temperature for 5 min, by which time the solution became >80% clear. After 15 min TLC showed >95% conversion to a less polar product and product began to solidify out of the solution. After 30 min the colorless solid was collected using vacuum filtration (710 mg). Volatiles in the mother liquor were removed using rotary evaporator and the resulting colorless solid was recrystallized from MeOH (104 mg) to give **55** (814 mg, 85%): characterization data were in agreement with those reported in method 1.

8-bromosangivamycin (56).

Method A. NBS (267.1 mg, 1.5 mmol) was added to a stirred solution of **19b** (309.2 mg, 1 mmol) in anhydrous DMF (8 mL) in a flame dried flask and the resulting pale-yellow solution was stirred at room temperature for 1 h, by which time TLC showed >90% conversion to a less polar product. Volatiles were removed from the clear, brown solution

using high vacuum rotary evaporator on a hot water bath (<80 °C). The resulting brown gummy oil was column chromatographed (15%, MeOH/EtOAc) to give **56** as an off-white solid (244.5 mg, 63%): mp: 174-175 °C; UV (MeOH): λ_{max} 286 nm (ϵ 14 100), λ_{min} 256 nm (ϵ 5500); ^1H NMR (DMSO- d_6): δ 8.09 (s, 1H, H2), 8.00 (br s, 1H, NH₂), 7.76 (br s, 1H, NH₂), 5.99 (d, J = 6.6 Hz, 1H, H1'), 5.55 (dd, J = 4.0 Hz, 1H, 5'-OH), 5.37 (d, J = 6.3 Hz, 1H, 2'-OH), 5.18 (d, J = 4.6 Hz, 1H, 3'-OH), 5.13 (q, J = 6.1 Hz, 1H, H2'), 4.20 ("q", J = 4.2 Hz, 1H, H3'), 3.95 (q, J = 3.5 Hz, 1H, H4'), 3.68 (dt, J = 3.4, 12.0 Hz, 1H, H5'), 3.49-3.55 (m, 1H, H5''); ^{13}C NMR: δ = 165.8 (C=O), 157.1, 152.2, 149.6, 112.5, 112.2, 90.5 (C1'), 86.2 (C4'), 71.1 (C2'), 70.8 (C3'), 62.23 (C5'); R_f (EtOAc:*i*PrOH:H₂O, 4:1:2) 0.45 (R_f **19b** 0.35); HRMS (ESI): m/z [M+H]⁺ calcd for C₁₂H₁₅BrN₅O₅⁺: 388.0251 and 390.0231; found 388.0265 and 390.0229.

Method B. DBH (101.6 mg, 1.1 mmol) was added to a clear, colorless solution of **19b** (100 mg, 0.0323 mmol) in MeOH (25 mL) in a flame dried flask and the resulting yellow solution was stirred at room temperature for 30 min, by which time TLC showed >90% conversion to a less polar product. Volatiles were removed from the clear, yellow solution using rotary evaporator. The resulting off-white solid was purified using column chromatography (10%, MeOH/EtOAc) to give **56** as a colorless solid (49.2 mg, 39%): characterization data were in agreement with those reported in method 1.

Ethyl 2-(phenylthio)octanoate (72). Thiophenol (260 μL , 280 mg, 2.54 mmol) was added to a stirred solution of NaH (60%, dispersion in paraffin liquid; 100.4 mg, 4.18 mmol) in anhydrous DMF (4 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 30 minutes and at ambient temperature for 30 minutes, by which time bubbles (H₂ gas) ceased. The reaction flask was chilled again and Ethyl 2-bromooctanoate **71b** (540 μL ,

630 mg, 2.51 mmol) was added at 0 °C. The resultant clear, colorless solution was stirred at 0 °C for 20 min and at ambient temperature for 2 hours, by which time TLC showed exclusive conversion to a slightly more polar spot. Volatiles were evaporated and co-evaporated with toluene (1 x) (vacuum pump) and the resulting pale gum was partitioned between CHCl₃ (20 mL) and NH₄Cl/H₂O (20 mL). The aqueous layer was extracted with CHCl₃ (2 x 5 mL) and the combined organic phase was washed with NaHCO₃/H₂O (25 mL), brine (25 mL), and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (10% EtOAc in hexanes) to give **72**²¹⁶ (633 mg, 90%) as a pale oil: ¹H NMR (CDCl₃) δ 7.49-7.46 (m, 2H, Ph), 7.35-7.25 (m, 3H, Ph), 4.16-4.09 (m, 2H, CH₂), 3.66 (dd, *J* = 6.6, 8.4 Hz, 1H, H₂), 1.96-1.87 (m, 1H, H₃), 1.82-1.73 (m, 1H, H_{3'}), 1.51-1.37 (m, 2H, H₄, H_{4'}), 1.36-1.26 (m, 6H, H₅, H_{5'}, H₆, H_{6'}, H₇, H_{7'}), 1.19 (t, *J* = 7.1 Hz, 3H, CH₃), 0.90 ("t", *J* = 6.8 Hz, 3H, CH₃); ¹³C NMR (CDCl₃) δ 172.4, 133.8, 132.7, 128.9, 127.7, 61.0, 50.9, 31.7, 31.5, 28.8, 27.2, 22.5, 14.1, 14.0.

Ethyl 2-((4-chlorophenyl)thio)hexanoate (73). Treatment of ethyl 2-bromohexanoate **71a** (500 μL, 610.5 mg, 2.74 mmol) with 4-chlorothiophenol/NaH/DMF as described for the preparation of **72** gave **73**²¹⁷ as a pale yellow oil (763.6 mg, 97%): ¹H NMR (CDCl₃) δ 7.41-7.37 (m, 2H, Ph), 7.30-7.26 (m, 2H, Ph), 4.17-4.09 (m, 2H, CH₂), 3.61 (dd, *J* = 6.6, 8.4 Hz, 1H, H₂), 1.94-1.84 (m, 1H, H₃), 1.81-1.69 (m, 1H, H_{3'}), 1.51-1.27 (m, 4H, H₄, H_{4'}, H₅, H_{5'}), 1.19 (t, *J* = 7.1 Hz, 3H, CH₃), 0.91 ("t", *J* = 7.1 Hz, 3H, CH₃); ¹³C NMR (CDCl₃) δ 172.1, 134.11, 134.10, 132.1, 129.0, 61.1, 51.0, 31.3, 29.4, 22.2, 14.1, 13.8.

Ethyl 2-((4-chlorophenyl)thio)octanoate (74). Treatment of ethyl 2-bromooctanoate **71b** (540 μL, 630 mg, 2.51 mmol) with 4-chlorothiophenol/NaH/DMF as described for

the preparation of **72** gave **74** as a colorless oil (664 mg, 84%): ^1H NMR (CDCl_3) δ 7.39-7.37 (m, 2H, Ph), 7.27-7.24 (m, 2H, Ph), 4.15-4.07 (m, 2H, CH_2), 3.60 (dd, $J = 6.7, 8.3$ Hz, 1H, H2), 1.92-1.81 (m, 1H, H3), 1.77-1.68 (m, 1H, H3'), 1.48-1.35 (m, 2H, H4, H4'), 1.33-1.27 (m, 6H, H5, H5', H6, H6', H7, H7'), 1.18 (t, $J = 7.1$ Hz, 3H, CH_3), 0.87 ("t", $J = 6.8$ Hz, 3H, H8, H8', H8"); ^{13}C NMR (CDCl_3) δ 172.0, 134.1, 134.0, 132.2, 129.0, 61.0, 51.0, 31.6, 31.5, 28.8, 27.2, 22.5, 14.1, 14.0.

Ethyl 2-((4-methoxyphenyl)thio)hexanoate (75). Treatment of ethyl 2-bromohexanoate **71a** (500 μL , 610.5 mg, 2.74 mmol) with 4-methoxythiophenol/NaH/DMF as described for the preparation of **72** gave **75**²¹⁸ as a colorless oil (739.3 mg, 96%): ^1H NMR (CDCl_3) δ 7.36-7.31 (m, 2H, Ph), 6.78-6.74 (m, 2H, Ph), 4.02 ("q", $J = 7.2$ Hz, 2H, CH_2), 3.41 (dd, $J = 6.6, 8.5$ Hz, 1H, H2), 1.82-1.75 (m, 1H, H3), 1.68-1.59 (m, 1H, H3'), 1.42-1.32 (m, 1H, H4), 1.30-1.21 (m, 3H, H4', H5, H5'), 1.10 (t, $J = 7.1$ Hz, 3H, CH_3), 0.82 (t, $J = 7.1$ Hz, 3H, CH_3); ^{13}C NMR (CDCl_3) δ 172.2, 160.1, 136.2, 123.4, 114.4, 60.7, 55.2, 51.6, 31.1, 29.3, 22.3, 14.1, 13.8.

Typical procedure for preparation of ethyl difluoroalkanoate (76 and 77). DBH was added to a stirred solution of ester **72** or **73** (0.5 mmol) and Py.9HF (3 mmol) in CH_2Cl_2 (2 mL) at ambient temperature. The resulting brown solution was stirred at ambient temperature or at 35 $^\circ\text{C}$ for the respective times as shown in Table 1. The reaction flask was cooled to room temperature, quenched by addition of ice-cold water, diluted with CH_2Cl_2 , and neutralized with drop-wise addition of conc. NH_4OH . Organic layer was separated and aqueous layer was back extracted (2 x CH_2Cl_2). Combined organic layer was washed with 1N HCl, brine, dried (MgSO_4) and concentrated in vacuo to give crude difluorinated product as a brown oil, with data as reported.²¹⁹ **76**²¹⁹ had: ^1H NMR

(CDCl₃) δ 4.25 (q, $^3J_{\text{H,H}} = 7.2$ Hz, 2H, CH₂), 2.09-1.88 (m, 2H, H3, H3'), 1.42-1.30 (m, 4H, H4, H4', H5, H5'), 1.28 (t, $^3J_{\text{H,H}} = 7.1$ Hz, 3H, CH₃), 0.85 (t, $^3J_{\text{H,H}} = 7.2$ Hz, 3H, H6, H6', H6''); ¹³C NMR (CDCl₃) δ 164.4 (t, $^2J_{\text{F,C}} = 33.2$ Hz, C1), 116.4 (t, $^1J_{\text{F,C}} = 249.8$ Hz, C2), 63.0 (CH₂), 34.5 (t, $^2J_{\text{F,C}} = 23.2$ Hz, C3), 23.5 (t, $^3J_{\text{F,C}} = 4.3$ Hz, C4), 22.2 (C5), 13.9 (CH₃), 13.7 (C6); ¹⁹F NMR (CDCl₃) δ -105.92 ppm (t, $^3J_{\text{F-H}} = 16.8$ Hz); GC-MS ($t_{\text{R}} = 7.5$ min) 151 (1.1), 124 (11.3), 116 (18.2), 87 (44.9), 67 (20.9), 55 (3.6);

77²¹⁹ had: ¹H NMR (CDCl₃) δ 4.25 (q, $^3J_{\text{H,H}} = 7.2$ Hz, 2H, CH₂), 2.03-1.91 (m, 2H), 1.45-1.18 (m, 11H), 0.81 ("t", $^3J_{\text{H,H}} = 6.8$ Hz, 3H, H6, H6', H6''); ¹³C NMR (CDCl₃) δ 164.5 (t, $^2J_{\text{F,C}} = 33.0$ Hz, C1), 116.4 (t, $^1J_{\text{F,C}} = 249.7$ Hz, C2), 62.7 (CH₂), 34.5 (t, $^2J_{\text{F,C}} = 23.2$ Hz, C3), 31.4, 28.7, 22.4, 21.4 (t, $^3J_{\text{F,C}} = 4.3$ Hz, C4), 13.9; ¹⁹F NMR (CDCl₃) δ -105.89 ppm (t, $^3J_{\text{F-H}} = 17.0$ Hz).

5-*O*-Benzyl-2,3-dideoxy-2-fluoro-2-(phenylsulfanyl)ribose (84) and 5-*O*-Benzyl-2,3-dideoxy-2-fluoro-2-(phenylsulfinyl)ribose (85). **83** (31 mg, 0.1 mmol) was treated with Py.9HF (185 μ L, 0.8 mmol) and DBH (114 mg, 0.4 mmol) in anhydrous CH₂Cl₂ (3 mL) according to general desulfurization-difluorination procedure gave 40 mg of yellow oil after aqueous workup: ¹⁹F-NMR (CDCl₃) δ -150.86 ("t", 1F, $J = 26.4$ Hz), -149.33 (dd, 0.4F, $J = 20.3, 27.3$ Hz), -139.24 to -138.93 (m, 0.6 F); HRMS (ESI) m/z 355.2857 [M+Na]⁺, calcd for C₁₈H₁₇FNaO₃S⁺ 355.0775; m/z 371.2611 [M+Na]⁺, calcd for C₁₈H₁₇FNaO₄S⁺ 371.0724.

2'-*S*-(4-Chlorophenyl)-2'-thiouridine (87). Slight modification of published procedure.¹³³ PPh₃ (464.3 mg, 1.77 mmol) was added to a stirred solution of 4-chlorothiophenol (1.28 g, 8.84 mmol) in anhydrous DMF (10 mL) and the resulting solution was stirred at ambient temperature for 30 minutes. 2,2'-anhydro-1- β -D-

arabinofuranosyluracil²²⁰ (1 g, 4.42 mmol) was added to the reaction mixture and the resulting solution was refluxed (153 °C) for 18 h. Volatiles were evaporated, co-evaporated with toluene (2 x). The resultant brown gum was triturated with hexanes (3 x) and the liquid was pipetted out. The remaining brownish gummy solid was triturated with ether and the resultant pale green solid was vacuum filtered, washed with minimal amount of ice-cold methanol to give 2'-S-(4-Chlorophenyl)-2'-thiouridine (**87**; 1.1 g, 67 %) as a colorless solid: ¹H NMR (DMSO-*d*₆)¹³³ δ 11.14 (br s, 1H, NH), 7.61 (d, *J* = 8.1 Hz, 1H, H6), 7.35 (d, *J* = 8.5 Hz, 2H, Ph), 7.28 (d, *J* = 8.5 Hz, 2H, Ph), 6.33 (d, *J* = 9.0 Hz, 1H, H1'), 5.91 (d, *J* = 5.6 Hz, 1H, 3'OH), 5.50 (d, *J* = 8.1 Hz, 1H, H5), 5.11 (t, *J* = 5.0 Hz, 1H, 5'OH), 4.34 (t, *J* = 5.2 Hz, 1H, H3'), 3.94-3.92 (m, 1H, H4'), 3.89 (dd, *J* = 5.3, 9.0 Hz, 1H, H2'), 3.59 ("t", *J* = 4.4 Hz, 2H, H5', H5"); ¹³C NMR (DMSO-*d*₆) δ 162.5 (C4), 150.4 (C2), 139.8 (C6), 133.1 (Ph), 132.3 (Ph), 132.0 (Ph), 128.8 (Ph), 102.3 (C5), 87.8 (C1'), 86.7 (C4'), 72.1 (C3'), 61.4 (C5'), 54.5 (C2').

3',5'-di-*O*-Acetyl-2'-S-(4-chlorophenyl)-2'-thiouridine (89a). DMAP (18.3 mg, 0.15 mmol) was added to a stirred suspension of **87** (1.1 g, 2.97 mmol) in Ac₂O (842 μL, 909 mg, 8.91 mmol) and the resulting suspension was stirred at ambient temperature overnight (15 h). MeOH was added, the reaction mixture was stirred at ambient temperature for 30 min, and volatiles were evaporated (vacuum pump, <25 °C). MeOH was added and evaporated, and the resulting gum was partitioned between CHCl₃ (50 mL) and H₂O (50 mL). The aqueous layer was extracted with CHCl₃, and the combined organic phase was washed with NaHCO₃/H₂O, 1N HCl, brine, and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was dried on vacuum pump to give 3',5'-di-*O*-Acetyl-2'-S-(4-chlorophenyl)-2'-thiouridine (**89a**; 1.34 g, 99%) as a colorless

foam, with sufficient purity to proceed to next step: ^1H NMR (CDCl_3) δ 10.12 (br s, 1H, NH), 7.27 (d, $J = 8.3$ Hz, 2H, Ph), 7.22 (d, $J = 8.2$ Hz, 1H, H6), 7.14 (d, $J = 8.4$ Hz, 2H, Ph), 6.14 (d, $J = 8.5$ Hz, 1H, H1'), 5.63 (d, $J = 8.2$ Hz, 1H, H5), 5.38 ("d", $J = 6.0$ Hz, 1H, H3'), 4.30-4.19 (m, 3H, H4', H5', H5''), 3.81 ("dd", $J = 6.3, 8.2$ Hz, 1H, H2'), 2.07 (s, 3H, CH_3), 2.00 (s, 3H, CH_3); ^{13}C NMR (CDCl_3) δ 170.3, 169.9 (2 x C=O), 163.2 (C4), 150.4 (C2), 139.2 (C6), 134.6 (Ph), 134.2 (Ph), 130.6 (Ph), 129.4 (Ph), 103.4 (C5), 89.0 (C1'), 81.0 (C4'), 73.7 (C3'), 63.5 (C5'), 53.5 (C2'), 20.7, 20.6 (2 x s, 2 x CH_3).

N3-Benzyl-3',5'-di-O-benzyl-2'-S-(4-chlorophenyl)-2'-thiouridine (89b). NaH (60%, dispersion in paraffin liquid; 216 mg, 9 mmol) was added to a stirred solution of **87** (556.2 mg, 1.5 mmol) in anhydrous DMF (6 mL) at 0 °C. The resulting suspension was stirred at 0 °C for 30 minutes and at ambient temperature for 30 minutes, by which time bubbles (H_2 gas) ceased. The reaction flask was chilled again and benzyl bromide (0.93 mL, 1.3 g, 7.8 mmol) was added at 0 °C. Bubbles were observed for a few minutes once the ice-bath was removed and the resultant pale solution was stirred at ambient temperature for 4 hours, by which time TLC showed a major (90%) less polar spot. Volatiles were evaporated and co-evaporated with toluene (1 x) (vacuum pump) and the resulting syrup was partitioned between CHCl_3 (20 mL) and $\text{NH}_4\text{Cl}/\text{H}_2\text{O}$ (20 mL). The aqueous layer was extracted with CHCl_3 (2 x 5 mL) and the combined organic phase was washed with $\text{NaHCO}_3/\text{H}_2\text{O}$ (25 mL), brine (25 mL), and dried (MgSO_4). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (20% EtOAc in hexanes) to give **89b** (827 mg, 86%) as a colorless foam: ^1H NMR (CDCl_3) δ 7.53-7.51 (m, 2H, Ph), 7.43-7.27 (m, 14H, Ph, H6), 7.15-7.11 (m, 2H, Ph), 6.93-6.90 (m, 2H, Ph), 6.47 (d, $J = 8.3$ Hz, 1H, H1'), 5.33 (d, $J = 8.1$ Hz, 1H, H5), 5.05-4.97 (m, 2H, benzylic),

4.71-4.64 (m, 2H, benzylic), 4.56 ("d", $J = 11.0$ Hz, 1H, benzylic), 4.50 ("d", $J = 11.0$ Hz, 1H, benzylic), 4.33-4.30 (m, 2H, H3', H4'), 3.81-3.77 (m, 2H, H2', H5'), 3.60 (dd, $J = 1.7, 10.4$ Hz, 1H, H5"); ^{13}C NMR (CDCl_3) δ 162.0, 150.9, 137.4, 137.2, 137.1, 136.8, 133.9, 133.8, 131.7, 129.5, 129.1, 128.8, 128.6, 128.4, 128.1, 128.0, 127.8, 127.7, 102.3 (C5), 90.2 (C1'), 82.3 (C4'), 80.3 (C3'), 73.9 (CH_2), 72.4 (CH_2), 70.6 (C5'), 55.7 (C2'), 44.1 (CH_2).

***N*3-Benzyl-3',5'-di-*O*-benzyl-2'-*S*-(4-methoxyphenyl)-2'-thiouridine (90b).** Treatment of **88**¹³⁴ (1.47 g, 4.0 mmol) with NaH (576 mg, 24 mmol) and BnBr (2.5 mL, 3.6 g, 20.8 mmol) as described for **87** $\rightarrow$ **89b** gave **90b** as a colorless foam (2.2 g, 86%): ^1H NMR (CDCl_3) δ 7.58 ("d", $J = 6.9$ Hz, 2H, Ph), 7.40-7.23 (m, 13H, Ph), 7.14-7.11 (m, 3H, Ph, H6), 6.56 (d, $J = 8.8$ Hz, 1H, H1'), 6.33 (d, $J = 8.7$ Hz, 2H, Ph), 5.11 (d, $J = 8.1$ Hz, 1H, H5), 5.04 ("d", $J = 13.5$ Hz, 1H, benzylic), 4.95 ("d", $J = 13.5$ Hz, 1H, benzylic), 4.64 (s, 2H, benzylic), 4.49 ("d", $J = 11.0$ Hz, 1H, benzylic), 4.39 ("d", $J = 11.0$ Hz, 1H, benzylic), 4.29 (d, $J = 5.3$ Hz, 1H, H4'), 4.25 (br s, H3'), 3.70-3.65 (m, 2H, H2', H5'), 3.60 (s, 3H, OCH_3), 3.54-3.51 ("m", 1H, H5"); ^{13}C NMR (CDCl_3) δ 170.8, 162.0, 159.8, 151.1, 137.4, 136.9, 135.3, 129.8, 128.6, 128.5, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 123.3, 114.6, 102.0 (C5), 90.4 (C1'), 82.0 (C3'), 80.9 (C4'), 73.8 (CH_2), 72.2 (CH_2), 70.8 (C5'), 56.3 (C2'), 55.4 (OCH_3), 44.1 (CH_2).

***N*3-Benzyl-3',5'-di-*O*-benzyl-2'-deoxy-2'-[(4-methoxyphenyl)sulfinyl]uridine [93 (*R/S-S*)].** MCPBA (750.7 mg of 70% reagent, 4.35 mmol) in CH_2Cl_2 (70 mL) was added drop-wise to a stirred solution of **90b** (2.2 g, 3.45 mmol) in CH_2Cl_2 (35 mL) at -50 $^\circ\text{C}$, and the temperature was allowed to rise to -30 $^\circ\text{C}$ in ~ 20 minutes. TLC showed $> 80\%$ conversion to a more polar product. The solution was poured into $\text{NaHCO}_3/\text{H}_2\text{O}$ (10 mL).

The aqueous layer was extracted with CHCl_3 (2 x 5 mL) and the combined organic phase was washed with H_2O (20 mL), brine (25 mL), and dried (MgSO_4). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (25% EtOAc in hexanes) to give **93** (2' *R/S*-S, ~1:1) (1.8 g, 80%) as a colorless foam: ^1H NMR (CDCl_3) δ 7.51-7.28 (m, 17.5H, Ph, H6), 7.10 (d, $J = 7.8$ Hz, 0.5H, H6), 7.03 (d, $J = 8.0$ Hz, 0.5H, H1'), 6.83 (d, $J = 8.0$ Hz, 0.5H, H1'), 6.49 (d, $J = 9.6$ Hz, 1H, Ph), 6.44 (d, $J = 8.1$ Hz, 1H, Ph), 5.36 (d, $J = 7.7$ Hz, 0.5H, H5), 5.04 (d, $J = 8.1$ Hz, 0.5H, H5), 4.96-4.26 (m, 8H, benzylic, H2', H3'), 3.86-3.43 (m, 6H, CH_3 , H4', H5', H5'');

^{13}C NMR (CDCl_3) for major isomer (extracted data): δ 162.9, 161.6, 150.6, 137.11, 137.10, 137.0, 136.5, 132.5, 129.8, 128.8, 128.6, 128.44, 128.38, 128.2, 127.9, 127.2, 114.6, 102.5 (C5), 82.9 (C1'), 82.1 (C4'), 80.9 (C3'), 74.0 (CH_2), 73.4 (CH_2), 72.7 (C5'), 70.9 (C2'), 55.6 (OCH_3), 44.1 (CH_2).

3',5'-di-*O*-Acetyl-2'-deoxy-2'-[(4-chlorophenyl)sulfonyl]uridine (91a). MCPBA (70% reagent, 250 mg, 1.45 mmol) was added to a stirred solution of **89a** (227.4 mg, 0.5 mmol) in CH_2Cl_2 (15 mL) and the resultant colorless solution was stirred at ambient temperature for 12h. TLC showed complete conversion to a more polar product. The reaction mixture was poured into $\text{NaHCO}_3/\text{H}_2\text{O}$ (10 mL). The aqueous layer was extracted with CHCl_3 (2 x 5 mL) and the combined organic phase was washed with H_2O (20 mL), brine (25 mL), and dried (MgSO_4). Volatiles were evaporated *in vacuo* to give **91a** (241 mg, 99%) as a colorless foam. Data for the crude sample: ^1H NMR (CDCl_3) δ 9.51 (br s, 1H, NH), 7.86 (d, $J = 8.6$ Hz, 2H, Ph), 7.55 (d, $J = 8.6$ Hz, 2H, Ph), 7.17 (d, $J = 8.2$ Hz, 1H, H6), 6.15 (d, $J = 6.2$ Hz, 1H, H1'), 5.72-5.66 (m, 2H, H5, H3'), 4.6 (t, $J = 6.7$ Hz, 1H, H2'), 4.42 (q, $J = 4.6$ Hz, 1H, H4'), 4.36 (dd, $J = 3.7, 12.5$ Hz, 1H, H5'), 4.25 (dd, $J = 4.9, 12.2$ Hz, 1H,

H5"), 2.09 (s, 3H, CH₃), 2.07 (s, 3H, CH₃); ¹³C NMR (CDCl₃) δ 170.4, 169.9 (2 x C=O), 162.9 (C4), 149.6 (C2), 141.7 (C6), 141.2 (Ph), 136.9 (Ph), 130.2 (Ph), 130.1 (Ph), 103.5 (C5), 87.7 (C1'), 80.9 (C4'), 71.2 (C3'), 65.1 (C5'), 62.4 (C2'), 20.71, 20.70 (2 x CH₃).

***N*3-Benzyl-3',5'-di-*O*-benzyl-2'-deoxy-2'-[(4-chlorophenyl)sulfonyl]uridine (91b).**

MCPBA (250.2 mg of 70% reagent, 1.45 mmol) in CH₂Cl₂ (6 mL) was added drop-wise to a stirred solution of **89b** (320.6 mg, 0.50 mmol) in CH₂Cl₂ (4 mL) and the resulting clear solution was stirred at ambient temperature overnight. Saturated NaHCO₃/H₂O (10 mL) was added, stirring was continued for 10 min, and the organic layer was separated. The aqueous layer was extracted with CHCl₃ (2 x 5 mL) and the combined organic phase was washed with H₂O (20 mL), brine (25 mL), and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (20% EtOAc in hexanes) to give **91b** (269.3 mg, 80%) as a colorless foam: ¹H NMR (CDCl₃) δ 7.59-7.56 (m, 2H, Ph), 7.55-7.51 (m, 2H, Ph), 7.48-7.28 (m, 14H, Ph, H6), 6.98-6.94 (m, 2H, Ph), 6.66 (d, *J* = 8.6 Hz, 1H, H1'), 5.37 (d, *J* = 8.2 Hz, 1H, H5), 5.08 ("d", *J* = 13.7 Hz, 1H, benzylic), 5.02 ("d", *J* = 13.7 Hz, 1H, benzylic), 4.68 ("d", *J* = 11.5 Hz, 1H, benzylic), 4.63 (dd, *J* = 1.8, 5.4 Hz, 1H, H3'), 4.58 ("d", *J* = 11.5 Hz, 1H, benzylic), 4.53 ("d", *J* = 10.9 Hz, 1H, benzylic), 4.45 ("d", *J* = 11.0 Hz, 1H, benzylic), 4.24 ("d", *J* = 1.9 Hz, 1H, H4'), 4.09 (dd, *J* = 5.4, 8.6 Hz, 1H, H2'), 3.70 (dd, *J* = 2.3, 10.6 Hz, 1H, H5'), 3.46 (dd, *J* = 2.0, 10.6 Hz, 1H, H5"); ¹³C NMR (CDCl₃) δ 161.8, 150.7, 140.7, 137.4, 137.1 (C6), 136.8, 136.6, 136.4, 130.2, 129.5, 129.1, 128.8, 128.6, 128.5, 128.3, 128.23, 128.16, 127.8, 103.0 (C5), 84.8 (C1'), 82.5 (C4'), 79.7 (C3'), 74.0 (CH₂), 73.1 (CH₂), 70.1 (C5'), 68.9 (C2'), 44.3 (CH₂).

***N*3-Benzyl-3',5'-di-*O*-benzyl-2'-deoxy-2'-[(4-methoxyphenyl)sulfonyl]uridine (92b).**

Treatment of **90b** (32 mg, 0.05 mmol) with MCPBA (26 mg of 70% reagent, 0.15 mmol) in CH₂Cl₂ (3 mL) as described for **89b** → **91b** gave **92b** (27 mg, 81%) as a colorless foam: ¹H NMR (CDCl₃) δ 7.61-7.60 (m, 2H, Ph), 7.51-7.48 (m, 2H, Ph), 7.43-7.25 (m, 13H, Ph), 7.10 (d, *J* = 8.2 Hz, 1H, H₆), 6.75 (d, *J* = 9.0 Hz, 1H, H_{1'}), 6.39-6.35 (m, 2H, Ph), 5.16 (d, *J* = 8.1 Hz, 1H, H₅), 5.07 ("d", *J* = 13.5 Hz, 1H, benzylic), 4.99 ("d", *J* = 13.5 Hz, 1H, benzylic), 4.80 ("d", *J* = 11.7 Hz, 1H, benzylic), 4.65-4.62 (m, 2H, benzylic, H_{3'}), 4.50 ("d", *J* = 10.8 Hz, 1H, benzylic), 4.39 ("d", *J* = 10.8 Hz, 1H, benzylic), 4.22 ("d", *J* = 1.6 Hz, 1H, H_{4'}), 4.05 (dd, *J* = 5.2, 9.0 Hz, 1H, H_{2'}), 3.67-3.63 (m, 4H, OCH₃, H_{5'}), 3.45 (dd, *J* = 2.0, 10.5 Hz, 1H, H_{5''}); ¹³C NMR (CDCl₃) δ 171.1, 163.9, 161.9, 150.7, 137.2 (C₆), 137.0, 136.9, 136.6, 130.6, 130.4, 129.8, 128.8, 128.5, 128.4, 128.2, 128.1, 127.9, 114.2, 102.7 (C₅), 85.0 (C_{1'}), 82.6 (C_{4'}), 80.0 (C_{3'}), 73.9 (CH₂), 73.1 (CH₂), 70.4 (C_{5'}), 68.4 (C_{2'}), 55.8 (OCH₃), 44.3 (CH₂).

***N*3-Benzyl-3',5'-di-*O*-benzyl-2'-fluoro-2'-*S*-(4-methoxyphenyl)-2'-thiouridine (**94b**).**

DAST (0.15 mL, 183 mg, 1.14 mmol) was added to a mixture of **93** (2'-*R/S*-*S*, ~1:1) (326.4 mg, 0.5 mmol) and SbCl₃ (23 mg, 0.1 mmol) in CH₂Cl₂ (10 mL), and the resulting pale solution was stirred at ambient temperature overnight (13 h). Cold saturated NaHCO₃/H₂O (10 mL) was added carefully, stirring was continued for 30 min, the organic layer was separated. The aqueous layer was extracted with CHCl₃ (2 x 5 mL) and the combined organic phase was washed with H₂O (20 mL), brine (25 mL), dried (MgSO₄) and evaporated *in vacuo* to give yellow oil. Gradient flash chromatography (0 → 30% EtOAc in hexanes) gave **94b** as a mixture of diastereomers (2'-*R/S*-*S*, ~4:1) (pale yellow foam 186.6 mg, 57%): ¹H NMR (CDCl₃) δ 7.55-7.26 (m, 18H, Ph, H₆), 6.84 ("d", *J* = 8.7 Hz, 2H, Ph), 6.51 (d, *J* = 14.7 Hz, 0.8H, H_{1'}), 6.40 (br s, 0.2H, H_{1'}), 5.68 (d, *J* =

8.1 Hz, 0.8H, H5), 5.41 (d, $J = 8.2$ Hz, 0.2H, H5), 5.14-5.01 (m, 1H, benzylic), 4.93-4.87 (m, 1H, benzylic), 4.68-4.43 (m, 4H, benzylic), 4.23-4.18 (m, 2H, H3', H4'), 3.91-3.79 (m, 4H, CH₃, H5'), 3.72-3.60 (m, 1H, H5''); ¹⁹F NMR (CDCl₃) δ -132.53 ppm (br s, 0.8F), -131.16 ppm (br s, 0.2F).

¹³C NMR (CDCl₃) for major isomer (extracted data): δ 162.3, 161.0, 150.2, 138.8, 137.6, 137.1, 136.6, 135.1, 135.0, 129.0, 128.59, 128.57, 128.4, 128.3, 128.1, 127.80, 127.6, 118.84, 118.82, 114.8, 109.1 (d, $^1J_{C2'-F} = 237.1$ Hz, C2'), 102.0, 90.5 (d, $^2J_{C1'-F} = 47.9$ Hz, C1'), 80.1 (C4'), 79.6 (d, $^2J_{C3'-F} = 18.1$ Hz, C3'), 73.5 (CH₂), 73.1 (CH₂), 67.5 (C5'), 55.4 (CH₃), 44.1 (CH₂).

N3-Benzyl-3',5'-di-O-benzyl-2'-deoxy-2'-fluoro-2'-[(4-

methoxyphenyl)sulfonyl]uridine (95b). MCPBA (241.6 mg of 70% reagent, 1.4 mmol) in CH₂Cl₂ (5 mL) was added drop-wise to a stirred solution of **94** (327.4 mg, 0.50 mmol) in CH₂Cl₂ (3 mL) and the resulting clear solution was stirred at ambient temperature overnight. Saturated NaHCO₃/H₂O (10 mL) was added, stirring was continued for 10 min, and the organic layer was separated. The aqueous layer was extracted with CHCl₃ (2 x 5 mL) and the combined organic phase was washed with H₂O (20 mL), brine (25 mL), and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (20% EtOAc in hexanes) to give **95b** as a mixture of diastereomers (2'-*R/S*-S, ~ 3:1) (colorless foam, 298.7 mg, 87%): ¹H NMR (CDCl₃) δ 7.90 (d, $J = 8.4$ Hz, 0.5H, Ph), 7.78 (d, $J = 8.3$ Hz, 1.5H, Ph), 7.54 (d, $J = 7.9$ Hz, 1H, H6), 7.55-7.12 (m, 15H, Ph), 6.95 ("d", $J = 8.9$ Hz, 1.5H, Ph), 6.89 ("d", $J = 8.5$ Hz, 0.5H, Ph), 6.70 (br s, 0.25H, H1'), 6.30 (d, $J = 18.6$ Hz, 0.75H, H1'), 5.73 (d, $J = 8.1$ Hz, 0.75H, H5), 5.27 (br s, 0.25H, H5), 5.06 (d, $J = 13.7$ Hz, 0.75H, benzylic), 5.00 ("d", $J = 13.7$ Hz, 0.25H,

benzylic), 4.92-4.80 (m, 2H, benzylic, H3'), 4.58 (d, $J = 11.6$ Hz, 1H, benzylic), 4.49-4.27 (m, 3H, benzylic), 4.11-4.06 (m, 1H, H4'), 3.90 (s, 2.25H, CH₃), 3.86 (s, 0.75H, CH₃), 3.81-3.87 (m, 0.25H, H5'), 3.75 (dd, $J = 2.3, 11.2$ Hz, 0.75H, H5'), 3.54 ("dd", $J = 3.7, 11.1$ Hz, 1H, H5"); ¹⁹F NMR (CDCl₃) δ -158.89 ppm ("t", $J = 16.3$ Hz, 0.75F), -156.50 ppm (d, $J = 19.5$ Hz, 0.25F).

¹³C NMR (CDCl₃) for major isomer: δ 165.0, 150.2, 139.3 (C6), 137.3, 136.8, 136.2, 131.38, 131.37, 129.1, 128.9, 128.6, 128.51, 128.50, 128.44, 128.36, 128.3, 128.1, 127.8, 127.7, 126.5, 114.5, 108.3 (d, $^1J_{C2'-F} = 237.0$ Hz, C2'), 101.8 (C5), 87.8 (d, $^2J_{C1'-F} = 38.1$ Hz, C1'), 79.5 (C4'), 75.5 (d, $^2J_{C3'-F} = 15.2$ Hz, C3'), 73.8 (CH₂), 73.5 (CH₂), 67.1 (C5'), 55.9 (CH₃), 44.1 (CH₂).

5-Bromo-3',5'-di-*O*-acetyl-2'-*S*-(4-chlorophenyl)-2'-thiouridine (96a) and 5-Bromo-3',5'-di-*O*-acetyl-2'-deoxy-2'-[(4-chlorophenyl)sulfinyl]uridine [97 (*R/S*-*S*)]. Py.9HF (352 μ L, 1.53 mmol) was added to a chilled solution of DBH (20.8 mg, 0.073 mmol) in dry CH₂Cl₂ (1 mL) at -78 °C. The resulting pale solution was stirred at -78 °C for 10 minutes and a solution of substrate **89a** (30mg, 0.066 mmol) in dry CH₂Cl₂ (2 mL) was added via syringe. The resultant orange solution was stirred at -78 °C for 2h and was brought to -30 °C over 45 minutes. The reaction mixture was diluted with CH₂Cl₂ (5 mL), washed with NaHCO₃/H₂O (5 mL), H₂O (5 mL), brine (5 mL), dried (MgSO₄). Volatiles were evaporated in vacuo and the yellow residue was column chromatographed (30% → 60% EtOAc in hexanes) to give **96a** (14.4 mg, 41%) as a colorless oil. Also isolated were **97** (6.5 mg, 18%, faster moving isomer) as a colorless oil and **97** (10.9 mg, 30%, slower moving isomer) as a colorless oil.

96a had: ^1H NMR (CDCl_3) δ 8.48 (br s, 1H, NH), 7.09-7.06 (m, 2H, Ph), 7.02-6.99 (m, 2H, Ph), 6.98 (s, 1H, H6), 5.94 (d, $J = 8.1$ Hz, 1H, H1'), 5.12 (dd, $J = 2.6, 5.9$ Hz, 1H, H3'), 4.12 (dd, $J = 4.0, 12.9$ Hz, 1H, H5'), 4.06-4.02 (m, 2H, H4', H5''), 3.47 (dd, $J = 5.9, 8.0$ Hz, 1H, H2'), 1.893-1.891 ("m", 6H, 2 x CH_3); ^{13}C NMR (CDCl_3) δ 170.03 (C=O), 169.98 (C=O), 158.3, 149.5, 138.2 (C6), 135.2, 134.3 (Ph), 130.4, 129.7 (Ph), 98.0 (C5), 89.3 (C1'), 81.3 (C4'), 73.6 (C3'), 63.5 (C5'), 54.5 (C2'), 20.9 (CH_3), 20.6 (CH_3).

97 (faster moving isomer) had: ^1H NMR (CDCl_3) δ 8.38 (br s, 1H, NH), 7.56-7.50 (m, 4H, Ph), 7.10 (s, 1H, H6), 5.89 (d, $J = 4.0$ Hz, 1H, H1'), 5.51 (t, $J = 8.2$ Hz, 1H, H3'), 4.45 (dd, $J = 2.8, 12.3$ Hz, 1H, H5'), 4.40-4.36 (m, 1H, H4'), 4.23 (dd, $J = 4.8, 12.3$ Hz, 1H, H5''), 3.92 (dd, $J = 4.2, 8.2$ Hz, 1H, H2'), 2.29 (s, 3H, CH_3), 2.12 (s, 3H, CH_3); ^{13}C NMR (CDCl_3) δ 170.6 (C=O), 170.4 (C=O), 157.9, 148.1, 140.6 (C6), 138.5, 138.4, 130.1 (Ph), 125.5 (Ph), 97.3 (C5), 85.3 (C1'), 80.3 (C4'), 72.1 (C3'), 67.3 (C2'), 62.1 (C5'), 20.8 (CH_3), 20.6 (CH_3).

97 (slower moving isomer) had: ^1H NMR (CDCl_3) δ 8.55 (br s, 1H, NH), 7.59-7.56 (m, 2H, Ph), 7.42-7.39 (m, 2H, Ph), 7.15 (s, 1H, H6), 6.13 (d, $J = 8.4$ Hz, 1H, H1'), 5.51 (dd, $J = 2.6, 6.2$ Hz, 1H, H3'), 4.30 (dd, $J = 3.2, 12.1$ Hz, 1H, H5'), 4.25 (q, $J = 2.9$ Hz, 1H, H4'), 4.17 (dd, $J = 2.9, 12.1$ Hz, 1H, H5''), 3.72 (dd, $J = 6.2, 8.3$ Hz, 1H, H2'), 2.13 (s, 3H, CH_3), 2.11 (s, 3H, CH_3); ^{13}C NMR (CDCl_3) δ 169.8 (C=O), 169.7 (C=O), 157.5, 148.7, 139.8 (C6), 139.0, 137.6, 130.1 (Ph), 127.1 (Ph), 98.2 (C5), 83.1 (C1'), 81.6 (C4'), 73.4 (C3'), 69.8 (C2'), 63.4 (C5'), 21.0 (CH_3), 20.6 (CH_3).

3',5'-di-*O*-Acetyl-5-iodo-2'-*S*-(4-chlorophenyl)-2'-thiouridine (96b). NIS (68 mg, 0.42 mmol) was added to a stirred solution of **89a** (46 mg, 0.1 mmol) and DAST (80 μL , 0.6 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 $^\circ\text{C}$ and the resulting purple solution was stirred

at 0 °C for 6 h and at ambient temperature for 32 h (total reaction time: 36 h). The reaction was quenched by addition of sat. Na₂S₂O₃ (5 mL), diluted with CH₂Cl₂ (5 mL). Organic layer was separated and aqueous layer was back extracted (2 x CH₂Cl₂). Combined organic layer was washed with NaHCO₃/H₂O (10 mL), brine (10 mL), dried (MgSO₄), and filtered. Volatiles were evaporated *in vacuo* and the brown residue was column chromatographed (25% → 50% EtOAc in hexanes) to give **96b** (42.5 mg, 73%) as a colorless oil.

Analogous treatment of **89a** (46 mg, 0.1 mmol) with NIS (68 mg, 0.42 mmol) and DAST (80 µL, 0.6 mmol) at 35 °C for 24 h gave **96b** (35 mg, 60%) as a pale-yellow oil. ¹H NMR (CDCl₃) δ 9.07 (br s, 1H, NH), 7.60 (s, 1H, H6), 7.39-7.35 (m, 2H, Ph), 7.31-7.28 (m, 2H, Ph), 6.23 (d, *J* = 8.4 Hz, 1H, H1'), 5.43 (dd, *J* = 2.2, 5.8 Hz, 1H, H3'), 4.44-4.31 (m, 3H, H4', H5', H5''), 3.77 (dd, *J* = 5.9, 8.3 Hz, 1H, H2'), 2.21 (s, 3H, CH₃), 2.19 (s, 3H, CH₃); ¹³C NMR (CDCl₃) δ 171.3, 170.0 (2 x C=O), 159.2 (C4), 149.8 (C2), 143.3 (C6), 135.2 (Ph), 134.3 (Ph), 130.4 (Ph), 129.8 (Ph), 89.0 (C1'), 81.3 (C4'), 73.7 (C3'), 69.7 (C5), 63.6 (C5'), 54.5 (C2'), 21.1, 20.7 (2 x s, 2 x CH₃).

3-*N*-benzyl-3',5'-di-*O*-benzyl-5-bromo-2'-deoxy-2'-fluoro-2'-*S*-(4-chlorophenyl)-2'-thiouridine (98). **89b** (255 mg, 0.4 mmol) was treated with Py.9HF (920 µL, 4 mmol) and DBH (572 mg, 2 mmol) in anhydrous CH₂Cl₂ (10 mL) according to general desulfurization-difluorination procedure gave 405 mg of yellow oil after aqueous workup. Column chromatography (10% → 70% EtOAc in hexanes) gave a major fraction of 118 mg as a pale-yellow oil: ¹⁹F-NMR (CDCl₃) δ -127.53 to -127.24 (m, 1F), -130.84 to -130.62 (m, 1F).

3-*N*-benzyl-3',5'-di-*O*-benzyl-5-iodo-2'-*S*-(4-methoxyphenyl)-2'-thiouridine (100)

and 3-*N*-benzyl-3',5'-di-*O*-benzyl-2'-fluoro-2'-*S*-(4-methoxyphenyl)-2'-thiouridine

(94b). NIS (95 mg, 0.42 mmol) was added to a stirred solution of 18 (128 mg, 0.2 mmol) and Py.9HF (138 μ L, 0.6 mmol) in anhydrous CH₂Cl₂ (3 mL) at -78 °C in a polypropylene vessel and the resulting brown solution was brought to ~5 °C overnight (16 h). Stirring was continued at ambient temperature for 7 h (total reaction time: 23 h). The reaction was quenched by addition of ice-cold water (10 mL), diluted with CH₂Cl₂ (5 mL), neutralized with drop-wise addition of conc. NH₄OH. Organic layer was separated and aqueous layer was back extracted (2 x CH₂Cl₂). Combined organic layer was washed with 1N HCl (10 mL), brine (10 mL), dried (MgSO₄), and filtered. Volatiles were evaporated *in vacuo* and the brown residue was column chromatographed (5% → 20% EtOAc in hexanes) to give **100** (44 mg, 29%) as a colorless oil and **94b** (16 mg, 12%) as a pale-yellow oil (2'-*R/S*-S, ~ 1:1).

Analogous treatment of 18 (128 mg, 0.2 mmol) with NIS (95 mg, 0.42 mmol) and DAST (160 μ L, 1.2 mmol) followed by aqueous workup (sat. Na₂S₂O₃, sat. NaHCO₃, brine, MgSO₄) and column chromatography gave **94b** (58 mg, 44%) as a pale-yellow oil (2'-*R/S*-S, ~ 1:1).

100 had: ¹H NMR (CDCl₃) δ 7.66-7.63 (m, 2H, Ph), 7.56 (s, 1H, H6), 7.44-7.31 (m, 13H, Ph), 7.12-7.10 (m, 2H, Ph), 6.51 (d, *J* = 8.9 Hz, 1H, H1'), 6.33-6.30 (m, 2H, Ph), 5.15 ("d", *J* = 13.4 Hz, 1H, benzylic), 5.02 ("d", *J* = 13.3 Hz, 1H, benzylic), 4.68-4.52 (m, 4H, benzylic), 4.33-4.25 (m, 2H, H4', H3'), 3.76-3.67 (m, 2H, H2', H5'), 3.66 (s, 3H, OCH₃), 3.51 (dd, *J* = 2.0, 10.5 Hz, 1H, H5'"); ¹³C NMR (CDCl₃) δ 159.8, 159.1, 150.8, 142.2 (C6), 137.22, 137.16, 136.2, 135.2, 130.4, 128.8, 128.6, 128.4, 128.2, 128.1, 128.06,

127.8, 127.7, 123.0, 114.7, 90.2 (C1'), 82.1 (C3'), 80.7 (C4'), 73.8 (CH₂), 72.3 (CH₂), 70.5 (C5), 68.9 (C5'), 56.4 (C2'), 55.5 (OCH₃), 46.0 (CH₂).

3-*N*-benzyl-3',5'-di-*O*-benzyl-5-bromo-2'-fluoro-2'-*S*-(4-methoxyphenyl)-2'-

thiouridine (101). Treatment of **94b** (131 mg, 0.2 mmol) with DBH (172 mg, 0.6 mmol) and Py.9HF (280 μ L, 1.2 mmol) at -78 °C according to the procedure described for **89a** $\rightarrow$ **96a/97** followed by column chromatography (15% EtOAc in hexanes) gave **101** as a mixture of diastereomers (2'-*R/S*-S, $\sim$ 65:35) (pale-yellow oil, 82 mg, 56%): ¹H NMR (CDCl₃) δ 7.84 (s, 1H, H6), 7.81 (d, J = 2.1 Hz, 0.2H, Ph), 7.66 (d, J = 2.2 Hz, 0.8H, Ph), 7.49-7.23 (m, 17H, Ph), 6.78 (d, J = 8.7 Hz, 0.8H, Ph), 6.75 (d, J = 8.7 Hz, 0.2H, Ph), 6.44 (d, J = 13.9 Hz, 0.8H, H1'), 6.27 (br s, 0.2H, H1'), 5.09 ("d", J = 13.6 Hz, 1H, benzylic), 4.92-4.80 (m, 1H, benzylic), 4.73-4.65 (m, 1H, benzylic), 4.62-4.41 (m, 3H, benzylic), 4.18-4.09 (m, 2H, H3', H4'), 3.92 (s, 0.6H, CH₃), 3.91 (s, 2.4H, CH₃), 3.82-3.72 (m, 1H, H5'), 3.61-3.53 (m, 1H, H5"); ¹⁹F NMR (CDCl₃) δ -133.52 ppm (br s, 0.8F), -131.57 ppm (br s, 0.2F).

¹³C NMR (CDCl₃) for major isomer (extracted data): δ 158.4, 157.2, 149.4, 138.1, 137.79, 137.76, 137.3, 136.8, 135.9, 134.1, 134.0, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 120.24, 120.23, 112.2, 112.0, 108.7 (d, ¹ $J_{C2'-F}$ = 239.1 Hz, C2'), 96.9, 90.4 (d, ² $J_{C1'-F}$ = 47.3 Hz, C1'), 80.3 (C4'), 79.7 (d, ² $J_{C3'-F}$ = 17.7 Hz, C3'), 73.5 (CH₂), 73.1 (CH₂), 67.0 (C5'), 56.4 (CH₃), 45.7 (CH₂).

5'-*O*-Acetyl-2',3'-dideoxy-2',3'-didehydro-2'-[(4-chlorophenyl)sulfonyl]uridine (or)

1-[5'-*O*-Acetyl-2',3'-dideoxy-2'-(4-chlorophenyl)sulfonyl- β -D-glycero-pent-2'-

enofuranosyl]uracil (102). mCPBA (70% reagent, 250 mg, 1.45 mmol) was added to a stirred solution of **89a** (227.4 mg, 0.5 mmol) in CH₂Cl₂ (15 mL) and the resultant

colorless solution was stirred at ambient temperature for 12h. TLC showed complete conversion to a more polar product. The reaction mixture was poured into NaHCO₃/H₂O (10 mL). The aqueous layer was extracted with CHCl₃ (2 x 5 mL) and the combined organic phase was washed with H₂O (20 mL), brine (25 mL), and dried (MgSO₄). Volatiles were evaporated *in vacuo* to give **91a** (241 mg, 99%) as a colorless foam. Purification of this material on silica gel chromatography (50% EtOAc in hexanes) gave **102** contaminated with 40% of **91a** (184 mg, 1.5:1 ratio of **102:91a**): ¹H NMR (CDCl₃) δ 10.04 (s, 0.6H, NH), 9.99 (s, 0.4H, NH), 7.89-7.85 (m, 0.8H, Ph), 7.84-7.81 (m, 1.2H, Ph), 7.57-7.53 (m, 2H, Ph), 7.36 (d, *J* = 8.1 Hz, 0.6H, H₆), 7.28-7.27 ("m", 0.6H, H_{1'}), 7.19 (d, *J* = 8.2 Hz, 0.4H, H₆), 7.01 ("d", *J* = 1.9 Hz, 0.6H, H_{3'}), 6.21 (d, *J* = 6.4 Hz, 0.4H, H_{1'}), 5.72-5.66 (m, 1.4H, H_{3'}, H₅), 5.15-5.11 (m, 0.6H, H_{4'}), 4.64 (t, *J* = 6.8 Hz, 0.4H, H_{2'}), 4.46-4.22 (m, 2.4H, H_{4'}, H_{5'}, H_{5''}), 2.09 (s, 1.8H, CH₃), 2.08 (s, 1.2H, CH₃), 2.07 (s, 1.2H, CH₃).

3-*N*-Benzyl uracil (103) and **3,5-di-*O*-benzyl-1,2-dideoxy-1,2-didehydro-2-[(4-chlorophenyl)sulfonyl]ribose (104)**. A stirred solution of **91b** (30 mg, 0.045 mmol) and selectfluor (24 mg, 0.067 mmol) in dry DMF (2 mL) was added to a chilled suspension of KH (30 wt% dispersion in mineral oil; 2.5 mg, 0.0613 mmol) in dry THF (5 mL) at -78 °C. TLC after 30 minutes showed complete consumption of starting material and two new spots. The reaction mixture was slowly warmed to 0 °C. Crude reaction mixture was partitioned between CHCl₃ (5 mL) and ice-cold NH₄Cl/H₂O (5 mL). The aqueous layer was extracted with CHCl₃ (2 x 2 mL) and the combined organic phase was washed with NaHCO₃/H₂O (10 mL), brine (10 mL), and dried (MgSO₄). Volatiles were evaporated *in*

vacuo and the residue was column chromatographed (5% → 50% EtOAc in hexanes) to give **103** (5.7 mg, 63%) as colorless oil and **104** (14.9 mg, 71%) as a colorless foam.

Analogously, treatment of substrate (30 mg, 0.045 mmol) in dry THF (2 mL) with a chilled suspension of KH (30 wt% dispersion in mineral oil; 2.5 mg, 0.0613 mmol) in dry THF (5 mL) at 0 °C for 15 minutes followed by aqueous workup and column chromatography also gave **103** and **104** in similar yields.

103 had: ²²¹ ¹H NMR (CDCl₃) δ 9.48 (1H, br s, NH), 7.45-7.42 (m, 2H, Ph), 7.34-7.24 (m, 3H, Ph), 6.50 (dd, *J* = 3.7, 7.5 Hz, 1H, H6), 5.79 (d, *J* = 7.7 Hz, 1H, H5), 5.10 (s, 2H, CH₂).

104 had: ¹H NMR (CDCl₃) δ 7.71 (d, *J* = 8.6 Hz, 2H, Ph), 7.38 (s, 1H, H1), 7.31-7.13 (m, 10H, Ph), 6.99-6.97 ("m", 2H, Ph), 4.81 (d, *J* = 2.9 Hz, 1H, H3), 4.73-4.70 (m, 1H, H4), 4.45-4.39 (m, 2H, CH₂), 4.37-4.30 (m, 2H, CH₂), 3.42 (dd, *J* = 5.4, 10.3 Hz, 1H, H5), 3.34 (dd, *J* = 5.7, 10.3 Hz, 1H, H5'); ¹³C NMR (CDCl₃) δ 160.7 (C1), 140.6 (Ph), 139.4 (Ph), 137.2 (Ph), 137.0 (Ph), 129.2 (Ph), 128.9 (Ph), 128.6 (Ph), 128.4 (Ph), 128.1 (Ph), 128.0 (Ph), 127.9 (Ph), 127.8 (Ph), 118.8 (C2), 90.5 (C4), 80.6 (C3), 73.7 (CH₂), 71.0 (CH₂), 68.7 (C5). HRMS (ESI) *m/z* 493.0846 [M+Na]⁺, calcd for C₂₅H₂₃ClNaO₅S⁺ 493.0847.

[4-[(4-Methoxyphenyl)sulfonyl]furan-2-yl]methyl acetate (105), (106) and 5'-O-Acetyl-2',3'-dideoxy-2',3'-didehydro-2'-[(4-methoxyphenyl)sulfonyl]uridine (107).

TDAE (0.24 mL, 1.04 mmol) was added to a stirred solution of **91a** (100 mg, 2.07 mmol) in DMF (2 mL) at -78 °C and the reaction mixture was brought to ambient temperature over 1h 15 minutes and a 10% HCl solution (2 mL) was added. The crude was extracted with EtOAc (3 x) and the combined organic phase was dried (Na₂SO₄). Volatiles were

evaporated *in vacuo* and the residue was column chromatographed (Petroleum ether (PE) 100% → EtOAc/PE 2/1 → 100% EtOAc) to give **105** (34.5 mg, 54%), **106** (21 mg, 24%), and **107** (35 mg, 40%) as colorless oils.

105 had: ^1H NMR (CDCl_3) δ 7.93 (d, J = 0.8 Hz, 1H, H1), 7.85-7.89 (m, 2H, Ph), 6.96-7.00 (m, 2H, Ph), 6.56 (s, 1H, H3), 4.97 (s, 2H, H5, H5'), 3.85 (s, 3H, OCH_3), 2.05 (s, 3H, Ac); ^{13}C NMR (CDCl_3) δ 170.3 (C=O), 163.8 (Ph), 152.4 (C4), 145.7 (C1), 132.9 (C2), 131.1 (Ph), 129.8 (Ph), 114.7 (Ph), 108.7 (C3), 57.4 (C5), 55.8 (OCH_3), 20.8 (CH_3); MS (ESI) m/z 328.17 $[\text{M}+\text{NH}_4]^+$, calcd for $\text{C}_{14}\text{H}_{18}\text{NO}_6\text{S}^+$ 328.08.

106 had: ^1H NMR (CDCl_3) δ 9.00 (br, 1H, NH), 7.76 (d, J = 8.8 Hz, 2H, Ph), 7.58 (s, 1H, H1'), 6.97-6.99 (m, 3H, Ph, H6), 5.69 (br s, 1H, OH), 5.62 (dd, J = 1.2, 8 Hz, 1H, H5), 4.93-4.79 (m, 1H, H3'), 4.27 (dd, J = 4.4, 12.4 Hz, 1H, H5'), 4.21 (dd, J = 4.8, 12.4 Hz, 1H, H5''), 3.86 (s, 3H, OCH_3), 1.95 (s, 3H, Ac); ^{13}C NMR (CDCl_3) δ 170.4 (C=O), 164.2 (Ph), 162.6 (C4), 160.3 (C6, C2), 150.3 (C4'), 140.2 (C2'), 131.5 (Ph), 129.9 (Ph), 116.9 (C1'), 114.9 (Ph), 103.7 (C5), 88.7 (C3'), 63.4 (C5'), 55.6 (OCH_3), 20.5 (CH_3). MS (ESI) m/z 440.04 $[\text{M}+\text{NH}_4]^+$, calcd for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_8\text{S}^+$ 440.11.

107 had: ^1H NMR (CDCl_3) δ 9.23 (br, 1H, NH), 7.79-7.75 (m, 2H, Ph), 7.24 (d, J = 8.4 Hz, 1H, H6), 7.18 (t, J = 1.6 Hz, 1H, H3'), 7.03-6.99 (m, 3H, Ph, H1'), 5.55 (dd, J = 2.0, 8.4 Hz, 1H, H5), 5.12-5.09 (m, 1H, H4'), 4.41 (dd, J = 4.0, 12.4 Hz, 1H, H5'), 4.29 (dd, J = 3.6, 12.4 Hz, 1H, H5''), 3.87 (s, 3H, OCH_3), 2.09 (s, 3H, Ac); ^{13}C NMR (CDCl_3) δ 170.1 (C=O), 164.9 (Ph), 162.9 (C4), 150.3 (C2), 142.1 (C6), 141.9 (C2'), 139.5 (C3'), 130.8 (Ph), 129.1 (Ph), 115.2 (Ph), 103.2 (C5), 87.5 (C1'), 82.8 (C4'), 63.9 (C5'), 56.1 (OCH_3), 20.8 (Ac). HRMS (ESI) m/z 423.0856 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{19}\text{N}_2\text{O}_8\text{S}^+$ 423.0857.

[4-[(4-Methoxyphenyl)sulfonyl]furan-2-yl]methyl acetate (105) and **uracil (105a)**. In a glove box, SED (31 mg, 0.1 mmol) was added to a solution of **91a** (50 mg, 0.1 mmol) in anhydrous DMF (4 mL) and stirred at ambient temperature for 15 minutes. Reaction flask was brought out of the glove box and water was added to the crude reaction mixture. The aqueous phase was extracted with CH₂Cl₂ (3 x), the combined organic phase was dried with Na₂SO₄ and volatiles were evaporated *in vacuo* to give **105** (29.4 mg, 95%) as a colorless oil.

Acidification of the aqueous phase (pH ~ 5-6) followed by a second extraction with CH₂Cl₂ (2 x) gave a white solid. ¹H NMR (MeOD) of the white solid revealed the presence of uracil **105a** along with other impurities.

3,5-di-O-Benzyl-1,2-dideoxy-1,2-didehydro-2-[(4-methoxyphenyl)sulfonyl]ribose (108), **2-[(benzyloxy)methyl]-4-[(4-methoxyphenyl)sulfonyl]furan (109)** and **3-N-benzyluracil (103)**. In a glove box, SED (22 mg, 0.067 mmol) was added to a stirred solution of **91b** (45 mg, 0.07 mmol) in DMF (4 mL) and the reaction mixture was stirred at ambient temperature for 15 minutes. Reaction flask was brought out of the glove box and water was added to the crude reaction mixture. The aqueous phase was extracted with CH₂Cl₂ (3 x) and the combined organic phase was dried with Na₂SO₄. Volatiles were evaporated *in vacuo* and the residue was column chromatographed (PE 100% → PE/EtOAc 9/1 → EtOAc 100%) to give **108** (22 mg, 70%) as a white gum and **103** (9.4 mg, 69%) as a white solid.

108 had: ¹H NMR (CDCl₃) δ 7.83-7.79 (m, 2H, Ph), 7.42 (s, 1H, H1), 7.37-7.31 (m, 3H, Ph), 7.26-7.24 (m, 5H, Ph), 7.09-7.07 (m, 2H, Ph), 6.86-6.84 (m, 2H, Ph), 4.86 (d, *J* = 3.2 Hz, 1H, H3), 4.77 (td, *J* = 3.2, 5.6 Hz, 1H, H4), 4.49 (d, *J* = 2.4 Hz, 2H, CH₂), 4.40 (s,

2H, CH₂), 3.81 (s, 3H, OCH₃), 3.48 (dd, *J* = 5.6, 10.4 Hz, 1H, H₅), 3.40 (dd, *J* = 5.6, 10.4 Hz, 1H, H₅); ¹³C NMR (CDCl₃) δ 163.3 (C^{Ph}), 159.7 (C1), 137.4 (C^{Ph}), 137.3 (C^{Ph}), 133.6 (C^{Ph}), 129.8 (2 CH^{Ph}), 128.7 (2 CH^{Ph}), 128.4 (2 CH^{Ph}), 128.1 (3 CH^{Ph}), 128.0 (CH^{Ph}), 127.9 (2 CH^{Ph}), 119.7 (C2), 114.2 (2 CH^{Ph}), 90.3 (C3), 80.7 (C4), 73.7 (CH₂), 70.8 (CH₂), 68.9 (C5), 55.7 (OCH₃); HRMS (ESI) *m/z* 484.1789 [M+NH₄]⁺, calcd for C₂₆H₃₀NO₆S⁺ 484.1788.

Analogous treatment of and **91b** (50 mg, 0.075 mmol) with **SED** (64 mg, 0.22 mmol) in DMF (4 mL) for 30 minutes at room temperature gave **109** as a mixture with **108** (7.4 mg, 2:1 ratio of **109:108**) as a colorless oil and **103** (13 mg, 86%) as a white solid.

109 had: ¹H NMR (200MHz, CDCl₃) δ 7.93 (s, 1H, H1), 7.91-7.84 (m, 2H, Ph), 7.36-7.30 (m, 5H, Ph), 7.01-6.96 (m, 2H, Ph), 6.49 (s, 1H, H3), 4.53 (s, 2H, CH₂), 4.42 (s, 2H, CH₂), 4.14-4.03 (m, 2H, H5), 3.86 (s, 3H, OCH₃); MS (ESI) *m/z* 376.17 [M+NH₄]⁺, calcd for C₁₉H₂₂NO₅S⁺ 376.12.

5'-O-Acetyl -2',3'-didehydro-2',3'-dideoxy-2'-fluorouridine (111) and **1-(5-O-Acetyl-2-deoxy-2-fluoro-β,D-arabinofuranosyl)uracil (114)**. TDAE (0.06 mL, 50.1 mg, 0.25 mmol) was added drop-wise *via* syringe to a stirred solution of **95a** (95 mg, 0.19 mmol) in dry THF (5 mL) at -70°C under sunlamp irradiation. After 1 h, Selectfluor (131 mg, 0.37 mmol) was added and the sunlamp was turned off. The resulting mixture was stirred for 1 h at -70°C and then overnight at ambient temperature. The reaction mixture was quenched with 3 mL of aqueous NH₄Cl and stirred for 30 min and then extracted with EtOAc. The combined organic layer was washed with Na₂CO₃/H₂O, brine and dried over

MgSO₄. Evaporation and column chromatography (5% MeOH in CHCl₃) gave **111** (10 mg: 18%) and **114** (5 mg: 10%).

111 was also obtained by another procedure (see Scheme 22). In a glove box, **SED** (68 mg, 0.24 mmol, 3 equiv.) was added to a stirred solution of **95a** (40 mg, 0.08 mmol) in anhydrous DMF (4 mL) and the resulting dark brown solution was stirred at 120 °C for 18 h. Reaction flask was brought out of the glove box, and 10% HCl solution was added to the crude reaction mixture. Aqueous layer was extracted with CH₂Cl₂ (2 x). Combined organic layer was dried (Na₂SO₄), filtered, and concentrated *in vacuo* to give a brown solid. Et₂O was added to the solid and was filtered. Filtrate was concentrated *in vacuo* to give **111** (<10 mg, ~ 46%) along with other impurities. ¹H NMR of the black solid (DMSO-*d*₆) showed similar peaks to the ones obtained for the oxidized form of the SED (SED²⁺).

111¹⁷³ had: ¹H NMR (CDCl₃) δ 8.47 (br s, 1H, NH), 7.52 (dd, *J* = 1.0, 8.1 Hz, 1H, H6), 6.90-6.88 (m, 1H, H1'), 5.78 (d, *J* = 8.1 Hz, 1H, H5), 5.69 (m, 1H, H3'), 5.06-5.02 (m, 1H, H4'), 4.35-4.21 (m, 2H, H5',5''), 2.11 (s, 3H, Ac); ¹⁹F NMR (CDCl₃) δ -133.76 (t, *J* = 4.6 Hz, F2'); MS (EI) *m/z* 271.09 [M+H]⁺. calcd for C₁₁H₁₂FN₂O₅⁺ 271.07.

114 had: ¹H NMR (CDCl₃) δ 8.32 (br s, 1H, NH), 7.51-7.53 (m, 1H, H6), 6.22-6.28 (m, 1H, H1'), 5.76 (d, *J* = 8.1 Hz, 1H, H5), 5.06 (d, *J* = 51.3 Hz, 1H, H2'), 4.43-4.29 (m, 3H, H3', H5', H5''), 4.21-4.19 (m, 1H, H4'), 2.12 (s, 3H, Ac); ¹³C NMR (150 MHz, CDCl₃) δ 170.9 (C=O), 162.7 (C4), 150.1 (C2), 140.9 (C6), 102.2 (C5), 94.4 (d, *J* = 191.8 Hz, C2'), 84.7 (d, *J* = 16.5 Hz, C1'), 82.9 (C4'), 75.4 (d, *J* = 26.5 Hz, C3'), 63.1 (C5'), 20.9 (CH₃); ¹⁹F NMR (CDCl₃) δ -199.91 (s, F2'); MS (EI) *m/z* 288.9 [M+H]⁺, calcd for C₁₁H₁₄FN₂O₆⁺ 289.08.

3-(benzyloxy)-2-[(benzyloxy)methyl]-4-[(4-methoxyphenyl)sulfonyl]furan (112). In a glove box, SED (62 mg, 0.22 mmol) was added to a solution of **95b** (50 mg, 0.073 mmol) in anhydrous DMF (4 mL) and the resulting solution was stirred at 120 °C for 3 h. Reaction flask was brought out of the glove box, and water was added to the crude reaction mixture. The aqueous phase was extracted with CH₂Cl₂ (3 x) and the combined organic phase was dried with Na₂SO₄. Volatiles were evaporated *in vacuo* and the residue was column chromatographed (PE/EtOAc 9/1 → EtOAc 100%) to give **112** (19.6 mg, 58%) as a white gum and **103** (12.4 mg, 84%) as a white solid.

Analogous treatment of **95b** (58.3 mg, 0.085 mmol) with SED (72 mg, 0.25 mmol) at ambient temperature for 4 h showed only trace amounts of **112** and **103** on TLC. Addition of degazed water (0.5 mL, 500 mg, 27.75 mmol) followed by heating at 80 °C for 30 minutes gave, after workup and silica gel chromatography, **112** (31.7 mg, 80%) and **103** (10.1 mg, 60%).

112 had: ¹H NMR (200MHz, CDCl₃) δ 7.94-7.87 (m, 2H, Ph), 7.83 (s, 1H, H1), 7.37-7.24 (m, 10H, Ph), 6.95-6.88 (m, 2H, Ph), 5.07 (s, 2H, CH₂), 4.41 (s, 2H, CH₂), 4.17 (s, 2H, CH₂), 3.84 (s, 3H, OCH₃); ¹³C NMR (50 MHz, CDCl₃) δ 163.6 (C3), 144.8 (C1), 142.4 (Ph), 140.7 (Ph), 137.4 (Ph), 136.2 (Ph), 132.8 (C4), 130.0 (Ph), 128.55(Ph), 128.53 (Ph), 128.48 (Ph), 128.0 (Ph), 127.9 (Ph), 125.6 (C2), 114.3 (Ph), 77.3 (CH₂), 72.4 (CH₂), 61.1 (CH₂), 55.7 (OCH₃). HRMS (ESI) *m/z* 482.1632 [M+NH₄]⁺ calcd for C₂₆H₂₈NO₆S⁺ 482.1632.

3-N-Methyl-2'-deoxy-2'-fluoro-2'-[(4-methoxyphenyl)sulfonyl]uridine (115). TDAE (0.036 mL, 0.155 mmol) was added drop-wise *via* syringe to a stirred solution of **95a** (15.4 mg, 0.031 mmol) in dry THF or DMF (0.5 ml) at -70°C under N₂. UV sunlamp

was switched on and after 1 h, CH₃I (44.4 mg, 0.31 mmol) was added. The resulting mixture (yellowish with white fine solid) was stirred for 1 h at -70°C and then overnight at ambient temperature. The reaction mixture was quenched with 3 ml of aqueous MeOH, stirred for 30 min and concentrated *in vacuo*. The crude product (100.8 mg), a yellowish solid, was washed with dichloromethane (2 x 5 mL), filtered and the filtrate concentrated *in vacuo* to recover 42.9 mg of a viscous orange. ¹⁹F-NMR of this crude product indicated no starting material (a triplet centred at - 157 ppm) and a triplet centred at - 161.42 ppm with additional resonances at in the region -122 to -126 ppm. Column chromatography (5% MeOH in CHCl₃) gave **115** (10.2 mg, ~ 79%) that was contaminated with an impurity, as the fastest moving fraction.

¹H NMR (300 MHz, CDCl₃) δ 7.75 (d, 2H, *J* = 6.9 Hz, Ph), 7.68 (d, 1H, *J* = 8.7 Hz, H6), 6.98 (d, 2H, *J* = 6.9 Hz, Ph), 6.40 (d, 1H, *J* = 20.4 Hz, H1'), 5.85 (d, 1H, *J* = 8.7 Hz, H5), 5.04 (m, 1H, H3'), 3.98-4.11 (m, 3H, H4' and H5',5''), 3.89 (s, 3H, OCH₃); 3.15 (s, 3H, NCH₃); ¹⁹F NMR (282 MHz, CDCl₃) δ -160.41 (t, *J* = 19.5 Hz, F2') and -160.75 (t, *J* = 19.8 Hz, F2') (ratio 1:0.16); MS (ESI) *m/z* 431.1 [M+ H]⁺, 453.1 [M+Na]⁺.

General Procedure for cyclic voltammetry. Electrochemical measurements were performed using an EG & G-Princeton Applied Research 263A all-in-one potentiostat, using a standard three-electrode setup with a glassy carbon electrode (working electrode, diameter = 3 mm), platinum wire auxiliary electrode and a non-aqueous Ag/Ag⁺ (0.01 M AgNO₃ + 0.1 M *n*-Bu₄NClO₄) system in acetonitrile as the reference electrode. All solutions under the study were 0.1 M in the supporting electrolyte *n*-Bu₄NPF₆ (Fluka puriss electrochemical grade) with the voltage scan rate of 0.2 V s⁻¹. Anhydrous DMF was obtained from Fisher Scientific. Solutions (2.5 mL) were thoroughly bubbled with

dry argon for 15 minutes to remove oxygen before any experiment and kept under positive pressure of argon. Under these experimental conditions the ferrocene/ferricinium couple, used as internal reference for potential measurements, was located at $E_{1/2} = + 0.05$ V in DMF.

4-Benzylamino-7-(β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (122a). BnBr (345 μ L, 496 mg, 2.9 mmol) was added to a stirred solution of tubercidin (**19a**; 266 mg, 1 mmol) in dried DMF (5 mL). After stirring for 48 hours at 40 °C, TLC showed almost complete conversion to a more polar product. Volatiles were evaporated to ~1 mL (< 40 °C, vacuum pump) and the resulting syrup was added dropwise to dried acetone (30 mL) with vigorous stirring. Et₂O (60 mL) was added to the suspension, which was chilled for 20 min at 0 °C and vacuum filtered. The hygroscopic precipitate was quickly dissolved in MeOH (10 mL) and Me₂NH/THF (2 M, 8 mL) was added. The resulting solution was stirred at reflux (65 °C, oil bath) for 20 h. TLC showed ~80% conversion to less polar spot, and volatiles were evaporated and coevaporated with MeOH (2 $\times$). The residue was dissolved in warm MeOH (10 mL), H₂O (60 mL) was added, and the solution was extracted with EtOAc (5 $\times$ 20 mL). The combined organic phase was dried (Na₂SO₄), concentrated *in vacuo*, coevaporated (2 $\times$ EtOH) and flash chromatographed (EtOAc) to give **122a** (238 mg, 67%) as a colorless oil: UV (MeOH) λ_{max} 276 nm, λ_{min} 244 nm; ¹H NMR (DMSO-*d*₆) δ 8.09-8.13 (m, 2H, NH, H2), 7.38 (d, J = 3.7 Hz, 1H, H6), 7.29-7.35 (m, 4H, Ph), 7.22-7.25 (m, 1H, Ph), 6.67 (d, J = 3.4 Hz, 1H, H5), 6.01 (d, J = 5.9 Hz, 1H, H1'), 5.27-5.32 (m, 2H, 2'-OH, 5'-OH), 5.12 (d, J = 4.3 Hz, 1H, 3'-OH), 4.70-4.78 (m, 2H, CH₂), 4.44 (q, J = 5.7 Hz, 1H, H2'), 4.09 ("q", J = 4.1 Hz, 1H, H3'), 3.90 (q, J = 3.4 Hz, 1H, H4'), 3.60-3.65 (m, 1H, H5'), 3.50-3.56 (m, 1H, H5''); ¹³C NMR (DMSO-*d*₆) δ

156.1, 151.4, 149.5, 140.1, 128.2 (Ph), 127.1 (Ph), 126.6, 122.3 (C6), 103.4, 99.2 (C5), 87.6 (C1'), 85.1 (C4'), 73.7 (C2'), 70.7 (C3'), 61.8 (C5'), 43.1 (CH₂); MS (ESI) *m/z* 357 (100%, MH⁺), HRMS (ESI) *m/z* 357.1581 (MH⁺), calcd for C₁₈H₂₉N₄O₄ 357.1563.

4-(4-Nitrobenzylamino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (122b).

Method A. Tubercidin (**19a**; 266 mg, 1.0 mmol) was treated with 4-nitrobenzyl bromide (648 mg, 3.0 mmol) at 80 °C for 24 h as described above for **122a** followed by addition of MeOH (15 mL) and Me₂NH/THF (2 M, 8 mL). The reaction mixture was stirred at reflux for 20 h; TLC showed ~85% conversion to a less polar product **122b** (244 mg, 56%, yellow oil): UV (MeOH) λ_{max} 278 nm, λ_{min} 240 nm; ¹H NMR (DMSO-*d*₆) δ 8.30 (t, *J* = 6.1 Hz, 1H, NH), 8.19 (“d”, *J* = 8.8 Hz, 2H, Ph), 8.12 (s, 1H, H2), 7.58 (d, *J* = 8.8 Hz, 2H, Ph), 7.43 (d, *J* = 3.7 Hz, 1H, H6), 6.69 (d, *J* = 3.5 Hz, 1H, H5), 6.04 (d, *J* = 6.3 Hz, 1H, H1'), 5.28-5.30 (m, 2H, 2 × OH), 5.14 (s, 1H, OH), 4.80-4.91 (m, 2H, CH₂), 4.45 (“d”, *J* = 4.4 Hz, 1H, H2'), 4.11- 4.13 (m, 1H, H3'), 3.92 (q, *J* = 3.5 Hz, 1H, H4'), 3.64 (dt, *J* = 3.9, 11.8 Hz, 1H, H5'), 3.52-3.57 (m, 1H, H5''); ¹³C NMR (DMSO-*d*₆-D₂O) δ 155.8, 151.0, 148.8, 147.9, 146.3, 127.9 (Ph), 123.4 (Ph), 122.8 (C6), 103.6, 99.3 (C5), 87.5 (C1'), 84.9 (C4'), 73.5 (C2'), 70.4 (C3'), 61.6 (C5'), 42.7 (CH₂); MS (ESI): *m/z* 402 (100%, MH⁺). Anal. Calcd for C₁₈H₁₉N₅O₆•1.25 H₂O (423.89): C, 51.00; H, 5.11; N, 16.52. Found: C, 51.05; H, 5.06; N, 16.03.

Method B. Step a. Ac₂O (377 μL, 408 mg, 4 mmol) was added to a stirred suspension of **19a** (266 mg, 1 mmol) in dried pyridine (5 mL) at 0 °C (ice bath) and stirring was continued at 0 °C for 12 h and then at ambient temperature for 9 h (total reaction time: 21 h). MeOH was added, the reaction mixture was stirred at ambient temperature for 30 min, and volatiles were evaporated (vacuum pump, <25 °C). MeOH was added and

evaporated, and the resulting gum was partitioned between CHCl_3 (50 mL) and 2% $\text{AcOH}/\text{H}_2\text{O}$ (50 mL). The aqueous layer was extracted with CHCl_3 , and the combined organic phase was washed with $\text{NaHCO}_3/\text{H}_2\text{O}$, brine, and dried (MgSO_4). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (EtOAc) to give 2',3',5'-tri-*O*-acetyltubercidin (**123**; 337 mg, 86%) as a colorless foam with data as reported.²²²

Step b. NaNO_2 (66 mg, 0.95 mmol) was added to a solution of **123** (150 mg, 0.38 mmol) in freshly prepared ~55% HF-pyridine¹⁹² (1.9 mL) at $-10\text{ }^\circ\text{C}$ in a sealed polypropylene vessel. The mixture was stirred at $-10\text{ }^\circ\text{C}$ for 15 min, and TLC showed almost complete conversion to a less polar spot. Ice/ H_2O was added and the mixture was extracted with CH_2Cl_2 . The combined organic phase was washed with $\text{NaHCO}_3/\text{H}_2\text{O}$, brine, and dried (Na_2SO_4). Volatiles were removed *in vacuo*, and the resulting brown oil was column chromatographed (30% EtOAc in hexanes) to give 7-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-4-fluoropyrrolo[2,3-*d*]pyrimidine (**124**; 124 mg, 82%) as a colorless oil: UV (MeOH) λ_{max} 258 nm, λ_{min} 233 nm; ^1H NMR (CDCl_3) δ 8.53 (d, $^4J_{\text{H-F}} = 0.5$ Hz, 1H, H2), 7.37 (d, $J = 3.8$ Hz, 1H, H8), 6.66 (d, $J = 3.8$ Hz, 1H, H7), 6.45 (d, $J = 6.0$ Hz, 1H, H1'), 5.74 (t, $J = 5.8$ Hz, 1H, H2'), 5.55 (dd or m, $J = 4.1, 5.6$ Hz, 1H, H3'), 4.32-4.42 (m, 3H, H4', H5', H5''), 2.12 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.02 (s, 3H, CH_3); ^{19}F NMR (CDCl_3) δ -64.81 ppm (s); ^{13}C NMR (CDCl_3) δ 170.2, 169.6, 169.4 (3 x C=O), 162.3 (d, $^1J_{\text{C-F}} = 253.8$ Hz, C4), 155.1 (d, $^3J_{\text{C-F}} = 11.9$ Hz, C7a), 151.1 (d, $^3J_{\text{C-F}} = 14.5$ Hz, C2), 125.7 (d, $^4J_{\text{C-F}} = 2.6$ Hz, C6), 105.4 (d, $^2J_{\text{C-F}} = 33.4$ Hz, C4a), 99.6 (d, $^3J_{\text{C-F}} = 4.9$ Hz, C5), 86.1 (C1'), 79.9 (C4'), 73.1 (C2'), 70.1 (C3'), 63.3 (C5'), 20.7, 20.5, 20.3 (3 x s, 3 x CH_3); MS (ESI): m/z 396 (100%, MH^+).

Step c. Freshly distilled Et₃N (113 μ L, 82 mg, 0.81 mmol) was added to a stirred suspension of **124** (93 mg, 0.23 mmol) and 4-nitrobenzylamine hydrochloride (67 mg, 0.35 mmol) in MeOH (3 mL) and stirring was continued for 5 h at ambient temperature. Volatiles were evaporated *in vacuo* and the residue was column chromatographed (50% EtOAc in hexanes) to give 2',3',5'-tri-*O*-acetyl-4-*N*-(4-nitrobenzyl)tubercidin (**125**; 58 mg, 47%) as a colorless oil: UV (MeOH) λ_{max} 277 nm, λ_{min} 238 nm. ¹H NMR (CDCl₃) δ 8.36 (s, 1H, H2), 8.17 (d, *J* = 8.7 Hz, 2H, Ph), 7.51 (d, *J* = 8.7 Hz, 2H, Ph), 7.12 (d, *J* = 3.768 Hz, 1H, H6), 6.42-6.46 (dd, 3.8, 6.0 Hz, 2H, H1', H5), 5.73 (t, *J* = 5.8 Hz, 1H, H2'), 5.54-5.59 (m, 2H, H3', NH), 4.95 (d, *J* = 6.1 Hz, 2H, CH₂), 4.31-4.40 (m, 3H, H4', H5', H5''), 2.14 (s, 6H, 2 $\times$ CH₃), 2.04 (s, 3H, CH₃); ¹³C NMR (CDCl₃) δ 170.4, 169.7, 169.5 (3 $\times$ C=O), 156.0 (C4), 152.3 (C2), 150.8 (C7a), 147.3 (Ph), 146.7 (Ph), 128.0 (Ph), 123.9 (Ph), 121.5 (C6), 103.8 (C4a), 99.4 (C5), 85.4 (C1'), 79.5 (C4'), 73.1 (C2'), 70.8 (C3'), 63.5 (C5'), 44.2 (CH₂), 20.8, 20.6, 20.4 (3 $\times$ s, 3 $\times$ CH₃). MS (ESI): *m/z* 528 (100%, MH⁺).

Step d. NH₃/MeOH (5 mL) was added to a stirred solution of **125** (54 mg, 0.1 mmol) in MeOH (1 mL) and stirring was continued at ambient temperature for 20 h. Volatiles were evaporated and the residue was column chromatographed with a 2 $\rightarrow$ 4% gradient of the upper phase of EtOAc/*i*-PrOH/H₂O (4:1:2) in EtOAc to give **122b** (35 mg, 85%) as a yellow oil.

5-Carboxamido-4-(4-nitrobenzylamino)-7-(β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (122c**).** Sangivamycin (**19b**; 155 mg, 0.5 mmol) was treated with 4-nitrobenzyl bromide (162 mg, 1.5 mmol) for 26 h at 40 $^{\circ}$ C as described for **19a** $\rightarrow$ **122a** and the alkylated intermediate was then stirred with Me₂NH/THF (2 M, 6 mL) and

MeOH (12 mL) for 45 h at ambient temperature. Me₂NH/THF (2 M, 2 mL) was added and the mixture was stirred at reflux for 32 h (total reaction time: 77 h). TLC showed ~80% conversion to the less polar **122c**, which was obtained as off-white crystals (101 mg, 45%) after recrystallization from EtOH: mp 154-158 °C (dec.); UV (MeOH) 286 nm (ϵ 23 100), $\lambda_{\min}$ 229 nm (ϵ 8700); ¹H NMR (DMSO-*d*₆) δ 10.29 (t, *J* = 6.0 Hz, 1H, NH), 8.17-8.21 (m, 4H, Ph, H2, H6), 8.11 and 7.47 (2 $\times$ s, 2 $\times$ 1H, CONH₂), 7.59 (brd, *J* = 8.8 Hz, 2H, Ph), 6.06 (d, *J* = 6.0 Hz, 1H, H1'), 5.43 (d, *J* = 6.3 Hz, 1H, 2'-OH), 5.20 (d, *J* = 5.0 Hz, 1H, 3'-OH), 5.09 (t, *J* = 5.8 Hz, 1H, 5'-OH), 4.90 ("d", *J* = 6.2 Hz, 2H, CH₂), 4.37 (q, *J* = 5.8 Hz, 1H, H2'), 4.10 ("q", *J* = 4.5 Hz, 1H, H3'), 3.93 (q, *J* = 4.0 Hz, 1H, H4'), 3.61-3.67 (m, 1H, H5'), 3.53-3.58 (m, 1H, H5"); ¹³C NMR (DMSO-*d*₆) δ 166.5, 156.5, 152.6, 150.4, 148.0, 146.4, 128.0 (Ph), 125.7, 123.6, 110.9, 101.7, 87.2 (C1'), 85.3 (C4'), 73.9 (C2'), 70.5 (C3'), 61.9 (C5'), 42.9 (CH₂); MS (ESI) *m/z* 445 (100%, MH⁺). Anal. Calcd for C₁₉H₂₀N₆O₇•1.5 H₂O (471.42): C, 48.41; H, 4.92; N, 17.83. Found: C, 48.59; H, 4.66; N, 17.65.

5-Cyano-4-(4-nitrobenzylamino)-7-(β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine

(122d). Toyocamycin (**19c**; 146 mg, 0.5 mmol) was heated with 4-nitrobenzyl bromide (364 mg, 1.5 mmol) in dried DMF (3 mL) at 40 °C for 63 h (TLC showed ~85% conversion to a less polar product). Volatiles were evaporated to ~1 mL (< 40 °C, vacuum pump) and this material was added dropwise to 20 mL of vigorously stirred dried acetone. Et₂O (40 mL) was added to the stirred suspension, which was chilled at 0 °C for 20 minutes and the precipitate was collected by vacuum filtration. Recrystallization from MeOH gave **122d** (99 mg, 46%) as colorless crystals: mp 214-216 °C; UV (MeOH) $\lambda_{\max}$ 274, 236 nm (ϵ 22 100, 18 900), $\lambda_{\min}$ 250, 228 nm (ϵ 12 800, 18 000); ¹H NMR (DMSO-

d_6) δ 8.35 (s, 1H, H2), 8.19-8.23 (m, 3H, H6, Ph), 7.59 (d, J = 8.6 Hz, 2H, Ph), 6.87 (s, 1H, NH), 5.93 (d, J = 5.6 Hz, 1H, H1'), 5.47 (d, J = 6.0 Hz, 1H, 2'-OH), 5.35 ("s", 2H, CH₂), 5.21 (d, J = 5.0 Hz, 1H, 3'-OH), 5.09 (t, J = 5.4 Hz, 1H, 5'-OH), 4.31 (q, J = 5.5 Hz, 1H, H2'), 4.08 (q, J = 4.6 Hz, 1H, H3'), 3.92 (q, J = 3.7 Hz, 1H, H4'), 3.62-3.68 (m, 1H, H5'), 3.53-3.58 (m, 1H, H5"); ¹³C NMR (DMSO- d_6 -D₂O) δ 152.6, 149.4, 146.7, 144.9, 142.7, 129.4, 128.5 (Ph), 123.5 (Ph), 115.2, 105.0, 87.7 (C1'), 85.8 (C4'), 85.5, 74.6 (C2'), 70.1 (C3'), 61.1 (C5'), 48.8 (CH₂); MS (ESI): m/z 427 (100%, MH⁺). Anal. Calcd for C₁₉H₁₈N₆O₆•0.5 H₂O (435.39): C, 52.41; H, 4.40; N, 19.30. Found: C, 52.17; H, 4.29; N, 19.30.

8-azidotoyocamycin (129). NaN₃ (158.1 mg, 2.43 mmol) was added to a stirred solution of **55** (300 mg, 0.810 mmol) in anhydrous DMF (10 mL) in a flame dried flask and the resulting colorless solution was stirred at room temperature in the dark for 16 h, by which time the solution turned a brown-red color and UV showed 6 nm bathochromic shift in the $\lambda_{\max}$, indicating the conversion to product. Volatiles were removed from the clear, brown-red solution using high vacuum rotary evaporator on a hot water bath (<80 °C) while in a dark room. The resulting brown gummy oil was purified using column chromatography in the dark (5%, MeOH/EtOAc) to give **129** as an off white solid (123.2 mg, 46%): HRMS (ESI): m/z [M+Na]⁺ calcd for C₁₂H₁₂N₈NaO₄⁺: 355.0874; found 355.0952.

Click product (132). Cyclooctyne **130** (9.04 mg, 0.060 mmol) was added to a stirred solution of **129** (20 mg, 0.060 mmol) in ACN and H₂O (3:1) in a flask and the resulting clear, colorless solution was stirred at room temperature for 4 h, by which time TLC showed >80% conversion to product to a more polar product. Volatiles were removed

using high vacuum rotary evaporator and a hot water bath (<80 °C). The resulting yellow oil was purified using column chromatography (10%, EtOAc/MeOH) giving **132** as an off white solid (17 mg, 59%). This solid was then re-purified using RP-HPLC (20% CAN in H₂O) to give **132** as an off white gummy solid (9.6 mg, 33%). *R_f* (EtOAc:*i*PrOH:H₂O, 4:1:2) 0.49; HRMS (ESI): *m/z* [M+H]⁺ calcd for C₂₂H₂₇N₈O₅⁺: 483.2099; found 483.2087.

Click product (133). Cyclooctyne **131** (16.6 mg, 0.060 mmol) was added to a stirred solution of **129** (20 mg, 0.060 mmol) in ACN and H₂O (3:1) in a flask and the resulting clear, pale red solution was stirred at room temperature for 4 h, by which time TLC showed >80% conversion to product to a more polar product almost on the baseline. Volatiles were removed using high vacuum rotary evaporator and a hot water bath (<80 °C). The resulting pale red oil was purified using column chromatography (17.5%, EtOAc/MeOH) giving **133** as an off white gummy solid (17.1 mg, 47%). *R_f* (EtOAc:*i*PrOH:H₂O, 4:1:2) 0.13; HRMS (ESI): *m/z* [M+H]⁺ calcd for C₃₀H₂₉N₁₀O₅⁺: 609.2317; found 609.2352.

2',3',5'-tri-*O*-acetyltoyocamycin. (138). Ac₂O (380 μL, 4 mmol) was added to a stirred solution of **19c** (292 mg, 1 mmol) in anhydrous pyridine (5 mL) at 0 °C (ice bath) and stirring was continued at 0 °C for 1 h and then at ambient temperature for 3 h (total reaction time: 4 h). MeOH was added, the reaction mixture was stirred at ambient temperature for 30 min, and volatiles were evaporated (vacuum pump, <25 °C). MeOH was added and evaporated, and the resulting gum was partitioned between CHCl₃ (25 mL) and 2% AcOH/H₂O (25 mL). The aqueous layer was extracted with CHCl₃, and the combined organic phase was washed with NaHCO₃/H₂O, brine, and dried (MgSO₄).

Volatiles were evaporated *in vacuo* and the residue was column chromatographed (70% EtOAc in hexanes) to give 2',3',5'-tri-*O*-acetyltoyocamycin (**138**; 405 mg, 97%) as a colorless foam: ¹H NMR (CDCl₃) δ 8.24 (s, 1H, H2), 7.72 (s, 1H, H8), 6.37 (s, 2H, NH₂), 6.30 (d, *J* = 5.4 Hz, 1H, H1'), 5.66 (d, *J* = 5.5 Hz, H2'), 5.48 (d, *J* = 5.1 Hz, H3'), 4.38 ("q", *J* = 3.9 Hz, H4'), 4.33-4.32 ("m", 2H, H5', H5"), 2.08 (s, 3H, CH₃), 2.06 (s, 3H, CH₃), 2.00 (s, 3H, CH₃).

8-Bromo-2',3',5'-tri-*O*-acetyltoyocamycin (139). DBH (157 mg, 0.55 mmol) was added to a stirred solution of **138** (209 mg, 0.5 mmol) in CH₂Cl₂ (4 mL) and the resulting brown solution was stirred at ambient temperature for 18 hours. The reaction mixture was partitioned between CH₂Cl₂ (16 mL) and NaHCO₃/H₂O (25 mL). The aqueous layer was extracted with CH₂Cl₂, and the combined organic phase was washed with brine and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (50% EtOAc in hexanes) to give **139** (195 mg, 79%) as a brown oil with data as reported.²²³

2',3',5'-tri-*O*-(tert-butyldimethylsilyl)toyocamycin (141). Imidazole (1.02 g, 15 mmol) and TBDMSCl (1.09 g, 7.2 mmol) was added to a stirred solution of **19c** (583 mg, 2 mmol) in anhydrous DMF (6 mL) and the resulting suspension was stirred at ambient temperature for 24 h and then at 35 °C for 12 h, by which time TLC showed ~40% unchanged substrate and two less polar spots. A second portion of imidazole (1.02 g, 15 mmol) and TBDMSCl (1.09 g, 7.2 mmol) were added and the resulting suspension was stirred at 35 °C for 24 h (total reaction time: 60 h), by which time TLC showed 80% conversion to a major less polar spot. Volatiles were evaporated and co-evaporated with toluene (high vacuum rotavap, <40 °C). Crude pale gum was partitioned between CHCl₃

(50 mL) and brine (50 mL). The aqueous layer was extracted with CHCl₃, and the combined organic layer was dried over anhydrous MgSO₄. Volatiles were evaporated *in vacuo* and the residue was column chromatographed (25% EtOAc in hexanes) to give **141** (736 mg, 58%) as a colorless oil: ¹H NMR (CDCl₃) δ 8.30 (s, 1H, H2), 8.13 (s, 1H, H8), 6.27 (s, 2H, NH₂), 6.18 (d, *J* = 4.1 Hz, 1H, H1'), 4.32 (d, *J* = 4.2 Hz, H2'), 4.21 (d, *J* = 4.4 Hz, H3'), 4.12-4.10 ("m", 1H, H4'), 4.02 (dd, *J* = 2.8, 11.6 Hz, 1H, H5'), 3.78 (dd, *J* = 1.8, 11.6 Hz, 1H, H5''), 0.97 (s, 9H, *t*But), 0.89 (s, 9H, *t*But), 0.80 (s, 9H, *t*But), 0.16 (s, 3H, CH₃), 0.15 (s, 3H, CH₃), 0.06 (s, 3H, CH₃), 0.05 (s, 3H, CH₃), -0.04 (s, 3H, CH₃), -0.14 (s, 3H, CH₃).

8-Iodo-2',3',5',-tri-*O*-(tert-butyldimethylsilyl)toyocamycin (142). LDA (2 M solution in THF, 560 μL, 1.1 mmol) was added to a stirred solution of **141** (139.5 mg, 0.22 mmol) in dry THF (2 mL) at -78 °C. After ~1 h at -78 °C, iodine (84 mg, 0.33 mmol). The resultant brown solution was stirred at -78 °C for 2 h and at ambient temperature for 48 hours (total reaction time: 51h). Reaction mixture was portioned between EtOAc (10 mL) and 2% AcOH/H₂O (10 mL). Aqueous layer was extracted with EtOAc. Combined organic layer was washed with brine (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo*, and column chromatographed (10% EtOAc in hexanes) to give **142** (27.3 mg, 16%) and **141** (82.3 mg, 58%) as brown oils.

142 had: ¹H NMR (CDCl₃) δ 8.40 (s, 1H, H2), 8.07 (s, 1H, NH₂), 6.15 (d, *J* = 4.5 Hz, 1H, H1'), 4.78 (s, 1H, NH₂), 4.27 (d, *J* = 4.3 Hz, H2'), 4.16 (d, *J* = 4.3 Hz, H3'), 4.06-4.03 ("m", 1H, H4'), 3.94 (dd, *J* = 3.0, 11.5 Hz, 1H, H5'), 3.72 (dd, *J* = 2.1, 11.5 Hz, 1H, H5''), 0.91 (s, 9H, *t*But), 0.84 (s, 9H, *t*But), 0.74 (s, 9H, *t*But), 0.10 (s, 3H, CH₃), 0.09 (s, 3H,

CH₃), 0.00 (s, 3H, CH₃), 0.01 (s, 3H, CH₃), -0.12 (s, 3H, CH₃), -0.24 (s, 3H, CH₃); HRMS (ESI) *m/z* 745.4651 [M-CH₃+H]⁺, calcd for C₂₉H₅₂IN₅O₄Si₃ 745.9249.

7-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)tubercidin (144). Tert-butyl hydroperoxide (85 μL, 0.75 mmol) and iodine (38 mg, 0.15 mmol) were added to a stirred suspension of **19a** (100 mg, 0.38 mmol) and benzotriazole (89.5 mg, 0.75 mmol) in anhydrous DMF (2mL). The resulting dark brown solution was stirred at 35 °C for 72 h, by which time TLC showed ~10% conversion to a less polar product. A second portion of TBHP (85 μL, 0.75 mmol) was added and the resulting dark brown solution was stirred at 35 °C for 24 h (total reaction time: 96 h). Volatiles were evaporated and co-evaporated with toluene (high vacuum rotavap, <50 °C). The resulting brown gum was column chromatographed (5% MeOH in EtOAc) to give **144** (26 mg, 18%) as a brown gum. This brown gum was then injected into a semi-preparative HPLC column (Phenomenex Gemini RP-C18 column; 5μ, 25 cm x 1 cm) *via* a 5 mL loop and was eluted with an isocratic mobile phase mixture 20% CH₃CN in H₂O at a flow rate of 1.5 mL/min to give **144** (*t_R* = 21 min) as a white powder: UV (MeOH) λ_{max} 278 nm (ε = 16700), λ_{min} 240 nm (ε = 8000); ¹H NMR (DMSO-*d*₆) δ 8.50 (d, 1H, *J* = 8.3 Hz, Ph), 8.22 (s, 1H, H2), 7.70-7.67 (m, 1H, Ph), 7.62-7.52 (m, 4H, Ph, NH₂), 7.04 (s, 1H, H8), 5.58 (dd, *J*=3.5,8.8, 3H, 5'-OH), 5.23 (d, *J*=6.9 Hz, 1H, H1'), 5.20 (d, *J*=6.4 Hz, 1H, 2'-OH), 4.92 ("dd", *J*=6.4,11.2 Hz, 3'-OH, H2'), 3.82 ("brs", 1H, H3'), 3.75 ("d", *J*=3.7, 6.2 Hz, H4'), 3.43 (dt, *J*=3.6,11.9 Hz, H5'), 3.28-3.22 (m, 1H, H5"); ¹³C NMR (DMSO-*d*₆): δ 158.22, 153.13 (C2), 148.82, 144.52, 135.13, 129.42 (Ph), 125.63, 125.10 (Ph), 119.70 (Ph), 110.51 (Ph), 102.04, 99.48 (C8), 88.20 (C1'), 85.97 (C4'), 71.31 (C2'), 70.77 (C3'), 62.12 (C5'); HRMS (ESI): *m/z* 384.1391 [M+H]⁺, calcd for C₁₇H₁₇N₇O₄ 384.1415.

5. CONCLUSION

In conclusion, I developed a novel desulfurization-difluorination method for the synthesis of α,α -difluoro esters from the corresponding α -arylthio esters, wherein the thiol group is present on the secondary internal carbon. 1,3-Dibromo-5,5-dimethylhydantoin (DBH) was used as the oxidant (Br^+) and Py.9HF was the fluoride ion (F^-) source. Arylthio esters with hydrogen as well as a ring deactivating chloro substituent at the para position of the aromatic ring were converted to the corresponding gem-difluoro esters under mild reaction conditions. However, the presence of a ring activating methoxy substituent on the para position of the aromatic ring caused the reaction to be sluggish and the difluorination was observed in low yields. Expansion of this desulfurization-difluorination methodology towards the thioether substrates having aldehyde, ketone, and unactivated alkane functional groups on the adjacent carbon was unsuccessful. Also, treatment of lactones under similar reaction conditions gave low yield conversion to the corresponding α -fluoro sulfides and α -fluoro sulfoxides. Attempted synthesis of *gem*-difluorouridine derivatives from the corresponding 2'-arylthio uridine or 2'-fluoro-2'-arylthio uridine precursors was unsuccessful and resulted mostly in halogenation of the pyrimidine ring at the C-5 and monofluorination at C2', without desulfurization. However, treatment of the fully benzylated uridine substrate bearing a *p*-methoxyphenylthioether substituent at the C2' with combination of NIS and DAST gave a moderate but encouraging yield (44%) of the corresponding monofluorinated thioether without any appreciable oxidation of thioether and iodination of the pyrimidine ring.

Chemical reduction protocols in combination with cyclic voltammetry were employed for reductive desulfonylation of 2'-arylsulfonyl-2'-deoxyuridines as a possible

alternative process towards incorporation of fluorine and other substituents at the C2'. A critical impact on the outcome of these reactions was the choice of the protecting groups. Thus, 3',5'-*O*-diacetylated substrates **91a** were found to decompose readily into the corresponding vinyl sulfone **102** during silica gel column purification, whereas the fully benzylated substrate **91b** was prone to uracil elimination in the presence of strong base. The cleavage of the C2'-S was found to occur at a relatively high cathodic potential close to - 2.4 V *vs* SCE, with the expulsion of the corresponding sulfinate, meaning that strong chemical reducing agents or selective electrochemical reductions were required. Interestingly the protecting group (Bn or Ac) had no impact on the reduction potential. Reactions of 2'-arylsulfonyl-2'-deoxy-2'-fluorouridines with using Organic Electron Donors (OEDs) under different conditions (excess of reducing agents, temperature, *uv* light activation) gave products resulting from the elimination of either acetate anion from C3' position or uracil from C1' upon reduction of the C2'-S bond leading to furan-type products. Interestingly, less powerful reducing-agent TDAE under UV light activation was found to cleave the C2'-sulfonyl bond to give 2',3'-unsaturated 2'-fluorouridine (18%) and 2'-fluoro-2'-deoxyuridine (10%) products. Tentative trapping of the resulting C2'-anion under these *uv* light conditions either with Selectfluor or MeI were however unsuccessful.

Bromination at C5-position of pyrimidine nucleosides such as uridine, 2'-deoxyuridine, arabinouridine, cytidine, and *N*4-benzoylcytidine were achieved under mild reaction conditions using DBH. Also, transformation of purine nucleosides such as adenosine, 2'-deoxyadenosine, guanosine, and 2'-deoxyguanosine to their corresponding 8-brominated counterparts with DBH. Although purine nucleosides required higher

amounts of reagents and produced brominated products in lower yields when compared to that of the pyrimidine counterparts (47-98% vs 70-98%). Increase in temperature and presence of Lewis acids such as TMSOTf and TsOH increased the rate of bromination reaction and reduced the equivalents of DBH reagent required. The DBH bromination protocol is bench friendly, with most conversions taking place at ambient temperature and producing the brominated products in moderate to high yields. Also, acid labile protection groups such as isopropylidene, and base labile protection groups such as acetyl, benzoyl, and silyl were found to be stable during the course of bromination. In addition, I developed novel NBS or DBH mediated bromination methodology for the synthesis of previously known 8-bromotoyocamycin and 8-bromosangivamycin. This methodology offers mild and improved synthetic alternative from previous reaction conditions. I also found that DBH-bromination of various unprotected nucleosides proceeded efficiently in MeOH, avoiding the need to use high-boiling and expensive solvent DMF.

Novel 6-*N*-benzyltubercidin and 6-*N*-(4-nitrobenzyl) analogues of tubercidin and sangivamycin were synthesized by initial alkylation at N1 with respective benzyl bromide followed by Me₂NH mediated Dimroth rearrangement. However, no evidence of formation of an initial N1-alkylated cationic intermediate was observed on TLC upon treatment of toyocamycin with 4-nitrobenzyl bromide. Isolated less polar product indicated direct alkylation on the exocyclic amino group to give 4-*N*-(4-nitrobenzyl)toyocamycin. Alternatively, 6-*N*-(4-nitrobenzyl)tubercidin was also prepared by (i) conversion of acetylated tubercidin to its 6-fluorocounterpart via Balz-Schiemann type fluoro-diazotization with NaNO₂/Py.9HF (55% HF); (ii) S_NAr displacement of the 6-fluoro group by 4-nitrobenzylamine; (iii) deacetylation with NH₃/MeOH. The 6-*N*-(4-

nitrobenzyl) derivatives of tubercidin and sangivamycin inhibited cross-membrane transport of labelled uridine by the human equilibrative nucleoside transporter hENT1 at $>1\text{ }\mu\text{M}$ and $\sim 120\text{ nM}$, respectively. Inhibition of the proliferation of L1210, HeLa, and PC-3 tumor cells in culture was observed with 6-*N*-benzyltubercidin and the 6-*N*-(4-nitrobenzyl) derivatives of sangivamycin and toyocamycin at $0.92\text{--}9.4\text{ }\mu\text{M}$ concentrations.

Novel 8-azidotoyocamycin was synthesized by treatment of 8-bromotoyocamycin with sodium azide. But the azide product was light sensitive and decomposed after exposure to light. Strain promoted click chemistry of 8-azidotoyocamycin with cyclooctynes gave the corresponding 8-triazolyl derivatives in moderate yields. Iodine mediated CH arylation of tubercidin with benzotriazole gave the corresponding 7-substituted benzotriazolyl product, which showed emission at 420 nm with a quantum yield of 0.002 s.

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PUBLICATIONS AND PRESENTATIONS

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