UNIVERSITE MONTPELLIER 2
SCIENCES ET TECHNIQUES DU LANGUEDOC

T H E S E

pour obtenir le grade de

DOCTEUR DE L'UNIVERSITE MONTPELLIER 2

***Spécialité** : CHIMIE ORGANIQUE, MINERALE et INDUSTRIELLE*
***Formation Doctorale** : Chimie des Biomolécules: Synthèse, Structure et Réactivité*
***Ecole Doctorale :** Sciences Chimiques (ED 459)*

présentée et soutenue publiquement

par

Ahmed KHALIL
Maître Assistant, Université Zagazig, Egypte

Le 3 Décembre 2010

SYNTHESE ET ETUDE D'ANALOGUES NUCLEOSIDIQUES FLUORES EN POSITION 2′ OU 3′

SYNTHESIS AND STUDY OF A SERIES OF 2′- OR 3′-FLUORINATED NUCLEOSIDE ANALOGUES

JURY

M. PERIGAUD Christian	Professeur, Université Montpellier II	Président
M. BENHIDA Rachid	Directeur de Recherche CNRS, Nice Sophia Antipolis	Rapporteur
M. MEDEBIELLE Maurice	Directeur de Recherche CNRS, Université Lyon I	Rapporteur
M. GOSSELIN Gilles	Directeur de Recherche CNRS, Idenix, Montpellier	Invité
M. MATHE Christophe	Maître de Conférences, Université Montpellier II	Directeur de Thèse

THESE DE DOCTORAT NOUVEAU REGIME

TITRE DE LA THESE: Synthèse et étude d'analogues nucléosidiques fluorés en position 2′ ou 3′.

AUTEUR: Ahmed KHALIL

ANNEE: 2010

DISCIPLINE: Chimie Organique, Minérale et Industrielle

LIEU: Institut des Biomolécules Max Mousseron, UMR 5247, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier 5 (France).

RESUME:
Dans le premier chapitre de cette thèse, nous nous sommes intéressés aux virus de l'immunodéficince humaine et des hépatites B et C ainsi qu'aux thérapies utilisées dans le traitement de ces affections. Nous avons introduit l'importance des nucléosides fluorés, et nous avons donné quelques exemples de nucléosides fluorés utilisés en chimiothérapie antivirale et antitumorale. Dans le second chapitre, nous avons présenté une synthèse rapide de 2',3'-didésoxy-3'-fluoro-β-D-thréo-nucléosides portant les bases pyrimidiques naturelles et substituées en position N3 par un groupement nitro ou amino. Les composés obtenus ont été évaluées contre divers virus à ADN et ARN (y compris le VIH) dans des expériences de culture cellulaire. Dans le troisième chapitre, nous nous somme intéressés à la synthèse de différents 2′,3′-didésoxy-2′-fluoro-3′-(N-hydroxyimino), (N-methoxyimino) and (hydroxyl-amino) nucléosides en série pyrimidine. Les composés obtenus ont été évaluées contre divers virus à ADN et ARN dans des expériences de culture cellulaire.

MOTS-CLES: analogues de nucléoside, 3'β-fluoronucléosides, 2'-fluoronucléosides, trifluorure de diéthylaminosulfure (DAST), antiviraux.

TITLE: Synthesis and study of a series of 2′- and 3′-fluorinated nucleoside analogues.

ABSTRACT:
In the first chapter, we presented the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), as well as the therapies used to treat these diseases. In a second part, we discussed about the importance of the incorporation of fluorine atom into nucleoside analogues, and in a third part of this chapter, we presented the recent literature sources of the synthesis and biological activity of fluorinated nucleosides. In the second chapter, we designed and synthesized a series of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides by direct and rapid methodology and evaluated them for their inhibitory effects on a number of RNA and DNA viruses in cell culture experiments. None of these nucleoside derivatives showed any antiretroviral activity nor cytotoxicity. In the third chapter of this manuscript, we synthesized a new series of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino),(N-methoxyimino) and (hydroxylamino)pyrimidine nucleosides and also evaluated for their inhibitory effects on a number of RNA and DNA viruses, without finding any activity or cytotoxicity.

KEYWORDS: nucleoside analogues, 3'β-fluoronucleosides, 2'-fluoronucleosides, Diethylaminosulfur trifluoride (DAST), antiviral agents.

Table of contents

Acknowledgement……...…….…...6

Abbreviations……..7

General introduction……..9

Chapter I: Nucleoside analogues and antiviral chemotherapy…..…… 10

I. Chemotherapy of viral infections…...……..11

I.1. The human deficiency virus (HIV)…….....11

I.1.1 HIV virion structure and genome……...11

I.1.2 HIV Life Cycle……...13

I.1.3 Metabolism of Nucleoside reverse transcriptase inhibitors (NRTIs)…….15

I.1.4 Inhibition of reverse transcriptase……..18

I.1.5 Structure of the approved NRTIs……...…19

I.1.6 NRTIs under development…….....21

I.2 Hepatitis B Virus (HBV)…….22

I.2.1 The HBV Genome……..23

I.2.2 The HBV life cycle…….24

I.2.3 Anti-HBV nucleoside analogues……26

I.3 Hepatitis C Virus (HCV)……27

I.3.1 The HCV Genome…….....27

I.3.2 The HCV life cycle……...….28

I.3.3 Treatment of Chronic Hepatitis C…….…….....31

I.3.4 Anti-HCV Nucleoside analogues under development…….....32

I.4 Conclusion……....33

II. The incorporation of fluorine into nucleoside analogues…….....….34

II.1 Unique properties of fluorine and their relevance in medicinal chemistry……..…35

II.1.1 Mimic Effect of fluorine……...….37

II.1.2 Lipophilicity of fluorine and fluorine-containing groups……..…….. 37

III Synthesis and biological activity of fluorinated nucleosides……..…….....39

III.1 Synthesis of Fluorinated Nucleosides…….……40

III.1.1 Synthesis of 1′-fluoronucleosides…….……40

III.2.1 Synthesis of 2′-fluoronucleosides…….……42

III.2.1.1 Synthesis of 2′α-fluoronucleosides……..…….42

III.2.1.2 Synthesis of 2′β-fluoronucleosides……..……..…50

III.2.1.3 Synthesis of 2′,2′-difluoronucleosides……..…….58

III.3.1 Synthesis of 3′-monofluorinated nucleosides……..…….……...59
III.3.1.1 Synthesis of 3′α-fluoronucleosides……..…….59
III.3.1.2 Synthesis of 3′β-fluoronucleosides……..…….64
III.1.4 4′-monofluorinated nucleosides…….....69
III.1.5 5′-monofluorinated nucleosides……...73
III.1.6 6′-monofluorinated nucleosides…….....78
IV Conclusion…….….....80
Chapter II: Synthesis of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides……..81
I. Introduction and background……..82
II. Synthesis……...85
II.1 Synthesis of 2'-deoxy-3-nitrouridine 7 and 2'-deoxy-3-nitrothymidine 8 …..……..85
II.2 Synthesis of 5'-*O*-acetyl-2'-deoxy-3-nitrouridine 9 and 5'-*O*-acetyl-2'-deoxy-3-nitrothymidine 10……..…86
II.3 Synthesis of 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 13 and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 14……...…88
II.4 Yield improvement of 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 11 and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 12…….89
II.5 Ring modification of 2'-deoxy-3-nitrothymidine 8 and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 14 to *N*-NH_2 derivatives 15 and 16…….90
II.6 Synthesis of 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil 19 and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine 20…….....91
II.7 Structure elucidation of compounds 19 & 20……..93
II.8 Synthesis of 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)cytosine 22……...….97
III. Biological Evaluation……...……..98
IV. Conclusion…….……...…..107
Experimental Part……...108
Chapter III: Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino),(N-methoxyimino) and (hydroxylamino)-pyrimidine nucleosides…….....130
I. Introduction and background……..……...…131
II. Synthesis……...…...133
II.1 The Synthesis of 2′-deoxy-2′-fluorouridine 27 and 2′-deoxy-2′-fluorocytidine 30.....133

II.2 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 34 (E/Z) and 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pento-furanosyl] uracil 35 (E/Z).....134
II.3 Stereoselective reduction of 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 34 (E/Z)……..……..……..135
II.4 Synthesis of 1-[2-amino-2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] cytosine dihydrochloride 38…….……..138
II.5 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] cytosine 40 (E/Z)……..140
II.6 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(N-hydroxyimino)-*β*-D-*glycero*-pento-furanosyl]cytosine 44 (E/Z)…….…….…..141
II.7 Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine 45……...142
II.8 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] N^4-methoxycytosine 47 (E/Z)…….143
II.9 Synthesis of 2′-deoxy-2′-fluoro-N^4-methoxycytidine 49……...…145
II.10 ^{19}F NMR study of the nucleosides *N*-Oximes……..……....…146
III. Biological Evaluation…….……..150
IV. Conclusion…….....…159
General Conclusion……..……...161
Experimental Part……...…162
Résumé…….189
References……208

ACKNOWLEDGEMENT

I would especially like to thank God for giving me the strength and endurance to complete this work.

I would like to thank Professor Christian Périgaud for welcoming me in his lab, and for giving me the opportunity to develop this experience abroad. I would like to thank my advisor, Dr. Christophe Mathé for the encouragement, motivation and valuable advice. He has provided immense support, guidance and leadership through these years.

I am thankful to my thesis committee members: Dr. Maurice Medebielle, Dr. Rachid Benhida, Dr. Gilles Gosselin and Prof. Christian Périgaud for accepting to spend their precious time in reviewing my thesis and giving their valuable suggestions.

I would like to give my gratitude to everyone who helped and encouraged me through these years and made the completion of this work possible.

I would like to acknowledge the Ministry of Higher Education and Scientific Research, Egypt for the financial support.

And, finally, I would like to thank my family members specially my wife and my sons, Mohamed and Marwan, and dear friends, for their love and support.

ABBREVIATION

Ac	acetyl
Ac_2O	acetic anhydride
AIBN	azobisisobutyronitrile
AIDS	acquired Immunodeficiency syndrome
aq.	aqueous
Bn	benzyl
BSTFA	bis-trimethylsilytrifluoroacetamide
Bz	benzoyl
CMV	cytomegalovirus
DAST	diethylaminosulphur trifluoride
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethylazodicarboxylate
Deoxo-Fluor	bis(2-methoxyethyl)aminosulfur trifluoride
DHP	dihydropyran
DIBAL-H	diisobutylaluminum hydride
DMAP	4-dimethylamino pyridine
DMF	*N*,*N*- dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dTMP	thymidine-5'-monophosphate
EDCI	1-(3-dimethylaminopropyl)-3-ethyl-carbodi -imide hydrochloride
eq.	equivalent
ESI-MS	electrospray Ionization Mass Spectrometry
et al.	et alitas (lat.)
FDA	food and drug administration
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human Immuno-deficiency Virus
HMDS	hexamethyldisilazane
HSV-1	herpes Simplex-1 Virus
HSV-2	herpes Simplex-2 Virus
LDA	lithium diisopropyl amide
LiHMDS	lithium Hexamethyldisilazide
Lindlar	Pd on $CaCO_3$/PbO
***m*-CBA**	meta-chlorobenzoic acid
***m*-CPBA**	meta-chloroperbenzoic acid
Me	methyl
MMTr	monomethoxytrityl
Ms	methanesulphonyl
MS	molecular sieves
MsCl	methyl sulphonyl chloride
MTHP	methoxytetrahydropyran
NBS	*N*-bromosuccinimide
NFSI	*N*-Fluorodi(benzenesulfonyl)amine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser Effect
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
RNA	ribonucleic acid
S_N	nucleophilic substitution
TBAF	tetra-n-butylammonium fluoride
TBDMS	tertbutyldimethylsilane
TEA	triethylamine
TEMPO	tetramethyl-1-piperidinyloxy
TFA	trifluoro acetic acid
THF	tetrahydrofurane
TMSOTf	trimethylsilyltrifluoromethane sulfonate
Tr	trityl
TsCl	toluenesulfonyl chloride
UV	ultraviolet
VZV	varicella-zoster virus

General Introduction

After the human immunodeficiency virus (HIV) was discovered as the etiological agent of acquired immune deficiency syndrome (AIDS), at present, there are some 60 antiviral drug preparations that have been approved by the US FDA, almost half of which are used for the treatment of HIV infections. The remaining half are used for the treatment of HBV, herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), influenza and HCV infections. Nucleoside analogues are still prominent drugs in the management of several viral infections.

The viral infections are still representing the major cause of morbidity and mortality in the world. In order to discover new chemical structures able to stop viral progressive process and eradication of their diseases are very important. The synthesis and study of new series of nucleoside analogues are still one of the promising researches in the development of new structures that may interfere selectively with viral proliferation.

In the first chapter of this manuscript, we present an introduction including genome structure and life cycle of human immunodeficiency (HIV), hepatitis B (HBV), and hepatitis C (HCV) as well as the nucleoside analogues used to treat these viral infections. The importance of incorporation of fluorine atom into nucleoside analogues and the recent literature sources of the synthesis and biological activity of the fluorinated nucleosides were also discussed.

In the second chapter of this manuscript we designed and synthesized a series of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides by rapid and direct methodology by protection of *N*-3 by nitro group to prevent formation of anhydronucleosides followed by fluorination with DAST, and evaluated their inhibitory effects on a number of RNA and DNA viruses.

In the third chapter of this manuscript, we synthesized a new series of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino), (*N*-methoxyimino) and (hydroxylamino)-pyrimidine nucleosides and also evaluated their inhibitory effects on a number of RNA and DNA viruses.

Chapter I

Nucleoside analogues and antiviral chemotherapy

I. Chemotherapy of viral infections:

I.1. The human deficiency virus (HIV)

In 1985, two years after the identification of human immunodeficiency virus (HIV)[1], which discovered by Luc Montagnier and Françoise Barré-Sinoussi (Nobel Prize in Medicine, 2008), and one year after the initial evidence about its etiological link to AIDS, was reported in Samuel Broder's group at the National Cancer Institute together with collaborators from Burroughs-Welcome company, 3′-azidothymidine (AZT, zidovudine) as the first nucleoside inhibitor with *in vitro* anti-HIV activity. The discovery of the anti-HIV activity of AZT was an important moment, providing the first proof of concept that the replication of HIV could be controlled by chemotherapy and thereby establishing the foundation of antiretroviral drug discovery research. Furman *et al.*[2] from Burroughs-Welcome first showed that AZT acts through its triphosphate metabolite by inhibiting reverse transcriptase (RT), the key enzyme of HIV responsible for the synthesis of proviral DNA. Thus, AZT became the first nucleoside HIV reverse transcriptase inhibitor (NRTI). Over the course of 25 years that followed after this seminal discovery, seven nucleosides and one nucleotide have been approved by the United States Food and Drug Administration for the treatment of HIV infection starting with the approval of AZT in 1987 and followed by didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), tenofovir disoproxil fumarate [TDF; prodrug for the oral delivery of the nucleotide analogue tenofovir (TFV)] and, most recently in 2003, emtricitabine (FTC). [3,4,5]

I.1.1 HIV virion structure and genome

Mature HIV virions have spherical morphology of 100-120 nm in diameter and consist of a lipid bilayer membrane that surrounds a dense truncated cone-shaped nucleocapsid (core) which contains the genomic RNA molecules, the viral protease (PR), reverse transcriptase (RT), integrase (IN), Vpu, Vif, Vpr and Nef, and some cellular factors. The lipid bilayer is of cellular origin and includes approximately 72 spikes of the viral Env glycoproteins and some cellular proteins. The principal structural proteins forming the core are, MA (matrix protein or p17): forms a shell associated directly to the inner side of the membrane through the N terminus; CA (capsid protein or p24): the N-terminal domains of CA assemble in hexameric rings to constitute the capsid ; NC: is implicated in formation and stabilization of the genomic RNA dimers and in the nucleocapsid assembly; p6: this domain of the p55 is required for the last stages of viral assembly, efficient release from the cell and internalization of the Vpr protein into the assembled virion (Figure **1**).[6]

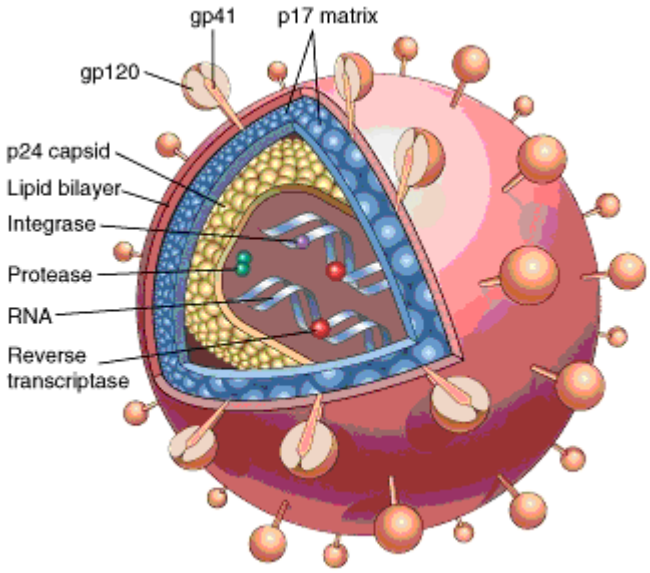


Figure (**1**): HIV-1 Virion (Elsevier Science 2002)

The HIV-1 genome (Figure **2**) consists of two identical 9.2 kb single stranded RNA molecules within the virion, whereas the persistent form of the HIV-1 genome is proviral double-stranded DNA within infected cells. Numbering positions in HIV are according to HXB2CG. Four main regions can be distinguished in the HIV genome: (1) LTR: regulatory regions located at both edges of the RNA molecules, flanking the coding region. Each LTR contains an U3, R and U5 region. The U3 contains binding sites for cellular transcription factors. The R region contains the trans-activation response element (TAR) implicated in Tat-mediated trans-activation. (2) The gag-pol gene encodes two polyprotein precursors. The Gag polyprotein includes the proteins of the nucleocapsid, and the Gag-Pol polyprotein comprises some structural proteins and three viral enzymes: protease (PR), reverse transcriptase (RT) and integrase (IN). (3) The env gene encodes the gp160 polypeptide precursor containing the exterior gp120 and the transmembrane gp41. (4) HIV accessory genes are: tat, rev, nef, vif, vpr and vpu (for HIV-1) or vpx (for HIV-2).[6]

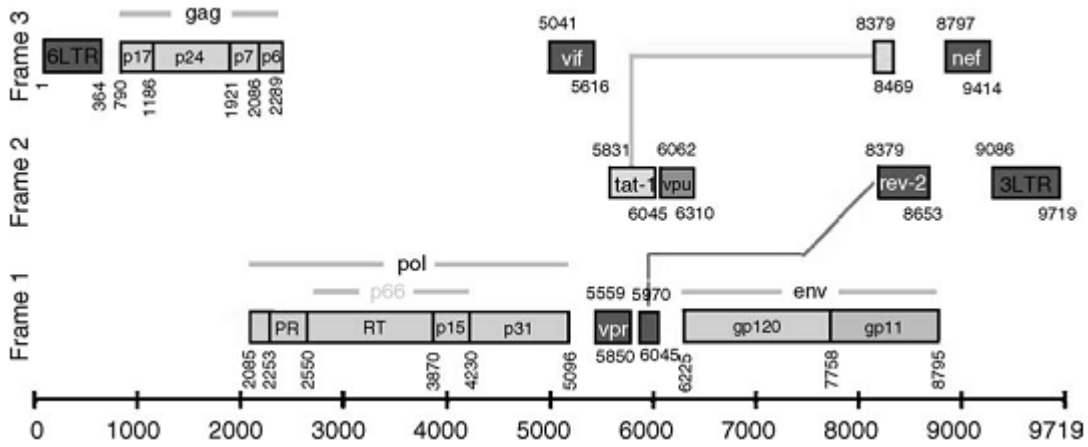


Figure (**2**): Genome organization of HIV

I.1.2 HIV Life Cycle (Figure 3):

Entry begins when the gp120 of the viral envelope spikes (in green) binds to the CD4 receptor on the surface of susceptible cells. Changes in gp120 lead to coreceptor (CCR5 or CXCR4) engagement and fusion mediated by the envelope gp41, followed by internalization of the viral core. After uncoating, reverse transcription leads to the generation of double-stranded DNA (dsDNA) from the single-stranded viral RNA genome (ssRNA). The provirus DNA is transported to the nucleus and integrated into the host chromosomal DNA (host DNA). Then DNA is transcribed by cellular polymerases to generate full-length and spliced messenger RNA, as well as full-length progeny virion RNA. Viral messages are translated in the cytoplasm and the newly produced viral proteins, together with genomic RNA, assemble into immature virions. New viral particles are released (budding) and become infectious after proteolytic maturation by the HIV protease enzyme. The targets of approved antiretroviral agents are underlined, namely: virus entry, fusion, reverse transcription, integration and proteolytic maturation.[7]

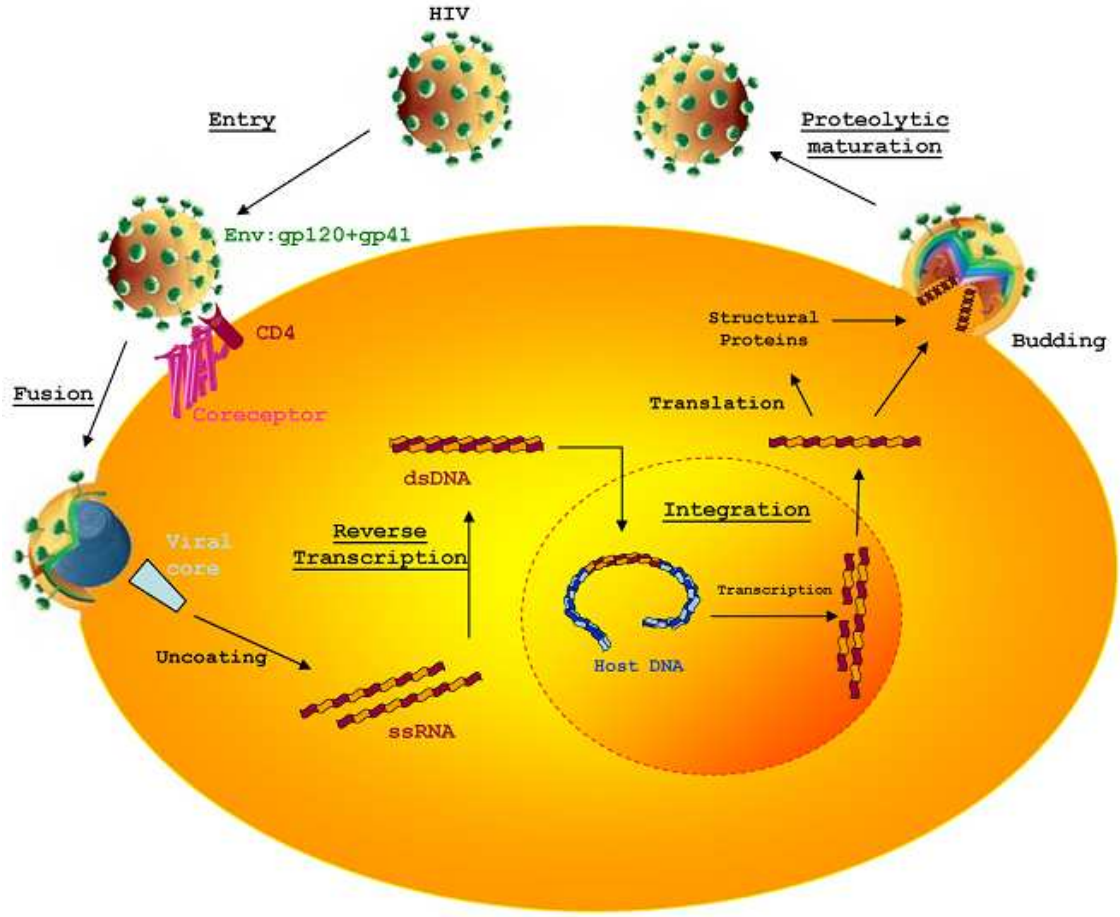


Figure (**3**): HIV Life Cycle

As disease progression is associated with higher HIV RNA levels in blood (viral load), an important objective of antiretroviral therapy is to reduce viral loads below the limit of detection of approved assays. Almost of the anti-HIV drugs target viral enzymes and proteins that are indispensable for the virus to complete its replication cycle, but none alone is able to achieve an undetectable viral load for sustained periods of time. Experience has shown that combinations of three or more active drugs, including at least two classes of antiretroviral, may achieve maximal suppression of plasma viral load and delay the selection of drug resistance mutations.[7]

I.1.3 Metabolism of Nucleoside reverse transcriptase inhibitors (NRTIs):

The absolute dependence on host cell enzymatic process for activation is a unique element in the pharmacology of NRTIs. A general scheme for the reversible activation of NRTIs is presented in figure **4**. General concepts of metabolism will be illustrated as follow:

- Cellular permeation:

Activation of NRTIs is first dependent on cellular entry by passive diffusion or carrier-mediated transport (Figure **4**). Transport plays an important role in the disposition of NRTIs due to their hydrophilicity and somewhat limited membrane permeability. Multiple members of the solute carrier superfamily containing over 350 transporters can transport NRTIs. Of particular interest for nucleoside analogues are the equilibrative and concentrative families of nucleoside transporters. Conversely, members of the ATP binding cassette family have been shown to efflux nucleoside and nucleotide analogues out of the cells. A surprising result was obtained when it was found that the multi-drug resistance related protein 4 (MRP4) could be a resistance factor for the acyclic dAMP analogue adefovir, currently used as an anti-HBV agent. Since this initial discovery, monophosphate forms of several NRTIs have been shown to be transported by MRP4 and the related transporters MRP5 and MRP8. Unphosphorylated nucleoside analogues have also been found to be transported by the ATP binding cassette family transporters breast cancer resistant protein and P-glycoprotein. In addition to affecting the distribution to sites of viral replication, the combined action of solute carrier-mediated influx and ATP binding cassette-mediated efflux in kidney proximal tubules can result in the elimination of NRTIs from the body.[8]

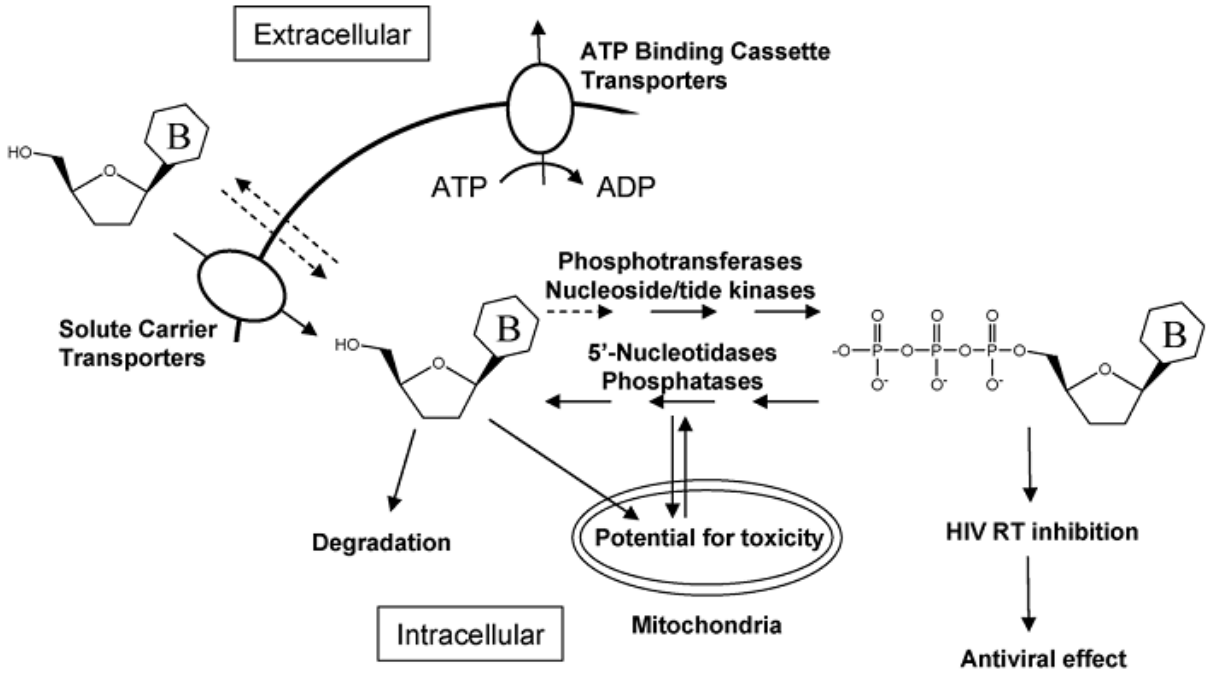


Figure (**4**): General scheme for the reversible activation of NRTIs

- Phosphorylation:

Once inside the cells (Figure **4**), the distribution of phosphorylated metabolites of NRTIs is dictated by the balance between phosphorylating and dephosphorylating enzymes. The first, and often rate limiting on phosphorylation step for nucleoside analogues are most commonly catalyzed by the four deoxynucleoside kinases involved in salvage of endogenous nucleosides: cytosolic deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1), and mitochondrial deoxyguanosine kinase (dGK) and thymidine kinase 2 (TK2). Likely reflecting the requirement of a constant supply of dNTPs to support DNA repair and mitochondrial DNA replication, many of the enzymes involved in deoxynucleoside salvage pathways are active throughout the cell cycle. One notable exception is the S-phase specific expression of TK1. The dependence of d4T and AZT on TK1 for activation results in markedly reduced anti-HIV activity in resting cells. The activity of nucleoside kinases are balanced by 5′-nucleotidases, a diverse set of enzymes that dephosphorylate NMPs. Interestingly, 5′-nucleotidases have been found to display phosphotransferase activity and can catalyze the monophosphorylation of ddI and ABC with IMP and AMP, respectively, serving as phosphate donors. Addition of the second phosphate group to nucleoside monophosphate analogues is completed by the NMP kinases thymidylate kinase, uridylate-cytidylate kinase, adenylate kinases and the guanylate kinase. A variety of enzymes are able to catalyze the final

phosphorylation step for NRTIs, including NDP kinase, phosphoglycerate kinase, pyruvate kinase and creatine kinase, resulting in formation of respective antivirally active triphosphate analogues. The requirement for multi-step intracellular activation processes and the generation of highly polar and impermeable triphosphate analogue species, results in a large difference between the readily measured plasma concentrations of NRTIs and the concentrations of the active metabolites in lymphoid cells and tissues.[9] Although correlations between the in vitro and in vivo intracellular levels of active NRTI metabolites have yet to be established, accurate determination of the intracellular pharmacokinetic profile of the active triphosphate is critical for understanding optimal dosing frequency, drug interactions, and toxicity potential of each individual NRTI. In recent years, this type of analysis became a standard part of preclinical and clinical development of new NRTIs.[10]

- Catabolism:

In addition to dephosphorylation, some NRTIs are subject to catabolism. While ddC, 3TC, FTC and TFV are not markedly metabolized and a majority of the oral dose is excreted unchanged in the urine, AZT, ddI, d4T and ABC are primarily excreted as metabolites (Table **1**). Unlike most drugs, NRTIs do not significantly interact with cytochrome P450 enzymes. AZT is a slight exception due to the minor formation of 3′-aminothymidine containing metabolite through the non-specific action of cytochrome P450 and their reductases.[11] In addition to interactions with cellular nucleoside and nucleotide metabolizing enzymes that result in formation of their active triphosphates, ddI and d4T are also catabolized by cellular nucleoside degradation pathways. Both ddI and d4T have their heterocyclic bases rapidly removed by depurination and depyrimidation, respectively. While the identity of the enzyme catalyzing a thymidine phosphorylase-like degradation of d4T has not been unambiguously determined, ddI has been shown to be degraded by purine nucleoside phosphorylase. NRTIs can also interact with general catabolic pathways. A majority of the administered AZT dose (74%) is excreted in the urine as its 5′-O-glucuronide due to metabolism by UDP-glucuronosyltransferase (UGT). ABC is also metabolized by UGT forming a 5′-*O*-glucuronide. In addition, the 5′-OH attached to the carbocyclic ring of ABC is also recognized by alcohol dehydrogenase (ADH). Combined, the Phase I (oxidation by ADH) and Phase II (conjugation by UGT) metabolism of ABC result in only 1.2% of the intact ABC dose being recovered in urine (Table **1**).[12, 13]

Table (**1**): Select metabolic and pharmacokinetic information for approved NRTIs.

NRTIs	Nucleoside kinase or phosphotransferase	Terminal half life (h)		Urinary recovery [a] (%)
		Plasma(NTRI)[a]	PBMC[b] (dNTP analogue)	
AZT	Thymidine kinase 1	0.5-3	7	14
ddI	IMP phosphotransferase	1.5	24	18
ddC	Deoxycytidine kinase	1-3		80
d4T	Thymidine kinase 1	1.2	7	39
3TC	Deoxycytidine kinase	5-7	22	71
ABC	AMP phosphotransferase	1.45	12-19; 20.6	1.2
TFV	Not applicable	~17	>60	70-80
FTC	Deoxycytidine kinase	~10	39	86

[a] Plasma terminal half life and urinary recovery (%) taken from manufactures prescribing information.[5]
[b] Peripheral blood mononuclear cells.

I.1.4 Inhibition of reverse transcriptase:

Following phosphorylation to their respective nucleoside triphosphate forms, NRTIs compete with endogenous dNTPs for incorporation by HIV RT. Active RT enzyme is a heterodimer with p51 and p66 subunits resulting from differential protease cleavage of the gag-pol polyprotein. The DNA- and RNA-dependent DNA polymerase activity is catalyzed by the p66 subunit and, coupled with RNAse H activity also residing in the p66 subunit, is responsible for the critical step of conversion of the minus stranded RNA viral genome into double stranded DNA for integration into the host cell genome. Like other polynucleotide polymerases, the overall structure of RT can best be described as a right hand with palm, fingers, and thumb subdomains. The incoming dNTP binds between the palm and the finger subdomains and the ribose and base make important contacts with residues including L74, Y115, M184 and Q151 (Figure **5**). A two-metal model has been proposed for the phosphoryl transfer reaction of polymerases including HIV RT. In the RT active site, two Mg^{2+} ions (Purple color) are coordinated by the catalytic triad of D110, D185 and D186 that, along with residues including K65, interact with triphosphate and 3′-terminus of the primer. Structural studies support an induced fit model where proper base pairing by the incoming dNTP results in formation of a closed polymerase, primer/template, and dNTP complex with the incoming dNTP appropriately aligned to be attacked by the 3′-OH at the terminus of the elongating primer strand. Kinetic studies showed a corresponding rate-limiting step that might reflect this conformation change. The dependence of the rate-limiting step on correct dNTP binding and base-pairing forms the basis for the fidelity of polymerization. Despite the structural diversity

present in NRTIs used clinically, their active triphosphates are able to effectively mimic the structural contacts of natural dNTPs in the HIV RT active site, allowing efficient incorporation. d4T-TP has been found to be incorporated as efficiently as endogenous TTP in certain sequence contexts. Once incorporated, the lack of a 3′-hydroxyl in all approved NRTIs results in obligate chain-termination of viral reverse transcripts.[14]

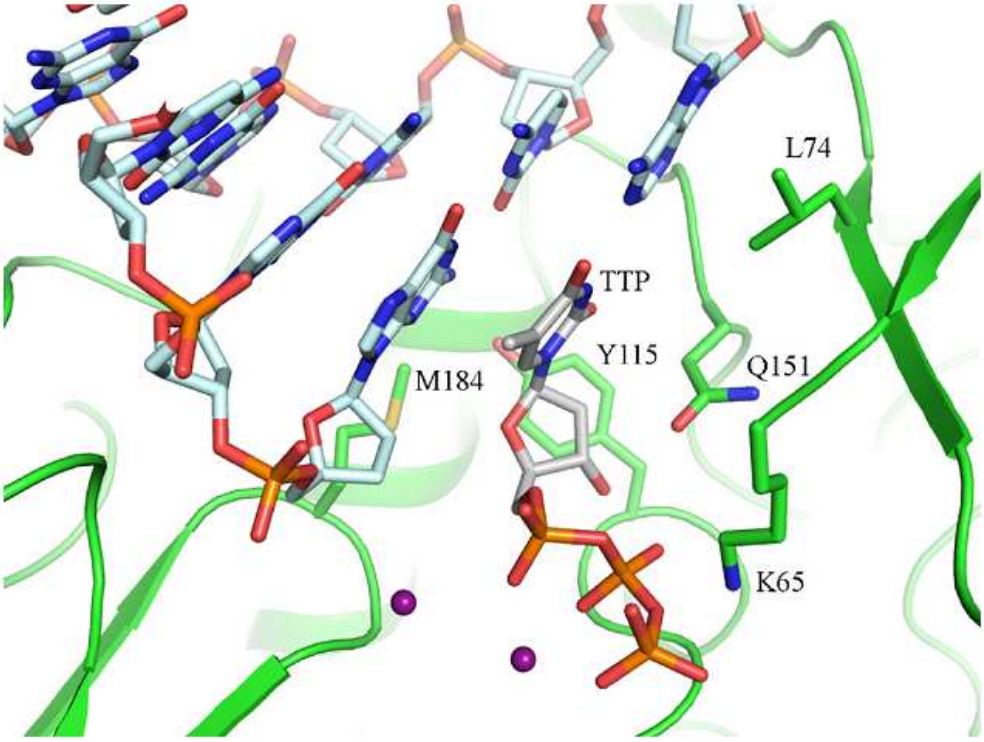


Figure (**5**): Nucleotide binding in the active site of HIV RT

1.1.5 Structure of the approved NRTIs:

There is structural diversity in the eight NRTIs that have been approved by regulatory agencies for HIV treatment (Figure **6**) and they are metabolized to analogues of all four natural dNTPs used during DNA synthesis. All currently approved NRTIs lack a 3′-hydroxyl and are obligate chain-terminators of DNA elongation. Besides simply removing the 3′-hydroxyl, as is the case for the 2′,3′-dideoxy nucleosides ddI and ddC, AZT contains a replacement of the 3′-hydroxyl with a 3′-azido functionality. Both d4T and ABC have unsaturation introduced into their ribose moieties resulting in 2′,3′-dideoxy-2′,3′-didehydro ribose ring analogues. Abacavir also replaces O4′ with a carbon resulting in a carbocyclic ring and 3TC and FTC replace C3′ with sulfur. The most marked ribose modifications are present in 3TC, FTC, and TFV. In addition to the oxathiolane ring, 3TC and FTC have the unnatural

L-enantiomeric configuration form. TFV, the only nucleotide analogue among approved NRTIs, has an acyclic linker attached to a modified phosphate moiety where a C-P phosphonate linkage replaces the normal O5′-P phosphate linkage. In contrast to the diverse chemical modifications of the ribose ring, very few base modifications are present in currently approved NRTIs. FTC contains a fluorine at the 5-position of its cytosine ring and ABC has a 6-modified diaminopurine ring that serves as a prodrug to guanine base; all the other approved NRTIs contain unmodified purine or pyrimidine bases.[5]

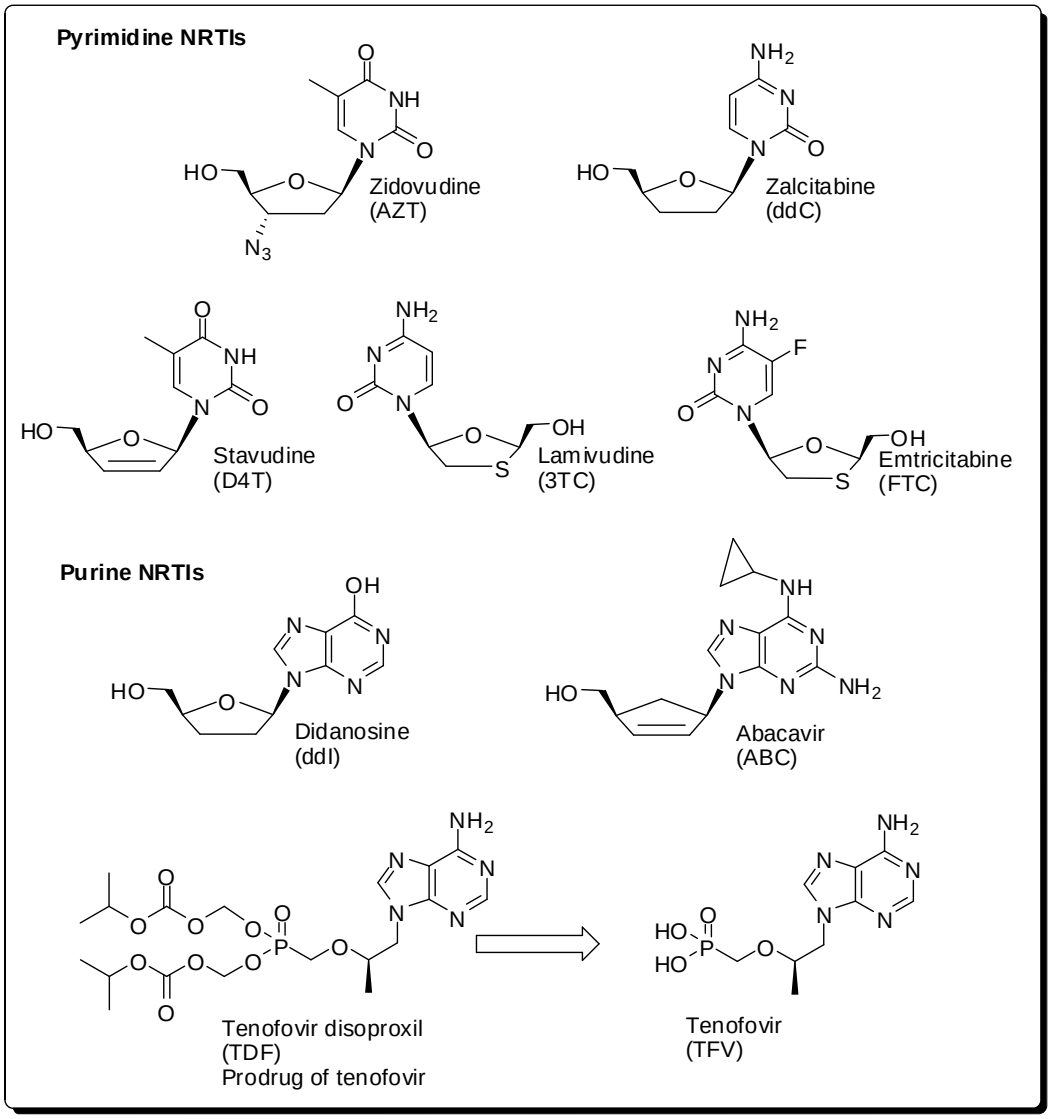


Figure (**6**): Structures of approved NRTIs

I.1.6 NRTIs under development:

Most of the currently used NRTIs have some safety and/or pharmacological limitations affecting their successful long-term use for the treatment of HIV-infected patients, either in general or in certain specific populations such as individuals genetically or medically predisposed to NRTI-related adverse effects, or those with NRTI resistance. Currently, there are multiple NRTIs in various stages of clinical development (Figure **7**). Some of them exhibit various attractive pharmacological properties such as favorable resistance profile or a novel mechanism of action against RT that could make them suitable for the treatment of patient populations in need of new agents.[5]

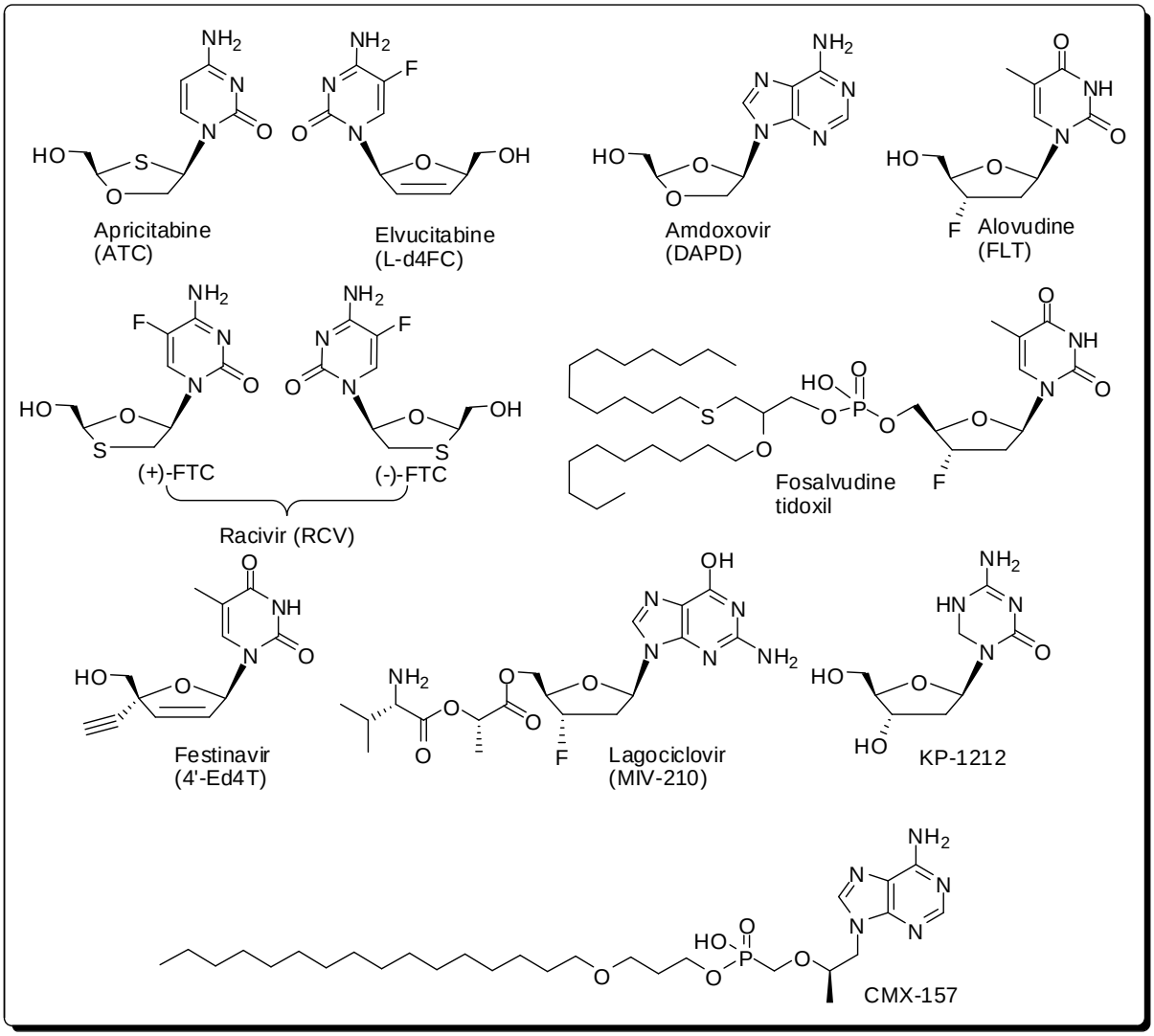


Figure (**7**): Structures of NRTIs currently in clinical development

I.2 Hepatitis B Virus (HBV):

It is estimated that about one-third of the global population – around 2 billion people have been infected with the hepatitis B virus at some stage in their lifetime. Of these, about 360 million people remain chronically infected carriers of the disease – most of them unaware they are infected but capable of spreading the disease to others. Hepatitis B virus is highly contagious. In highly endemic areas, most cases involve either mother-to-child (perinatal) or child-to-child transmission (mainly through cuts, bites, scrapes and scratches). The disease is also spread by unsafe injection practices, unsafe blood transfusions, and sexual contact. Hepatitis B infection can be detected through a blood test, even in people not showing any symptoms of the disease. The incubation period is 75 days on average, but may vary from about 30 to 180 days. HBV may be detected in serum 30–60 days following infection and persist for widely variable periods of time. The outcome of hepatitis B infection differ according to the age at which the person was infected. Acute hepatitis B (i.e. clinically apparent) occurs in 1% of perinatal cases, 10% of cases involving children under five and 30% of cases involving older age groups. A small portion of those with acute hepatitis B infection (0.1%-0.6%) develop fulminant hepatitis B which kills 70% of those affected. The development of chronic (i.e. Long-term) hepatitis B infection occurs mainly in children infected below the age of five years. As many as 90% of children infected perinatally and 30% of children infected before their fifth birthday go on to develop chronic hepatitis B infection – often showing no signs of infection until much later in life. Of the people infected at a later age, only 6% are likely to develop the chronic form of the disease. Most adults who get hepatitis B recover and are then protected for life against the disease. People with chronic hepatitis B infection have a 15%-25% risk of dying prematurely from hepatitis B-related cirrhosis or liver cancer – often during the most productive adult years. In 2003, there were an estimated 600 000 deaths from chronic hepatitis B-related diseases. Although a long-term treatment exists for chronic hepatitis B infection, it is very expensive and has only a 40%-50% success rate. It is also associated with severe side-effects and frequent relapses. Hepatitis B occurs in countries worldwide. In areas with a high prevalence of the disease – i.e., areas in sub-Saharan Africa, South-East Asia, the Eastern Mediterranean region, south and western Pacific islands, the Amazon basin and the Caribbean – as many as one in five of the population may be infected.[15]

I.2.1 The HBV Genome:

HBV is the prototype member of a family of small, enveloped DNA virus called hepadnaviruses, which infect a restricted number of mammals and birds. These viruses share a narrow host range and preferential tropism for hepatocytes. The genome of hepadnaviruses is a relaxed circular, partially double-stranded DNA genome that replicates via an RNA intermediate.[16] In figure (**8**) represented the circular, partially double-stranded HBV DNA genome, Both DNA strands of the duplex are held together by base pairing over 250–300 nucleotides at their 5′ extremities. The unit length-strand (minus strand) is covalently linked to the viral polymerase and bears a 9- nucleotide redundant sequence at its extremities. The complementary strand (plus strand) is linked to a capped oligoribonucleotide at its 5′ end. The plus strand is less than unit length and terminates at different positions resulting in the presence of a single-stranded region of variable length. Two 11-bp repeats, DR1 and DR2, located at the 5′ ends of the minus and plus strands, play a critical role in viral DNA replication. Four open reading frame carried by the minus strand are represented by large arrows.

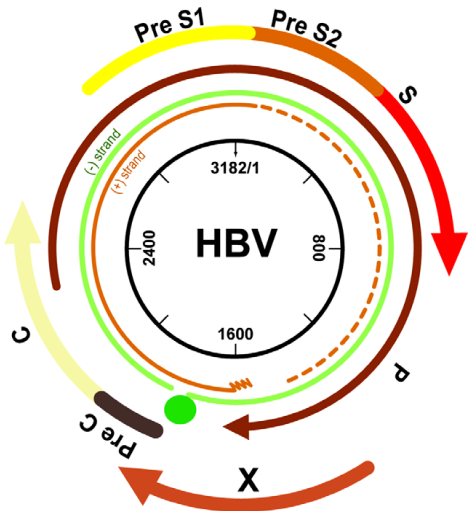


Figure (**8**): Genome of Hepatitis B Virus

The HBV genome is around 3.2 kb in length and presents a highly compact genetic organization with 4 overlapping open reading frames (ORFs) that cover the entire genome. The pre-S/S ORF encodes the three viral surface proteins, the pre-C/C ORF encodes the e antigen (HBeAg) and the core antigen (HBcAg), the P ORF encodes the terminal protein (TP) and the viral polymerase that possesses DNA polymerase, reverse transcriptase and RNaseH

activities. The X gene encodes a small protein that is essential for virus replication but whose function remains partially understood. Finally, a viral protein termed HBSP has been shown to be encoded by a spliced viral transcript.[17]

I.2.2 The HBV life cycle:

Although many steps of hepadnavirus replication have been elucidated (Figure **9**), the initial phases of hepatocyte infection, namely virion attachment, uncoating and entry remain poorly defined, and a cell-surface receptor has not been identified so far. These limitations may be explained by the low efficiency of HBV infection in few available in vitro systems. In the absence of candidate receptor, a less conventional hypothesis has been proposed, in which scavenging liver sinusoidal endothelial cells (LSEC), rather than hepatocytes themselves, mediate the initial uptake of the viral pathogen into the liver.[18]

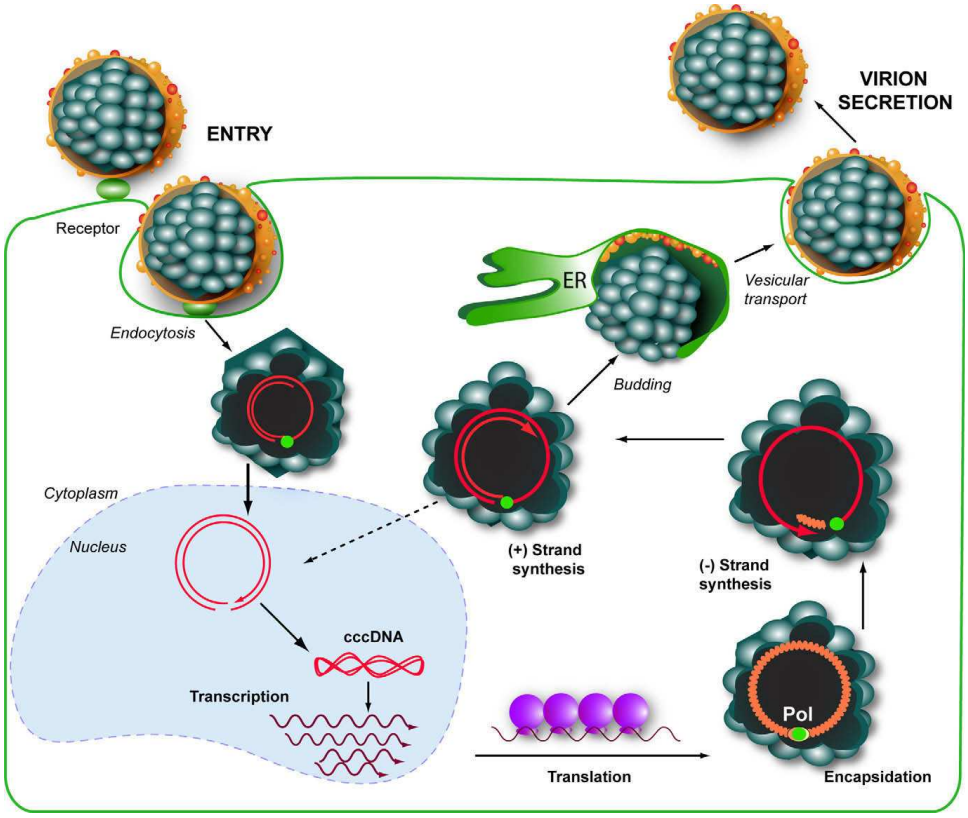


Figure (**9**): HBV life cycle

After viral entry, the nucleocapsid is released into the cytoplasm and transported along the microtubules to the nuclear membrane. Nuclear import of the nucleocapsid through the nuclear pore complex appears to be mediated by importins α and β and it is followed by

disintegration of the nucleocapsids and liberation of the HBV genome in the nuclear pore basket. In the nucleus, the relaxed circular, partially duplexed DNA genome is converted into a covalently closed circular molecule: the cccDNA. The cccDNA serves as template for transcription of all viral RNAs, including the pregenomic RNA (pgRNA) as well as subgenomic RNAs. This step is critical for amplification of the viral progeny and for maintaining productive infection in the host. Four promoters and two enhancers that are preferentially active in hepatocytes regulate the transcription of viral RNAs, and the HBx protein has been reported to play an essential role in activating HBV transcription. It has been shown that the cccDNA is organized into a minichromosome harboring a chromatin-like structure in the nuclei of infected cells. This structure is known to be resistant to antiviral agents and might be responsible for viral rebound upon withdrawal of antiviral therapy. Recently, a chromatin immuno precipitation-based methodology (ChIP/cccDNA assay) has been established to analyze the transcriptional regulation of the cccDNA minichromosome. These studies led to demonstrate that HBV replication is regulated by the acetylation status of the cccDNA bound H3/H4 histones, and that H3/H4 acetylation status is tightly correlated with the level of viremia in the infected patient. The regulatory protein HBx has been found to play a key role in HBV transcription and replication. Early studies have shown that HBx stimulates the activity of viral promoters and enhancers, and that the closely related virus WHV deficient for the expression of WHX cannot replicate in the animal host. More recently, it was found that the replication of X-deficient HBV genomes was strongly compromised in established cell lines and in the mouse liver, and that HBx provided in “trans” was able to restore HBV replication to wild-type levels. In most studies, this effect has been attributed to the trans-activator activity of nuclear HBx protein. It is worth noticing that HBV replication does not require a step of viral DNA integration into host chromosomes. In the cytoplasm, the pgRNA is selectively packaged into progeny capsids and reverse-transcribed by the viral polymerase into minus strand DNA followed by the synthesis of relaxed circular DNA (RC-DNA). Capsids containing RC-DNA can be either recycled for intracellular cccDNA amplification, or assemble with the viral surface proteins in the endoplasmic reticulum to form the viral particles that will be released from the cell. So far, inspection of the viral genome has not led to identify a dominant viral oncogene, and accordingly, introduction of the HBV genome into primary cells in culture or into transgenic murine models has not provided convincing evidence of cell transformation or liver tumourigenesis.[19]

I.2.3 Anti-HBV nucleoside analogues:

At present, several drugs are licensed by the United States Food and Drug Administration (FDA) for the treatment of chronic hepatitis B (CHB): IFN- and pegylated IFN-, three nucleoside analogues (lamivudine, entecavir and, since October 2006, telbivudine) and two nucleotide analogues (adefovir dipivoxil and tenofovir disoproxil) (Figure **10**). Because of high rates of resistance (as in the case of lamivudine), poor tolerability (as in the case of IFN) and possible side effects (i.e. nephrotoxicity with adefovir if used at supra-optimal doses), new therapeutic options are warranted. HBV DNA polymerase is the main target for the nucleoside or nucleotide analogues. Future therapy may lie in (i) drug combinations of existing anti-HBV drugs, (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (iii) small interfering RNAs (siRNAs) (as was shown for many other viruses, siRNAs can be used to inhibit HBV replication in vitro and in vivo), (iv) helioxanthin and related molecules, which inhibit viral nucleic acid and viral protein expression or (v) the prevention of nucleocapsid formation by heteroaryldihydropyrimidines (HAPs).[20]

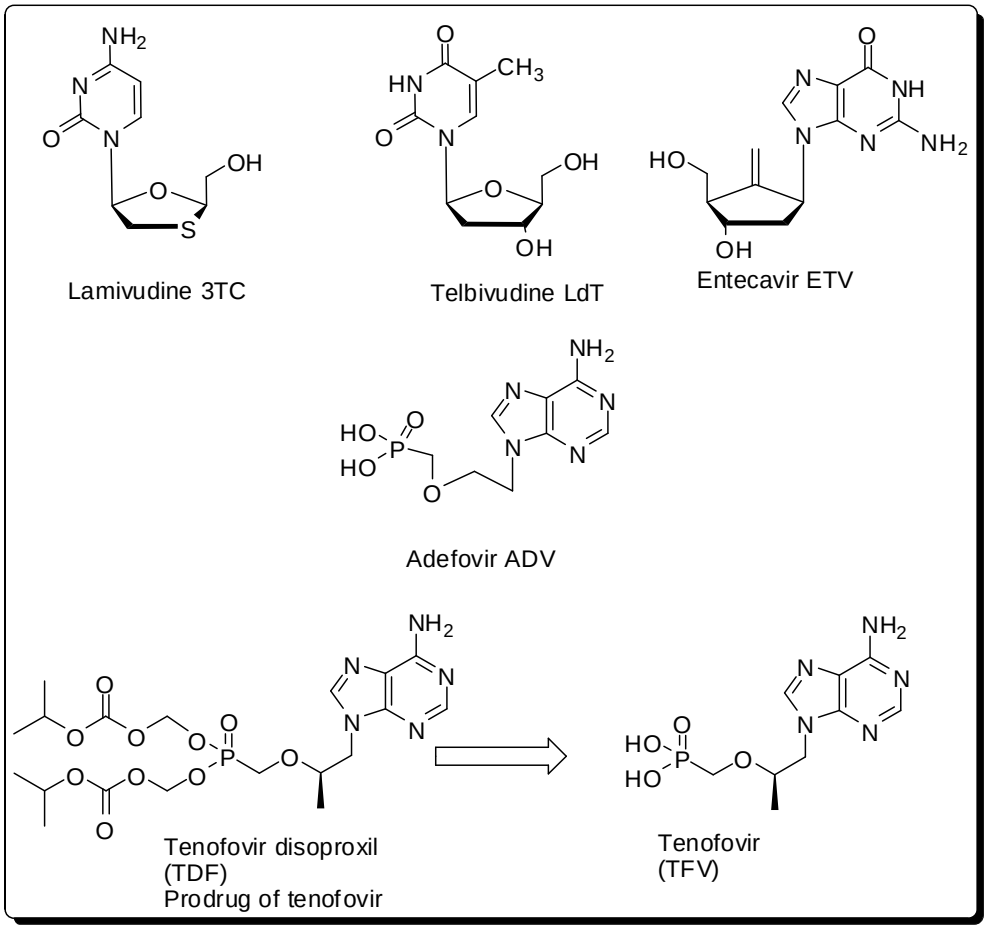


Figure (**10**): The chemical structure of potential HBV-polymerase inhibitors

I.3 Hepatitis C Virus (HCV):

Since its discovery in 1989, representing a turning-point in the search for infectious agents associated with post-transfusion non-A, non-B hepatitis, hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease and affects approximately 300 million people worldwide at the present time. Persistent infection with HCV is associated with the development of chronic hepatitis, hepatic steatosis, cirrhosis, and hepatocellular carcinoma.[21] In general, people with chronic hepatitis C are relatively asymptomatic and have few, if any, clinical manifestations prior to the development of cirrhosis. HCV is a small, enveloped RNA virus belonging to the Hepacivirus genus of the Flaviviridae family, which also includes several classical flaviviruses, including dengue virus and yellow fever virus, as well as pestiviruses, such as bovine viral diarrhea virus and the unassigned GB viruses.[22]

I.3.1 The HCV Genome:

HCV has never been characterized by Electron Microscopy (EM), and little is known about its structure and morphogenesis in host cells, due to the lack of an efficient *in vitro* culture system. In addition, the direct visualization of virus particles from infected serum or liver tissue has proved very difficult. Putative HCV particles were observed that varied in shape and size, between 30 and 60 nm in diameter. Serum HCV particles have also been found to be highly heterogeneous in terms of their buoyant density on sucrose or cesium chloride gradients, possibly due to association with antibodies or lipoproteins. This problem contributes to the tremendous difficulty involved in the direct observation of HCV particles from the serum samples of infected patients. HCV contains a single-stranded positive RNA genome, approximately 9600 nucleotides in length (Figure **11**). The HCV genome contains one long open reading frame encoding a polyprotein precursor of just over 3000 amino acid residues, flanked by structured 5′ and 3′ non-translated regions (NTRs). The 5′NTR contains an internal ribosome entry site (IRES), essential for cap independent translation of the viral RNA. The 3′NTR has a tripartite structure consisting of a short variable region, a poly U tract and a highly conserved 98-nucleotide RNA element essential for viral replication. Translation of the HCV open reading frame yields a polyprotein precursor that is co- and post-translationally processed by cellular and viral proteases to generate structural and non-structural proteins. The 10 HCV proteins making up the polyprotein are organized in the following order: NH2-C-E1-E2-p7-NS2- NS3-NS4a-NS4b-NS5a-NS5b-COOH (Figure **11**).[23]

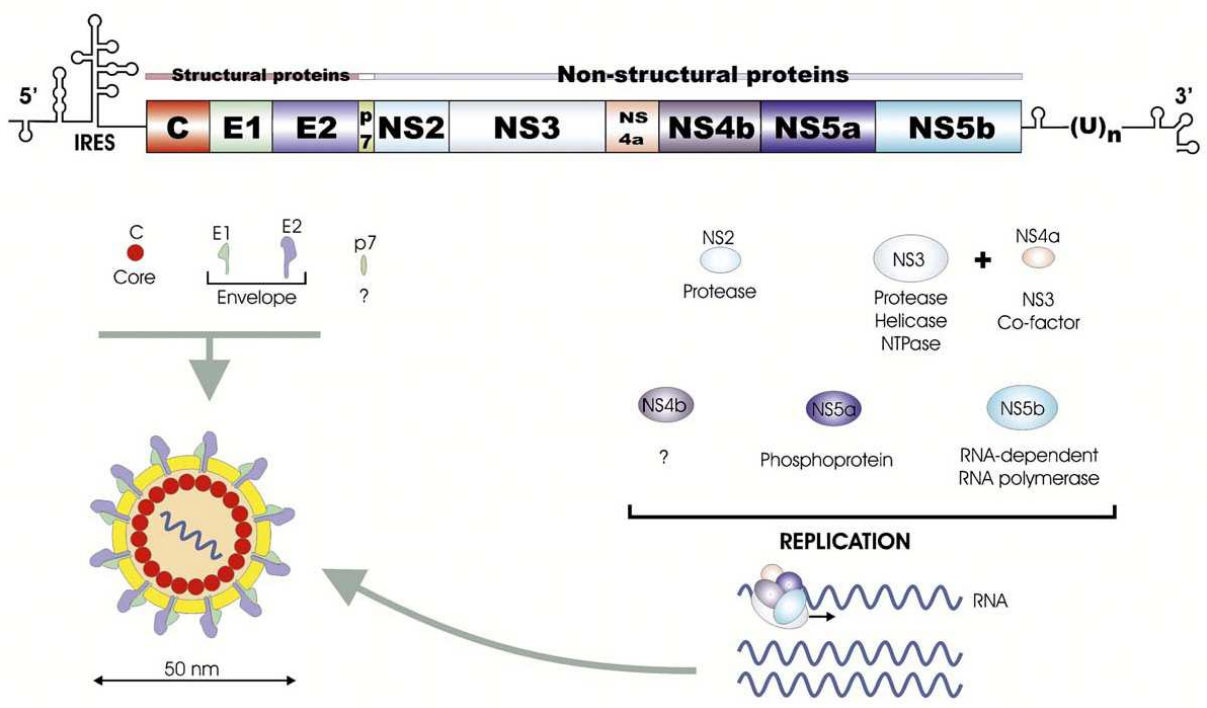


Figure (**11**): HCV genome organisation

I.3.2 The HCV life cycle:

After attachment, the HCV nucleocapsid is released in the cell cytoplasm as a result of a fusion process between viral and cellular membranes (Figure **12**). HCV entry into cells has been shown to be pH dependent and related to clathrin-mediated endocytosis. Entry is followed by a fusion step within an acidic endosomal compartment. HCV envelope glycoproteins are believed to belong to class II fusion proteins. However, in contrast with other class II fusion proteins, they do not appear to require cellular proteinase cleavage during their transport through the secretory pathway. The identity of the HCV fusion peptide remains controversial. E1 appeared as a good candidate because sequence analysis suggested the presence of a fusion peptide in its ectodomain. Nevertheless, E2 was shown to share structural homology with class II fusion proteins. In addition, the transmembrane domains of both proteins have been recently suggested to play an important role in fusion.

The HCV IRES has the capacity to form a stable pre-initiation complex by directly binding the 40S ribosomal subunit without the need of canonical translation initiation factors, an event that constitutes the first step of HCV polyprotein translation. The 40S subunit assembles with eIF3, and this ternary complex joins with eIF2, guanosine triphosphate, and the initiator transfer RNA (tRNA) to form a 48S particle in which the tRNA is positioned in the P site of

the 40S subunit, base paired to the start codon of the mRNA. Upon hydrolysis of guanosine triphosphate, eIF2 releases the initiator tRNA and dissociates from the complex. A second guanosine triphosphate hydrolysis step involving initiation factor eIF5B then enables the 60S ribosomal sub-unit to associate, forming a functional 80S ribosome that initiates viral protein synthesis (Figure **12**). A number of cellular proteins have been reported to interact with the 5′UTR including the polypyrimidine tract-binding protein, heterogeneous nuclear ribonucleoprotein L, La autoantigen (is an RNA-binding protein that is involved in initiation and termination of RNA polymerase III transcription), poly(rC)-binding protein 2, and NS1-associated protein 1. The biologic significance of these protein-RNA interactions remains unknown. In addition, HCV proteins have been reported to affect IRES translational efficiency, including the core protein and the nonstructural proteins NS4A and NS5B. The HCV 3′UTR has also been suggested to modulate IRES-dependent translation.

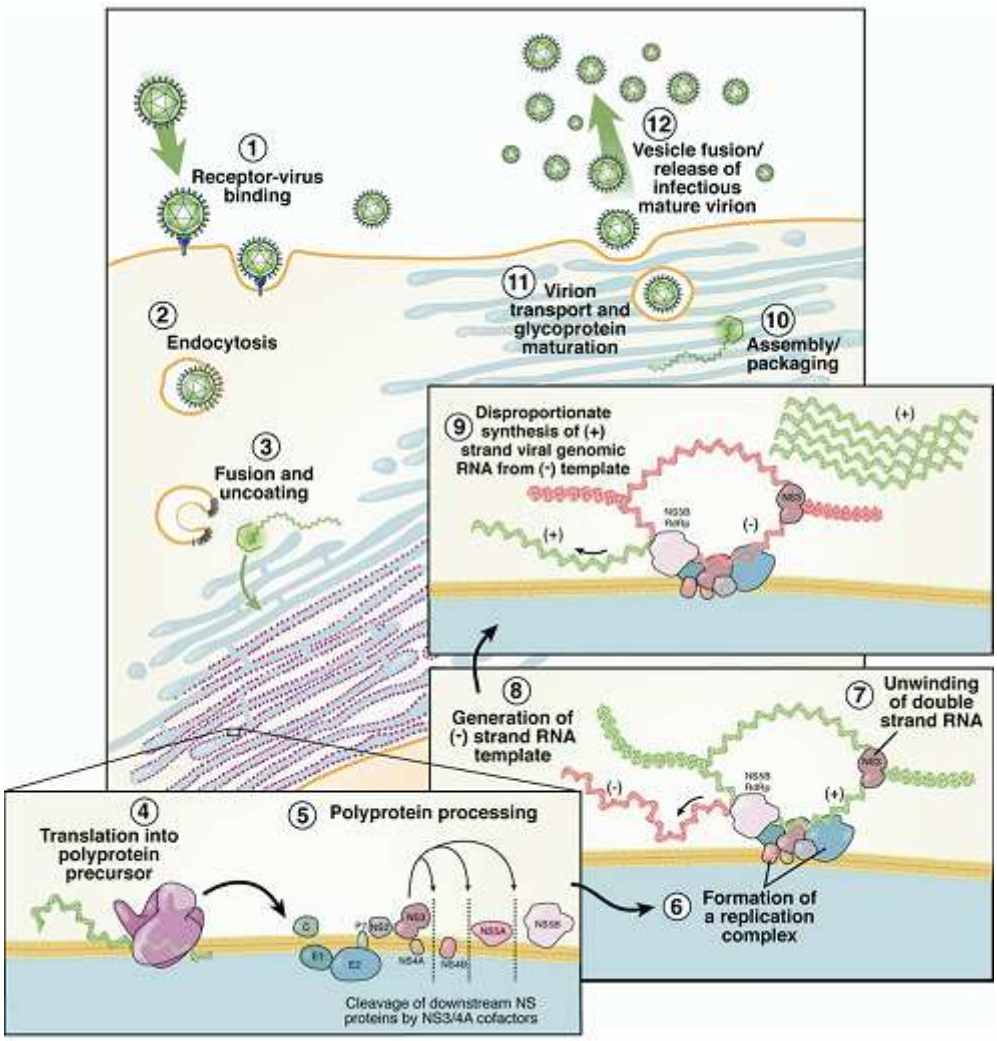


Figure (**12**): HCV life cycle

The large precursor polyprotein generated by HCV genome translation is targeted to the ER membrane for translocation of the E1 ectodomain into the ER lumen, a process mediated by the internal signal sequence located between the core and E1 sequences. Cleavage of the signal sequence by the host signal peptidase yields the immature form of the core protein (P23), which probably plays a role in virus assembly. The signal peptide is further processed by a host signal peptide peptidase to yield the mature form of the core protein (P21). The host signal peptidase also ensures cleavage at the E1-E2 junction in the ER lumen. Additional signal peptidase cleavages at the C-terminal end of E2 and between p7 and NS2 give rise to p7. E1 and E2 subsequently undergo several maturation steps, including N-glycosylation, conformation, and assembly of E1E2 heterodimers. Heterogeneous E1E2 aggregates are also produced, which could modulate viral particle formation. The zinc-dependent NS2-3 autoprotease ensures cis-cleavage of NS3 from NS2. NS3 needs to assemble with its cofactor NS4A to catalyze cis-cleavage at the NS3-NS4A junction and trans-cleavage at all downstream junctions including NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B (Figure **12**). The cleavage sites recognized by the NS3-NS4A proteinase have in common the following sequence: Asp/ GluXXXXCys/Thr-Ser/Ala, with trans-cleavages occurring downstream of a cysteine residue and the cis-cleavage occurring downstream of a threonine residue.

The precise mechanisms of HCV replication are still poorly known. By analoguey with other positive-strand RNA viruses, HCV replication is thought to be semi-conservative and asymmetric with 2 steps, both of which are catalyzed by the NS5B RdRp. The positive-strand genome RNA serves as a template for the synthesis of a negative-strand intermediate of replication during the first step. In this step, negative-strand RNA serves as a template to produce numerous strands of positive polarity that will subsequently be used for polyprotein translation, synthesis of new intermediates of replication, or packaging into new virus particles (Figure **12**). The positive-strand RNA progeny is transcribed in a 5- to 10-fold excess compared with negative-strand RNA. NS5B RdRp was initially thought to catalyze primer-dependent initiation of RNA synthesis, either through elongation of a primer hybridized to the RNA template or through a copy-back mechanism. More recently, the HCV RdRp has been shown to be able to initiate de novo RNA synthesis under certain experimental conditions. Initiation of RNA strand synthesis at the 3′end of the plus and minus strands involves domain I of the 5′UTR that can form a G/C-rich stem loop, the 3′UTR, and a cis-acting replication element (5BSL3.2) consisting of 50 bases located in a large predicted

cruciform structure at the 3′ end of the HCV NS5B-coding region. Initiation of RNA replication is triggered by an interaction among proteins of the replication complex, the 3′X region of the 3′UTR, and 5BSL3.2 that forms a pseudoknot structure with a stem loop in the 3′UTR. A phosphorylated form of polypyrimidine tract-binding protein has been found in the replication complex, and polypyrimidine tract-binding protein has been shown to interact with 2 conserved stem-loop structures of the 3′UTR, an interaction thought to modulate RNA replication.

Little is known about HCV assembly and release because of the lack of appropriate study models. HCV core proteins can efficiently self-assemble to yield nucleocapsid-like particles with a spherical morphology and a diameter of 60 nm, but the presence of a nucleic acid appears to be required. Viral particle formation is probably initiated by the interaction of the core protein with genomic RNA. The core-RNA interaction could play a major role in the switch from replication to packaging. Budding of virus-like particles in the ER lumen has been observed in different models and shown to lead to the formation of coated particles. Indeed, the HCV envelope glycoproteins E1 and E2 are associated with ER membranes through their transmembrane domains, suggesting that virus assembly could occur in the ER. Moreover, the presence of N glycan residues at the surface of HCV particles is also in keeping with a transit via the Golgi apparatus. Newly produced virus particles may leave the host cell by the constitutive secretory pathway.[24]

I.3.3 Treatment of Chronic Hepatitis C

The only therapeutic intervention able to halt this progressive process is eradication of HCV by Interferon (IFN)-based therapies. Since the empirical choice to use IFN in 1986, therapy for chronic hepatitis C has constantly evolved over the past decade, with the attainable sustained virological response (SVR) rates increasing through the years. The addition of the guanosine nucleoside analogue ribavirin (Rbv) to IFN can be considered the major breakthrough in the treatment of chronic hepatitis C (Figure **13**).[25] Through mechanisms of action that still remain largely unknown.[26]

The latest innovation in the treatment of chronic hepatitis C has been the pegylation of the IFN molecule (PegIFN) through the attachment of one or more polyethylene glycols to the IFN, a process that is able to modify the immunological, pharmacokinetic and

pharmacodynamic properties of the drug.[27] PegIFN has become the standard of care for chronic hepatitis C. Currently, two forms of pegylated IFN exist: PegIFNα2a (Pegasys®; Hoffmann-LaRoche, Basel, Switzerland) and PegIFNα2b (PegIntron®; Schering-Plough, Kenilworth, NJ, USA), which show significant differences in terms of pharmacokinetics and dynamics, that ultimately might translate into different efficacy rates.[28]

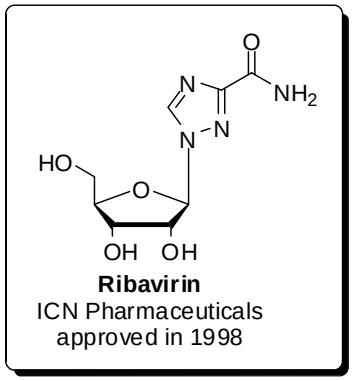


Figure (**13**): Structure of Ribavirin

I.3.4 Anti-HCV Nucleoside analogues under development:

*S*pecifically *T*argeted *A*ntiviral *T*herapy for H*C*V (STAT-C) includes orally available small molecules, such as protease and polymerase inhibitors. Early data have demonstrated the high antiviral activity of several STAT-C agents, although the emergence of resistance to such agents has also been identified. The use of protease or polymerase inhibitors in combination with pegylated interferon (PEG-IFN) and ribavirin increases antiviral activity while also suppressing the selection of resistant variants. Additional approaches for HCV therapy being investigated include compounds targeting intracellular host factors that play a role in viral replication or virion production. The ultimate goal in the long term is to use combinations of new drugs that can completely eradicate HCV with minimal toxicity and possibly avoid the need for interferon. Currently, there are multiple nucleosides analogues in various stages of clinical development acting as polymerase inhibitors (Figure **14**). In April 2009, Roche in collaboration with Pharmasset and interMune introduced RG7128 and RG7227, the first clinical trial of two direct HCV antiviral medications without interferon and ribavirin in the trial phase I.[29]

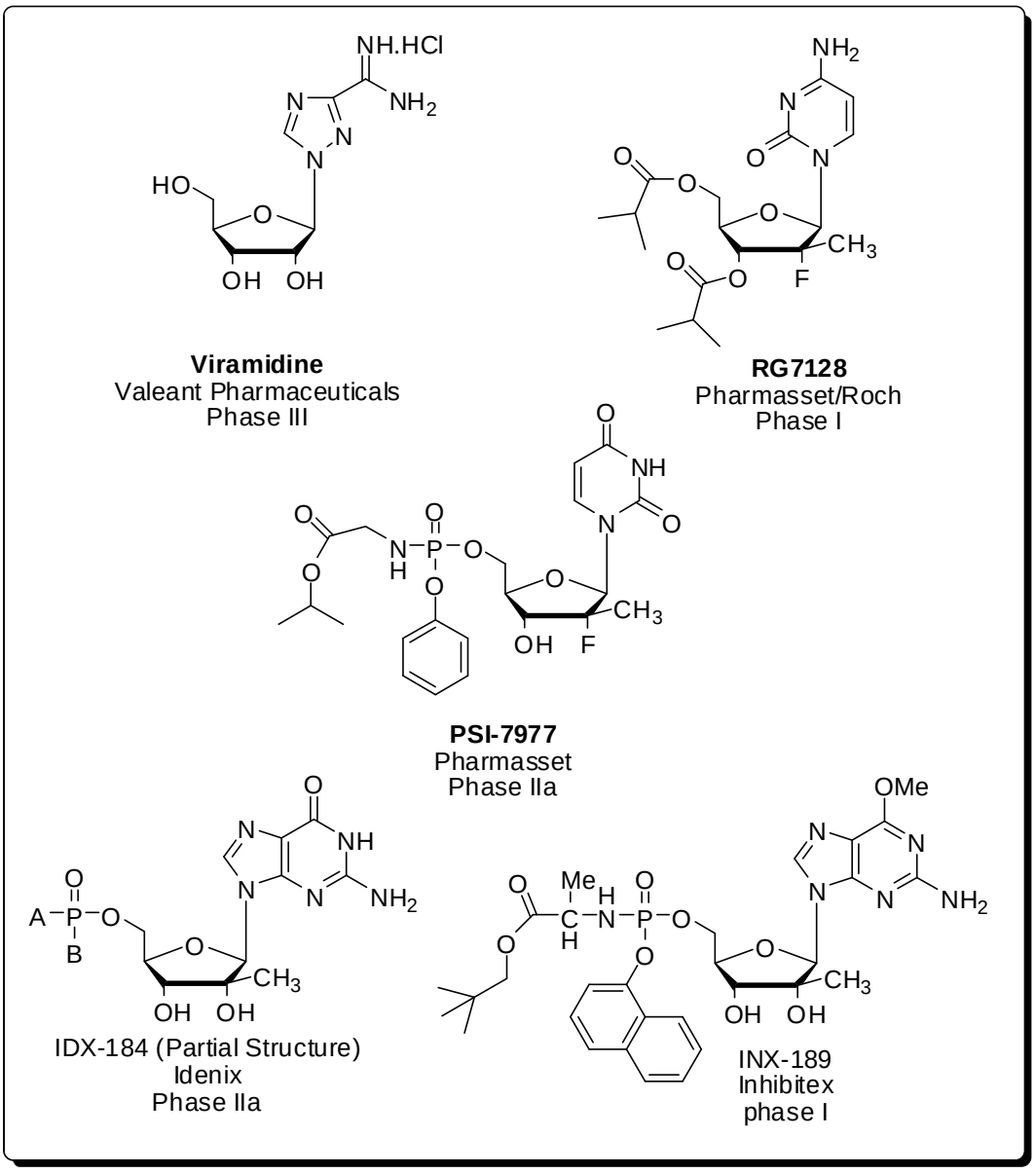


Figure (**14**): Structures of some nucleoside / nucleotide analogues currently in clinical development

I.4 Conclusion:

Pandemics associated with the infection by the HIV, HBV, and HCV illustrate the need to find new agents to increase the therapy efficacy of the existing treatments. The nucleoside analogues in this context occupy an important place as antiviral agents. In this regard, our interest has focused on the synthesis of a new series of nucleoside analogues characterized by the presence of fluorine atom on the sugar moiety. In the second and third parts of this chapter, we discussed the importance of the incorporation of fluorine atom into nucleosides and the recent literature sources of the synthesis and biological activity of the fluorinated nucleosides.

II. The incorporation of fluorine into nucleoside analogues:

Introduction:

In order to synthesize a variety of fluorine-containing biologically active compounds, development of efficient synthetic methods applicable to fluorine containing nucleoside analogues is necessary. There is strong demand for expansion of the availability of versatile fluorine containing synthetic intermediates to promote target-oriented synthesis as well as diversity-oriented synthesis. The successful diversity achieved by fluorinated nucleoside analogues for the treatment of several diseases caused by DNA and RNA viruses, we designed and synthesized new fluorinated nucleoside analogues to evaluate them as antiviral agents against DNA and RNA viruses (Figure **15**).

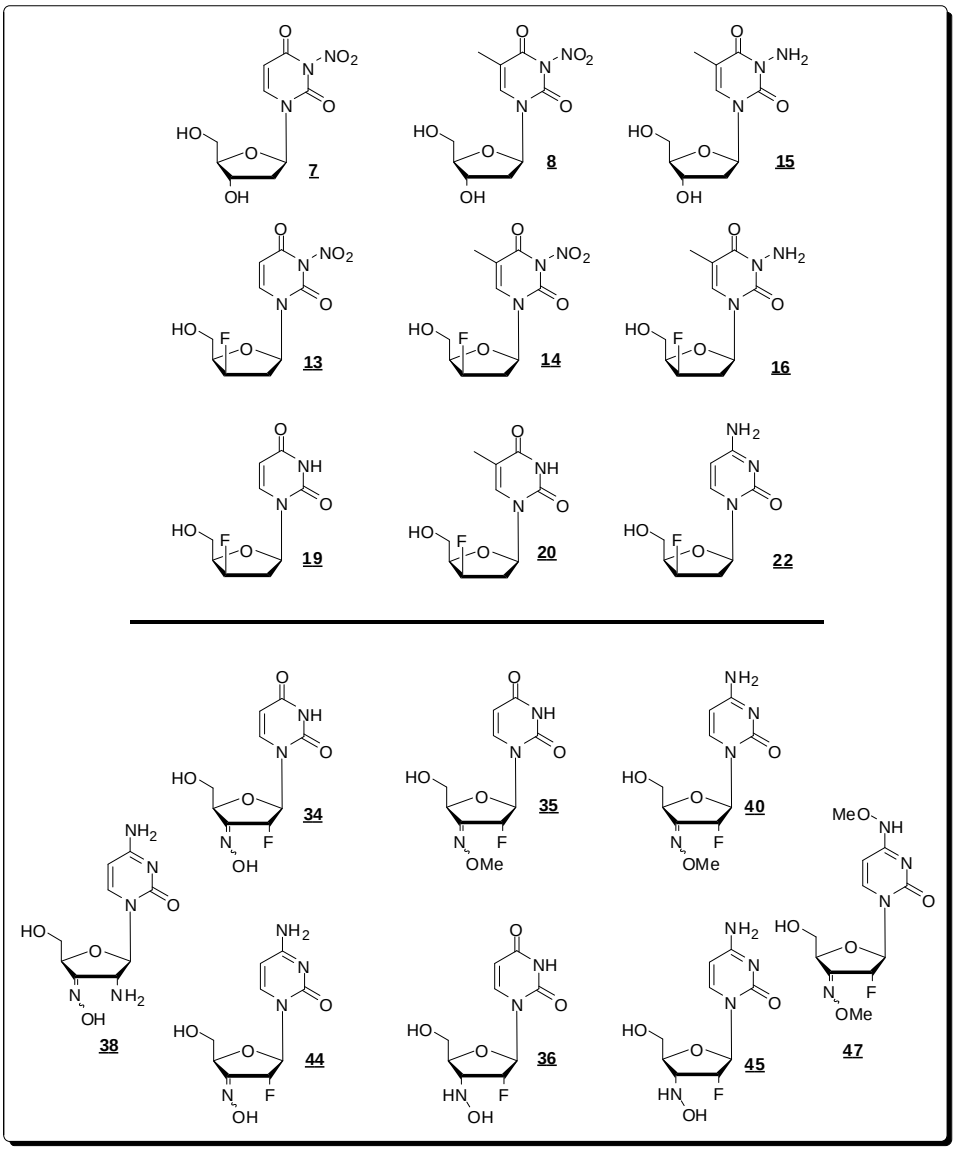


Figure (**15**): the structures of the fluorinated nucleosides synthesized in our work.

As a brief introduction, characteristic properties associated with the incorporation of fluorines to nucleoside analogues are described based on the most updated literature sources:

II.1 Unique properties of fluorine and their relevance in medicinal chemistry:

In spite of such scarcity, enormous numbers of synthetic fluorine containing compounds have been widely used in a variety of fields because the incorporation of fluorine atom(s) or fluorinated group(s) often furnishes molecules with quite unique properties that cannot be attained using any other element. Two of the most notable examples in the field of medicinal chemistry are 9α-fluorohydrocortisone (an anti-inflammatory drug) [30] and 5-fluorouracil (an anticancer drug) [31], discovered and developed in 1950s, in which the introduction of just a single fluorine atom to the corresponding natural products brought about remarkable pharmacological properties. Since then, more than half a century has passed, and the incorporation of fluorine into pharmaceutical and veterinary drugs to enhance their pharmacological properties has become almost standard practice. In 2006, the best- and the second-best-selling drugs in the world were Lipitor® (atorvastatin calcium) by Pfizer/Astellas ($ 14.4 billion/year) and Advair® (U.S.A.)/Seretide® (E.U.) (a mixture of fluticasone propionate and salmeterol; only the former contains fluorine) by GlaxoSmithKline ($6.1 billion/year) which contain one and three fluorine atoms, respectively. These fluorine-containing drugs are followed by risperidone (rank 10th, with one fluorine and $4.2 billion/year) for schizophrenia by Janssen, and lansoprazole (rank 17th, with a CF_3 moiety and $3.4 billion/year), a proton pump inhibitor, by Takeda/Abbott (Figure **14**).[32]

These huge successes of fluorine-containing drugs continue to stimulate research on fluorine in medicinal chemistry for drug discovery. It would not be an exaggeration to say that currently every new drug discovery and development program without exception explores fluorine-containing drug candidates. Accordingly, a wide variety of fluorine-containing compounds based either on known natural products or on new skeletons has been synthesized, or subjected to biological evaluation. In most cases, one to three fluorine atoms are incorporated in place of hydroxyl groups or hydrogen atoms. Representative examples include efavirenz (CF_3) (HIV antiviral) [33], fluorinated shikimic acids (CHF or CF_2) (antibacterial) [34], and epothilone B analogue (CF_3) (anticancer) [35] (Figure **16**).

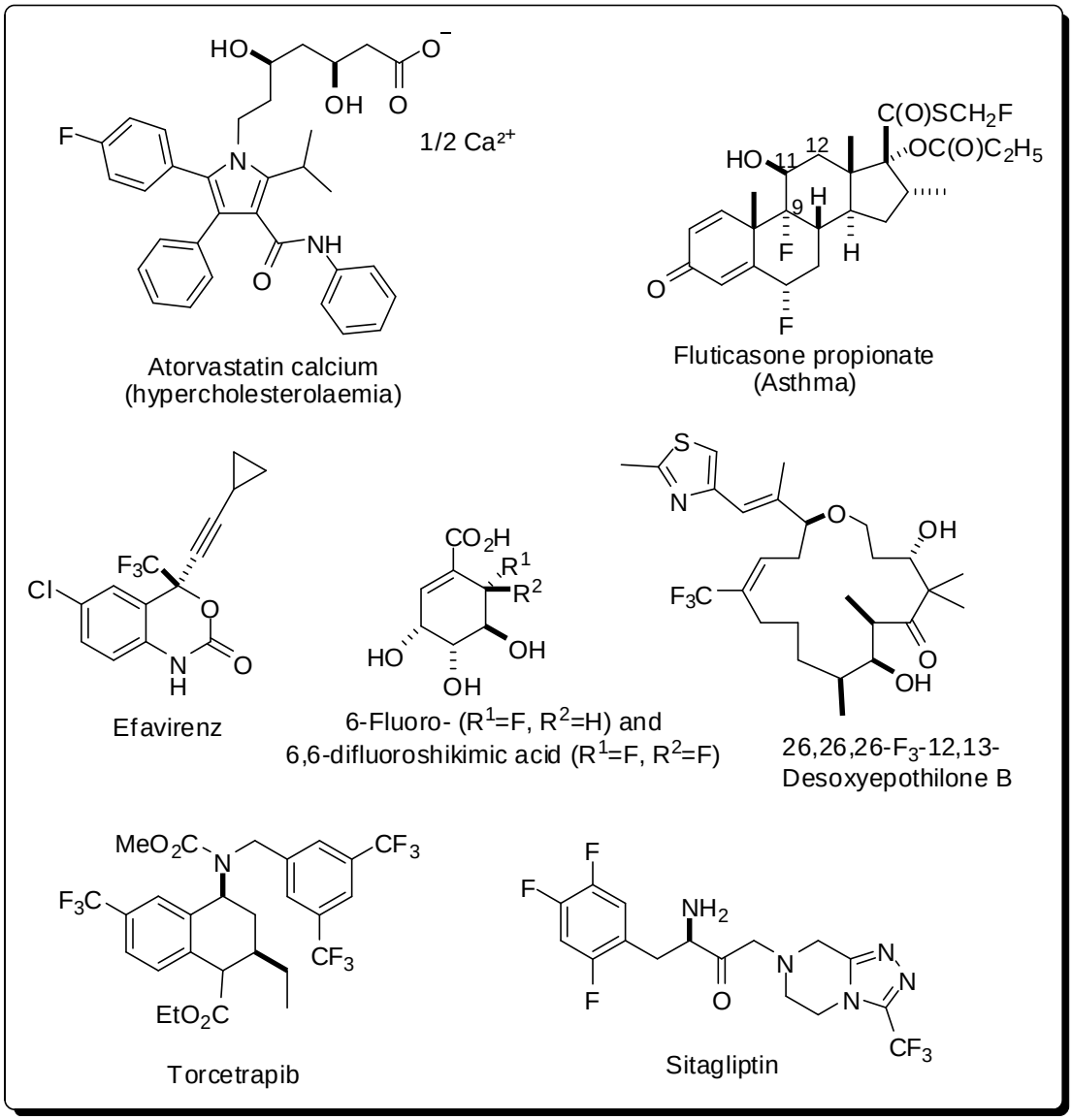


Figure (**16)**: Examples of fluorinated-containing drugs and drug candidates

In addition to these fluorine-containing drugs or drug candidates, a smaller number of fluorine-containing drugs include 6-9 fluorines in a molecule. For example, torcetrapib (a potent inhibitor of cholesterol ester transfer protein) possesses three CF_3 groups (Pfizer) [36], and sitagliptin (an antidiabetic for type 2 diabetes) has three fluorine atoms and one CF_3 group (Merck) [37] (Figure **16**).

II.1.1 Mimic Effect of fluorine:

Table **2** summarizes representative physical properties of fluorine in comparison with other selected elements. As showing table 2, the van der waals (vdW) radius of fluorine is 1.47 Å which is only 20% larger than that of hydrogen and much smaller than those of other halogens (Cl and Br are 46% and 54% larger than hydrogen, respectively). The C-F bond length in CH_3F is 1.382 Å, which is 0.295 Å longer than the methane's C-H bond, but 0.403 and 0.551 Å shorter than the C-Cl and C-Br bonds, respectively. Owing to this similarity in size to hydrogen, it has been shown that microorganisms or enzymes often do not recognize the difference between a natural substrate and its analogue wherein a C-H bond of the substrate is replaced with a C-F bond. This observation is the basis of what is regarded as the "mimic effect" of fluorine for hydrogen.[38]

Table (**2**): Representative physical data of selected elements

	Element (X)					
	H	C	O	**F**	Cl	Br
Electronegativity	2.20	2.55	3.44	**3.98**	3.16	2.96
van der waals radius (Å)	1.20	1.70	1.52	**1.47**	1.75	1.85
H_3C-X bond length (Å)	1.087	1.535	1.425	**1.382**	1.785	1.933
H_3C-X dissociation energy (kcal/mol)	103.1	88.0	90.2	**108.1**	81.1	67.9
Ionization potential (kcal/mol)	313.9	259.9	314.3	**402.2**	299.3	272.7
Electron affinity (kcal/mol)	17.42	29.16	33.73	**78.52**	83.40	77.63

II.1.2 Lipophilicity of fluorine and fluorine-containing groups:

The absorption and distribution of a drug molecule in vivo are controlled by its balance of lipophilicity and hydrophilicity as well as ionization. Enhanced lipophilicity together with change in amine pK_a often leads to increase in blood-brain barrier (BBB) permeability or binding free energy through favorable partition between the polar aqueous solution and the less-polar binding site. It is generally recognized that incorporation of fluorine or fluorinated groups increases the lipophilicity of organic compounds, especially aromatic compounds. Table **3** shows selected Hansch π_x parameters for monosubstituted benzenes. As Table shows, fluorobenzene is slightly more lipophilic than benzene, but chlorobenzene is much more lipophilic. In a similar manner, CF_3- Ph is 57% more lipophilic than CH_3-Ph. The comparison of CF_3-Y-Ph and CH_3-Y-Ph (Y = O, CO, CONH, SO_2) reveals that CF_3-Y- Ph is substantially more lipophilic than CH_3-Y-Ph. In these CF_3-containing compounds, a strongly electron -

withdrawing CF_3 group significantly lowers the electron density of the adjacent polar functional groups Y, which compromises the hydrogen-bonding capability of these functional groups with water molecules, and hence decreases hydrophilicity. Along the same lines, CF_3-benzenes with Lewis basic functionalities such as amine, alcohol, ether, carbonyl, and amide at the ortho or para position, decreases the hydrogen-bond accepting capability of these functionalities in an aqueous phase, which leads to increase in hydrophobicity and thus lipophilicity.[39]

Table (**3**): Hansch hydrophobicity parameters for monosubstituted benzenes

X in C_6H_5-X	π_x[a]	X in C_6H_5-X	π_x	X in C_6H_5-X	π_x
F	0.14	OCH_3	-0.02	$CH_3C(O)NH$-	-1.63
Cl	0.71	OCF_3	1.04	$CF_3C(O)NH$-	0.55
OH	-0.67	CH3C(O)-	-1.27	CH_3SO_2-	-1.63
CH_3	0.56	CF3C(O)-	0.08	CF_3SO_2-	0.55
CF_3	0.88				

[a] $\pi_{x:}$ log P_x- log P_H (Octanol-water); introduced by Crowin Hansch in the early 1960s, Hansch analysis consider both the physicochemical aspects of drug distribution from the point of application to the point of effect and the drug-receptor interaction, more positive π values indicate higher lipophilicity of the substituent.

III Synthesis and biological activity of fluorinated nucleosides:

Introduction:

There are several reasons for the introduction of fluorine atom(s) into drug-candidate compounds. Most of these involve the unique properties of the fluoro group, such as the similarity of its van der waal radius to that of hydrogen, its high electronegativity, and the stability of the C-F bond. The small size of fluoro group makes it possible to replace almost any C-H bond in organic compounds with a corresponding C-F bond. For instance, fluorinated nucleosides bearing C-F bonds in their sugar moiety would not cause significant steric hindrance while penetrating into a pocket of their target enzymes. The fluoro group is the most electronegative of all elements and has one of the highest ionization potentials. Therefore, the fluorine substituent in the sugar moiety brings about strong electron-withdrawing effect on C-F bonds, which induces intramolecular electrostatic interaction that can change the conformation of the molecule.[40] Since the C-F bond is stronger than the corresponding C-H bond, the C-F bond distance becomes short enough to protect its carbon center from various nucleophilic attacks. Moreover, the carbon center of the C-F bond resists oxidation due to the strong electronegative nature of fluorine. Thus, fluorinated compounds often exhibit potent biological activities, and have attracted considerable attention into the pharmaceutical field. In addition, the unique properties of the fluoro group also contribute to improving pharmacokinetics properties, such as ADME (absorption, distribution, metabolism, and excretion), and reducing undesired effects.[38]

Natural products bearing fluorine atoms are so rare that only five compounds have been discovered. Interestingly, a nucleoside analogue, nucleocidin, is one of these fluorinated naturally occurring compounds.[41] Its discovery motivated organic chemists to synthesize fluorinated nucleoside. Additionally, the practical uses of 5-fluorouracil[31], gemcitabine[42], and clofarabine[43] as anticancer drugs have also attracted interest. A fluorinated nucleoside, clevudine (L-FMAU, Levoir) [44], is expected to get FDA approval as an anti-hepatitis B virus agent in the near future. Since, fluoronucleosides have been shown to be useful in clinical studies, novel antiviral fluoronucleosides such as FddA (lodenosine) [45], MIV-210 (a prodrug of FddG) [46], and FLT (alovudine, MIV-310) [47], are currently under development.

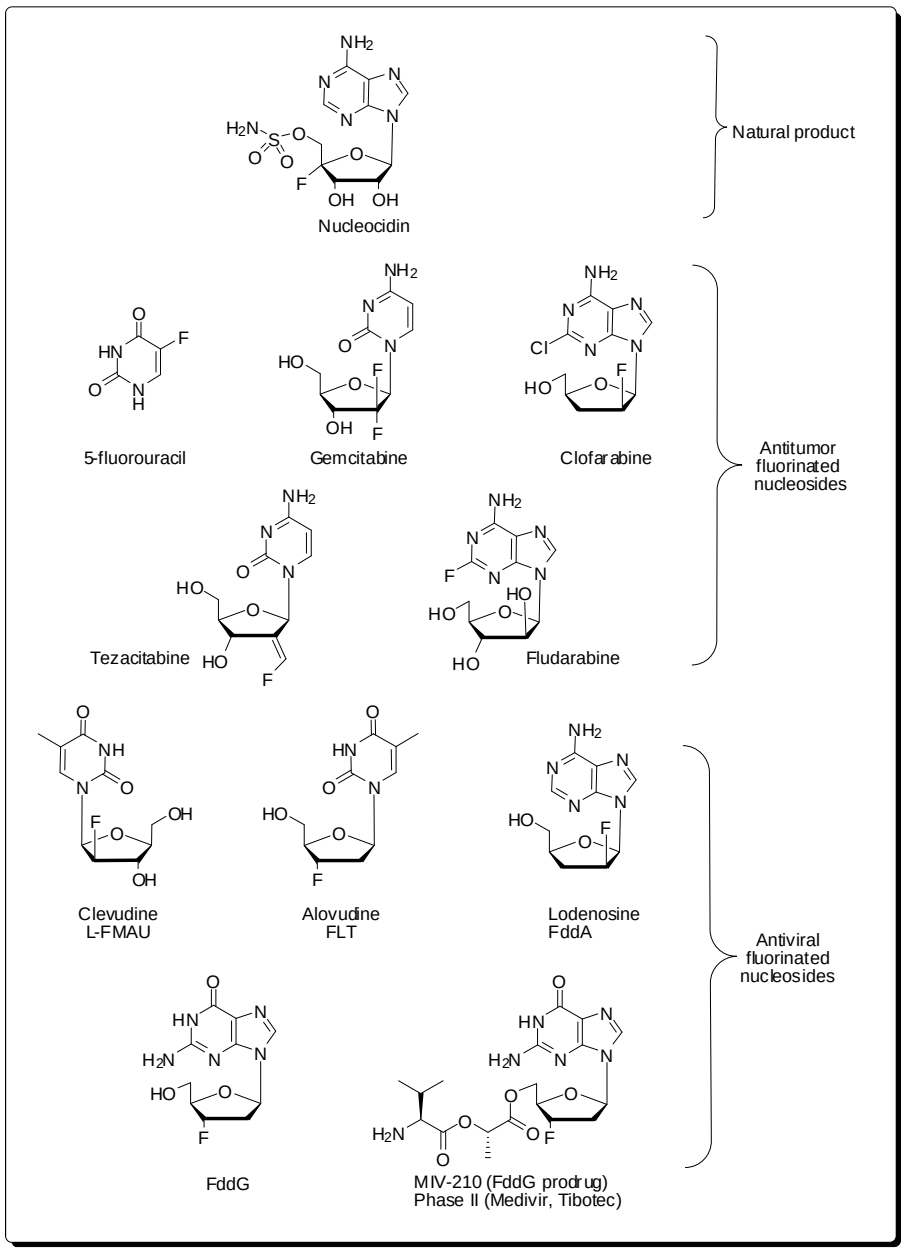


Figure (**15**): Structures of fluorinated nucleoside analogues: natural product, antitumoral and antiviral agents

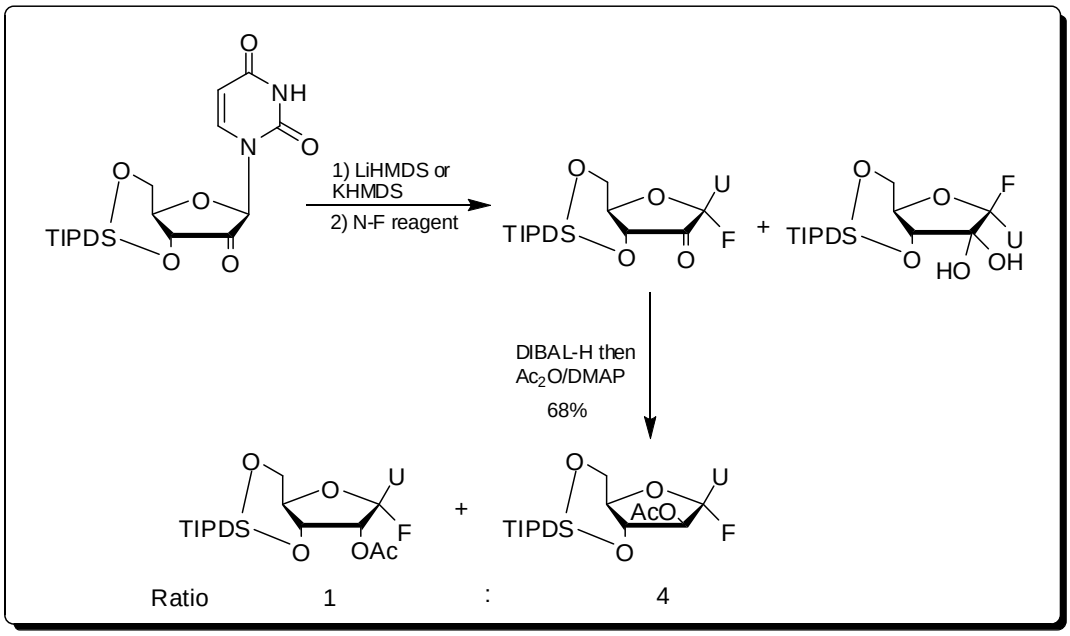
III.1 Synthesis of Fluorinated Nucleosides:

In this part, we describe the main syntheses of several fluorinated nucleoside analogues from common ribonucleosides or from a fluorinated glycone moiety:

III.1.1 Synthesis of 1′-fluoronucleosides:

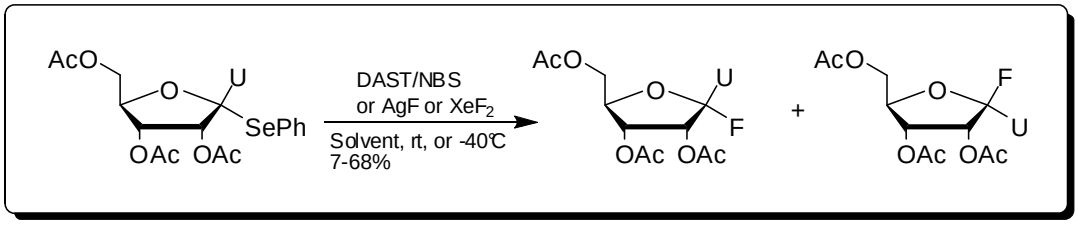
A large number of fluoronucleoside analogues, which having a fluorine atom on the sugar moiety, have been synthesized. However, the replacement of fluorine atoms at the 1′-position was seldom studied, because one might speculate that 1′-fluoronucleosides would be too

unstable to be synthesized. Recently, Shuto and co-workers reported the first synthesis of 1′-fluoronucleosides (Scheme **1**). In their synthesis, electrophilic fluorination of the 1′-lithium enolate prepared *in situ* via a treatment of 2′-ketouridine with LiHMDS afforded an anomeric mixture of the 1′-fluoro-2′-ketouridine derivatives in 57-88% yield. Reduction of the 2′-keto-moiety followed by protection of the resultant hydroxyl group gave the 1′-fluorouridine derivative and its arabino-type congener (5:6=1:4) in 68% yield. [48]



Scheme (**1**): Synthesis of 1′-fluorouridine derivatives according to Kodama *et al*

Shuto *et al.* also developed another route to pyrimidine 1′-fluorouracil nucleosides. This involved treatment of 2′,3′,5′-tri-*O*-acetyl-1′-phenylselenouridine with DAST/NBS, AgF or XeF_2 to provide the 1′-fluorouridine triacetate and its α-anomer in 7-68% yield (Scheme **2**). Deprotection of the synthesized 1′-fluoronucleosides under various conditions was unsuccessful, probably due to the instability of the product.[49]

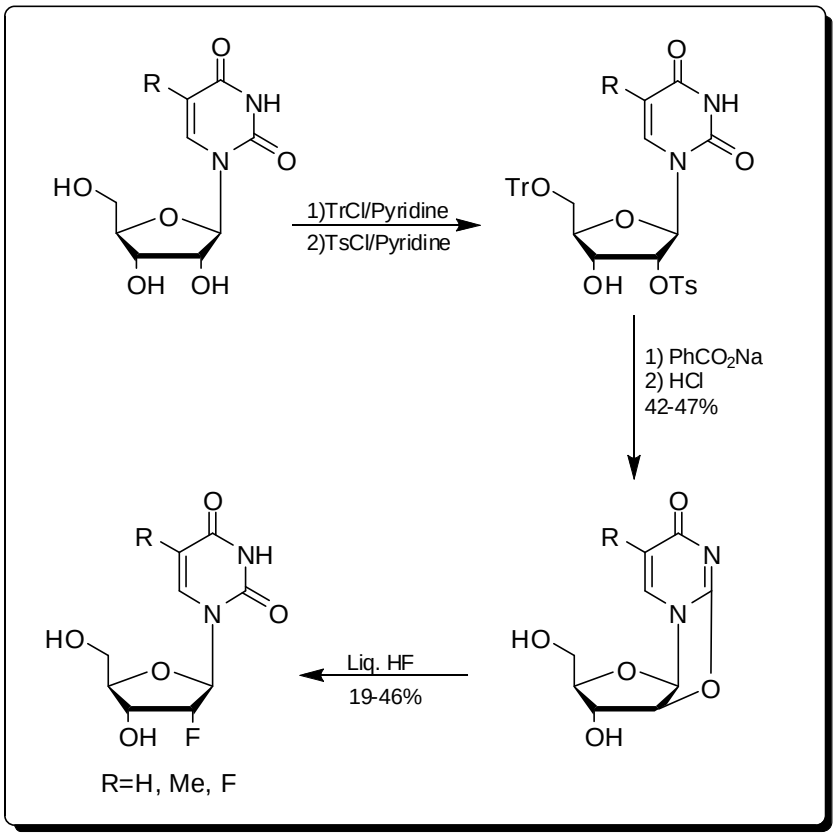


Scheme (**2**): Synthesis of 1′-fluorouridine derivatives according to Kodama *et al*

III.2.1 Synthesis of 2′-fluoronucleosides:

III.2.1.1 Synthesis of 2′α-fluoronucleosides:

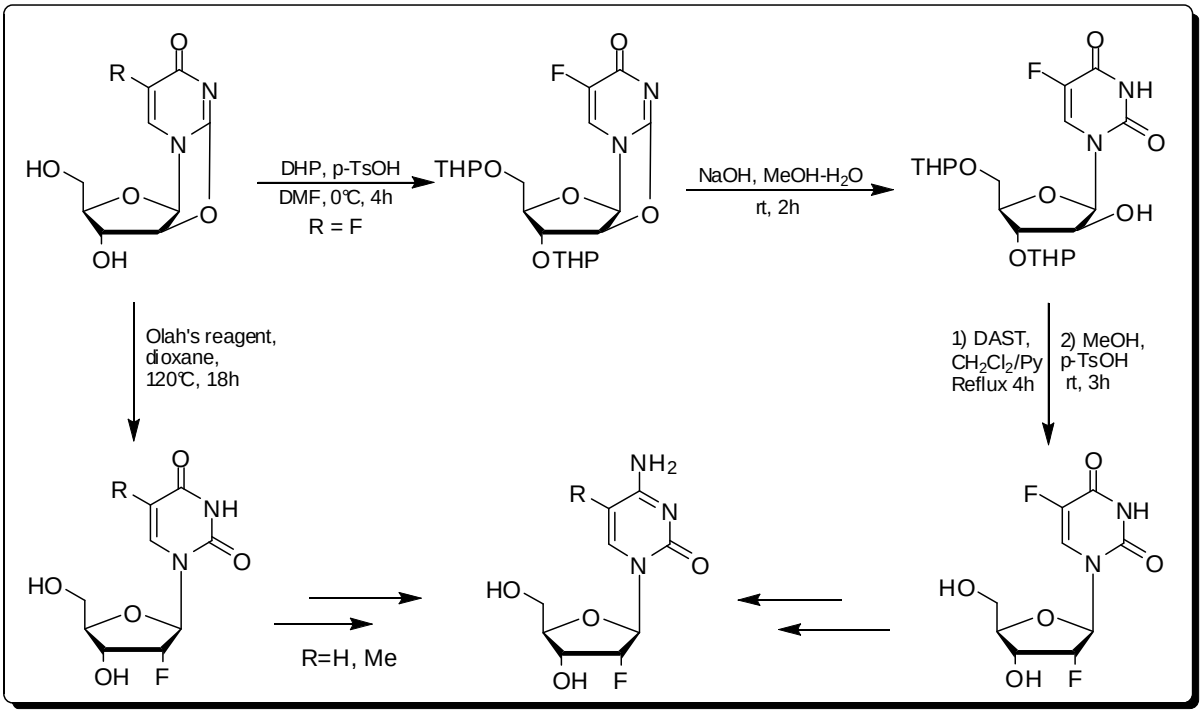
In 1964, Fox and co-workers first synthesized several 2′-deoxy-2′α-fluoronucleosides starting from some commercially available nucleosides (Scheme **3**). Selective protections of the hydroxyl groups in the starting materiel in two steps gave the 2′-tosyloxy derivatives, which were further subjected to treatment with $PhCO_2Na$ and HCl-mediated removal of the trityl protecting group to deliver the 2,2′-anhydro intermediates in 42-47% yield. Nucleophilic fluorination was carried out via treatment with liquid HF to afford the desired 2′α-monofluorinated nucleosides in 19-46% yield.[50]



Scheme (**3**): Synthesis of 2′α-fluorouridine derivatives according to Fox *et al*.

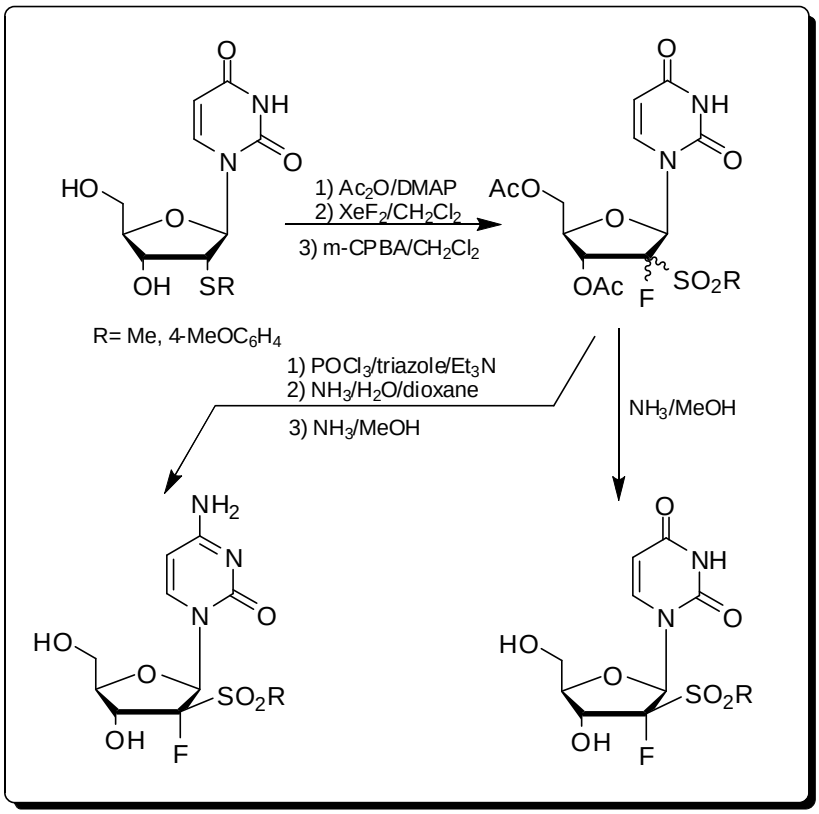
This was the first example of a nucleoside with fluorinated carbohydrate moiety, and the S_N^2 reaction of a 2,2′-anhydro nucleoside by anhydrous HF became the standard method to introduce a 2′α-F moiety into pyrimidine nucleosides.[50] Because HF itself is a hazardous reagent, the "tamed hydrofluoric acid", Olah's reagent (HF/Pyridine), was used by Shi *et al.*[51] to fluorinate 2,2′-anhydro nucleosides, and they also reported a DAST fluorination approach which gave (2′*R*)-D-2′-deoxy-2′,5-difluorouridine in excellent yield, and the latter afforded

(2′*R*)-D-2′-deoxy-2′,5-difluorocytidine, which showed potent anti-HCV and inhibitory activity to ribosomal RNA (Scheme **4**).



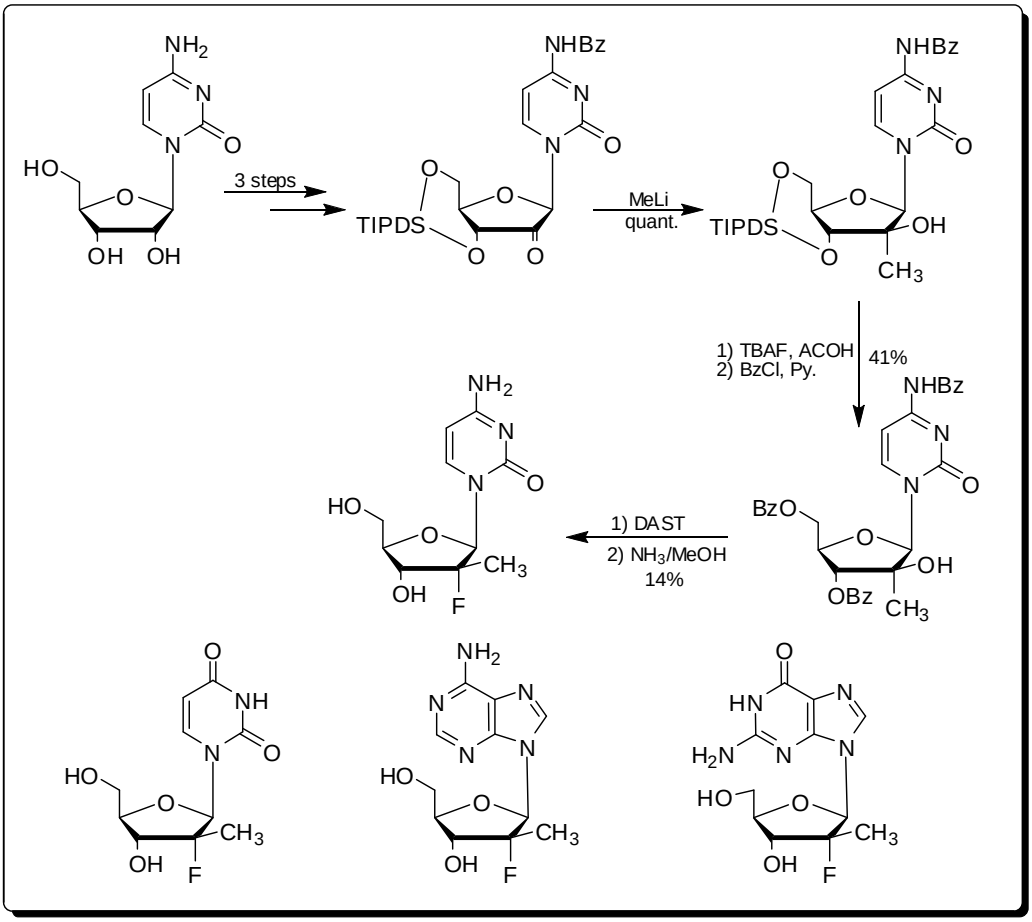
Scheme (**4**): Synthesis of 2′α-fluoronucleosides according to Shi *et al*.

Based on the fact that dFdUrd and dFdCyd exhibited potent anticancer activity against solid tumours, Robins and co-workers accomplished the synthesis of 2′-[alkyl(or aryl)sulfonyl]-2′-deoxy-2′α-fluoronucleosides, two analogues of dFdUrd and dFdCyd. Starting from 2,2′-anhydro-β-D-arabino-furanosyluracil, 2′-thiouridines were obtained via treatment with thiolate anions. Protection of the hydroxyl groups with acetyl groups followed by fluorination with XeF_2/CH_2Cl_2 and oxidation afforded the stable α- fluorosulfones. The compounds of α-fluorosulfones were further subjected to treatment with NH_3/MeOH or a base-transformation procedure to provide the 2′-[methyl(or 4-methoxylbenzyl)sulfonyl]-2′-deoxy-2′α-fluoronucleosides (Scheme **5**).[52]



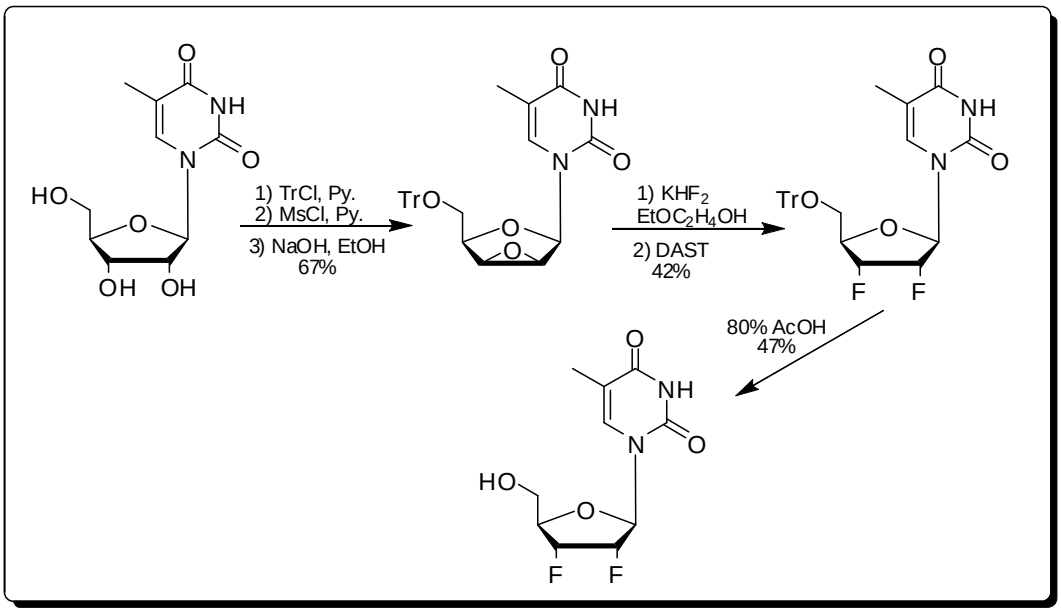
Scheme (**5**): Synthesis of α- fluorosulfones according to Robins *et al*.

Recently, Watanabe *et al.* designed and synthesized β-D-2′-deoxy-2′α-fluoro-2′-C-methylcytidine as a potential inhibitor of hepatitis C virus (HCV) replication (Scheme **6**). In their synthesis, cytidine was firstly converted into the 2′-ketone derivative in three steps. Treatment of 2′-keto compound with methyllithium gave 2′-C-methylcytidine derivative. Removal of the silyl group and benzoylation of the resultant hydroxyl groups provided the protected 2′-C-methylcytidine derivative, which was subjected to fluorination with DAST and removal of the benzoyl groups with NH_3/MeOH to furnish the target nucleoside.[53] Using a similar strategy, this group also carried out the synthesis of three other analogues, the uracil derivative and the purine nucleosides.[54]



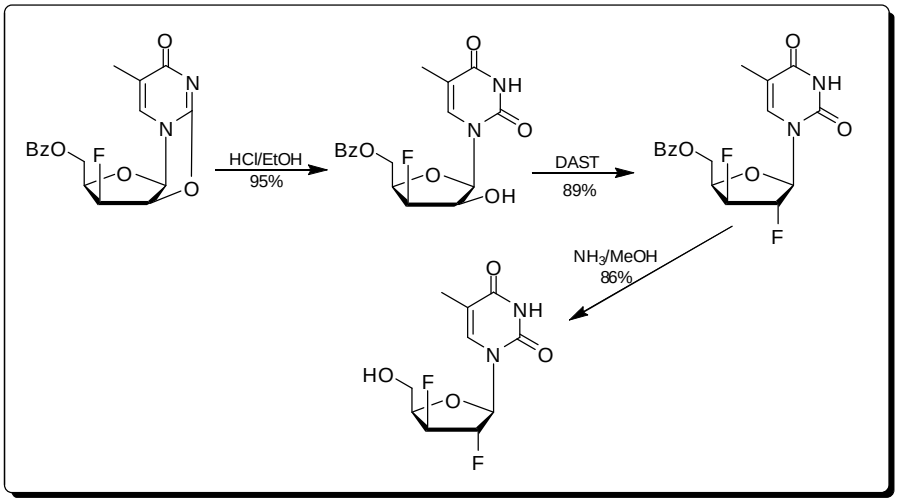
Scheme (**6**): Synthesis of β-D-2′-deoxy-2′α-fluoro-2′-C-methylnucleosides

Considering that 1-(2,3-dideoxy-2-fluoro-β-D-threo-pentofuranosyl)cytosine (F-ddC) and 3′-deoxy-3′-fluorothymidine (FLT) exhibited high activities against HIV-1, Watanabe and co-workers synthesized 2′,3′-dideoxy-2′,3′-difluoro-5-methyluridine in 1991,[55] which combined the characteristics of the nucleosides F-ddC and FLT (Scheme **7**). This compound was screened for its activity against HIV and feline TLV in vitro, without showing any significant activity. In their synthesis, the commercially available ribo-furanosylthymine was firstly converted into the 2′,3′-lyxo-epoxide in three steps. Treatment of the epoxide with KHF_2 in ethoxyethanol at 140 °C afforded a mixture of 2′-fluoro-xylo and 3′-fluoro-arabino nucleosides, which, without separation, were further treated with DAST to provide the difluoro-protected nucleoside. Removal of the trityl group with 80% AcOH at 100 °C gave the target nucleoside.



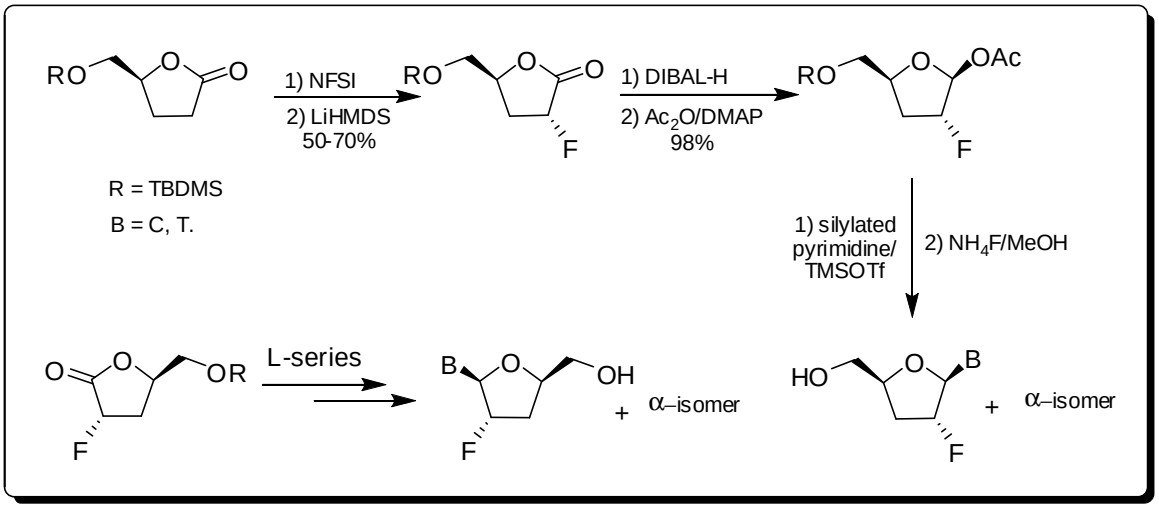
Scheme (**7**): Synthesis of 2′,3′-dideoxy-2′,3′-difluoro-5-methyluridine

Two years later, Gosselin's group[56] described the synthesis of 1-(2,3-dideoxy-2,3-difluoro-β-D-xylo-furanosyl)thymine (Scheme **8**). Exposure of the 2,2′-anhydro intermediate to HCl/EtOH provided the lyxoside in 95% yield, which was further fluorinated with DAST to give the xylo-difluoro nucleoside. Removal of the benzoyl group yielded the target nucleoside in 86% yield, which was tested for its in vitro inhibitory effects on the replication of a number of DNA and RNA viruses (including HIV) in several cell systems, without showing a marked antiviral effect or produced any detectable alteration of host-cell morphology at the highest concentration tested.



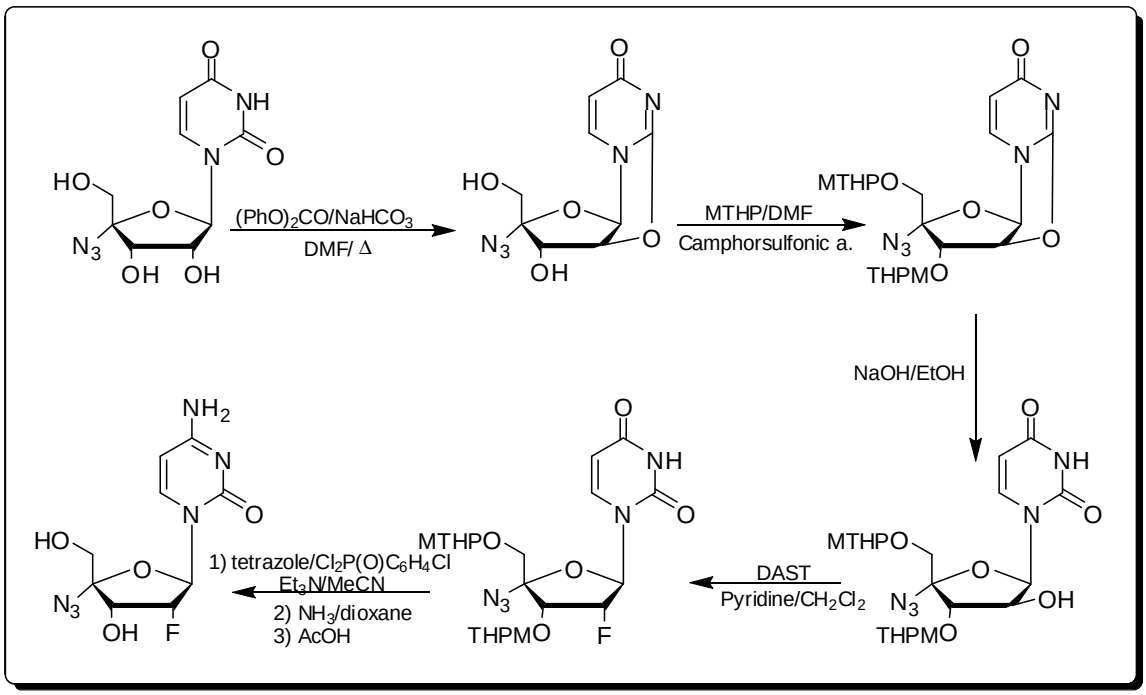
Scheme (**8**): Synthesis of 1-(2,3-dideoxy-2,3-difluoroβ-D-xylo-furanosyl)thymine

In 1998, Liotta and co-workers developed a highly diastereoselective method for the introduction of a fluorine atom into a carbohydrate sugar ring precursor and this methodology was successfully utilized to synthesize the 2′-fluoronucleosides (Scheme **9**). The electrophilic fluorination of the chiral lactone with NFSI/LiHMDS diastereoselectively afforded the mono-fluorinated product in 50-70% yield. The key intermediate was reduced with DIBAL-H followed by acetylation with Ac_2O/DMAP to produce the anomeric acetate. The last product was used for the synthesis of 2′α-fluoro-D-nucleosides by standard Vorbrüggen methodology with TMSOTf as the Lewis acid. The L-enantiomer nucleosides were also prepared, starting from the fluorinated lactone using a similar synthetic route.[57]



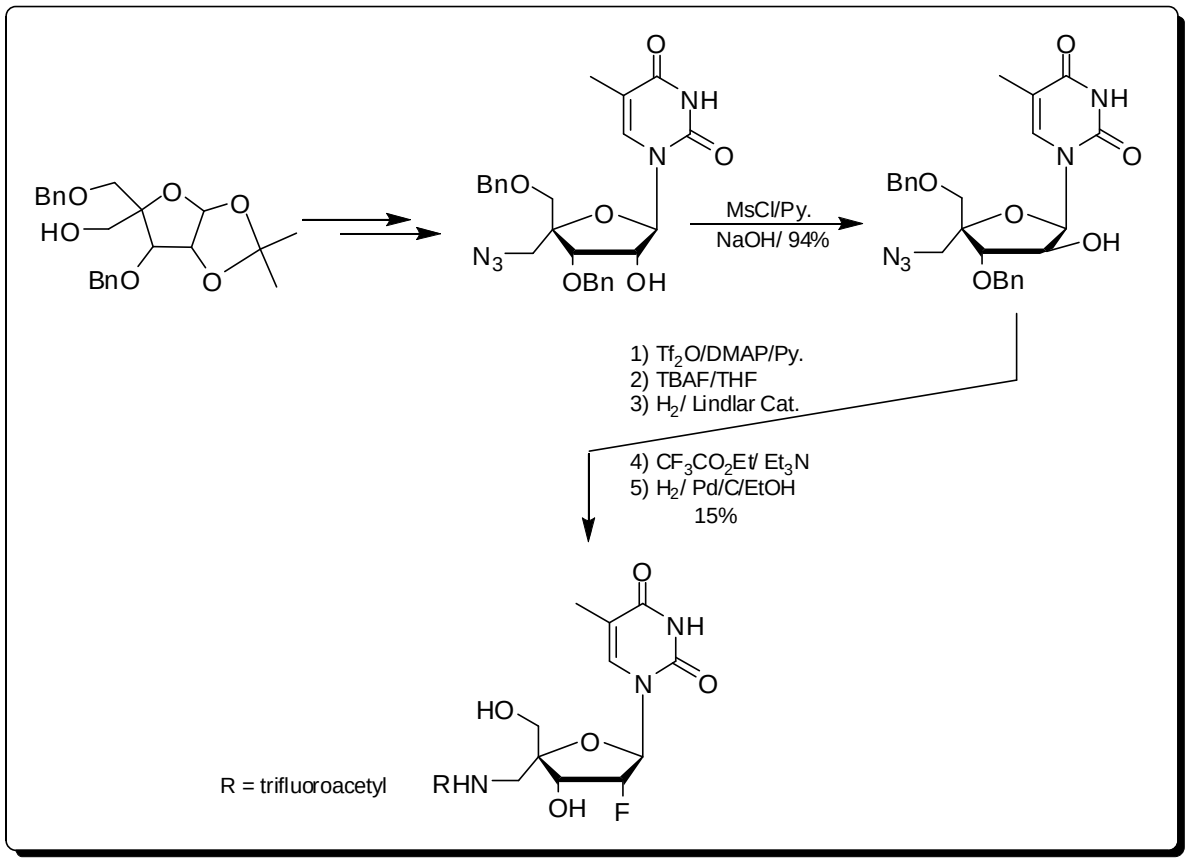
Scheme (**9**): Synthesis of 2′-fluoronucleosides according to McAtee *et al.*

Recently, Smith *et al.* described the synthesis of 4′-azido-2′-deoxy-2′-fluorocytidine by treatment of 4′-azidouridine with diphenylcarbonate yielded 2,2′-anhydro-4′-azidouridine in 85%. Protection with 4-methoxy-3,6-dihydro-2*H*-pyran, followed by hydrolysis with NaOH in EtOH, provided arabino-derivative in 56% yield over steps. Fluorination with DAST furnished protected 4′-azido-2′-deoxy-2′-fluorouridine as a single isomer in 56% yield. Standard conversion from uridine to cytidine was conducted with 1H-tetrazole and 4-chlorophenyldichlorophosphate followed by ammonia. Deprotection with acetic acid provided the target nucleoside in 26% yield over two steps, the potency against HCV replication is also observed ~3-fold lower as compared to 4′-azidocytidine.[58]



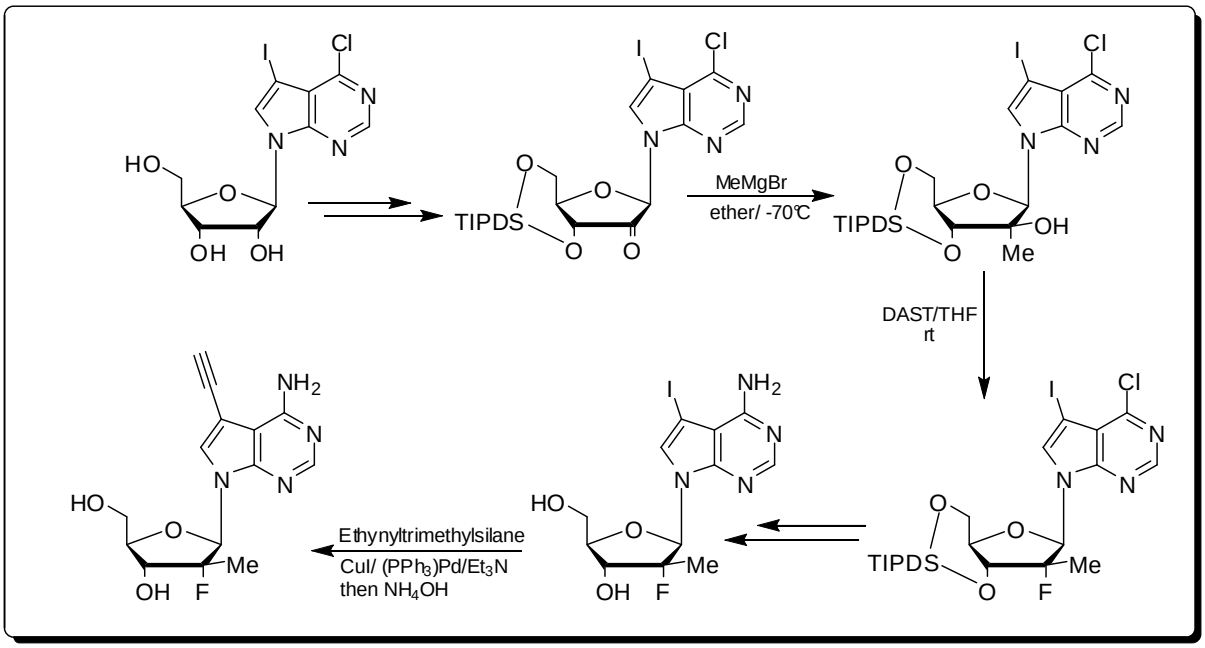
Scheme (**10**): Synthesis of 4′-azido-2′-deoxy-2′-fluorocytidine according to Smith *et al*.

In order to investigate the possibility of increasing the binding affinity and nuclease resistance of oligonucleotides containing C-substituted nucleotides towards RNA, Wengel and Pfundheller designed and synthesized the 4′-C-aminomethyl-2′-deoxy-2′α-fluorothymidine (Scheme **11**). The starting material, 3,5-di-*O*-benzyl-4-C-hydroxymethyl-1,2-di-*O*-isopropylidene-α-D-ribo-furanose, was first converted into the nucleoside via the standard transformation of functional groups and Vorbrüggen methodology. Reversing of the R configuration of the C-2′ hydroxyl group into the S configuration was accomplished by mesylation of the ribo-configurated compound followed by reaction with NaOH, and the protected arabino-configurated nucleoside was obtained in 94% yield. The target nucleoside was accessed through nucleophilic fluorination with TBAF, Lindlar reduction and subsequent removal of the protecting groups.[59]



Scheme (**11**): Synthesis of 4′-C-aminomethyl-2′-deoxy-2′α-fluorothymidine

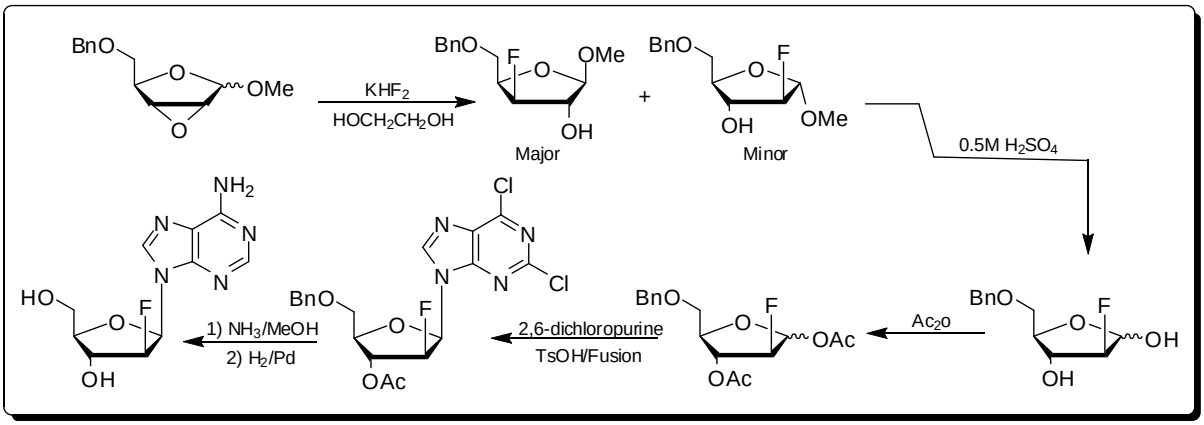
Starting from modified base nucleoside, which was converted into ketone via selective silylation and oxidation, Prhavc and co-workers, accomplished the synthesis of 7-deaza-7-ethynyl-2′-deoxy-2′-fluoro-2′-C-methyladenosine (Scheme **12**). The key steps of the synthesis were the selective methylation of ketone with MeMgBr and subsequent fluorination of the alcohol with DAST to give 2′-deoxy-2′-fluoro-2′-C-methyl derivative. The final fluorinated nucleoside was afforded by the coupling of the intermediate with ethynyltrimethylsilane, the corresponding nucleoside triphosphate was efficient chain terminator of the HCV NS5b polymerase with IC_{50} of 0.4 μM.[60]



Scheme (**12**): Synthesis of 7-deaza-7-ethynyl-2′-deoxy-2′-fluoro-2′-C-methyladenosine

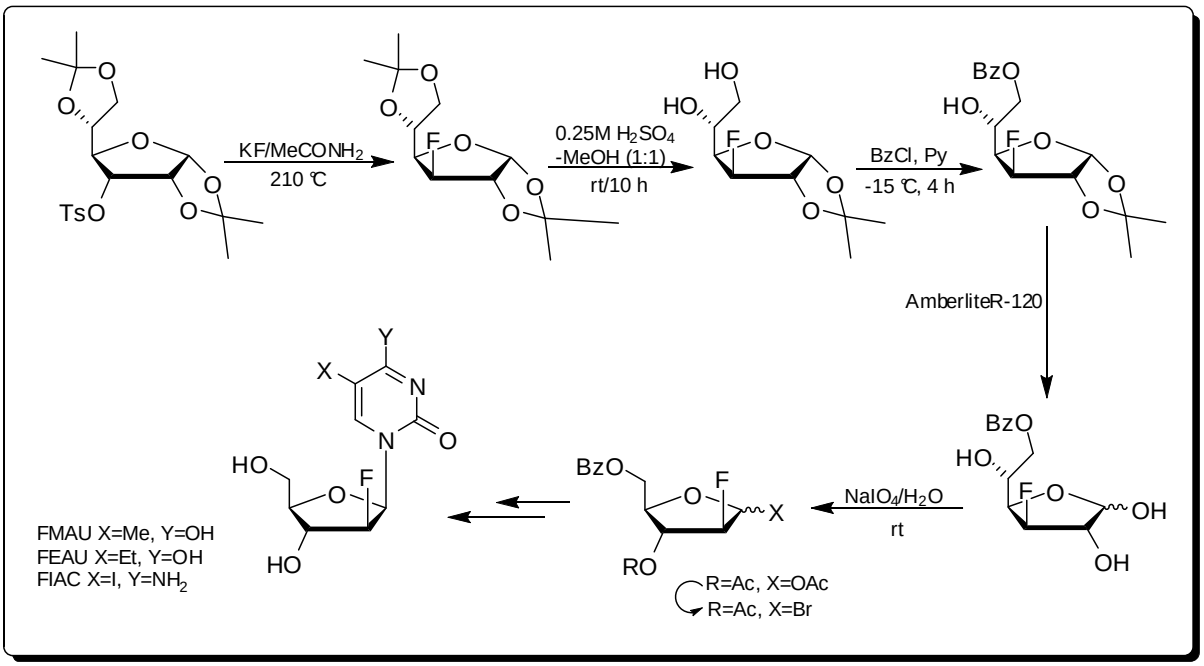
III.2.1.2 Synthesis of 2′β-fluoronucleosides:

Introduction of a fluorine atom at the 2′β-position of nucleoside analogues has produced variety of interesting antiviral agents. FIAC, FEAU, FMAU and their congeners were first synthesized in Fox's group (Schemes **13** and **14**) and showed potent activity against HSV and also demonstrated excellent activity against hepatitis B virus (HBV), Varicella zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). In addition, FMAU showed significant activity against murine leukemias. F-ara-A was first synthesized by Wright *et al.*[61] (Scheme **13**) as a fluoro derivative of β-D-arabinofuranosyladenine (ara-A), an agent approved by the FDA for HSV-1 and 2 treatment. Methyl 2,3-anhydro-5-*O*-benzyl-β-D-riboside was treated with KHF_2, and gave a mixture of the 3′- and 2′-fluoro-D-arabinofuranoside with the 3′β-F isomer as the major product. 2′β-Fluorinated sugar was used for coupling with 2,6-dichloropurine to give protected F-ara-nucleoside and subsequently afforded F-ara-A.



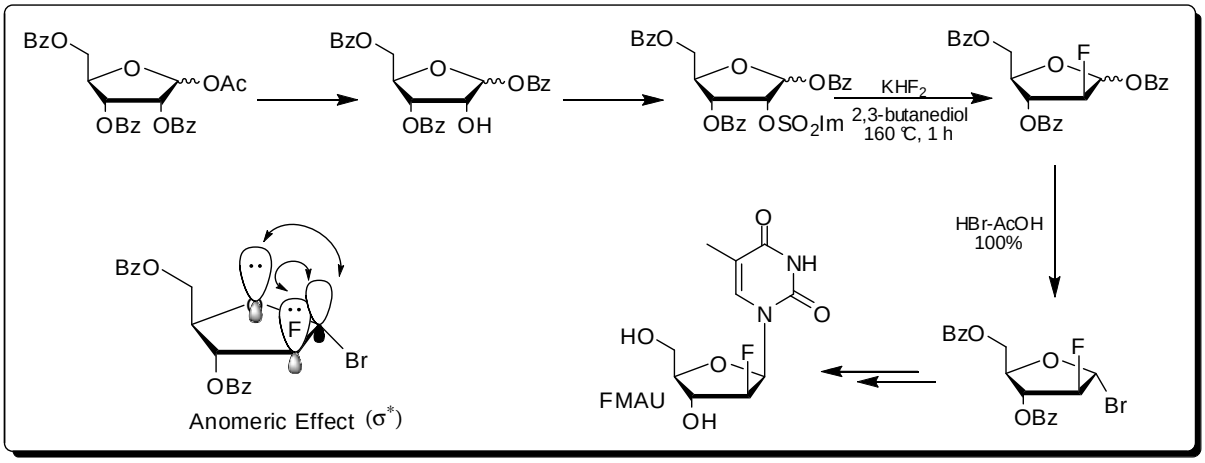
Scheme (**13**): Synthesis of F-ara-A according to Wright *et al.*

Due to the low yield of the 2-fluoro sugar according to Wright *et al.*, Watanabe *et al.*[62,63] subsequently developed a practical synthetic approach to 2-deoxy-2-fluoro-D-arabinose from a readily available D-glucose derivative (Scheme **14**). The crucial steps were the fluorination of protected D-glucofuranose by KF in acetamide as well as the conversion of 6-*O*-benzoyl-3-deoxy-3-fluoro-D-glucofuranose into 5-*O*-benzoyl-2-deoxy-2-fluoro-arabinofuranose by periodate-oxidation. A series of pyrimidine nucleosides, such as FMAU, FEAU and FIAU, were prepared in a large scale for biological evaluation, these compounds showed not only potent activity against HSV, but also an excellent activity against HBV and other viruses such as varicella zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV).



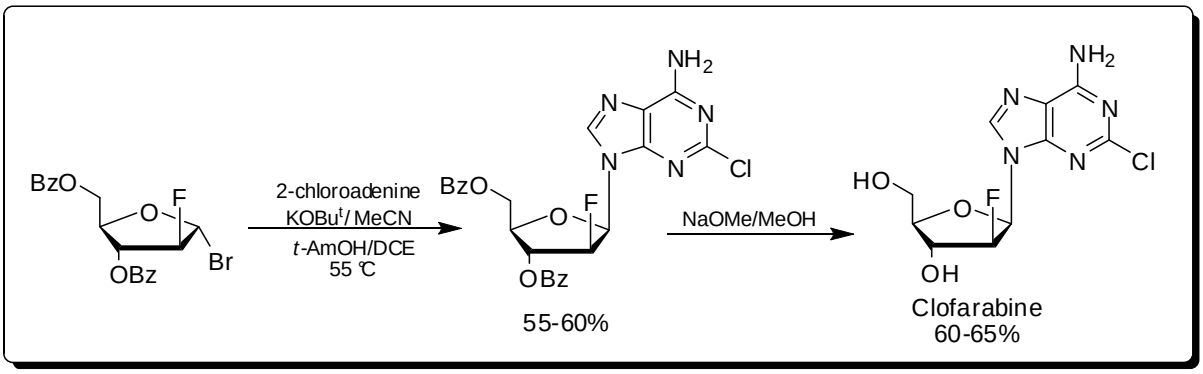
Scheme (**14**): Synthesis of FMAU, FEAU and FIAU according to Watanabe *et al.*

The potent antiviral activities of FIAC and FMAU prompted Tann *et al.*[64] to develop an efficient method for the construction of the 2′β-F arabino sugar (Scheme **15**). The direct displacement of 2-imidazoylsulfonate with KHF_2 gave 63% yield of 2′β-F sugar, and subsequent bromination with 30% HBr in AcOH produced a quantitative conversion to the 1-α-bromo sugar. The exclusive formation of the 1-α-glycosyl bromide, attributed to the stabilizing interaction between the unshared electron pairs on fluorine and oxygen with the σ* orbital for the axial C-Br bond, the so-called "anomeric effect",[65] leads to preferential formation of β-nucleosides, and the product is being used as a standard sugar for the preparation of 2′-deoxy-2′-fluoro-arabinonucleosides, in particular for pyrimidine nucleosides.



Scheme (**15**): Synthesis of FMAU according to Tann *et al.*

It is noteworthy that the condensation of bromosugar in scheme **14** or **15** with 6-chloropurines is usually difficult. In fact, the reactions of the bromosugar in scheme **14** with some purine bases, such as 6-chloropurine and 2,6-dichloropurine, give a mixture of four isomers (7-, or 9-substitued, α, β-anomers) from which the desired isomer was separated in low yield.[66] Montgomery *et al.*[67] synthesized and evaluated various 2-halo-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenines and found that clofarabine had potent anti-tumor activity. Recently, Bauta *et al.*[68] reported an efficient manufacturing process for clofarabine starting from commercially available bromosugar (Scheme **16**).

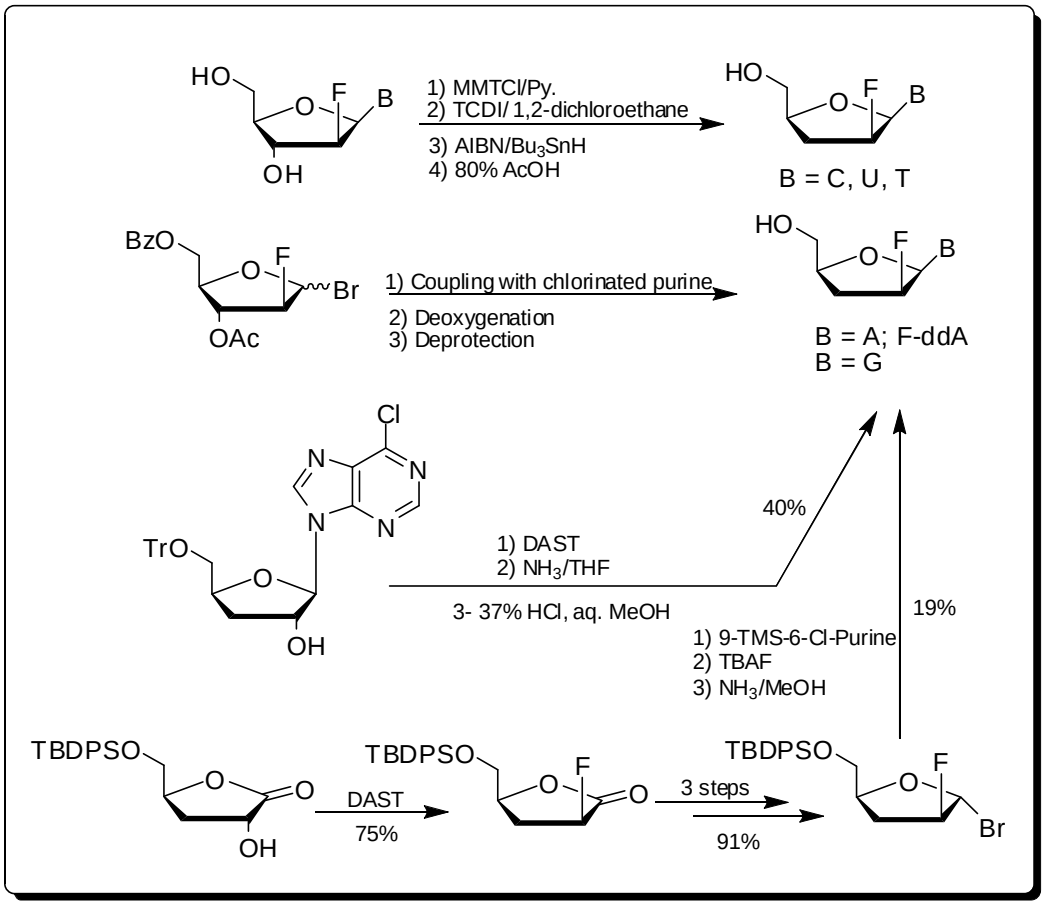


Scheme (**16**): Synthesis of Clofarabine according to Bauta *et al*.

2′,3′-Dideoxy nucleosides (ddNs) exhibit potent anti-HIV activity and several in this class of nucleosides, such as ddI and ddC, are in clinical use. However, the instability of these compounds in an acidic media hampers oral administration. Since the fluorine at the 2′-position of a carbohydrate moiety can provide acidic and enzymatic stabilities to the glycosidic bond of nucleosides, a number of 2′-F ddNs have been prepared and evaluated. The 2′α-F isomers of ara-ddI and ara-ddA were inactive, but the 2′β-F isomers, F-ara-ddI and F-ara-ddA, were just as potent as parent drugs.[69]

In 1990, Sterzycki and co-workers[70] synthesized the 2′,3′-dideoxy-2′-fluoro-arabino-furanosylpyrimidine nucleosides (including the highly bioactive F-ddC, Base = C), starting from the 2′-deoxy-2′-fluoro-arabino-furanosylpyrimidine nucleosides. Their synthesis was carried out by treatment of 2′-deoxy-2′β-fluoropyrimidine nucleosides with MMTCl, and the resultant protected esters were subjected to deoxygenation by reaction with 1,1′-thiocarbonyldiimidazole (TCDI), followed by reduction with Bu_3SnH and deprotection to provide the target nucleosides. In the same year, the purine nucleoside analogues[71,72] (F-ddA) were also prepared by the Marquez group via coupling of 3′-α-acetyl-2′β-fluoro bromo sugar with 2,6-dichloropurine or 6-chloropurine followed by transformation of base, deoxygenation and deprotection. It should be noted that, a decade later, Izawa's[73,74] group and Choudhury's[75] group improved the synthesis of F-ddA. The process of Izawa's group involved the fluorination of the protected 6-chloro-9-(3-deoxy-β-D-erythro-pentofuranosyl)-9H-purine with DAST followed by deprotection and base transformation. Choudhury's group utilized the highly syn-stereoselective fluorination of the lactone with DAST to obtain the fluorolactone, which was further converted into the bromo sugar in a straightforward fashion.

Introduction of the base moiety via coupling of the sugar with 9-TMS-6-Cl-purine and deprotection gave F-ddA in 19% yield (Scheme **17**).

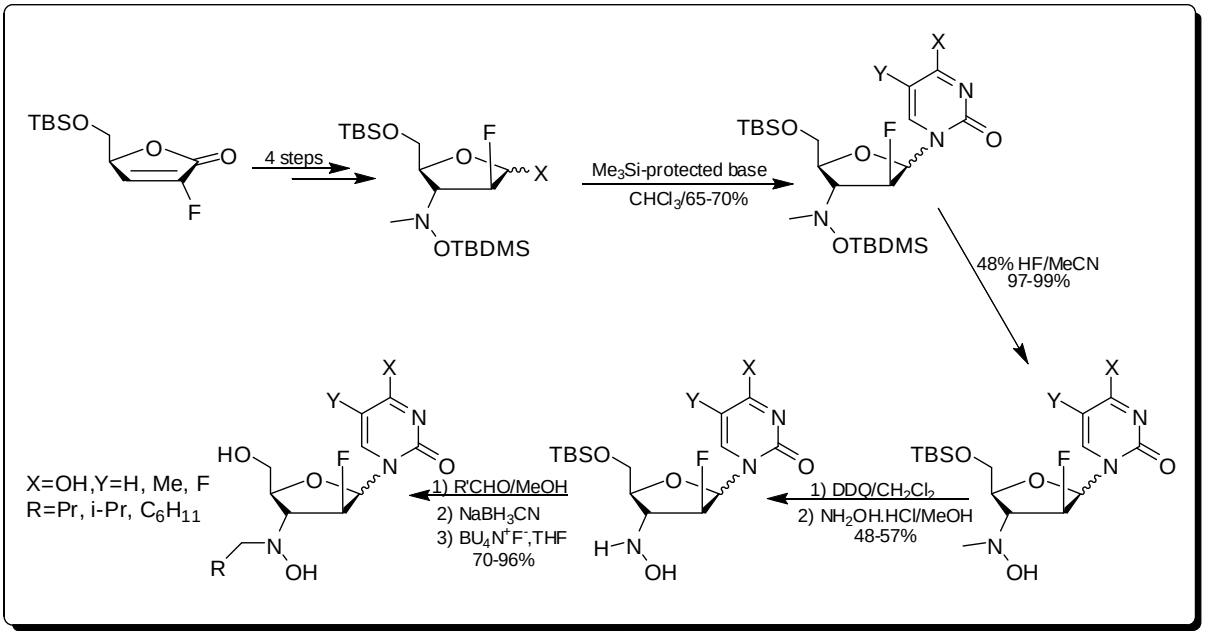


Scheme (**17**): Synthesis of 2′β-F-ddNs

To study the effect of 3′-C substitution in highly bioactive D-FMAU, a large number of 3′-substituted 2′-deoxy-2′-fluoro-arabino-furanosyl nucleoside analogues such as 3′-azido-2′,3′-dideoxy-2′-fluoro-5-methyl-arabino-uridine and 2′,3′-dideoxy-2′-fluoro-3′-C-hydroxymethyl-arabino-furanosylpyrimidine nucleosides were synthesized by the groups of Watanabe, Sterzycki and Hassan.[76]

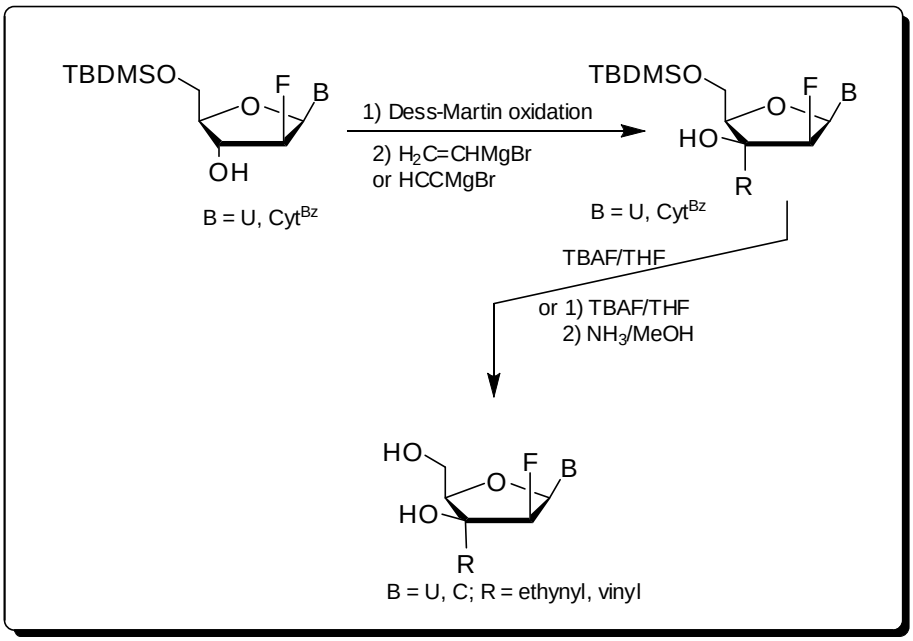
Recently, Zhao *et al.*[77] designed and synthesized a series of novel 3′-(alkyl(hydroxy)amino)-2′-fluoronucleoside analogues, which were prepared via conjugate addition of *N*-methylhydroxylamine to various 2-fluorobutenolides. The products were obtained as single isomers under absolute control of stereochemistry. The crucial *N*-demethylation was readily achieved by means of DDQ oxidation, followed by nitrone/oxime exchange reaction. Using

this procedure, a variety of alkyl groups could be efficiently introduced at the 3′-*N*-atom of the nucleoside analogues (Scheme **18**).



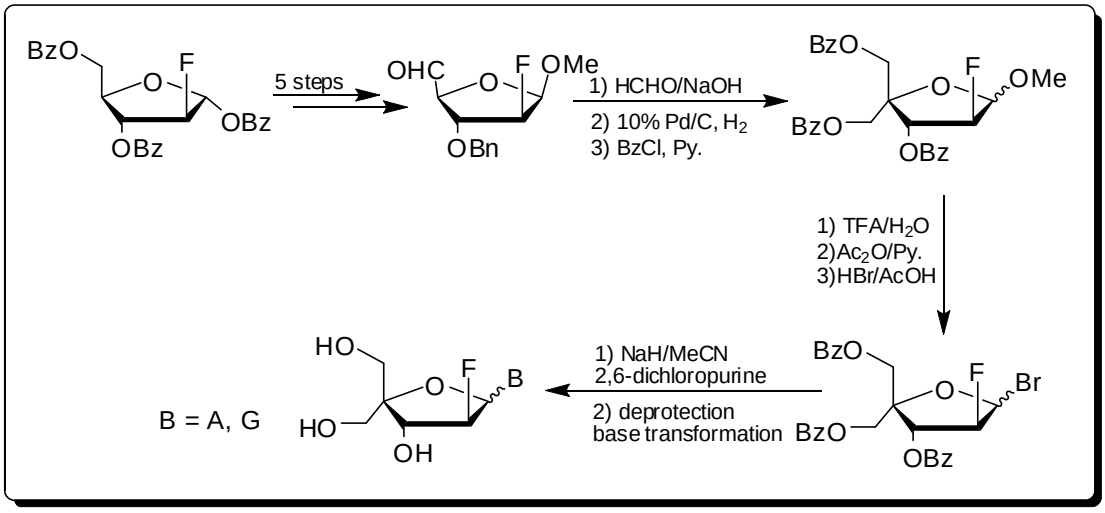
Scheme (**18**): Synthesis of 3′-(alkyl(hydroxy)amino)-2′-fluoronucleoside analogues

Secrist and co-workers[78] described the synthesis of several 2′-deoxy-3′-C-ethynyl and 3′-C-vinyl-2′-fluoro-β-D-lyxo-furanosyl nucleosides based on the knowledge that some 3′-alkyl or 3′-alkynyl branched nucleoside analogues have exhibited potent antiviral and anticancer activities. Their synthesis was accomplished via the key intermediates, the protected 2′-deoxy-2′-fluoro-β-D-arabino-furanosyl nucleoside analogues. Oxidation of the secondary alcohol with Dess-Martin periodinane followed by addition *in situ* of ethynylmagnesium bromide or vinylmagnesium bromide gave the lyxo-adducts in high stereoselectivities. Final removal of all of the protecting groups provided the target nucleosides, these nucleosides were examined *in vitro* against a panel of human tumor cell lines, no marked cytotoxicity was noted (Scheme **19**).



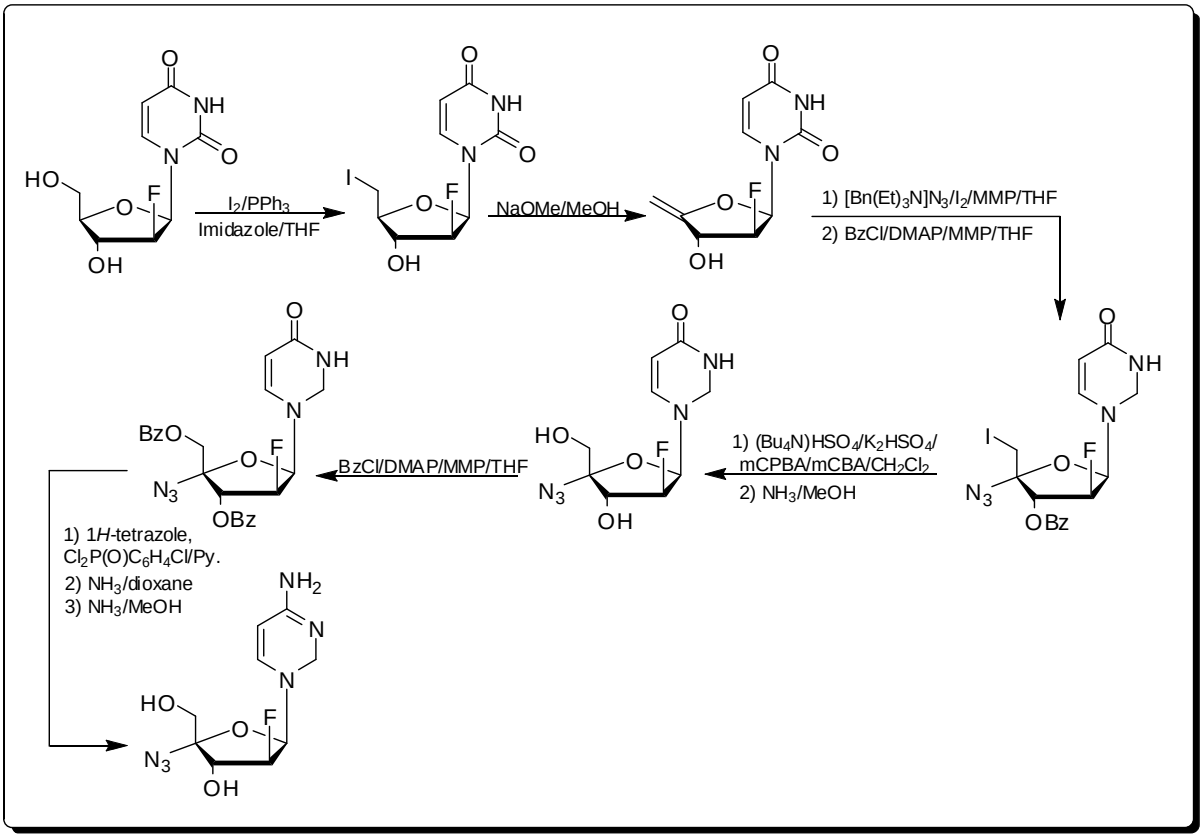
Scheme (**19**): Synthesis of 2′-deoxy-3′-C-ethynyl and 3′-C-vinyl-2′-fluoro-β-D-lyxo-furanosyl nucleosides

In addition to the aforementioned nucleosides, Secrist's group also carried out the synthesis of several 4′-C-hydroxymethyl-2′-fluoro-D-arabino-furanosylpurine nucleosides (Scheme **20**), which were further evaluated for their cytotoxicities on human tumour cell lines. In their synthesis, the aldehyde, which was prepared in five steps, reacted with HCHO/NaOH to afford the diol. Exposure of the compound to TFA/H_2O followed by acetylation and bromination of the resultant furanose delivered the bromo sugar. Using the sodium salt glycosylation procedure, the target nucleosides were provided after deprotection and base transformation.[79]



Scheme (**20**): Synthesis of 4′-C-hydroxymethyl-2′-fluoro-D-arabino-furanosylpurines

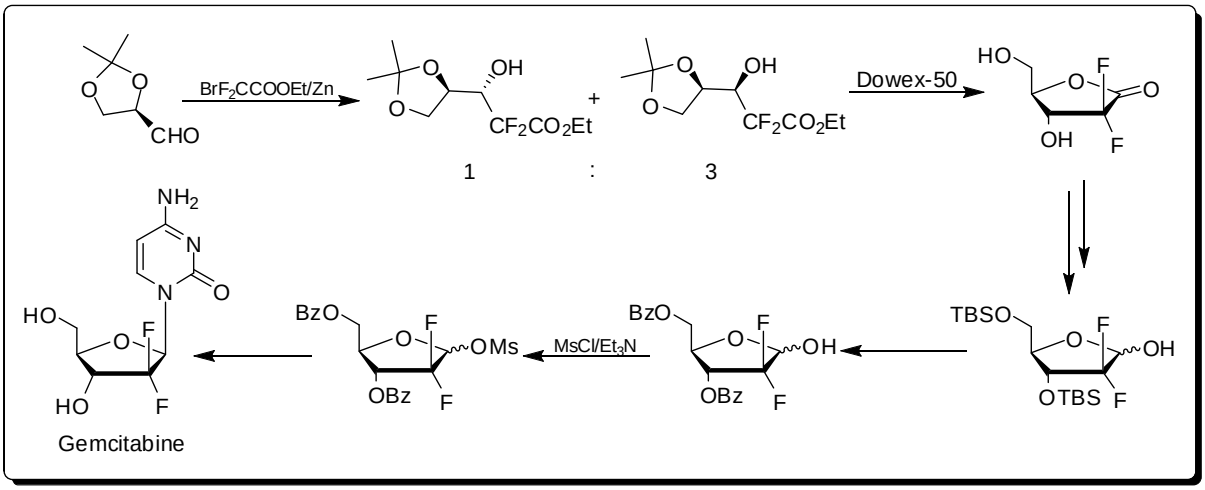
Recently, Smith *et al.* [58] discovered 4′-azido-2′-deoxy-2′-fluoroarabinocytidine to act as a highly potent and selective inhibitors of HCV replication (EC_{50} = 24 nM). Treatment of 2′-deoxy-2′-fluoroarabinouridine with iodine and triphenylphosphine and imidazole in THF gave the iodide nucleoside in 76% yield. Elimination of the hydrogen iodide was performed by sodium methoxide to furnish the corresponding olefin in 82% yield. Treatment of the corresponding olefin with iodine and benzyltriethylammonium azide, prepared from the corresponding chloride and sodium azide in MMP and THF, provided the corresponding 4′-azido in 93% yield as a single isomer. Oxidative nucleophilic substitution of the 5′-iodine with *m*-chlorobenzoic acid/*m*-chloroperoxybenzoic acid, followed by deprotection gave 4′-azido-2′-deoxy-2′-fluoroarabinouridine. Protection of the 3′,5′-hydroxy groups followed by standard conversion from uridine to cytidine furnished the target nucleoside in 88% yield (Scheme **21**).



Scheme (**21**): Synthesis of 4′-azido-2′-deoxy-2′-fluoroarabinocytidine

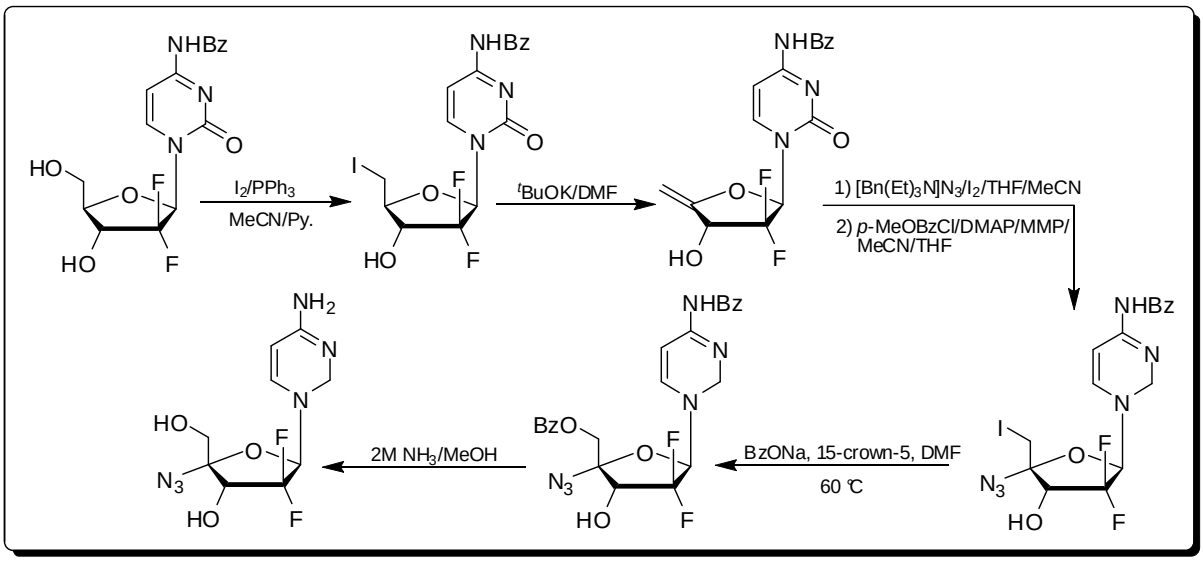
III.2.1.3 Synthesis of 2′,2′-difluoronucleosides:

Gemcitabine (2′-deoxy-2′,2′-difluorocytidine) is a clinically effective anti-cancer agent for the treatment of pancreatic cancer. It has also shown anti-tumor activity against a wide spectrum of human solid tumors. Researchers at Lilly described the synthesis of gemcitabine, by construction of the 2,2-difluoro-D-ribose moiety using a CF_2-containing building block. Thus, glyceraldehyde was coupled with ethyl bromodifluoroacetate under Reformatski conditions to give a mixture of R and S configuration. The major isomer was hydrolyzed to give a lactone, which was further converted to 2,2-difluoro-D-ribose. Due to the strong electron-withdrawing effect of the 2-gem-difluoro group, the intermediate was converted to the 1-*O*-mesylate, which was condensed with cytosine to afford gemcitabine (Scheme **22**).[42]



Scheme **22**: Synthesis of Gemcitabine according to Hertel *et al.*

The introduction of 4′-azido into Gemcitabine reported by Smith *et al.*[58], resulted in a highly interesting compound with significant antiviral potency in the HCV replicon system (EC_{50} = 66 nM) and no apparent cytotoxicity. 4′-azido-2′-deoxy-2′,2′-difluorocytidine was synthesized by a similar strategy of 4′-azido-2′-deoxy-2′-fluoroarabinocytidine by Smith's group (Scheme **23**).



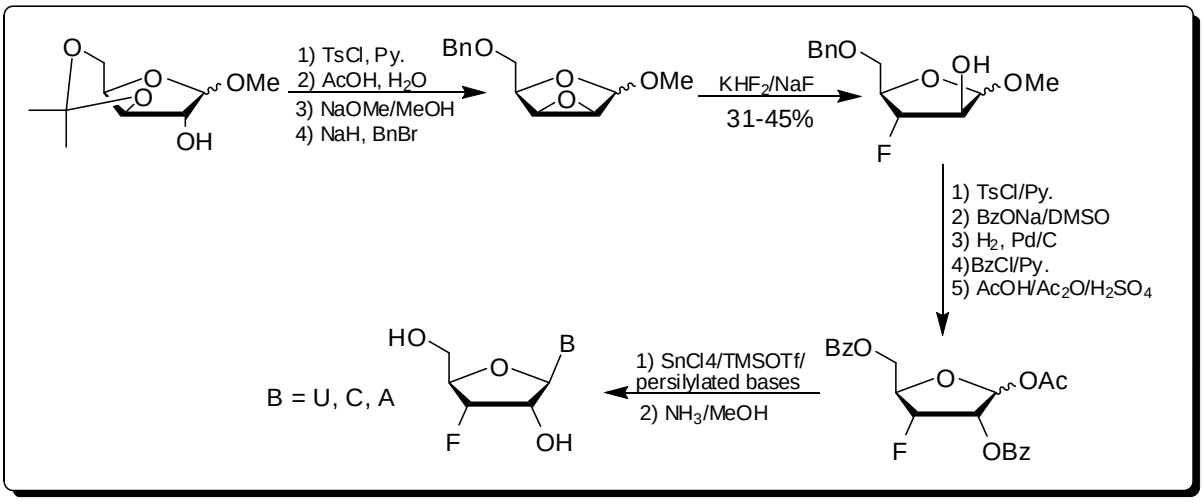
Scheme (**23**): Synthesis of 4′-azido-2′-deoxy-2′,2′-difluorocytidine

III.3.1 Synthesis of 3′-monofluorinated nucleosides:

The nucleoside analogues in which the hydroxy group at C-3′ was substituted by a fluorine atom exhibited a wide range of biological properties. The synthesis and investigation of the biological activity of 3′-deoxy-3′-fluoro nucleoside analogues have received relatively little attention. Since FLT was reported to be as potent as AZT against HIV in 1988, the synthesis and biological activity of 3′-monofluorinated nucleosides have been greatly developed in recent years.[76]

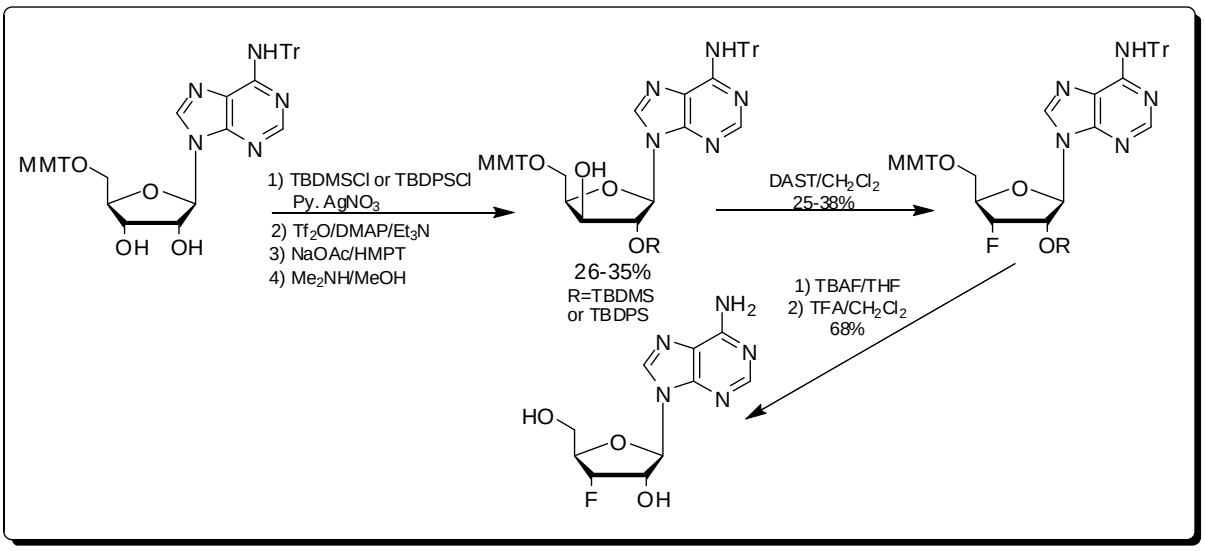
III.3.1.1 Synthesis of 3′α-fluoronucleosides:

Mikhailopulo and co-workers pioneered the synthesis of 3′-deoxy-3′-fluoro-D-ribo-furanosides as early as 1989. Starting from the methyl glycoside, the epoxide was provided in a straightforward fashion over four steps. Treatment of the epoxide with KHF_2/NaF gave the fluoride in 31-45% yield, and this was converted into the acetate after several operations of protecting groups involving the inversion of configuration of the C-2 position via nucleophilic substitution. The condensation between the sugar moiety and various silylated bases followed by deprotection provided the desired nucleosides, among the 3′-fluoro-substituted 3′-deoxynucleoside analogues, 3′-fluoro-3′-deoxyadenosine proved to be a markedly inhibitor to the replication of a number of viruses including vaccinia virus, polio virus-1, coxsackie virus B4, sindbis virus, reovirus-1, and semliki forest virus, its minimum inhibitory concentration (MIC) for these viruses ranged from 1 to 7 µg/ml (Scheme **24**).[80,81]



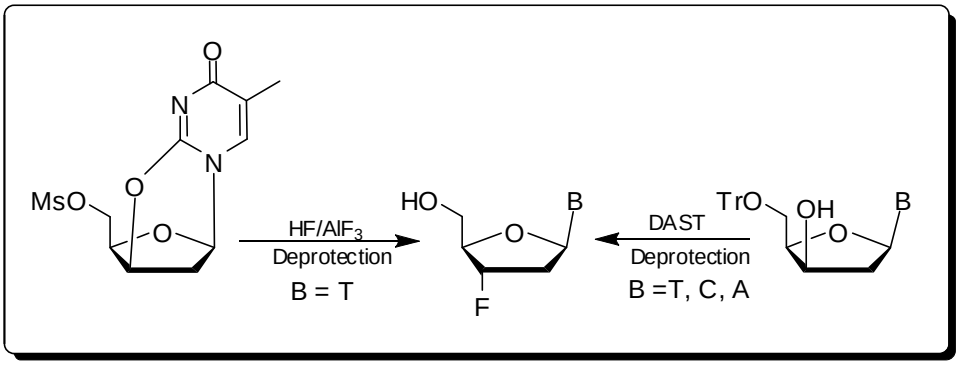
Scheme (**24**): Synthesis of 3′-deoxy-3′α-fluoro-β-D-nucleosides

A different route to 3′-deoxy-3′α-fluoroadenosine was also described in 1990.[82] Starting from the protected adenosine and through a suitable protected intermediate using triflate activation and nucleophilic displacement with NaOAc, the "xylo" epimers were obtained in two cases after protection of the 2′-position with different silyl groups. Treatment of the xylo derivatives with DAST gave the corresponding 3′α-fluoro derivatives with inversion of the C-3′ configuration. Desilylation by treatment with TBAF and subsequent removal of the trityl group with TFA afforded the 3′-deoxy-3′α-fluoroadenosine. In addition, a 3′α-fluoro-substituted guanine (B=G) was also accessed by the Imbach's[83] group and the Pankiewicz's[84] group, who used a similar strategy, namely inversion of the C-3′ configuration of the different protected guanine derivatives followed by fluorination with DAST (Scheme **25**).



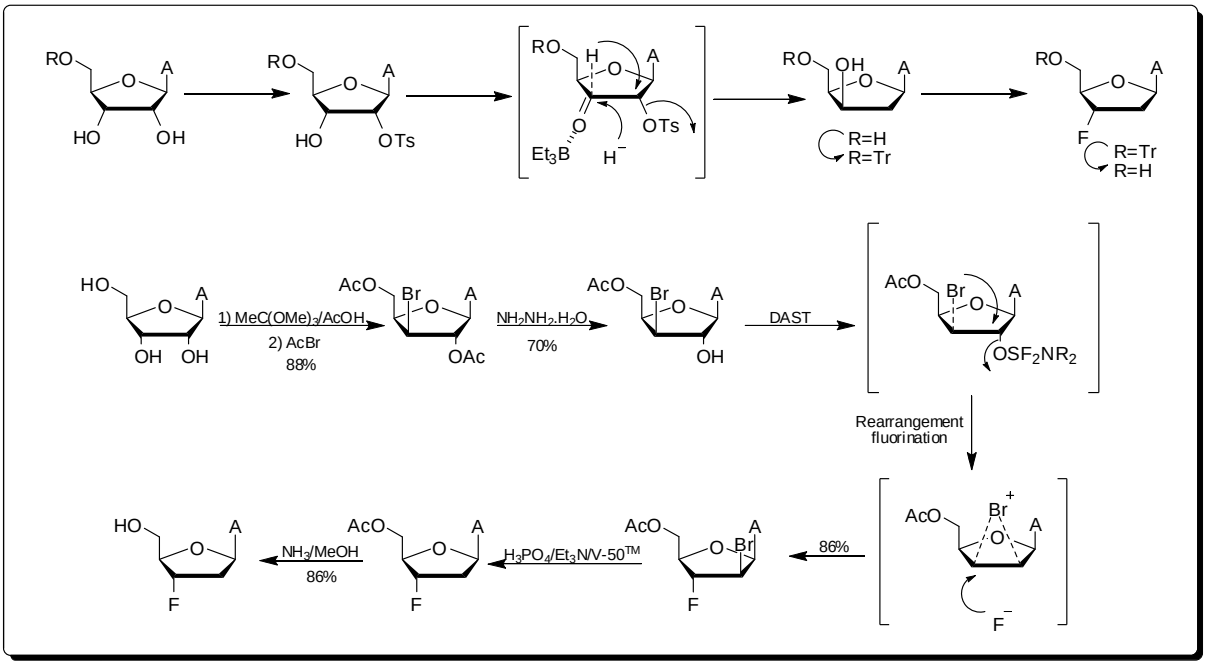
Scheme (**25**): Synthesis of 3′-deoxy-3′α-fluoroadenosine

Although 3′α-fluoro-3′-deoxythymidine (B=T) has been prepared via opening of the 2,3′-anhydro bond of 2,3′-anhydro-1-(2-deoxy-β-D-threo-pentofuranosyl)thymine with HF/AlF_3 and the reaction of 3′-*O*-mesylthymidine with KHF_2 by Langen *et al.*[85,86] Moreover, Herdewijn *et al.*[87] developed a more general procedure for the synthesis of 3′α-fluoro-2′,3′-dideoxynucleosides. They utilized the reactions between the different 2′-deoxynucleosides and DAST as the key step and, in all cases, the desired 3′α-fluoro nucleoside derivatives were obtained in moderate-to-good yields (Scheme **26**).



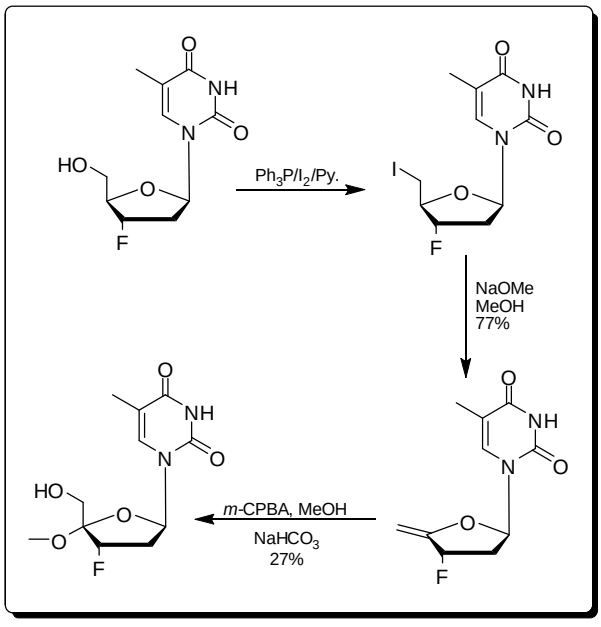
Scheme (**26**): Synthesis of 3′α-fluoro-2′,3′-dideoxynucleosides

Robins *et al.*[88] developed a facile sequence with a deoxygenative [1,2]-H shift rearrangement as the key step. The transformation from ribonucleoside to 2′-deoxy xylonucleoside consists of a [1,2]-hydride shift with the departure of a leaving group from the opposite face. Transient formation of a C=O group is followed by rapid transfer of a hydride-equivalent, which results in a double inversion of the stereochemistry at the two vicinal carbon atoms. Thus, 3′α-F-ddNs were prepared from ribonucleoside in six steps in good yield. Katayama *et al.*[89] also described another facile six-step synthesis of 3′α-F-ddNs using a similar deoxygenative rearrangement which involved a [1,2]-bromo shift during 3′α-fluorination (Scheme **27**).



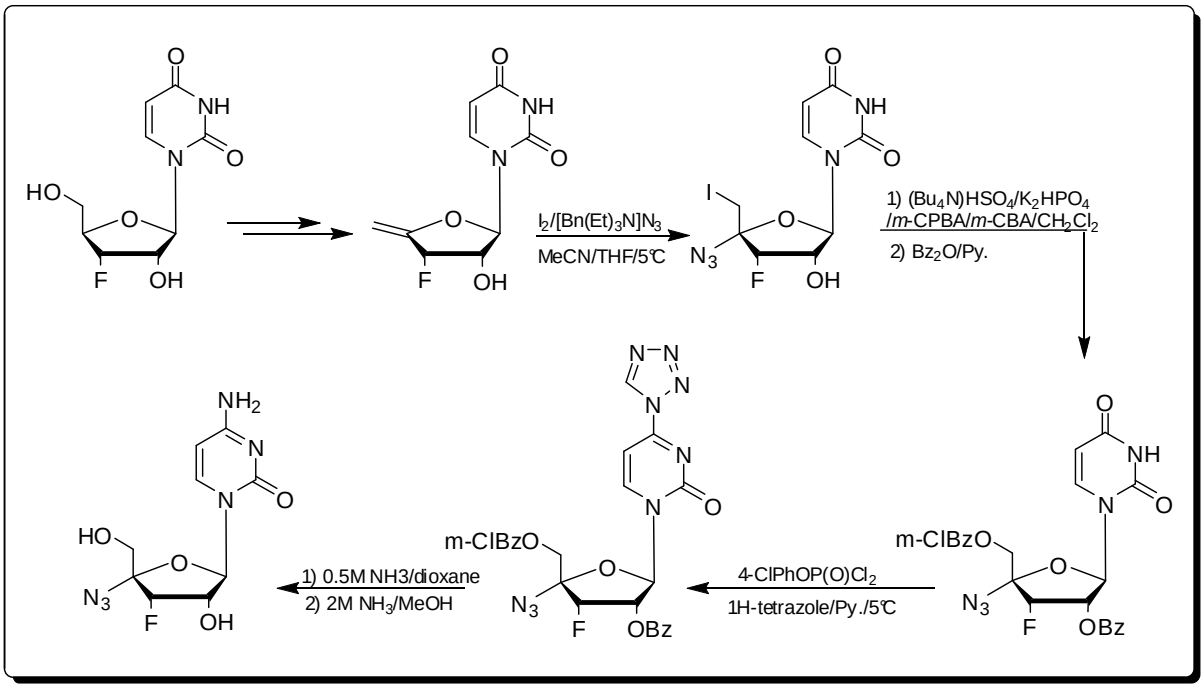
Scheme (**27**): Synthesis of 3′α-F-ddA by deoxygenative [1,2]-H shift rearrangement

Starting from 3′α-fluoro-3′-deoxythymidine, Prisbe and co-workers accessed the synthesis of a 4′-methoxy-substituted nucleoside. In their synthesis, reaction of the nucleoside with PPh_3/I_2/pyridine produced the iodide derivative, which was further subjected to dehydroiodination via treatment with NaOMe/MeOH to afford the terminal olefin in 77% yield. Final epoxidation with *m*-CPBA followed by *in situ* opening of the resultant epoxide provided 3′α-fluoro-4′-methoxy-3′-deoxythymidine in 27% yield (Scheme **28**).[90]



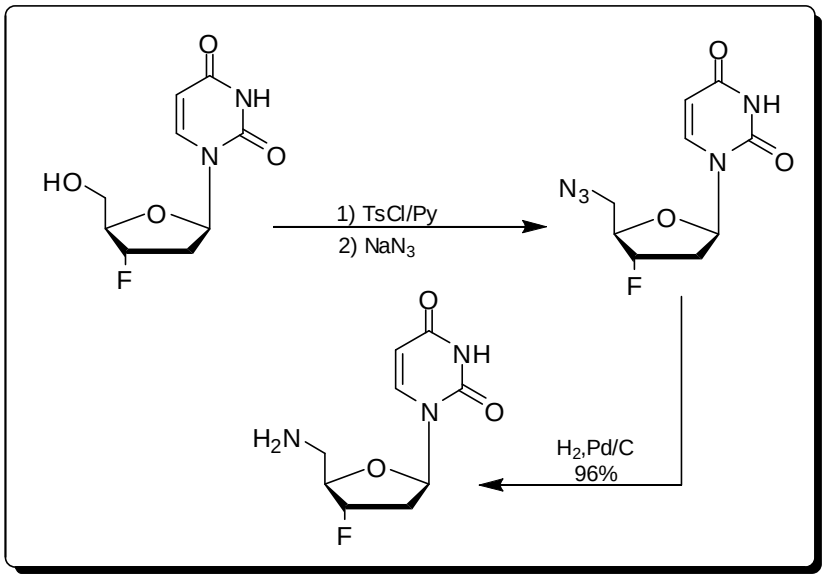
Scheme (**28**): Synthesis of 3′α-fluoro-4′-methoxy-3′-deoxythymidine

Using a similar strategy for the synthesis of 4′-azido-2′-deoxy-2′-fluoroarabinocytidine, Smith *et al* [58] reported the synthesis of 4′-azido-3′-deoxy-3′-fluorocytidine (Scheme **29**). They found that the 3′-fluoro analogue of 4′-azidocytidine were completely inactive as well as nontoxic in the replicon system. The 3′-hydroxy group was suggested from structural studies with deoxycytidine kinase to be important for productive binding to the kinase as hydrogen bonds are formed with E197 and Y86 in the human deoxycytidine kinase active site. Removal of the 3′-hydroxy group in nucleoside structure may prevent these compounds from binding productively to human dCK and thus from being phosphorylated to their triphosphate derivative.



Scheme (**29**): Synthesis of 4′-azido-3′-deoxy-3′-fluorocytidine

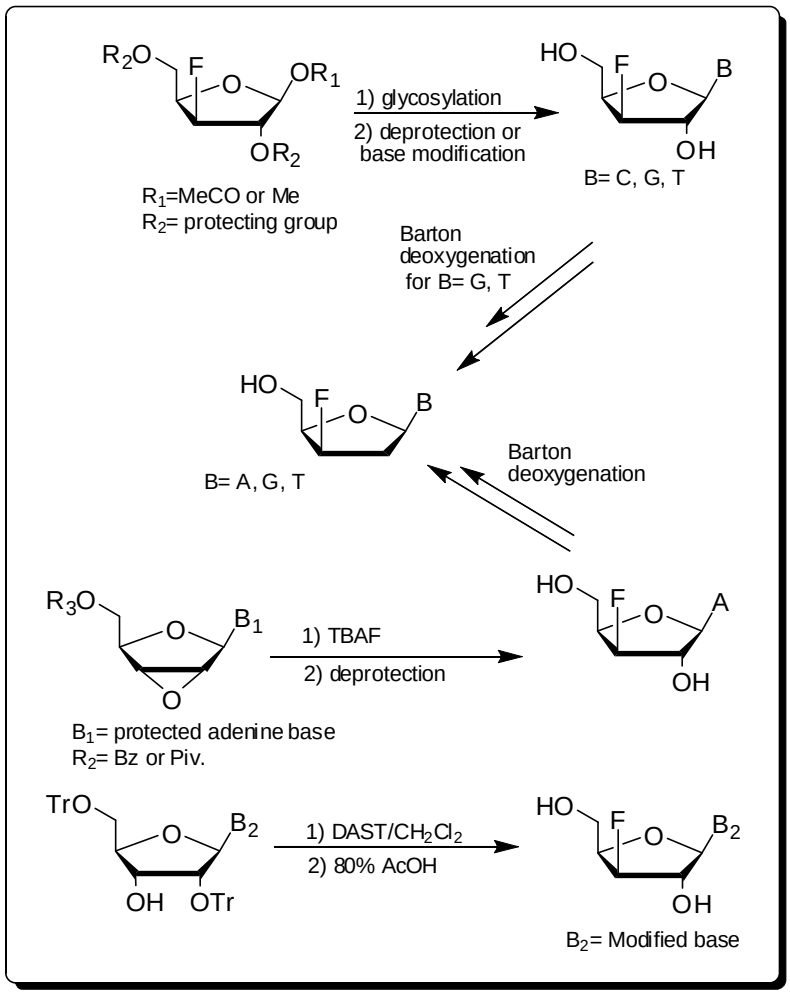
When the hydroxyl group in the 5′ position in 3′-fluoro-3′-deoxyuridine was replaced by an amine group, the resultant nucleoside was synthesized and further utilized to prepare the analogues of deoxyuridine monophosphate (dUMP), which has been shown to be an inhibitor of trypanosomal enzymes. Tosylation of 3′-fluoro-3′-deoxyuridine, followed by treatment with sodium azide generated the azide, which underwent catalytic hydrogenation to afford the desired nucleoside (Scheme **30**).[91]



Scheme (**30**): Synthesis of 5′-amino-3′-fluoro-3′,5′-dideoxyuridine

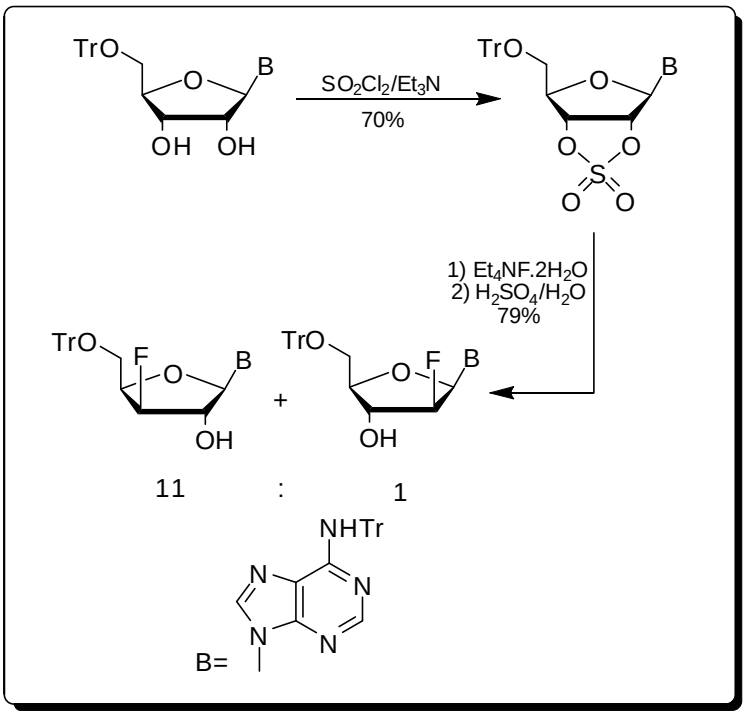
III.3.1.2 Synthesis of 3′β-fluoronucleosides:

Usually, most of the methodologies which were used to synthesize the 3′α-fluoronucleosides could also be successfully utilized for the preparation of the 3′β-fluoro nucleosides. 3′-Deoxy-3′-fluoro-β-D-xylo-furanosides bearing natural heterocyclic bases were synthesized in two traditional ways. The 3′-deoxy-3′-fluoro-β-D-xylo-nucleosides (B = G, C, T)[56,92,93] were prepared through glycosylation of the corresponding fluorinated furanoses, while the 3′-deoxy-3′-fluoro-β-D-xylo-nucleosides[94,95] (B= A, U) were obtained by means of fluorination of pre-modified nucleoside analogue. In addition, starting from the 3′-deoxy-3′-fluoro-β-D-xylo furanosides, the 2′,3′-dideoxy-3′-fluoro-β-D-xylo-furanosides were also obtained through a Barton-type reductive deoxygenation (Scheme **31**).[56]



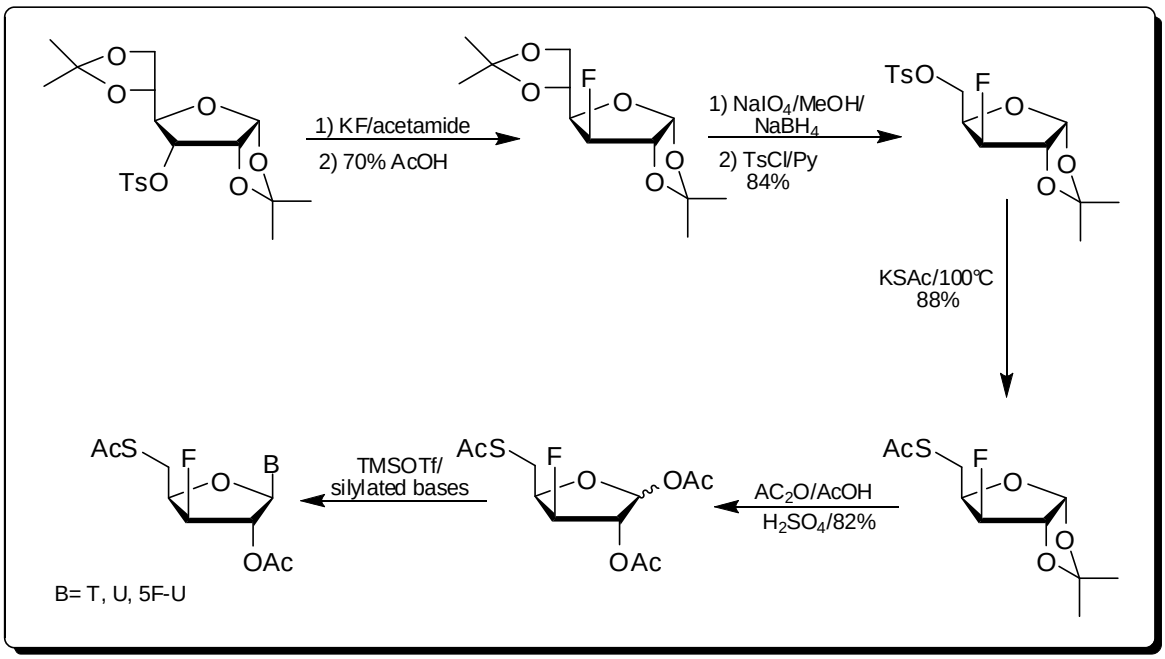
Scheme (**31**): Synthesis of 3′β-fluoro nucleosides

In 2002, Fuentes and co-workers found that nucleophilic opening of nucleoside-derived cyclic sulfates was a regio- and stereo-selective method for preparing 3′β-fluoro nucleoside derivatives. The nucleoside derivative, prepared by tritylation of adenosine, was treated with SO_2Cl_2/Et_3N to provide the cyclic sulfate compound as the sole product. The opening reaction of the cyclic sulfate compound by reaction with tetraethylammonium fluoride dihydrate in acetone gave two regioisomers in a ratio of 11:1. This good regioselectivity was mainly attributed to the larger steric hindrance at position C-2′ rather than at C-3′ (Scheme **32**).[96]



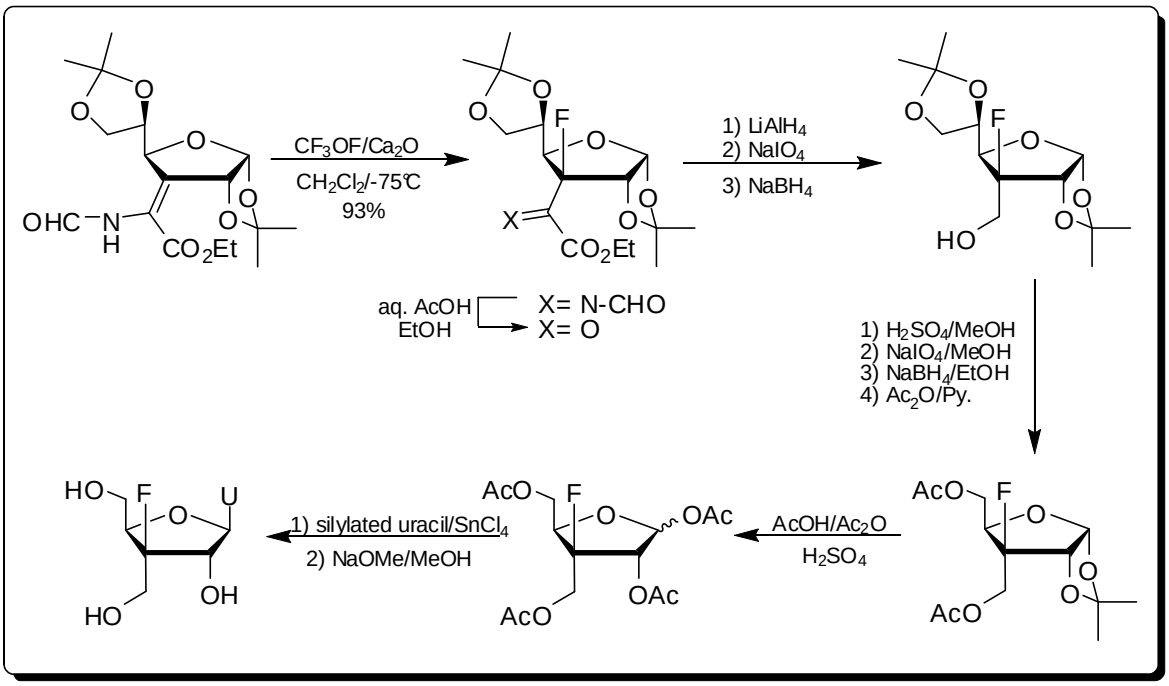
Scheme (**32**): Synthesis of 3′-Deoxy-3′β-fluoro-β-D-xyloadenosine

Recently, an efficient synthesis of 3-fluoro-5-thio-xylo-furanosyl nucleosides was described by Komiotis and co-workers. In their synthesis, the 3′β-fluorine atom was introduced via treatment of the tosylate with KF/acetamide. The resultant fluoride was subjected to periodate oxidation, borohydride reduction and sulfonylation to afford the 3′β-fluoro intermediate in 84% yield. After thioacetation of the product to form 3′β-fluoro-5-thio intermediate and acetolysis, the yielded acetate was condensed with silylated pyrimidine bases to give the target nucleosides. Biological assays demonstrated that the resultant nucleosides were good candidates for the development of potential antiviral agents, as significantly lower concentrations of these agents with respect to AZT were needed to neutralize rotavirus infectivity (Scheme **33**).[97]



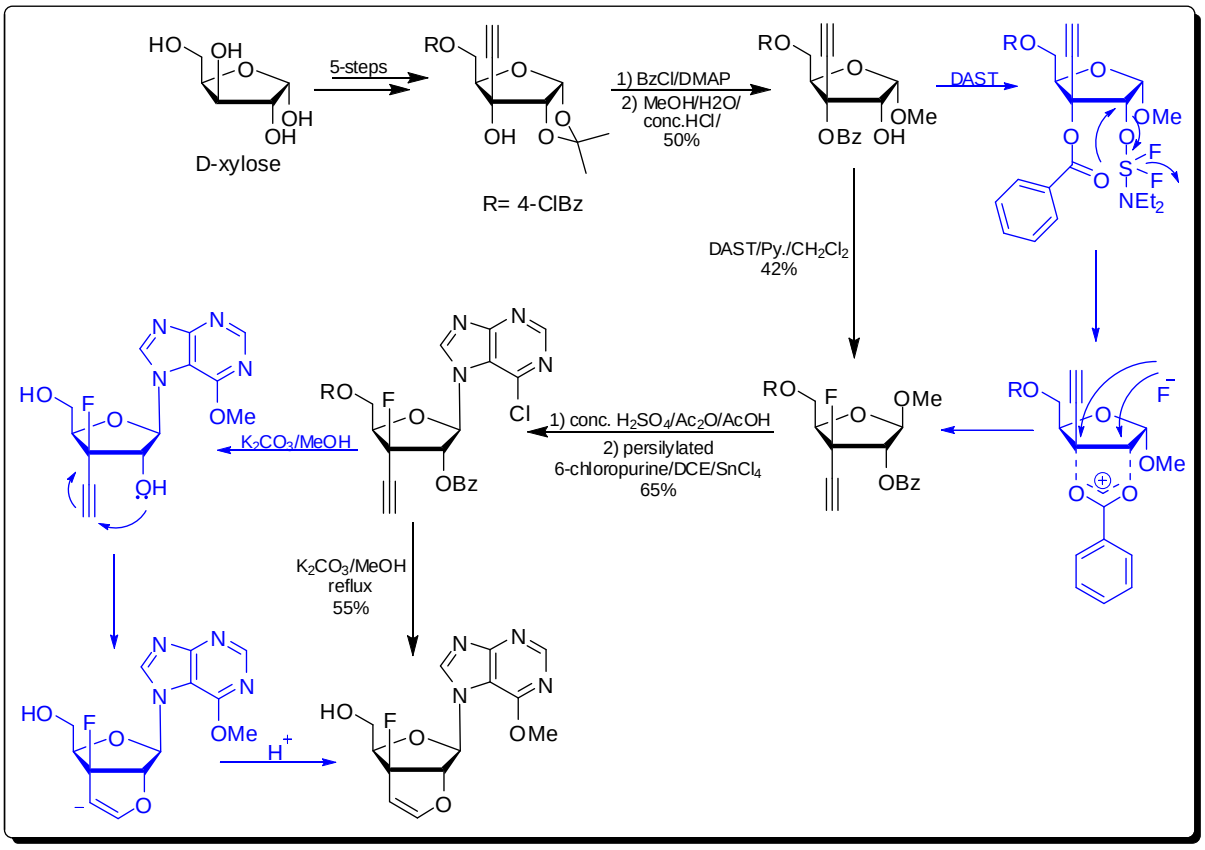
Scheme (**33**): Synthesis of 3-fluoro-5-thio-xylo-furanosyl nucleosides

Starting from the pre-modified α-D-glucofuranose, Brink *et al*.[98] completed the synthesis of 3′-deoxy-3′β-fluoro-3′-C-hydroxymethyl-β-D-uridine(Scheme **34**). Their method highlighted the stereo-specific introduction of fluorine at the branching point of a branched-chain sugar. Exposure of the pre-modified compound to trifluoro(fluoroxy)methane using dry and ethanol-free CH_2Cl_2 as the solvent yielded the fluorinated *N*-formylimine in high yield, which was converted into the more stable oxo-ester by mild hydrolysis. Reaction of oxo-ester intermediate with $LiAlH_4$ followed by glycol cleavage with $NaIO_4$ and further reduction of the resultant aldehyde group with $NaBH_4$ provided the primary alcohol compound. Subjecting to selective removal of the isopropylidene ketal and further usual transformation of the functional groups furnished the acetate intermediate. Hydrolysis of the resultant compound and simultaneous protection of the *in situ* generated hydroxyl groups afforded the anomeric mixture. Glycosylation of the anomeric mixture using the modified Hilbert-Johnson procedure followed by removal of the acetyl groups provided the desired nucleoside and its α isomer.



Scheme (**34**): Synthesis of 3′-deoxy-3′β-fluoro-3′-C-hydroxymethyl-β-D-uridine

Recently, Zhao *et al*.[99] accomplished the synthesis of a 3′-deoxy-3′-fluoro-2′-*O*,3′-C-vinylene-linked bicycle purine nucleoside(Scheme **35**). Their synthesis commenced with D-xylose, which was converted to the 3β-C-ethynyl sugar in five steps according to a reported procedure. Benzoylation of 3-C-vinylene intermediate and subsequent methanolysis with concentrated HCl gave the protected 1-methoxy-3-C-vinylene intermediate. A neighbouring-group-participating fluorination of mediated by DAST provided the 3-fluoro-3-deoxy-3α-C-ethynyl sugar in 42% yield. Acetolysis of the resultant product followed by condensation with persilylated 6-chloropurine furnished the purine derivative. Treatment of resultant compound with K_2CO_3 in MeOH at 65 °C finalized the synthesis of the fluorinated bicyclic nucleoside in 55% yield. Zhao *et al*. proposed that the formation of the bicyclic ring involved an intramolecular cycloaddition induced by fluorine.

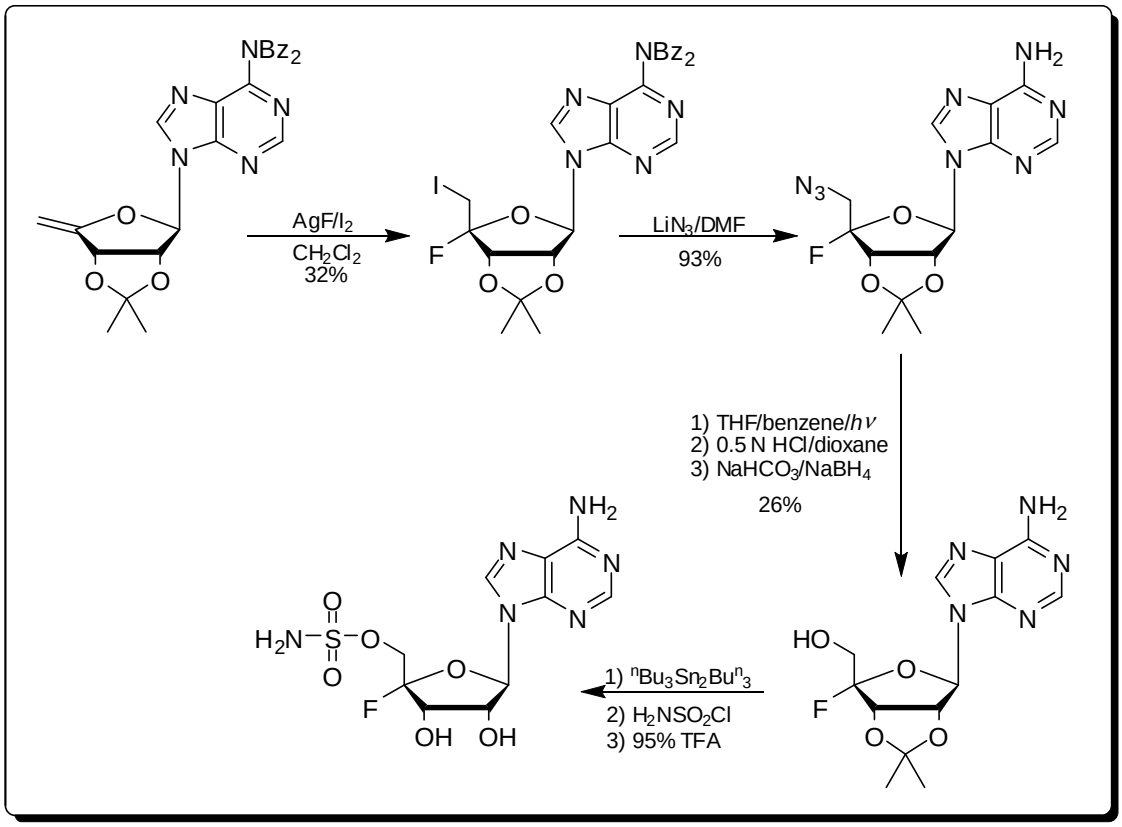


Scheme (**35**): Synthesis of 3′-deoxy-3′-fluoro-2′-O,3′-C-vinylene-linked bicycle-purine nucleoside and proposed mechanism by the authors [99]

III.1.4 4′-monofluorinated nucleosides:

Although the antitrypanosomal antibiotic and potent inhibitor of protein biosynthesis, nucleocidin, was isolated from Streptomyces calvus in 1957, its structure was confirmed by Morton about 10 years later.[41,100] So far, nucleocidin is the only fluorinated nucleoside analogue isolated from a natural organism. In 1976, Moffatt and co-workers first accomplished its synthesis, starting from the protected β-D-erythro-pent-4-enofuranosyladenine derivative in eight steps (Scheme **36**).[101] In their synthesis, reaction of the protected β-D-erythro-pent-4-enofuranosyladenine derivative with IF, generated *in situ* from AgF/I_2, gave the 4′-fluoro-5′-deoxy-5′-iodo nucleoside in 32% yield, which was further subjected to treatment with LiN_3/DMF to furnish the 4′-fluoro-5′-azido-nucleoside analogue in high yield. Conversion of the resultant compound into the 4′-fluoro-2′,3′-*O*-isopropylideneadenosine was fulfilled via photolysis followed by acidic hydrolysis and borohydride reduction. Exposure of 4′-fluoro-2′,3′-*O*-isopropylideneadenosine to $Bu_3Sn_2Bu_3$ and subsequent treatment with an excess of sulfamoyl chloride afforded the 4′-fluoro-5′-*O*-sulfamoyladeosine derivative, which was deprotected with 95% TFA to provide nucleocidin

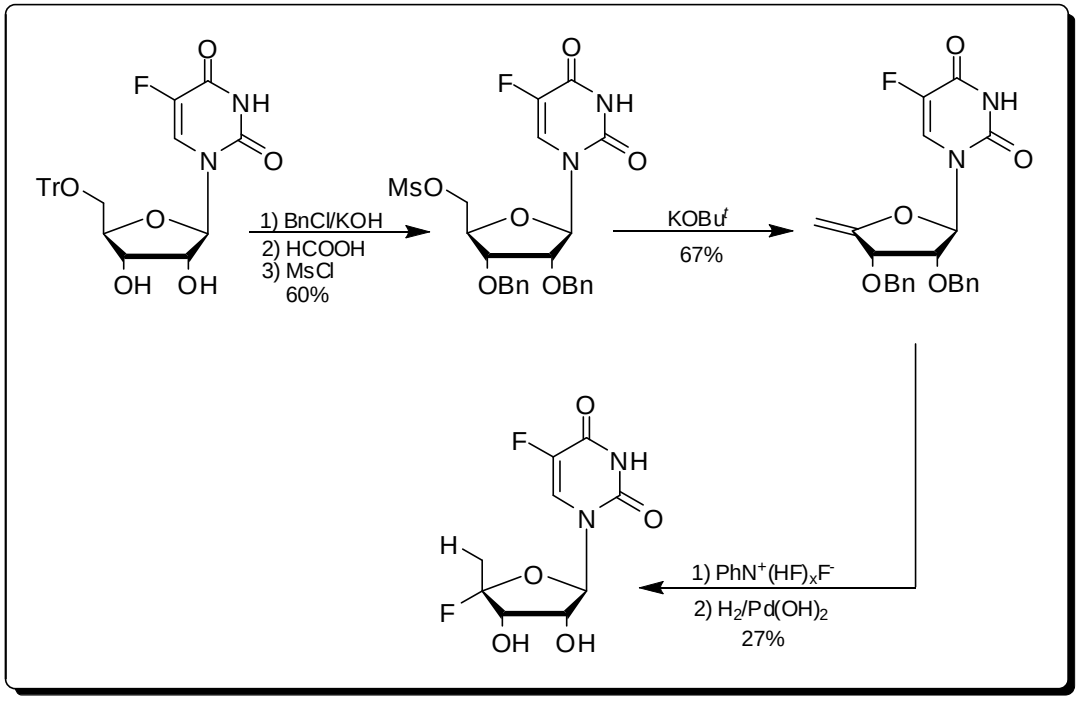
as a monohydrate in 77% yield. In addition, the uracil analogue and its corresponding phosphate ester analogue were also prepared by Moffatt's group using a similar route.[102] It should be noted that, in 1993, Maguire and co-workers modified the procedure of the Moffatt group to synthesize nucleocidin and other nucleocidin analogues.[103]



Scheme (**36**): Synthesis of Nucleocidin according to Moffatt *et al.*

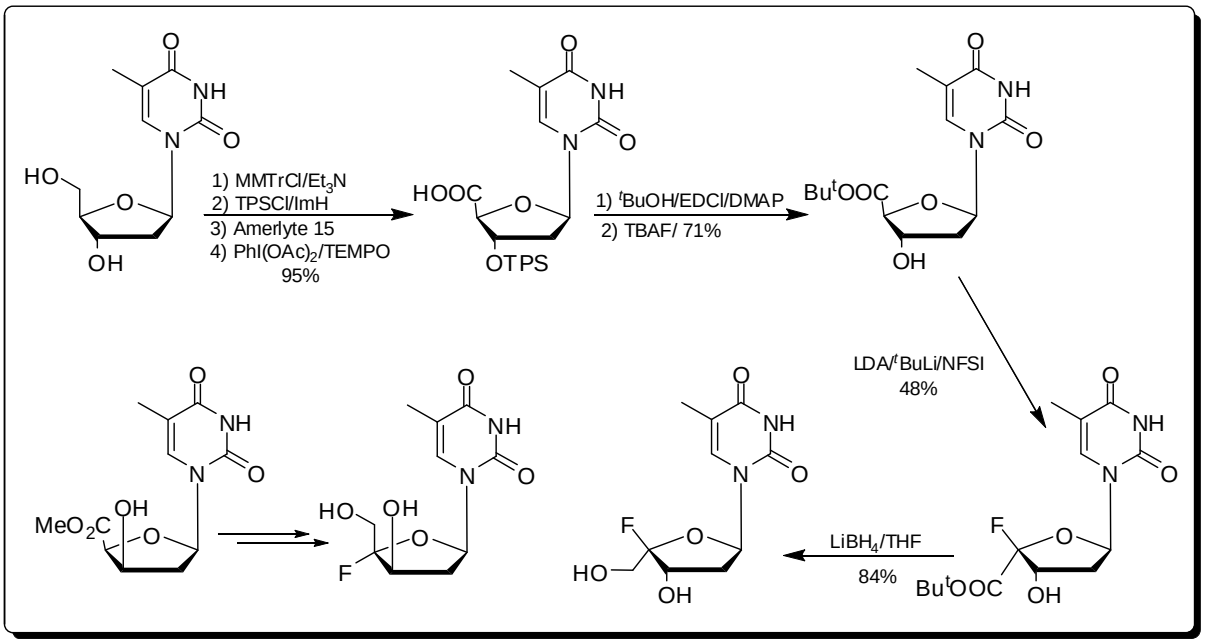
5′-Deoxy-5-fluorouridine (5′-dFUrd) is a prodrug of 5-fluorouracil (FUra) and has antitumour activity superior to that of the parent drug (FUra) in a variety of model tumour systems. However, research has also demonstrated that 5′-dFUrd was still a relatively poor substrate for uridine phosphorylase, which was attributed to the possibility that uridine phosphorylase could not efficiently cleave the glycosidic linkage to release the FUra under acidic conditions. In view of the fact that the fluorine atom at the 4′-position made the glycosidic linkage of the nucleoside unusually acid labile, Danenberg and co-workers synthesized the 5′-deoxy-4′,5-difluorouridine as a new prodrug of FUra. Beginning from 5′-*O*-trityl-5-fluorouridine, the 5′-*O*-methane-sulfonyl derivative was prepared in three steps in 60% yield. Exposure of this compound to KO^{t}Bu in dioxane gave the olefin, which was fluorinated with PhN^{+}(HF)xF^{-} and

subsequently deblocked via catalytic hydrogenation to yield the desired nucleoside (Scheme **37**).[104]



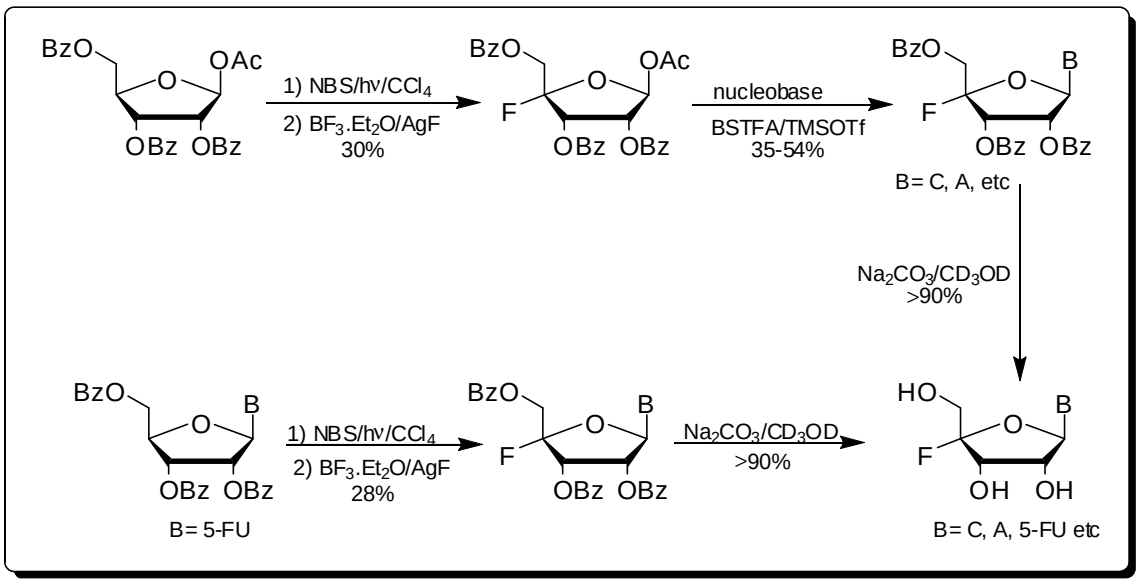
Scheme (**37**): Synthesis of 5′-deoxy-4′,5-difluorouridine

In 2001, Jung and Toyota described the synthesis of 2′-deoxy-4′-fluorothymidine using a novel method to stereoselectively introduce fluorine into the 4′ position. The *tert*-butyl ester of thymidylic acid, obtained from 2′-deoxythymidine in six steps and in 67% overall yield, was subjected to NFSI-mediated electrophilic fluorination to predominately afford the β-fluoro-3′-sulfonate in 48% yield. Reduction of the ester with $LiBH_4$ produced the desired nucleoside in good yield. Utilizing a similar synthetic route, the α-fluoro isomer was also accessed, starting from methyl ester substrate (Scheme **38**).[105]



Scheme (**38**): Synthesis of 2′-deoxy-4′-fluorothymidine according to Jung and Toyota

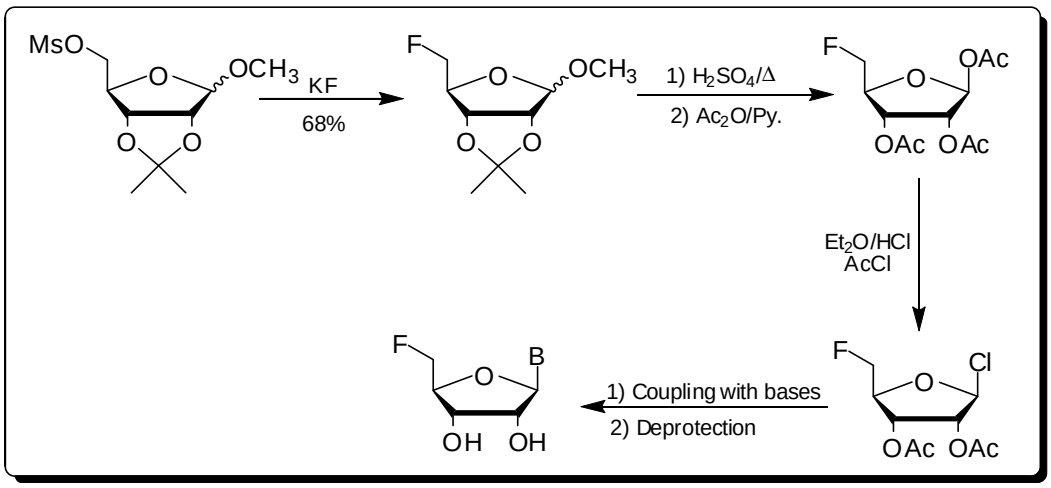
In only two-to-three steps via sequential bromination and fluorination of ribo-furanoses or nucleosides, Verdine's group described a concise synthesis of 4′-fluoro nucleosides in 2007. Their synthesis started with the preparation of 4-fluoro-β-D-ribo-furanose via treatment of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose with NBS under UV lamp followed by direct fluorination of the crude resultant bromide using silver tetrafluoroborate (generated *in situ* from $BF_3.Et_2O$ and AgF). Modified Hilbert-Johnson *N*-glycosylation of furanose with BSTFA and TMSOTf gave the protected 4′-fluoro nucleosides in medium yields. Another alternative approach to 4′-F nucleoside synthesis was achieved through NBS-mediated bromination of 5-FU ribo-furanose followed by fluorination with silver tetrafluoroborate and 2′,3′,5′-tri-*O*-benzoyl-4′,5-difluorouridine was obtained in 28% yield. The final stage of the 4′-F nucleoside synthesis involved the removal of all the benzoyl groups on the sugar moiety via treatment with methanolic Na_2CO_3 (Scheme **39**).[106]



Scheme (**39**): Synthesis of 4′-fluoro nucleosides according to Lee *et al*.

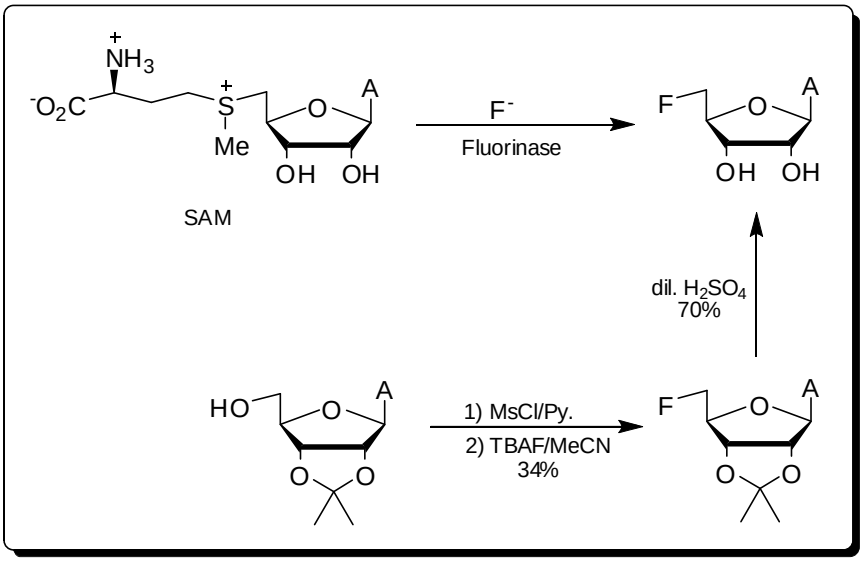
III.1.5 5′-monofluorinated nucleosides:

Nucleosides bearing fluorine(s) at C-5′ were designed and synthesized in order to hamper the possibility of phosphorylation of these nucleosides to the corresponding mono-, di-, and triphosphates in cells. It was then investigated if these 5′-deoxy-5′-fluoro compounds would show any activities, which would not be dependent on their conversion into the corresponding nucleotides. The synthesis of the 5′-deoxy-5′-fluoro-ribo-furanosides was described by Kissman and Weiss in 1958. The introduction of the 5′-fluorine was accomplished via treatment of the mesylate with KF to give the fluoromethyl derivative in 68% yield. Acidic hydrolysis of the resultant compound and subsequent acetylation afforded the triacetate, which was converted into the chloro sugar for nucleoside formation. From the key intermediate, a series of nucleosides were obtained through coupling with different purine bases or pyrimidine bases followed by deblocking.[107]



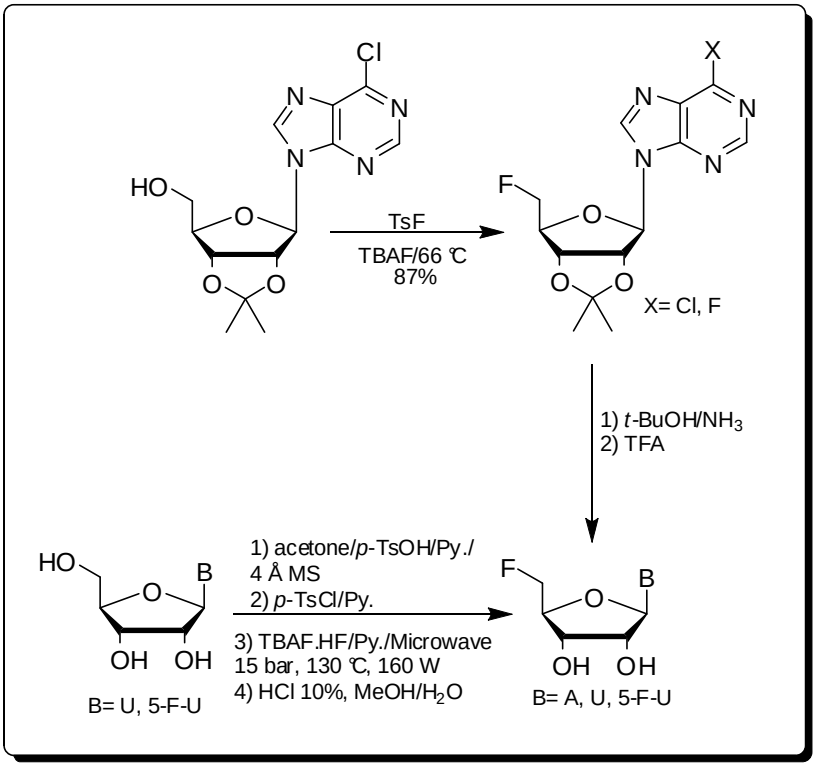
Scheme (**40**): Synthesis of 5′-deoxy-5′-fluoro nucleosides according to Kissman and Weiss

In 2002, O'Hagan and co-workers first identified a fluorinase enzyme from Streptomyces cattleya, which could mediate and catalyze the conversion of fluoride ion and (S)-adenosyl-L-methionine (SAM) into 5′-fluoro-5′-deoxyadenosine (5′-FDA).[108] This discovery of O'Hagan *et al.* opened up a new biotechnological opportunity for the preparation of organofluorine compounds. In the same year, O'Hagan's group also described a simple chemical synthetic route to 5′-FDA in three steps, starting from the ketal-protected adenosine (Scheme **41**).[109] A further study of 5′-FDA as a biosynthetic intermediate during fluorometabolite biosynthesis in S. cattleya was also reported later.[110,111]



Scheme (**41**): Synthesis and bio-synthesis of 5′-FDA

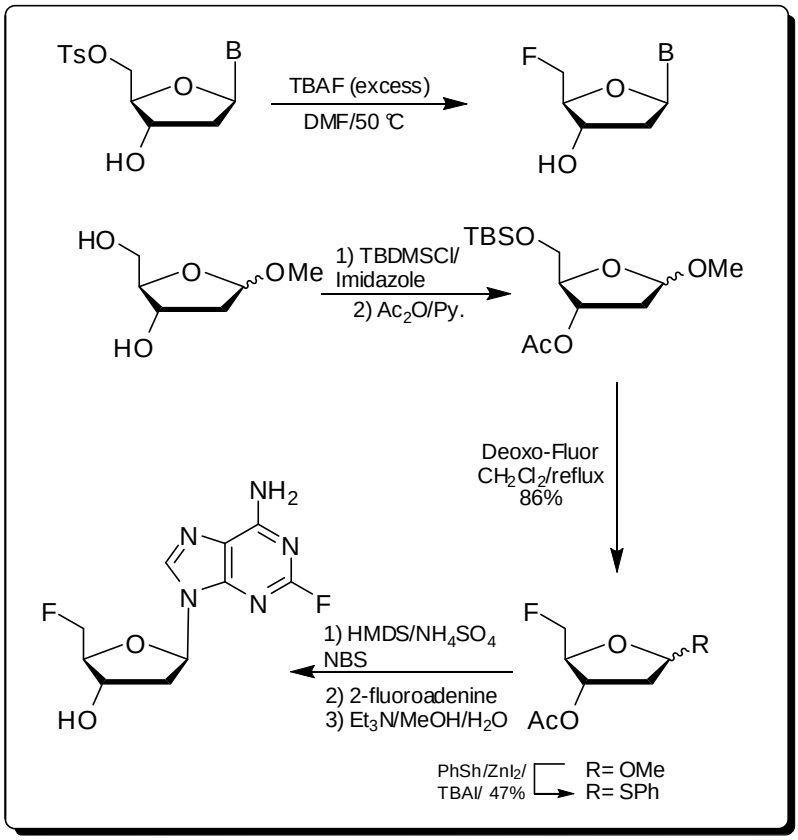
Several years later, an improved synthesis of 5′-FDA was described by Ashton and Scammells.[112] Beginning from the commercially available 6-chloropurine riboside, fluorination was accomplished by treatment with TsF/TBAF, and a mixture of 6-Chloro and 6-Fluoro was formed in 87% yield. Amination of this mixture followed by deprotection with TFA gave 5′-FDA in 78% yield. Compared with the 24% overall yield of O'Hagan's method, their synthesis high-lighted the yield (68% overall yield). In addition, recently Phuoc Le's group[113] utilized a microwave-assisted fluorination approach to obtain 5-deoxy-5-fluoro-substituted uracil nucleoside analogues starting from uridine and 5-Fluorouridine. By the application of microwaves, the reaction time of the fluorination was significantly reduced (Scheme **42**).



Scheme (**42**): An improved synthesis of 5′-FDA

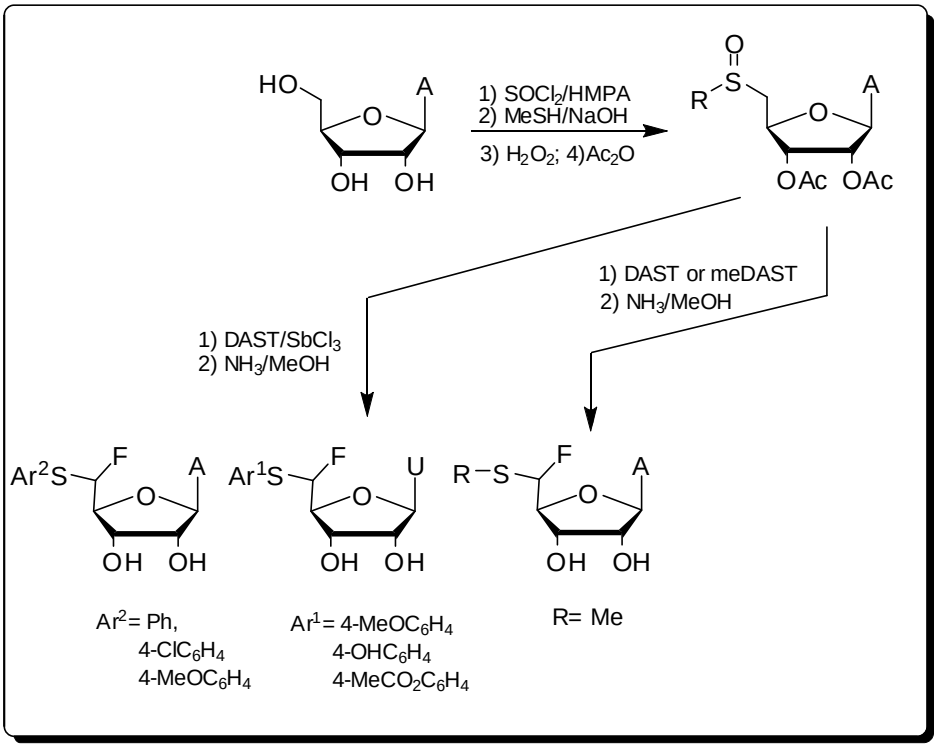
Kowollik and co-workers conveniently addressed the synthesis of 2′,5′-dideoxy-5′-fluoro-ribo-nucleosides by treatment of the corresponding 5′-*O*-tosyl nucleoside derivatives with excess TBAF in DMF at 50 °C.[114] Interestingly, designed as a potent P-site inhibitor of adenylyl cyclase, 2′,5′-dideoxy-2,5′-difluoroadenosine and its α anomer were also synthesized by Kirk's group in 2004.[115] Kirk's synthesis began with the conversion of the methyl 2-deoxy-ribo-furanoside to the 3-*O*-acetyl-5-*O*-(tert-butyldimethylsilyl) derivative in two steps,

and the key steps included the introduction of 5′-fluorine atom through fluorination with Deoxo-Fluor and glycosylation of the phenylthioriboside with 2-fluoroadenine (Scheme **43**).



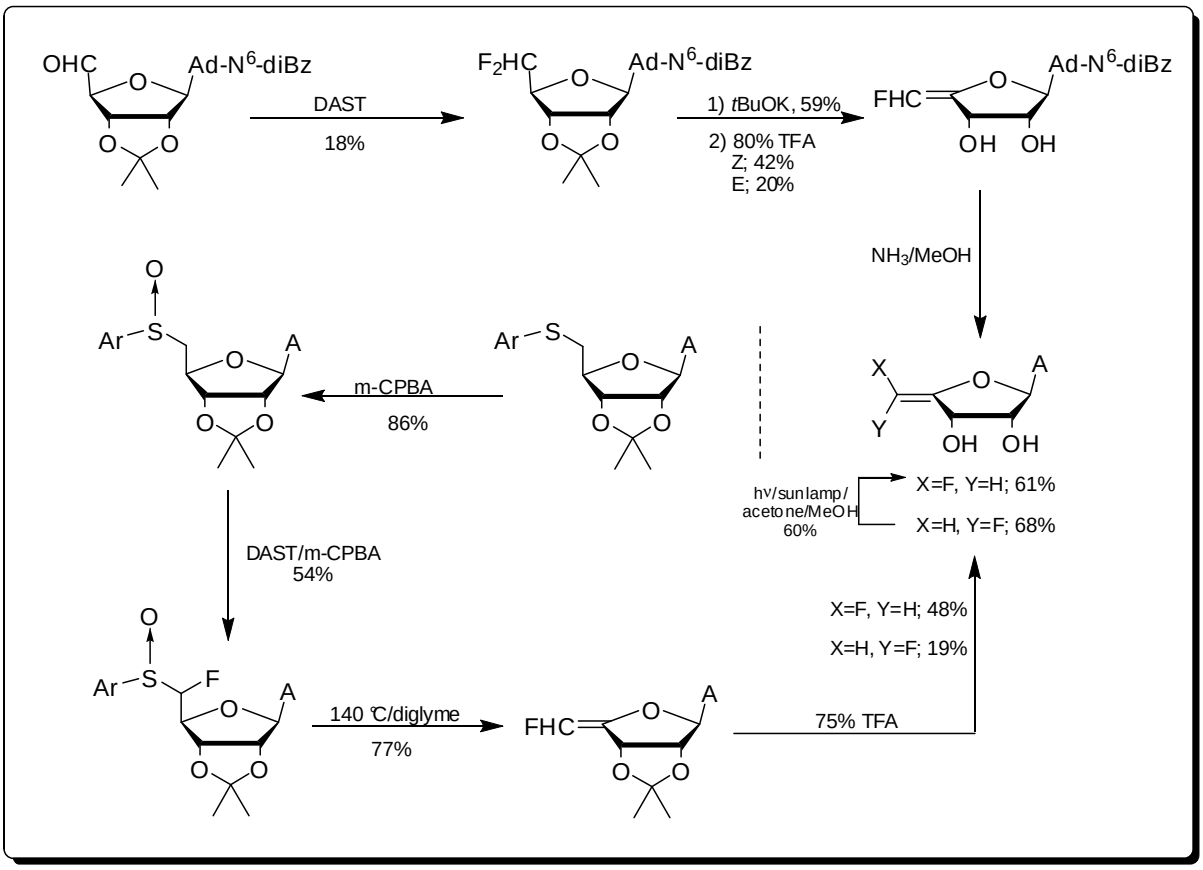
Scheme (**43**): Synthesis 2′,5′-dideoxy-2,5′-difluoroadenosine

Designed as an analogue of 5′-deoxy-5′-(methylthio)adenosine (MTA), which has been demonstrated to be a potent inhibitor of bovine liver AdoHcy hydrolase, 5′-deoxy-5′-fluoro-5′-(methylthio)adenosine was synthesized by Sufrin's group in 1989 and further evaluated for inhibitory activity towards MTA phosphorylase and for its biological effects in L120 and L5178Y murine leukemia cell lines. After a four-step conversion of adenosine into the MTA sulfoxide derivative, fluorination was accomplished through treatment with (meDAST) or DAST to provide the 5′-fluorinated diacetate, which was further subjected to deacetylation with NH_3/MeOH to give the desired nucleoside.[116] Later, using a similar synthetic route, Robins and co-workers[117,118] described an access to other analogues, 5′-aryl-5′-fluoro-5′-thioadenosine and 5′-aryl-5′-fluoro-5′-thiouridines starting from their corresponding thioethers or sulfoxides using DAST/$SbCl_3$ or XeF_2 as the fluorinating reagent.



Scheme (**44**): Synthesis of 5′-fluoro-5′-thio nucleosides

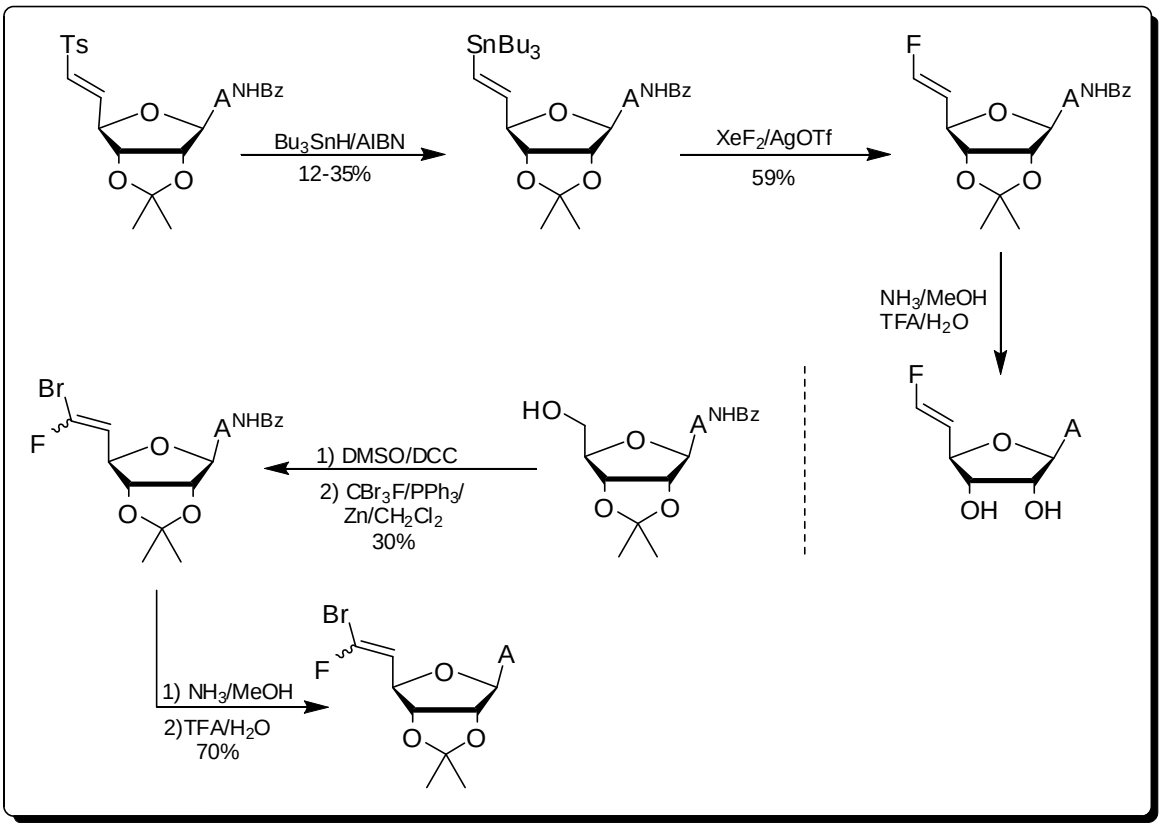
Based on the enzymatic pathway for the conversion of S-adenosyl-L-homocysteine (SAH) into adenosine, McCarthy *et al.* designed, synthesized and biologically evaluated a novel class of mechanism-based inhibitors of SAH hydrolase, the 4′,5′-unsaturated 5′-fluoroadenosine nucleosides. They developed two different routes to address the synthesis of 4′,5′-unsaturated 5′-fluoroadenosine nucleosides. The key steps in the first method included the fluorination of the 2,3-*O*-isopropylideneadenosine-5-aldehyde derivative with DAST in 18% yield and dehydrofluorination of the resultant compound with BuOK in DMSO. Removal of the protecting group with NH_3/MeOH finalized the synthesis of the desired nucleosides. Alternatively, isopropylideneadenosine was converted into the thioether, oxidation with *m*-CPBA and subsequent fluorination produced the α-fluoro sulfoxide in 47% yield, which was further transformed into the desired nucleosides by means of thermolysis followed by deprotection of with TFA (Scheme **45**).[119]



Scheme (**45**): Synthesis of 4′,5′-unsaturated 5′-fluoroadenosine according to McCarthy *et al.*

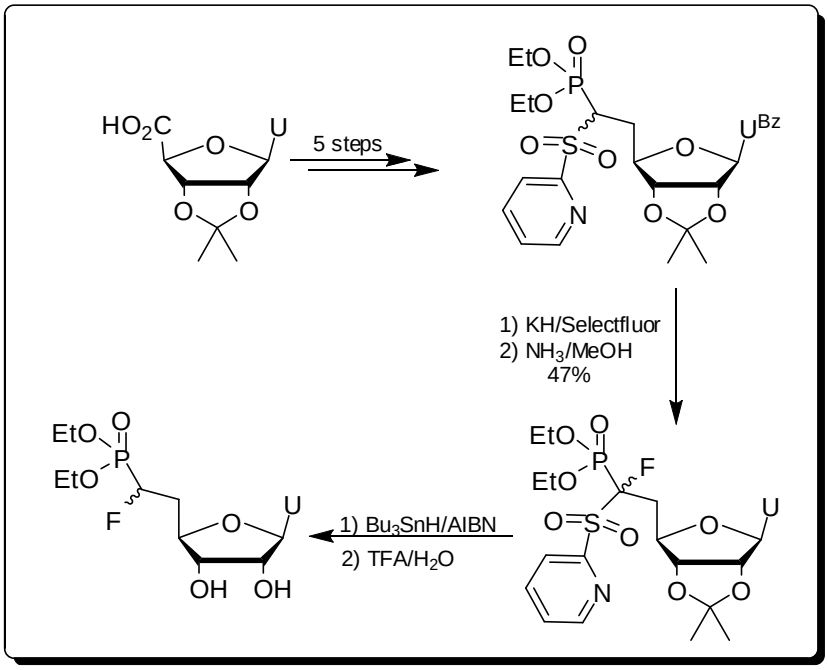
III.1.6 6′-monofluorinated nucleosides:

Designed as potential mechanism-based inhibitors against AdoHcy hydrolase and as antiviral reagents, Robins's group synthesized the 6′-fluorohomovinyl adenosine derivative and the 6′-bromo-6′-fluorohomovinyl adenosine derivative. The synthesis of the 6′-fluorohomovinyl nucleoside began with the conversion of the starting material, 6′-(E)-vinyl sulfone homonucleoside, into the vinyl 6′-stannane via treatment with Bu_3SnH/AIBN. Fluorodestannylation of the compound with XeF_2/AgOTf gave the protected 6′-fluoride, which was further subjected to deprotection to provide the (E)-5′,6′-didehydro-6′-deoxy-6′-fluorohomoadenosine. With the protected adenosine as the starting material, synthesis of the 6′-bromo-6′-fluorohomovinyl nucleoside involved Moffatt oxidation, treatment of the resultant 5′-carboxaldehyde with (bromofluoromethylene)triphenylphosphorane reagent, followed by deprotection to provide the desired nucleoside (Scheme **46**).[120,121]



Scheme (**46**): Synthesis of 6′-fluorohomovinyl adenosine nucleosides

Aside from the 6′-fluorohomovinyl derivative and the 6′-bromo-6′-fluorohomovinyl derivative, Robins and Wnuk also synthesized the 6′-deoxy-6′-fluorohomonucleoside 6′-phosphonate using their developed methodology, which highlighted the stannyl radical-mediated cleavage of π-deficient heterocyclic sulfones. In their synthesis, 2′,3′-*O*-isopropylideneuridine 5′-carboxylic acid was converted into the pyridin-2-yl sulfone in five steps, which was subjected to fluorination with Selectfluor followed by debenzoylation to yield the α-fluoro sulfone phosphonate in 47% yield. Exposure of resultant compound to Bu_3SnH/AIBN caused the cleavage of the sulfonyl linkage, and further removal of the isopropylidene group afforded the target nucleoside phosphonate. Their methodology provided a facile new route for the preparation of α-fluoro-α-[$^{2/3}$H] carbonyl compounds and phosphonates.[122]



Scheme (**47**): Synthesis of 6′-deoxy-6′-fluorohomouridine 6′-phosphonate

IV Conclusion

This literature review showed the importance of the synthesis of fluorinated nucleoside analogues and their applications as antiviral agents. So, we decided to synthesis a new series of nucleoside analogues characterized by the presence of fluorine atom in the positions 3′-β and 2′-α on the sugar moiety. In the second chapter of this manuscript we designed and synthesized a series of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides and evaluated their inhibitory effects on a number of RNA and DNA viruses. Finally, in the third chapter of this manuscript, we synthesized a new series of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino),(N-methoxyimino) and (hydroxylamino)-pyrimidine nucleosides and also evaluated their inhibitory effects on a number of RNA and DNA viruses.

Chapter II

Synthesis of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides

I. Introduction and background of chapter II:

A number of 3′-α-fluorinated ddNs display potent antiviral activity (Figure **16**). 3′-α-Fluoro-2′,3′-dideoxyuridine (FddU) demonstrated anti-HIV activity, and the 3′-α-F substitution of ddG (FLG) has higher anti-HIV activity in comparison to the parent nucleoside ddG in MT-4 and ATH8 cells.[87] FddT (FLT) is one of the most potent inhibitors of HIV reverse transcriptase (HIV-RT). Unfortunately, FLT produces toxic effects. However FddClU demonstrated significant anti-HIV activity with low cytotoxicity in human leukemic cells as well as in bone marrow progenitor cells. FddClU is also active against HIV strains resistant to AZT, ddI, ddC and 3TC. FddG and LFddC have also demonstrated their anti-HBV activity.[123]

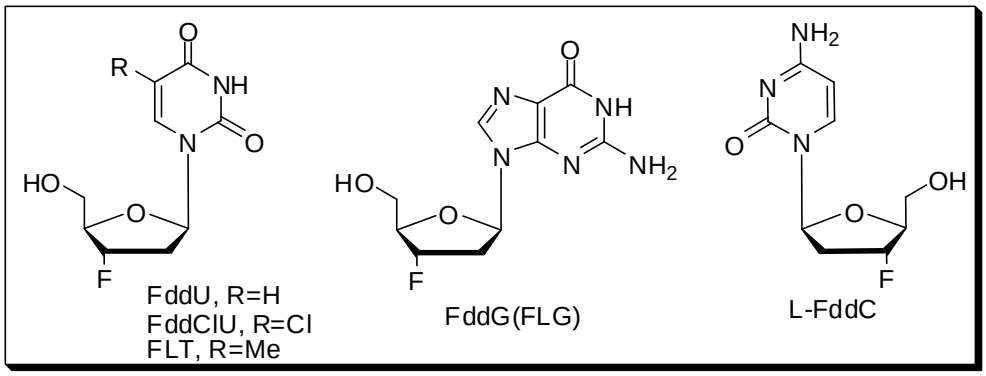
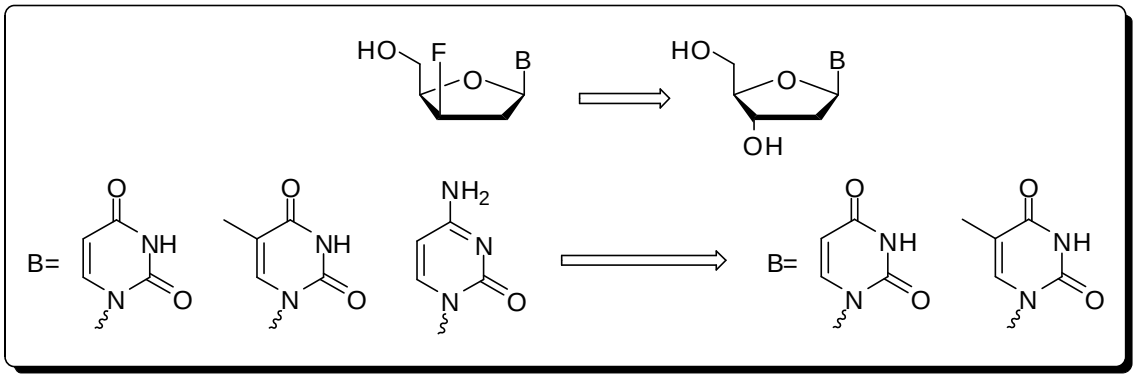


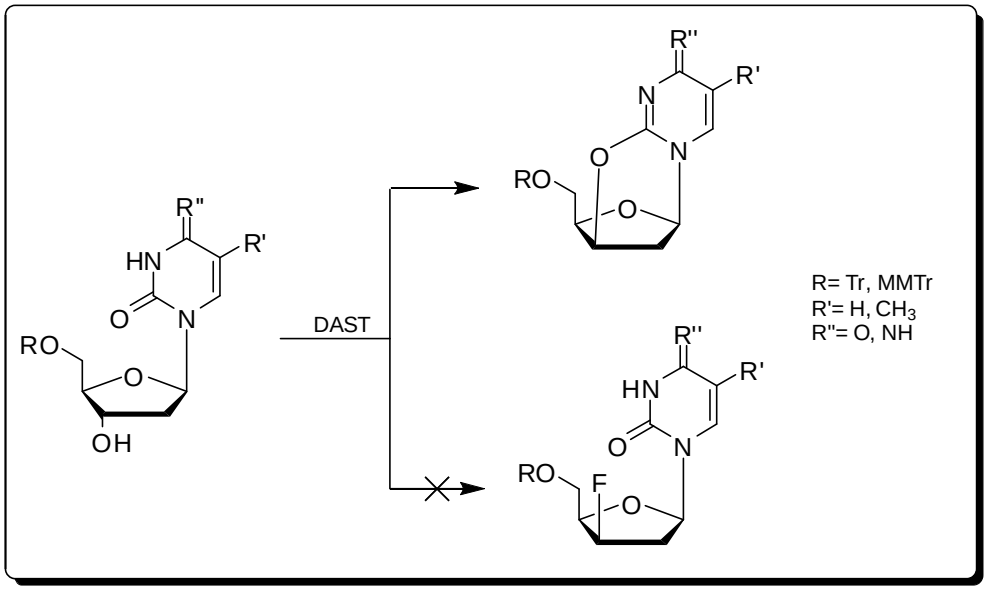
Figure (**16)**: Biologically active 3′-α-fluorinated 2′,3′-dideoxy nucleoside analogues.

Little attention has been paid to the synthesis of 3′-β-fluoro-substituted nucleosides owing to their difficulty and indirect preparation methods. So far, the 3′-β-fluoro-substituted nucleosides have been synthesized through glycosylation reaction between pre-formed 3-deoxy-3-fluoro-D-*xylo*-furanosides with heterocyclic bases or by introduction of a fluorine atom onto 2',3'-anhydro-D-ribofuranonucleosides.[76] The resulting 3-deoxy-3-fluoro-D-*xylo*-nucleosides were finally subjected to a Barton-type reductive deoxygenation to afford the corresponding 2',3'-dideoxy-3'-β-fluoro nucleoside analogues. These methodologies have been used to synthesize the nucleoside derivatives bearing adenine,[124] guanine and thymine[56] as heterocyclic bases in 13~15 steps. In order to have a rapid access to the hitherto unknow derivatives of uracil and cytosine and evaluate their antiviral properties, we envisioned their preparation from the corresponding natural 2'-deoxynucleoside derivatives (Scheme **48**). At this point, after selective protection in 5' position, a direct nucleophilic fluorination reaction using a fluorinating agent such as diethylaminosulfur trifluoride (DAST) would not give the corresponding C-3'-β-fluoro pyrimidine nucleoside.[94]



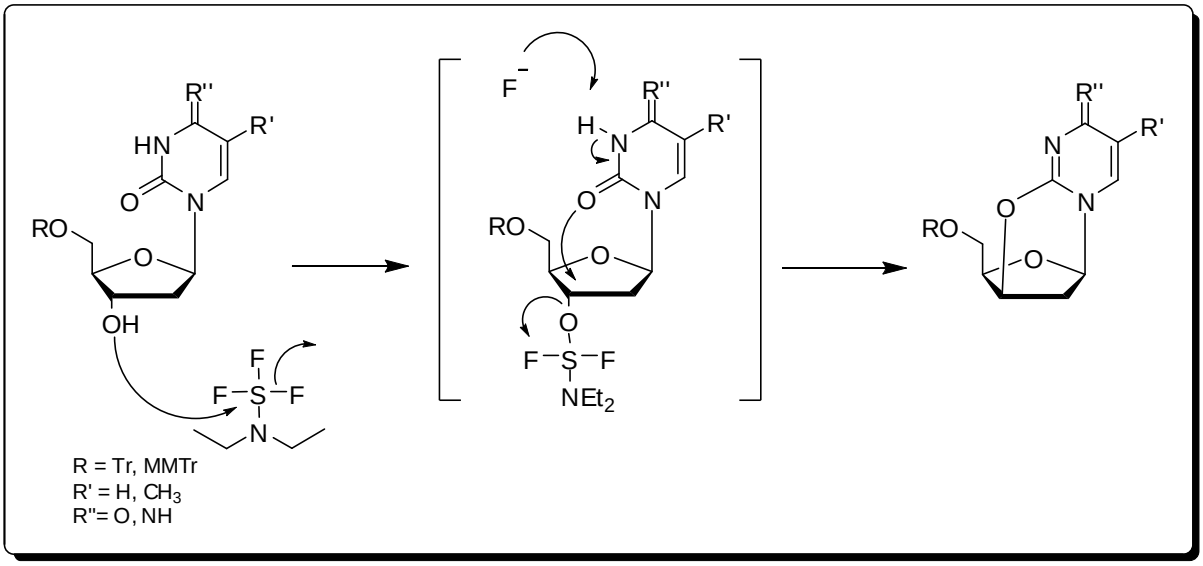
Scheme **(48)**: Synthesis of 3'-β-fluoro pyrimidine nucleosides

Literature review regarding, the methodology of the direct synthesis of the 3′-β-fluoro pyrimidine nucleosides achieved with nucleophilic fluorinating agents, Agyei-Aye *et al*. reported that the reaction of DAST with the appropriate 5'-protected pyrimidine nucleosides proceeded quickly at room temperature to give anhydronucleosides (Scheme **49**).[125]



Scheme **49**: The reaction of pyrimidine nucleosides with DAST.

Indeed, the intramolecular attack of the C-2 carbonyl group of the nucleobase on carbon 3', activated with a good leaving group [C-O-$SF_2(NEt_3)$], on the sugar moiety precedes the attack of a fluorine ion from the β-face of the nucleoside. Closure to the anhydro compounds may be promoted by the presence of F^- ions which could serve as a base to abstract the H-N of the pyrimidine, thus facilitating ring closure (Scheme **50**).



Scheme **50**: Mechanism according to Agyei-Aye *et al*.

In this regard, Serra *et al*. reported the use of nitro group as protective group for the *N*-3 position. The use of such protective electron-withdrawing group prevented the formation of anhydro nucleoside due to the formation of an unstable charged intermediate during nucleophilic substitutions upon 2'-*O*-triflyluridine[126] as well as 2',3'-cyclic sulfate pyrimidine nucleoside (Figure **17**).[127]

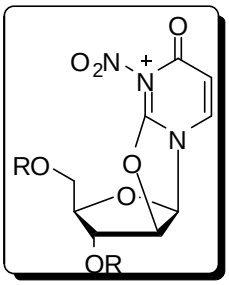


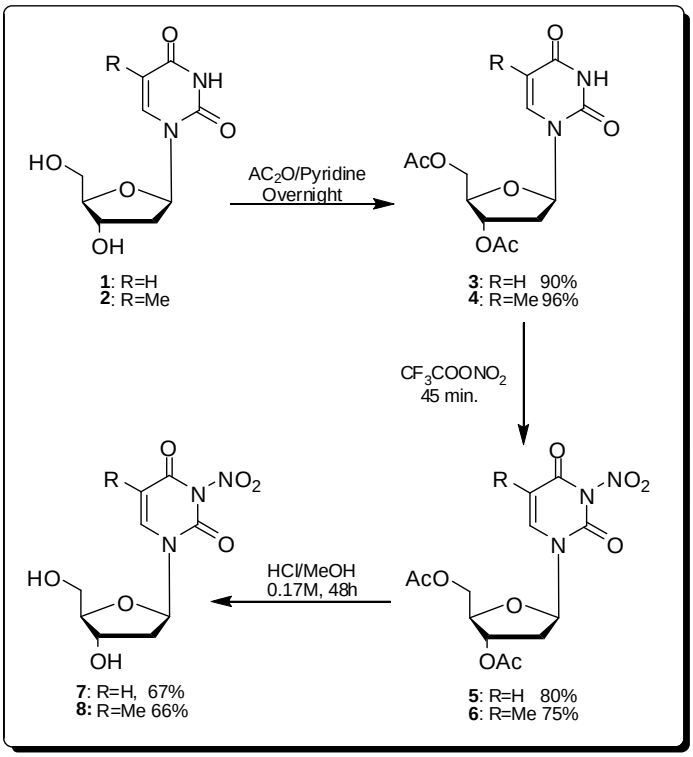
Figure **17**: unstable intermediate.

II The Synthesis:

II.1 Synthesis of 2'-deoxy-3-nitrouridine 7 and 2'-deoxy-3-nitrothymidine 8:

5'-*O*-acetyl-2'-deoxy-3-nitrouridine **9** and 5'-*O*-acetyl-2'-deoxy-3-nitrothymidine **10** were chosen as the key intermediates and were prepared in four steps from 2'-deoxyuridine **1** and 2'-deoxythymidine **2**, respectively. Acetylation[128] of 2'-deoxyuridine **1** and 2'-deoxythymidine **2** with acetic anhydride in pyridine at room temperature for 12 h gave 3',5'-di-*O*-acetyl-2'-deoxyuridine **3** and 3',5'-di-*O*-acetyl-2'-deoxythymidine **4** in 90% and 96% yield, respectively (Scheme **51**).

According to literature data, the *N*-nitration reaction by a treatment with HNO_3 nucleosides undergoes *O*-nitration, oxidation of primary alcohols to acids, and/or nitration at C5 (in the uridine case), depending on the reaction conditions and protecting groups. Additionally experiments on 2',3',5'-tri-*O*-acetyluridine, using either (i) nitronium benzoate (prepared from $AgNO_3$/PhCOCl in CH_3CN and filtering off the precipitate of AgCl), (ii) $Cu(NO_3)_2/Ac_2O$ or (iii) NO_2BF_4 in CH_3CN, at different temperatures, with or without 2,6-di-tert-butyl-4-methylpyridine, were unsuccessful, and starting materiel was mostly recovery.[129]



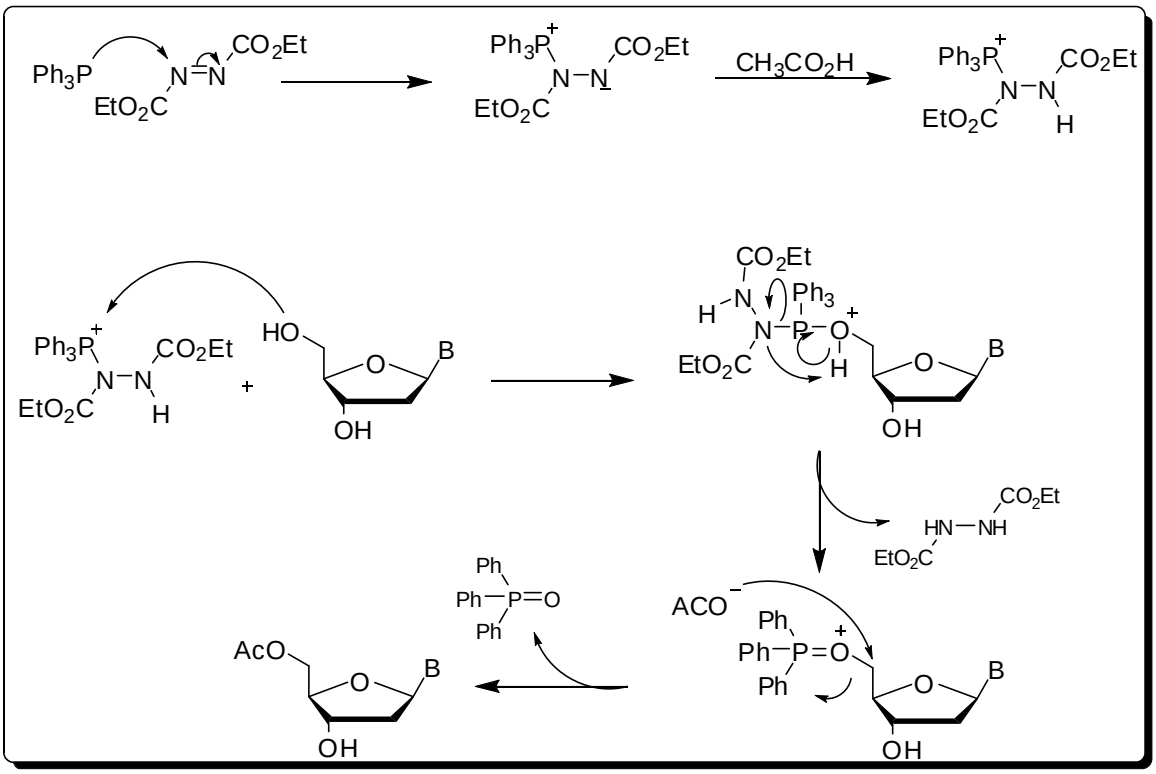
Scheme **51**: Synthesis of 3-nitropyrimidine nucleosides

In our case, the *N*-nitration reaction of 3',5'-di-*O*-acetyl-2'-deoxyuridine **3** and 3',5'-di-*O*-acetyl-2'-deoxythymidine **4** were carried out with nitronium trifluoroacetate in cold dichloromethane, generated from NH_4NO_3 (4 eq.) and $(CF_3CO)_2O$ (8 eq.), and provided the desired 3',5'-di-*O*-acetyl-2'-deoxy-3-nitrouridine **5** and 3',5'-di-*O*-acetyl-2'-deoxy-3-nitrothymidine[130] **6** in 80% and 75% yield, respectively.

The *N*-NO_2 group is stable in acidic media, and the alcohol-protecting groups can be removed by treatment with MeOH/HCl at room temperature. The protected nitro derivatives, **5** and **6**, were treated with HCl (0.17M) in MeOH at room temperature for 48 h and gave 2'-deoxy-3-nitrouridine **7** and 2'-deoxy-3-nitrothymidine[130] **8** in 67% and 66% yield, respectively (Scheme **51**). Disappearance of the NH proton, small shifts to downfield of the H5 and H6 protons, and large shifts to upfield of C2 ($\Delta\delta \approx -5$ ppm) and C4 ($\Delta\delta \approx -8$ ppm) are the main features of the NMR spectra of nitro derivatives in relation to their parent compounds. In the case of uridine, Ariza *et al*.[129] have observed a similar feature of *N*-nitro uridine derivatives in relation to uridine.

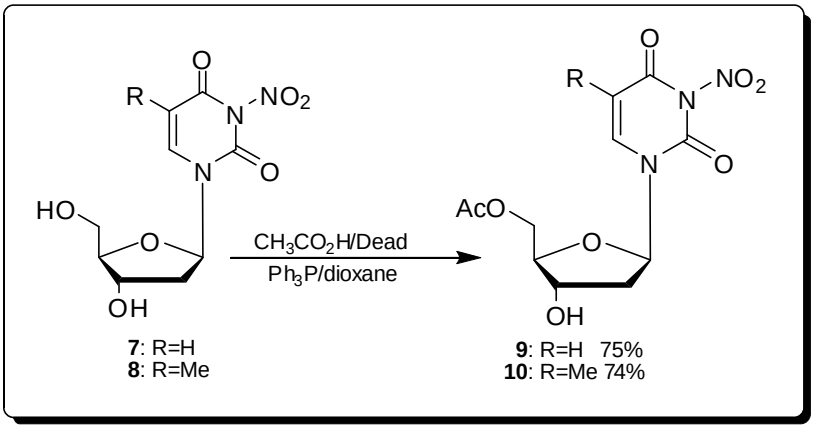
II.2 Synthesis of 5'-*O*-acetyl-2'-deoxy-3-nitrouridine 9 and 5'-*O*-acetyl-2'-deoxy-3-nitrothymidine 10:

Mitsunobu[131] reported that the selective acetylation of 5'-hydroxyl group of thymidine can be achieved in 55% yield by adding a dioxane solution of acetic acid (1 eq.) and diethyl azodicarboxylate (1 eq.) to thymidine and triphenylphosphine (1 eq.) suspention in dioxane. He and Bischofberger modified the procedure by adding the activating agent to the diol containing an excess of acetic acid. Thus, when a dioxane solution of diethyl azodicarboxylate (1.5 eq.) was added to a suspension containing deoxynucleosides, triphenylphosphine (1.5 eq.) in dioxane, the desired products 5'-acetyl deoxynucleosides were obtained in good yields (Scheme **52**). [132]



Scheme **52**: Mechanism according to Mitsunobu reaction.

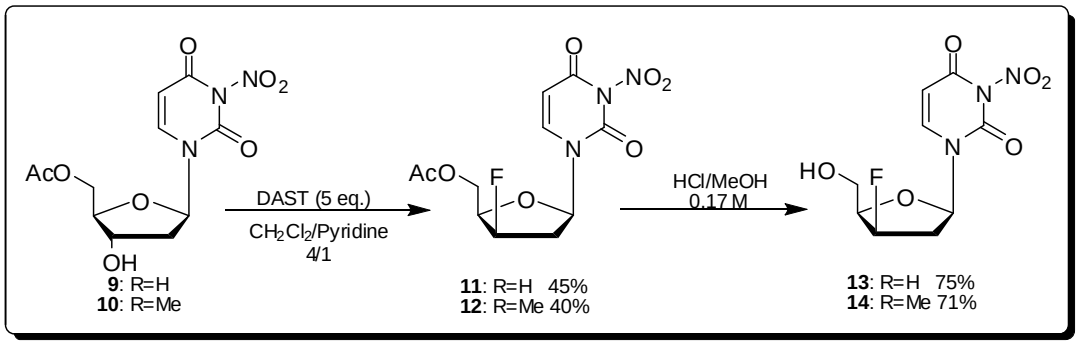
Selective acetylation of 2'-deoxy-3-nitrouridine **7** and 2'-deoxy-3-nitrothymidine **8** were achieved by modified Mitsunobu procedure and gave the key intermediates 5'-*O*-acetyl-2'-deoxy-3-nitrouridine **9** and 5'-*O*-acetyl-2'-deoxy-3-nitrothymidine **10** in 75% and 74% yield, respectively after purification by silica gel chromatography (Scheme **53**).



Scheme **53**: Synthesis according to He and Bischofberger.

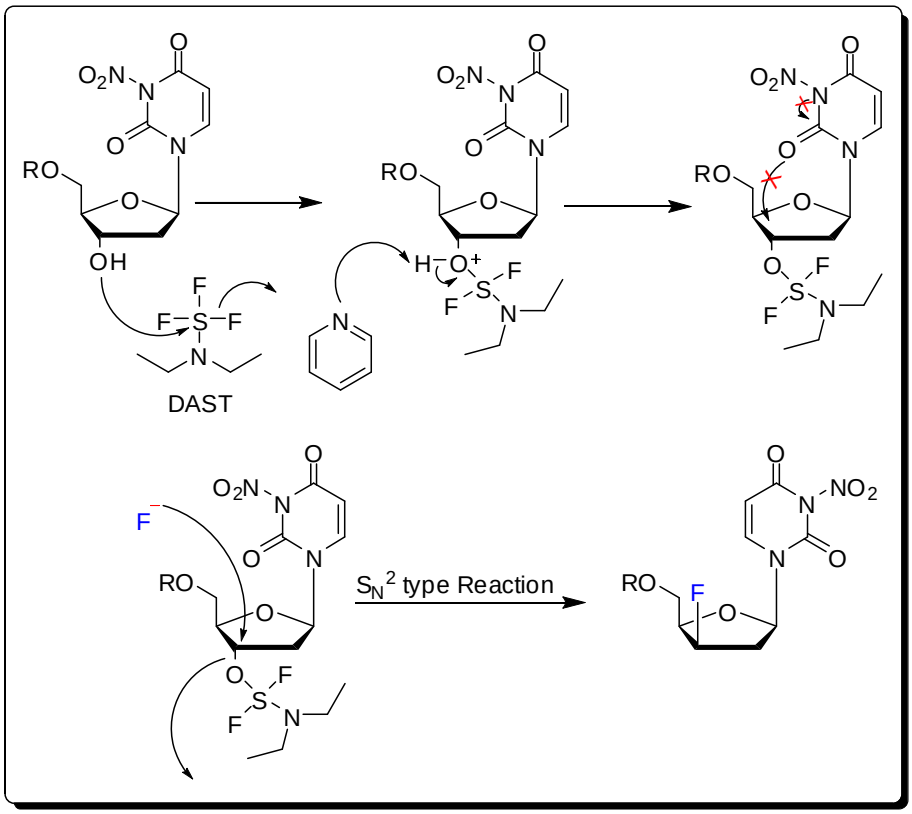
II.3 Synthesis of 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 13 and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 14:

The desired transformation of compounds **9** and **10** to 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil **11** and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine **12** proceeded with inversion of configuration (S_N^2 type reaction) by treating with DAST in dichloromethane/ pyridine in 45% and 40% yield, respectively after purification by silica gel chromatography (Scheme **54**).



Scheme **54**: Fluorination reaction followed by deprotection in acidic media.

Fluorodeoxygenation of 5'-*O*-acetyl-2'-deoxy-3-nitropyrimidine nucleosides with DAST involves the conversion of the hydroxyl function into a good leaving group [alkoxy(diethylamino)sulfur difluoride intermediate (C-O-SF_2NEt_2)] that is displaced by nucleophilic attack (S_N^2 type reaction) by the released fluoride. The fact that reaction occurs with inversion of configuration allows introduction of the C-F bond with stereochemical control and the nitro group avoids the formation of *O*-anhydronucleosides due to unstable charged intermediate (Scheme **55**).

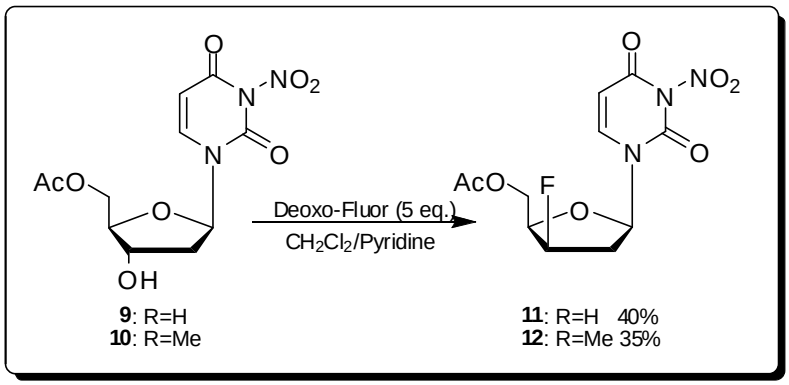


Scheme (**55**): The proposed mechanism of deoxyfluorination of N^3-nitropyrimidine nucleosides by DAST.

Finally, deprotection of the compounds **11** and **12** using HCl (0.17 M) in methanol provided 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil **13** and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine **14** in 75% and 71% yield, respectively (Scheme **54**).

II.4 Yield improvement for 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 11 and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 12:

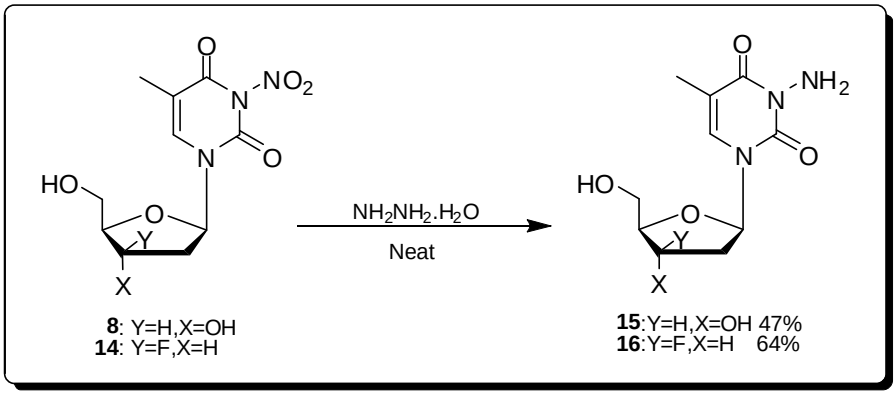
Our attempt to improve the yield of the fluorination reaction with DAST by using Deoxo-Fluor reagent due to thermal stability of Deoxo-Fluor[133] failed. Unfortunately, the yields were decreased 5% for two products. The compounds **9** and **10** were reacted with Deoxo-Fluor reagent (5 eq.) in dichloromethane/pyridine gave the compound **11** and **12** in 40% and 35% yield, respectively (Scheme **56**).



Scheme **56**: Fluorination reaction with Deoxo-Fluor reagent

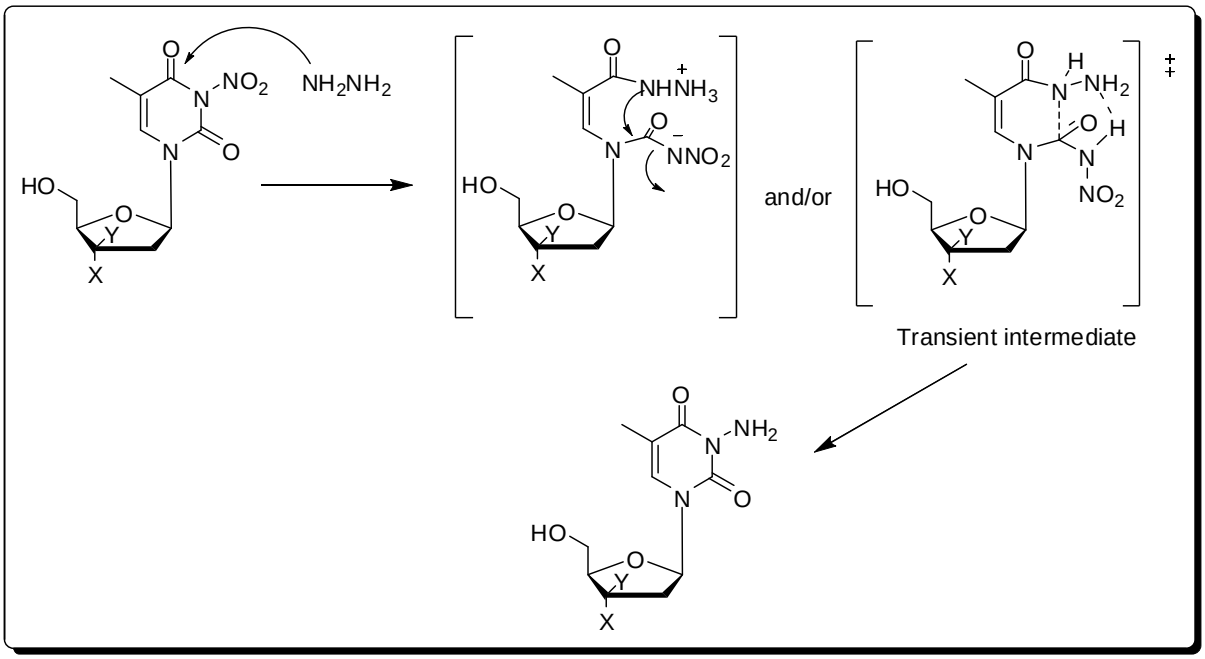
II.5 Ring modification of 2'-deoxy-3-nitrothymidine 8 and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 14 to *N*-NH_2 derivatives 15 and 16:

Having in hands the *N*-NO_2 derivatives **8** and **14**, we decide to modify the base moiety by introduction of NH_2 group instead of NO_2 group in position *N*-3 on the base moiety. It was reported[129] that the substitution of the imidic hydrogen by a nitro group strongly activates the C4-carbonyl group of uridine and thymidine. Activated nucleobases can then be easily modified by treatment with hydrazine to provide N^3-modified nucleosides through a ring-opening/ring closing mechanism. 2'-deoxy-3-nitrothymidine **8** and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine **14** reacted rapidly with hydrazine to give 2'-deoxy-3-aminothymidine[134] **15** and 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-aminothymine **16** in 47% and 64% yield, respectively (Scheme **57**).



Scheme **57**: Ring modification of *N*-NO_2 to *N*-NH_2.

Apparently, once the ring opening occurs, the cyclization toward the six membered ring is favored despite the fact that the nitrogen (N_a) in the CO-N_aH-N_bH$_2$ moiety should be better nucleophile than the (N_b) nitrogen. Bearing in mind that no additives are needed to facilitate the closing step, it is also plausible that the nitrogen (N_b) of the intermediate is largely protonated and/or that transient species participate in the cyclization step (Scheme **58**).

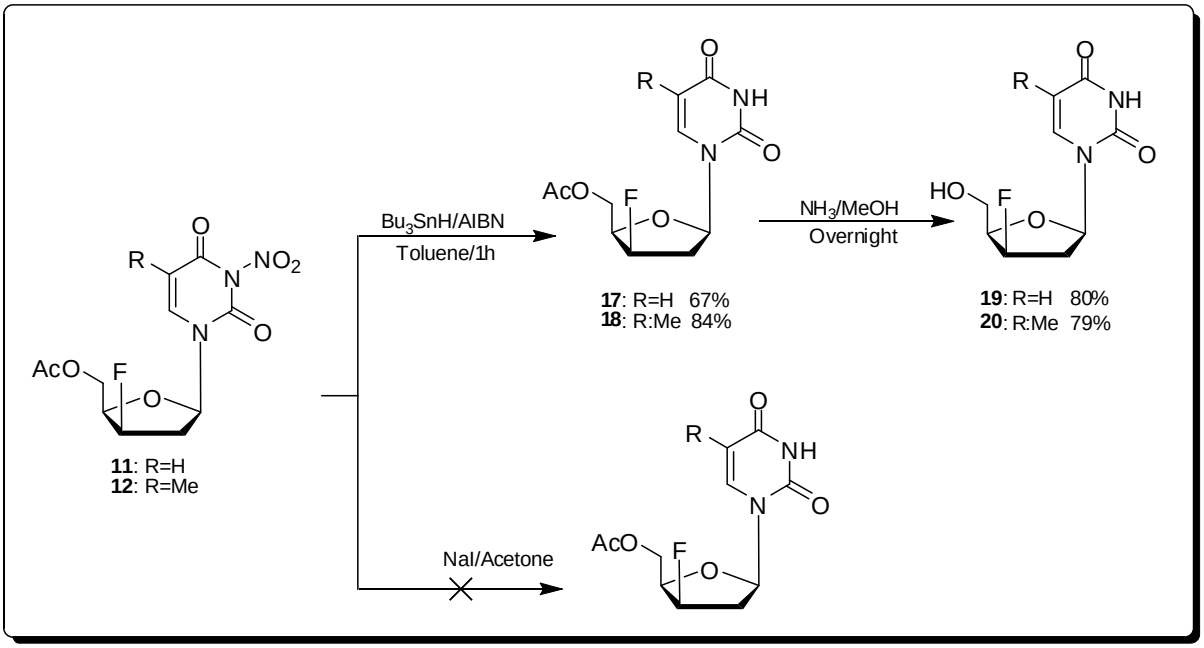


Scheme **58**: Mechanism according to Ariza et al.

II.6 Synthesis of 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil 19 and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine 20:

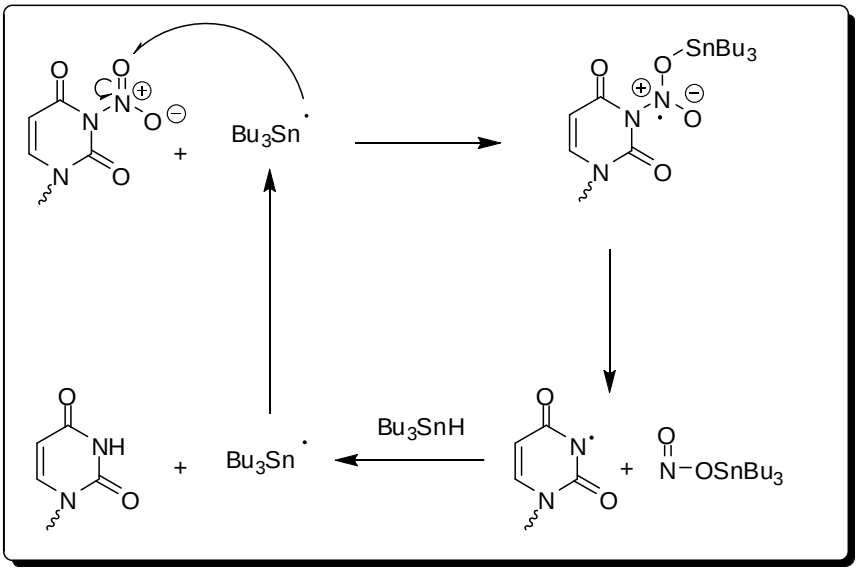
The denitration of *N*-NO$_2$ according to Serra *et al.*[127] using NaI/acetone failed. However, Imrie[135] reported that the denitration of *N*-nitroamines was achieved with tributyltin hydride in benzene or toluene in the presence of a radical initiator (e.g. AIBN). Thus, 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil **11** and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine **12** were reacted with Bu_3SnH/AIBN in toluene at 100°C for 1 h to give 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil **17** and 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine **18** in 67% and 84% yield, respectively. Deprotection of acetyl group in NH_3/MeOH led to 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil **19** and 1-(2,3-

dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine **20** in 80% and 79% yield, respectively (Scheme **59**).



Scheme **59**: Denitration according to Imrie followed by deprotection.

The initial reaction of the tributyltin radicals with compounds **11** and **12** is likely to involve addition to an oxygen atom of the nitro group to give the nitroxide radical. Subsequent decomposition of nitroxide radical to aminyl radicals followed by hydrogen abstraction from tributyltin hydride to provide denitration of the heterocyclic base (Scheme **60**).



Scheme **60**: Radical Mechanism according to Imrie.

II.7 Structure elucidation of compounds 19 & 20

The stereochemical assignment of **19** was studied by NOE correlations and NOESY as well as examination of the coupling constant $J_{H\text{-}H}$ and $J_{H\text{-}F}$. The NOESY spectrum of **19** gave enhancement between H-6 and H-5', H-6 and H-2' but no enhancement between H-6 and H-3' (Figure **18**). However, the results of NOE effects didn't show significant information about the position of the fluorine atom on the sugar moiety.

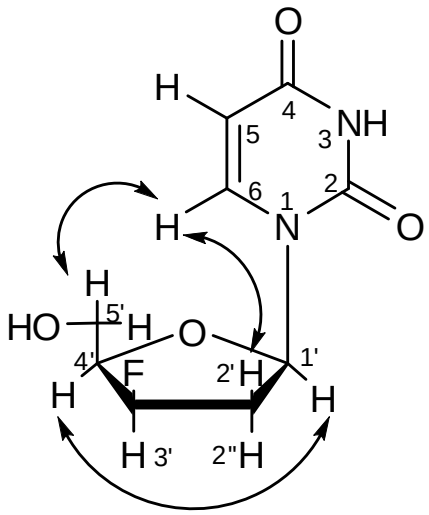


Figure (**18**): NOE correlations from NOESY spectrum of **19**.

In order to identify the position of the fluorine atom, we also analysed the coupling constants between $^{1}H\text{-}^{19}F$ and $^{1}H\text{-}^{1}H$ of **19**. Table **4** shows the $^{1}H\text{-}^{19}F$ and $^{1}H\text{-}^{1}H$ coupling constants of **19**, and the coupling constants of the H-2'/2"adjacent to the fluorine atom.

Table **(4)**: The $^{1}H\text{-}^{19}F$ and $^{1}H\text{-}^{1}H$ coupling constants of **19**

^{1}H NMR					
J (Hz)	H-3'	*J* (Hz)	H-2"	*J* (Hz)	H-2'
$J_{3'\text{-}F}$	54.3	$J_{2''\text{-}F}$	42.8	$J_{2'\text{-}F}$	28.0
$J_{3'\text{-}2''}$	4.2	$J_{2''\text{-}2'}$	16.4	$J_{2'\text{-}2''}$	16.4
$J_{3'\text{-}4'}$	2.4	$J_{2''\text{-}1'}$	8.0	$J_{2'\text{-}1'}$	2.3
		$J_{2''\text{-}3'}$	4.2		

The ^{19}F-^{1}H coupling constants and the splitting pattern of **19** are shown in table **5** and figure **19**, which reveals the coupling constant ^{19}F-^{1}H, with the splitting pattern doublet of doublets of doublets of doublets.

Table (**5**): the ^{19}F-^{1}H coupling constants of **19**

^{19}F NMR (dddd, -190.5 ppm)			
J	(Hz)	*J*	(Hz)
$J_{F\text{-}3'}$	54.7	$J_{F\text{-}4'}$	30.0
$J_{F\text{-}2''}$	43.0	$J_{F\text{-}2'}$	25.4

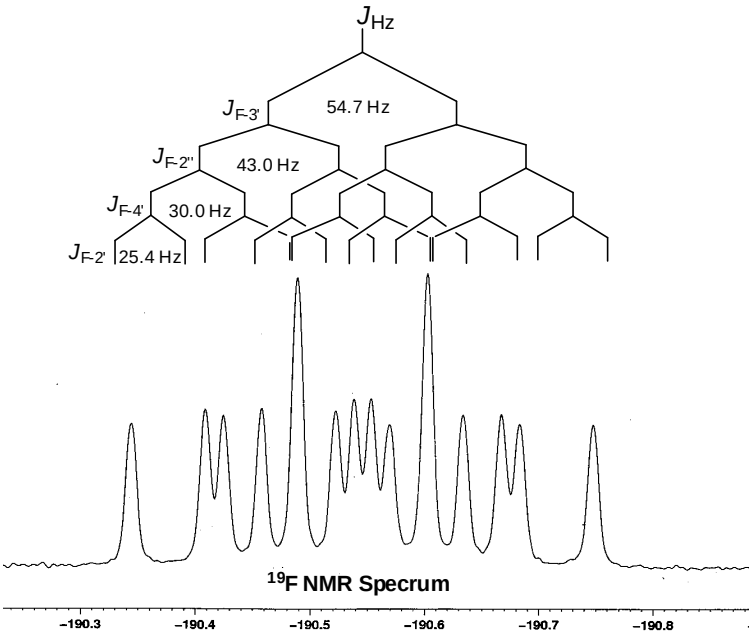


Figure **(19)**: the coupling constants and splitting pattern of **19.**

Indeed, the coupling constants ^{1}H-^{19}F and ^{1}H-^{1}H of **19** may be useful for assignment and conformation purposes since the magnitude of these heteronuclear coupling constants follow a Karplus-type relation as shown in figure **20**.

According to Karplus type relation, the coupling constant reaches a maximum when the dihedral angle is 180°, and reaches a minimum when the dihedral angle is 90°. In our case, the coupling constant H-2″-^{19}F ($J_{2''\text{-}F}$ = 42.8 Hz), (Table **4**), and ^{19}F-H-2″ ($J_{F\text{-}2''}$ = 43.0 Hz), (Table **5**), more higher than the coupling constant H-2′-^{19}F ($J_{2'\text{-}F}$ = 28.0 Hz), (Table **4**), and ^{19}F-H-2′ ($J_{F\text{-}2'}$ = 25.4 Hz), (Table **5**), is owing to the dihedral angle between H-2″ and the fluorine atom

around 180°, and between H-2′ and the fluorine atom around 60°, when the fluorine atom in the position β (Figure **20**).

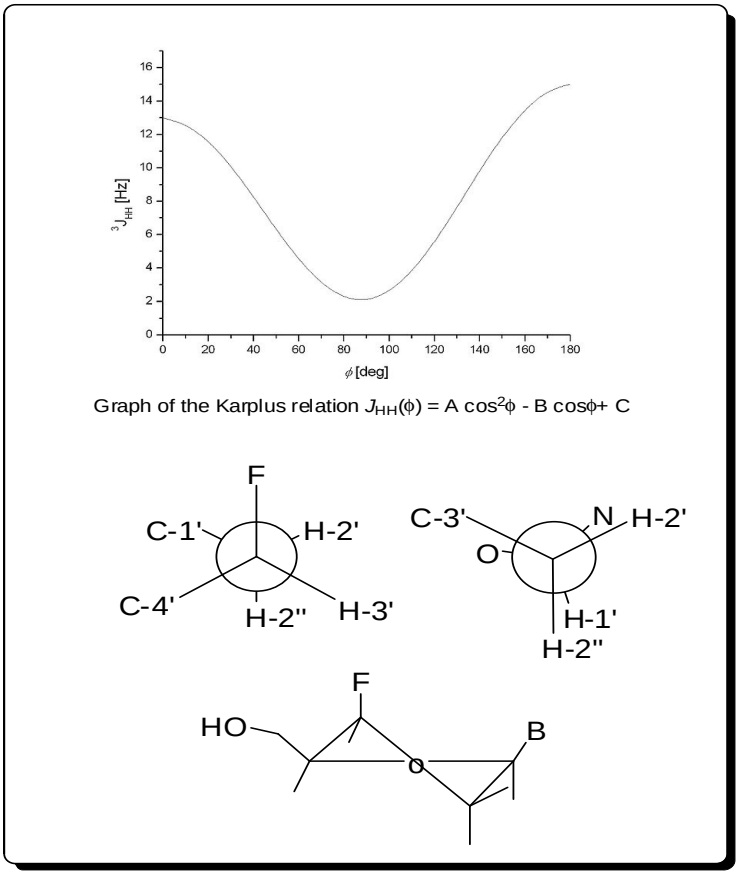


Figure (**20**): Karplus type relation

In agreement with these results, and the correlation between the coupling constants and the Karplus type relation analysis in the compound **19**, the fluorine atom is probably at the β face on the sugar moiety.

Finally, the structure of the compound **19** was unambiguously confirmed by X-ray crystallography and revealed the expected 3'-β-fluoro position as well as a 2'-exo/3'-endo (northern) puckering (Figure **21**).

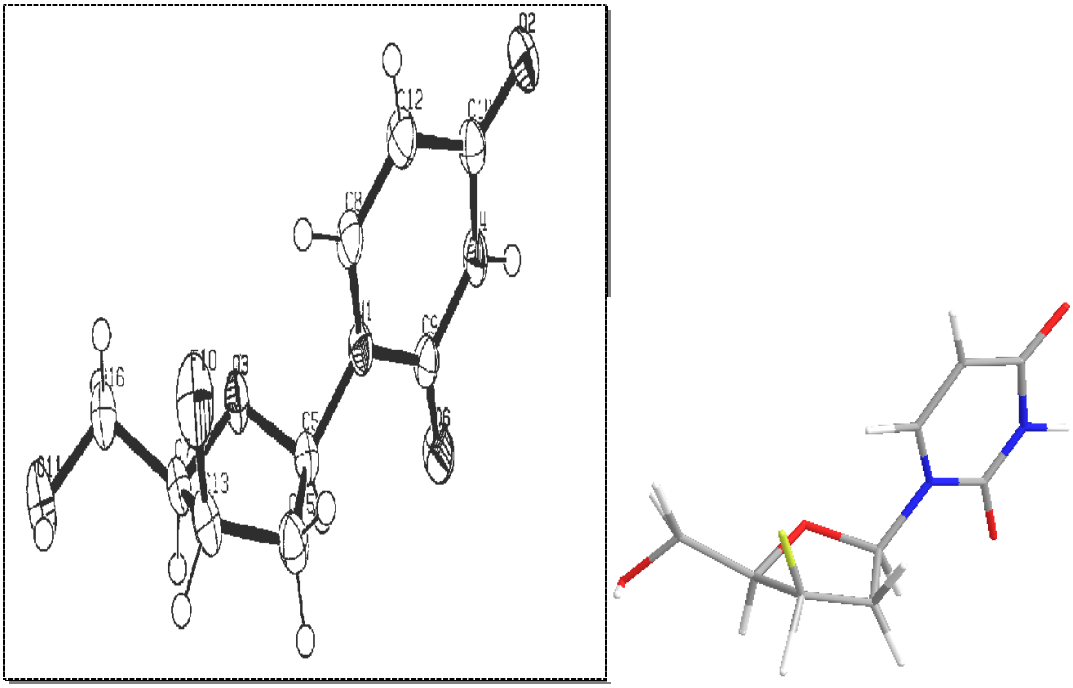


Figure **21**: X-ray crystallography of compound **19**.

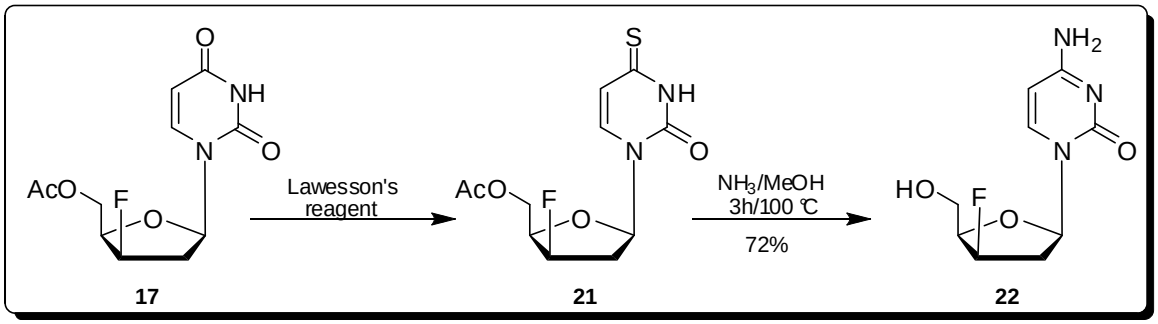
Additionally, the physicochemical properties of compound **20** were identical to those previously reported by Gosselin *et al.*[56]. Table **(6)** showed the coupling constant (*J*) between H and F in ^{1}H NMR and ^{19}F NMR of **20** that we synthesized by our methodology and the compound previously prepared by Gosselin *et al.*[56]

Table **(6)**: the coupling constants between H and F of compound **20**

J (Hz)	^{1}H NMR		*J* (Hz)	^{19}F NMR	
	Compound **20**	[Lit.][56]		Compound **20**	[Lit.][56]
$J_{3'\text{-}F}$	54.6	55.3	$J_{F\text{-}3'}$	54.6	54.7
$J_{3'\text{-}2''}$	4.5	4.5	$J_{F\text{-}2''}$	42.4	42.1
$J_{3'\text{-}4'}$	2.4	2.4	$J_{F\text{-}4'}$	30.8	29.8
$J_{2'\text{-}F}$	25.2	28.7	$J_{F\text{-}2'}$	25.1	25.1
$J_{2'\text{-}2''}$	15.9	16.0			
$J_{2'\text{-}1'}$	2.7	2.7			

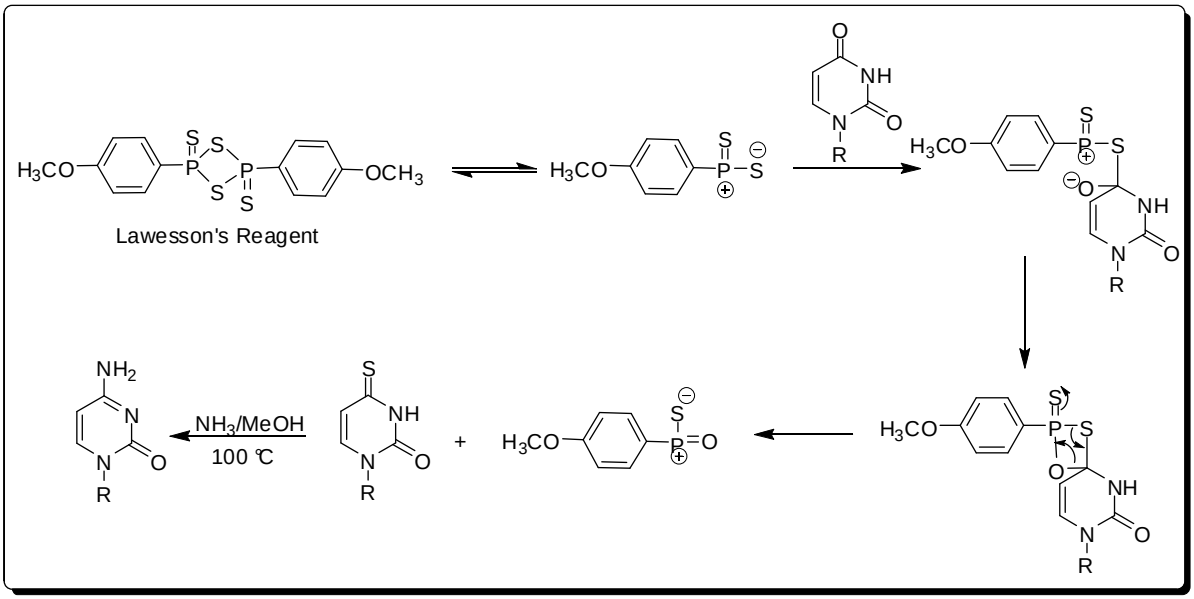
II.8 Synthesis of 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)cytosine 22:

Conversion of 1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil **17** to 1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)cytosine **22** was carried out via a treatment with Lawesson's reagent[136,137], followed by the treatment of 4-thioamide intermediate **21** with methanolic ammonia at 100 °C in stainless-steel bomb (Scheme **61**).



Scheme **61**: Ring transformation with Lawesson's reagent followed by aminolysis

The usual method of thionation is performed in refluxing 1,2-dichloroethane, in which the mechanism of Lawesson's reagent was suggested to involve dissociation equilibrium, which yields a highly reactive aryldithiophosphine ylide. This ylide can then react with carbonyl function group to form thiobetaine, which decomposes to the corresponding thioketone (Scheme **62**).



Scheme **62**: Mechanism of Lawesson's reaction

III. Biological Evaluation:

The nucleoside analogues **7, 8, 13, 14, 15, 16, 19, 20** and **22**, which were obtained during this chapter, were evaluated in laboratory:

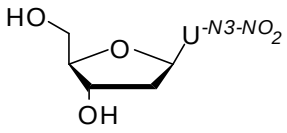
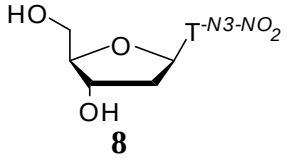
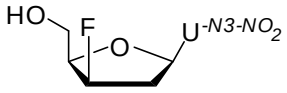
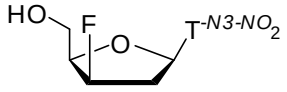
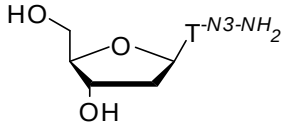
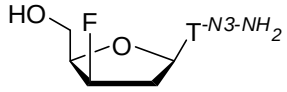
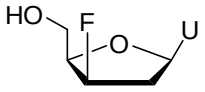
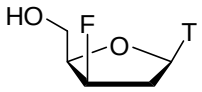
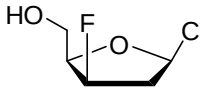
- **Prof. Jan Balzarini, Rega Institute, Leuven, Belgium.**

The activity and cytotoxicity were evaluated against the following viruses in cell cultures:

- Anti-HIV-1 and -HIV-2 in human T-lymphocyte (CEM) cells.
- Varicella-zoster virus (VZV) in human embryonic lung (HEL) cells.
- Herpes simplex, Vaccinia and Vesicular stomatitis viruses in HEL cell culture.
- Cytomegalovirus in human embryonic lung (HEL) cells.
- Feline Corona Virus (FIPV) and Feline Herpes in CRFK cell culture.
- Anti-influenza virus activity and cytotoxicity in MDCK cell cultures.
- Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus in HeLa cell cultures.
- Para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures.

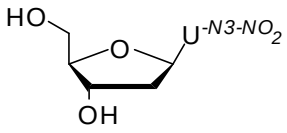
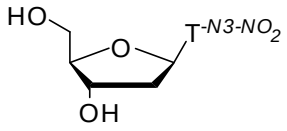
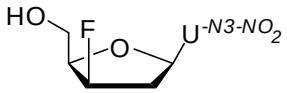
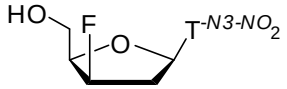
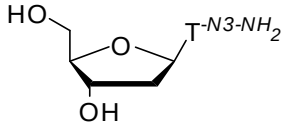
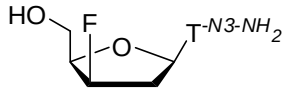
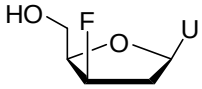
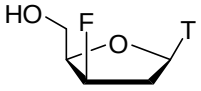
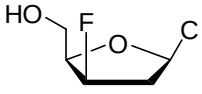
The results obtained are shown in tables (**7**), (**8**), (**9**), (**10**), (**11**), (**12**), (**13**) and (**14**).

Table (**7**): Anti-HIV-1 and -HIV-2 activities in human T-lymphocyte (CEM) cells.

	T-lymphocyte (CEM) cells	
	HIV-1 [a]EC_{50} (μM)	HIV-2 [a]EC_{50} (μM)
 **7**	>50	>50
 **8**	>250	210 ± 57
 **13**	>50	>50
 **14**	>50	>50
 **15**	>0.4	>0.4
 **16**	>250	>250
 **19**	>250	>250
 **20**	>250	>250
 **22**	≥250	≥250

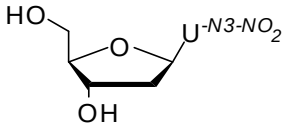
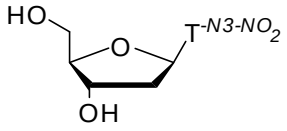
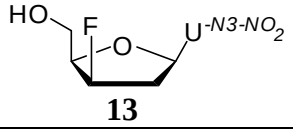
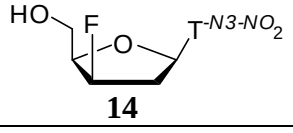
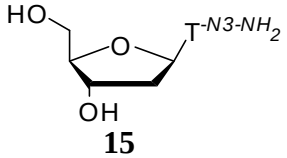
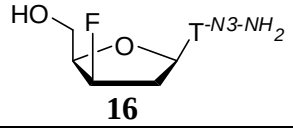
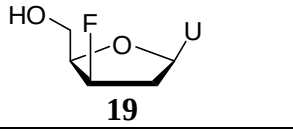
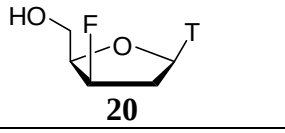
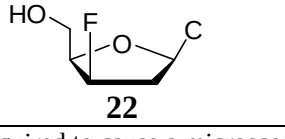
[a] EC_{50} = effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50 %.

Table (**8**): Activity against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells

	Varicella-Zoster virus (VZV) in HEL cells			
	Cytotoxicity (μM)		[a]EC_{50} (μM)	
	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]	TK^+ VZV Strain OKA	TK^- VZV Strain 07-1
 **7**	>100	>100	>100	>100
 **8**	>100	>100	>100	>100
 **13**	>100	57	>20	>20
 **14**	>100	70	>100	>100
 **15**	>100	>100	>100	>100
 **16**	>100	>100	>100	>100
 **19**	>100	>100	>100	>100
 **20**	>100	>100	>100	>100
 **22**	>100	>100	>100	>100

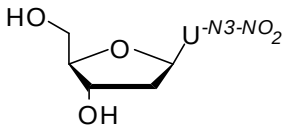
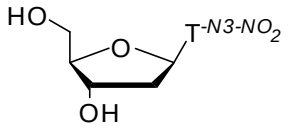
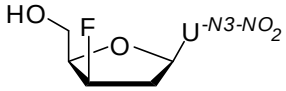
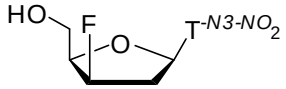
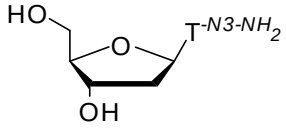
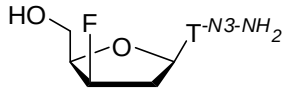
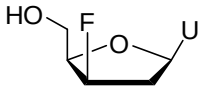
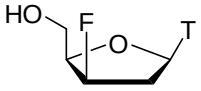
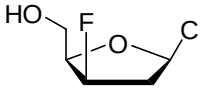
[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

Table (**9**): Cytotoxicity and antiviral activity against Herpes simplex, vaccinia and Vesicular stomatitis viruses in HEL cell culture.

	Herpes simplex, Vaccinia and Vesicular stomatitis viruses in HEL cell cultures.					
	EC_{50} [b] (μM)					
	Minimum Cytotoxic conc. [a] (μM)	Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
 **7**	>100	>100	>100	>100	>100	>100
 **8**	>100	>100	>100	>100	>100	>100
 **13**	>100	>100	>100	>100	>100	>100
 **14**	>100	>100	>100	>100	>100	>100
 **15**	>100	>100	>100	>100	>100	>100
 **16**	>100	>100	>100	>100	>100	>100
 **19**	>100	>100	>100	>100	>100	>100
 **20**	>100	>100	>100	>100	>100	>100
 **22**	>100	>100	>100	>100	>100	>100

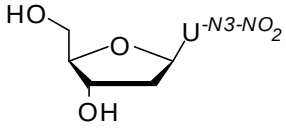
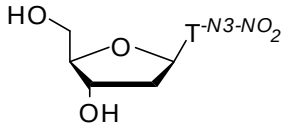
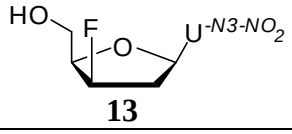
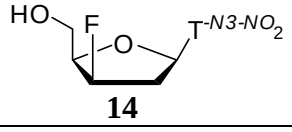
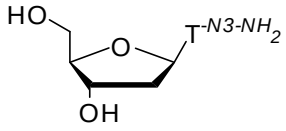
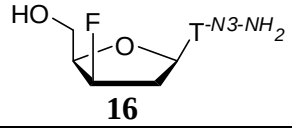
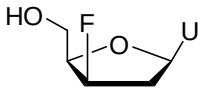
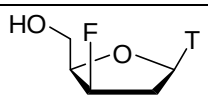
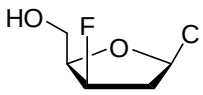
[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table (**10**): Activity against cytomegalovirus in human embryonic lung (HEL) cells.

	Cytomegalovirus HEL cells			
	Cytotoxicity (μM)		[a]EC_{50} (μM)	
	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]	AD-169 strain	Davis strain
 **7**	>100	>100	>100	>100
 **8**	>100	>100	>100	>100
 **13**	>100	57	>20	>20
 **14**	100	70	>20	76
 **15**	>100	>100	>100	>100
 **16**	>100	>100	>100	>100
 **19**	>100	>100	>100	>100
 **20**	>100	>100	>100	>100
 **22**	>100	>100	>100	>100

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

Table (**11**): Virus activity and Cytotoxicity in CRFK cell culture against Feline Corona Virus (FIPV) and Feline Herpes.

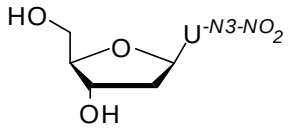
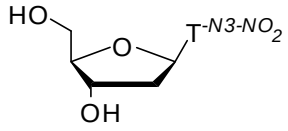
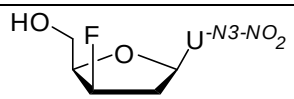
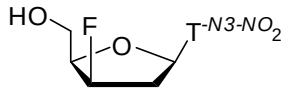
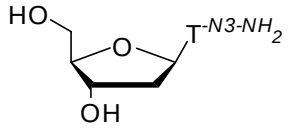
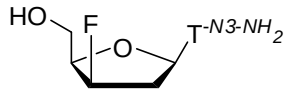
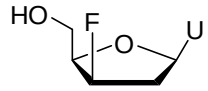
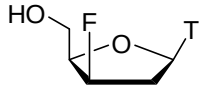
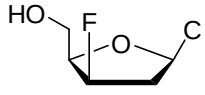
	Feline viruses in CRFK cell cultures		
	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
 **7**	>100	>100	>100
 **8**	>100	>100	>100
 **13**	>100	>100	>100
 **14**	>100	>100	>100
 **15**	>100	>100	>100
 **16**	>100	>100	>100
 **19**	>100	>100	>100
 **20**	>100	>100	>100
 **22**	>100	>100	>100

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

CRFK cells: Crandell-Rees Feline Kidney cells.

Table (**12**): Activity as anti-influenza virus activity and cytotoxicity in MDCK cell cultures.

	Anti-influenza virus activity and cytotoxicity in MDCK cell cultures.							
	CC_{50}[a] (μM)	Minimum cytotoxic conc.[b] (μM)	Antiviral EC_{50}[c]					
			Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
 **7**	>100	>100	>100	>100	>100	>100	>100	>100
 **8**	>100	>100	>100	>100	>100	>100	>100	>100
 **13**	>100	>100	>100	>100	>100	>100	>100	>100
 **14**	>100	>100	>100	>100	>100	>100	>100	>100
 **15**	>100	>100	>100	>100	>100	>100	>100	>100
 **16**	>100	>100	100	>100	100	>100	>100	>100
 **19**	>100	>100	>100	>100	>100	>100	>100	>100
 **20**	>100	>100	100	>100	100	>100	>100	>100
 **22**	>100	>100	>100	>100	>100	>100	>100	>100

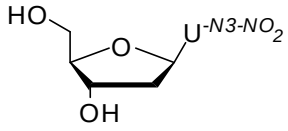
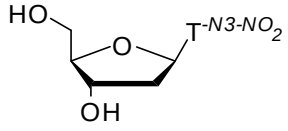
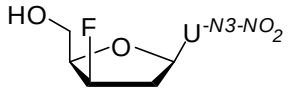
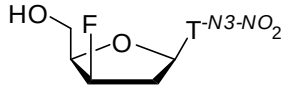
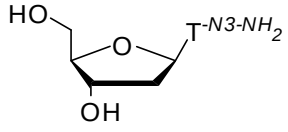
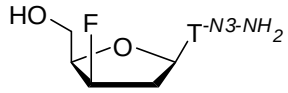
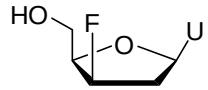
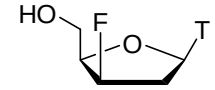
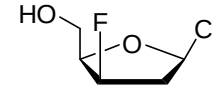
[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

MDCK cells: Madin Darby canine kidney cells.

Table (**13**): Cytotoxicity and antiviral activity against Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus in HeLa cell cultures.

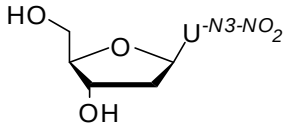
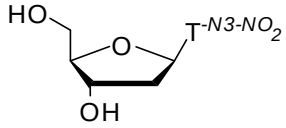
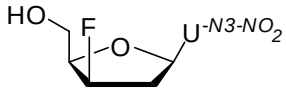
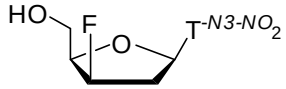
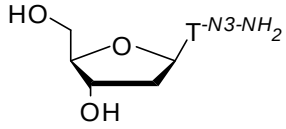
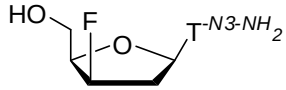
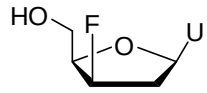
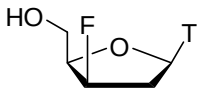
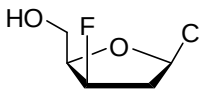
	Vesicular Stomatis, Coxasackie and Respiratory synctial viruses in HeLa cell cultures.							
	CC_{50} [a] (μM)	Minimum cytotoxic conc.[b] (μM)	Antiviral EC_{50} [c]					
			Vesicula stomatitis virus		Coxsackie virus B4		Raspiratory syncytical virus	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
 **7**	>100	>100	>100	>100	>100	>100	>100	>100
 **8**	>100	>100	>100	>100	>100	>100	>100	>100
 **13**	>100	100	>20	>20	>20	>20	>20	>20
 **14**	>100	>100	>100	>100	>100	>100	>100	>100
 **15**	>100	100	>20	>20	>20	>20	>20	>20
 **16**	>100	>100	100	>100	100	>100	>100	>100
 **19**	>100	>100	>100	>100	>100	>100	>100	>100
 **20**	>100	>100	100	>100	100	>100	>100	>100
 **22**	>100	>100	>100	>100	>100	>100	>100	>100

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table (**14**): Cytotoxicity and antiviral activity against Para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures.

	Viruses in Vero cell cultures					
	EC_{50} [b] (μM)					
	Minimum Cytotoxic conc. [a] (μM)	Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
 **7**	>100	>100	>100	>100	>100	>100
 **8**	>100	>100	>100	>100	>100	>100
 **13**	>100	>100	>100	>100	>100	>100
 **14**	>100	>100	>100	>100	>100	>100
 **15**	>100	>100	>100	>100	>100	>100
 **16**	>100	100	>100	>100	>100	>100
 **19**	>100	>100	>100	>100	>100	>100
 **20**	>100	100	>100	>100	>100	>100
 **22**	>100	>100	>100	>100	>100	>100

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

IV. Conclusion of chapter II:

A rapid synthesis of 2',3'-dideoxy-3'-fluoro-β-D-*threo*-pyrimidine nucleosides bearing the pyrimidine canonical bases of nucleic acids and N^3-nitro or N^3-amino pyrimidine bases has been developed in order to discover new nucleoside derivatives as potential antiviral drugs. The synthesis was achieved by substitution of imide-like proton by nitro group as a electron withdrawing group to avoid the formation of *O*-anhydronucleoside and increasing the chance of S_N2 type reaction. Direct deoxyfluorination using DAST led to 2',3'-dideoxy-3'-fluoro-β-D-*threo*-3-nitropyrimidine nucleosides followed by denitration or transformation of nitro group to amino group. Finally, deprotection of the protecting group to provide the final compounds. However, when the nucleosides **7, 8, 13, 14, 15, 16, 19, 20** and **22** were evaluated against DNA and several RNA viruses (including HIV) in cell culture experiments, none of the nucleoside derivatives showed any antiretroviral activity nor cytoxicity at the highest concentration tested (usually 100 µM).

Experimental Part

General Procedures and Instrumentation:

Thin layer chromatography was performed on precoated aluminium sheets of Silica Gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbency followed by charring with 5% ethanolic sulfuric acid and heating. Column chromatography was carried out on Silica Gel 60 (Merck, Art. 9385).

Evaporation of solvents was carried out on a rotary evaporator under reduced pressure. Melting points were determined in open capillary tubes on a Gallenkamp MFB-595-010 M apparatus and are uncorrected. UV spectra were recorded on an Uvikon 931 (Kontron) spectrophotometer. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75 MHz and ^{19}F NMR at 282 MHz in $(CD_3)_2SO$ at ambient temperature with a Brüker DRX 400.

Chemical shifts (δ) are quoted in parts per million (ppm) referenced to the residual solvent peak, $(CD_3)CD_2H)SO$ being set at δ_H 2.49 and δ_C 39.5 relative to tetramethylsilane (TMS).

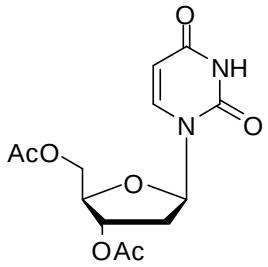
^{19}F chemical shifts are reported using trichlorofluoromethane as external reference. Deuterium exchange and COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in Hertz. 2D ^{1}H-^{13}C heteronuclear COSY were recorded for the attribution of ^{13}C signals.

Mass spectra were recorded on ESI Q-Tof Waters for the analysis of positive electrospray mode (ESI > 0).

Specific rotations were measured on a Perkin-Elmer Model 241 spectropolarimeter (path length 1 cm), and are given in units of 10^{-1} deg cm^2 g^{-1}.

Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France).

Organic extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$. All moisture-sensitive reactions were carried out under rigorous anhydrous conditions under an argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under reduced pressure.



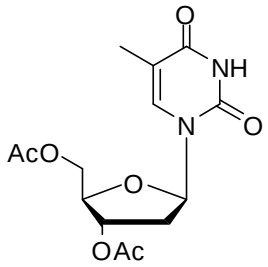
3',5'-di-*O*-acetyl-2'-deoxyuridine[128] **3**

Acetic anhydride (18 ml, 190 mmol) was added dropwise to a stirred solution of 2'-deoxyuridine **1** (8.8 g, 38.5 mmol) in dry pyridine (200 ml) at 0 °C. The reaction mixture was stirred at room temperature overnight. The solution was evaporated to dryness in *vacuo* to give an oily residue, which was dissolved in CH_2Cl_2 (100 ml), washed with saturated aqueous solution of $NaHCO_3$ and water. The separating organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **3** (9.39 g, 90%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.4 (s, 1H, NH), 7.65 (d, 1H, *J* = 8.1 Hz, H-6), 6.15 (t, *J* = 7.1 Hz,1H, H-1'), 5.7 (d, 1H, *J* = 8.1 Hz, H-5), 5.18 (m, 1H, H-3'), 4.22 (m, 2H, H-5', 5''), 4.18 (m, 1H, H-4'), 2.27-2.47 (m, 2H, H-2',2''), 2.07 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.5 and 170.3 (2xCO), 163.3 (C-4), 150.7 (C-2), 140.7 (C-6), 102.6 (C-5), 84.7 (C-1'), 81.5 (C-4'), 74.2 (C-3'), 63.9 (C-5'), 36.0 (C-2'), 21.1 (CH_3), 20.9 (CH_3).

MS ESI-QTof > 0: m/z 313.1 $(M+H)^+$.



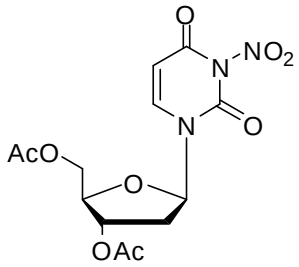
3',5'-di-*O*-acetyl-2'-deoxythymidine[128] **4**

Acetic anhydride (12.8 ml, 135.4 mmol) was added dropwise to a stirred solution of 2'-deoxythymidine **2** (6.6 g, 27.2 mmol) in dry pyridine (150 ml) at 0 °C. The reaction mixture was stirred at room temperature overnight. The solution was evaporated to dryness in *vacuo* to give an oily residue, which was dissolved in CH_2Cl_2 (100 ml), washed with saturated aqueous solution of $NaHCO_3$ and water. The separating organic layer was dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **4** (9.32 g, 96%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.38 (s, 1H, NH), 7.5 (s, 1H, H-6), 6.17 (t, J =7.2 Hz, 1H, H-1'), 5.18 (m, 1H, H-3'), 4.25 (m, 2H, H-5',5''), 4.16 (m, 1H, H-4'), 2.42 (m, 1H, H-2'), 2.29 (m, 1H, H-2''), 2.07 (s, 6H, 2x CH_3), 1.79 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 and 169.9 (2xCO), 163.5 (C-4), 150.3 (C-2), 135.7 (C-6), 109.9 (C-5), 83.9 (C-1'), 80.9(C-4'), 73.8 (C-3'), 63.6 (C-5'), 35.4 (C-2'), 20.7 (CH_3), 20.5 (CH_3), 12.1 (CH_3).

MS ESI-QTof > 0: m/z 327.2 $(M+H)^+$.



3',5'-di-*O*-acetyl-2'-deoxy-3-nitrouridine 5

Trifluoroacetic anhydride (22.4 ml, 158.83 mmol) was added to a suspension of finely powdered NH_4NO_3 (6.36 g, 79.42 mmol) in anhydrous CH_2Cl_2 (200 ml) at 0 °C. The mixture was vigorously stirred at room temperature until the solid was dissolved (ca. 1h) and then cooled again. Compound **3** (6.2 g, 19.6 mmol) in CH_2Cl_2 (50 ml) was added. The resulting solution was stirred for 45 min. and washed with cold $NaHCO_3$ 5% (2 X 50 ml), H_2O (50 ml), dried over anhydrous MgSO4, evaporated in *vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99/1) as eluent to give **5** (5.7 g, 80 %) as a white foam.

Melting Point: (EtOAc) 128-129 °C.

$[\alpha]_D^{20}$: +15.8 (c 0.82, DMSO)

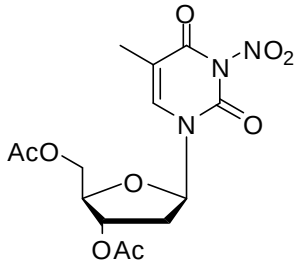
UV: (EtOH 95) λ_{max} 258 nm, (ε 8800).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.87 (d, J = 8.6 Hz, 1H, H-6), 6.15 (m, 2H, H-5, H-1′), 5.2 (m, 1H, H-3′), 4.14-4.32 (m, 3H, H-4′, H-5′, H-5″), 2.14 (m, 2H, H-2′), 2.05, 2.06 (s, 6H, 2x CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 and 169.9 (2xCO), 155.4 (C-4), 145.3 (C- 2), 141.6 (C-6), 100.5 (C-5), 86.6 (C-1′), 81.8 (C-4′), 73.3 (C-3′), 63.3 (C-5′), 36.1 (C-2′), 20.5, 20.7 (2xCH_3).

MS ESI-QTof > 0: m/z 358.1 $(M+H)^+$, 715.2 $(2M+H)^+$.

Analysis: Calculated for $C_{13}H_{15}N_3O_9$: C, 43.70; H, 4.23; N, 11.7
Found: C, 43.38; H, 4.52; N, 11.98.



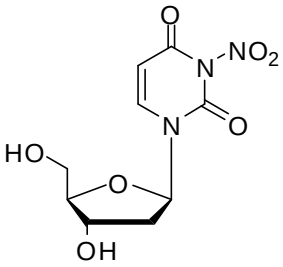
3',5'-di-*O*-acetyl-2'-deoxy-3-nitrothymidine[130] **6**

Trifluoroacetic anhydride (32 ml, 226 mmol) was added to a suspension of finely powdered NH_4NO_3 (9.12 g, 113.9 mmol) in anhydrous CH_2Cl_2 (200 ml) at 0 °C. The mixture was vigorously stirred at room temperature until the solid was dissolved (ca. 1h) and then cooled again. Compound **4** (9.28 g, 28.44 mmol) in CH_2Cl_2 (50 ml) was added, The resulting solution was stirred for 45 minute, then, washed with cold $NaHCO_3$ 5% (2 X 50 ml), H_2O (50 ml), dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99/1) as eluent to give **6** (8.16 g, 75%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.71 (s, 1H, H-6), 6.13 (t, J = 7.0 Hz, 1H, H-1'), 5.22 (m, 1H, H-3'), 4.28-4.22 (m, 3H, H-5',5'',4'), 2.5 (m, 1H, H-2'), 2.4 (m, 1H, H-2''), 2.07 (s, 6H, 2xCH_3), 1.93 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 and 169.9 (2xCO), 156.3 (C-4), 145.2 (C-2), 136.9 (C-6), 109.3 (C-5), 85.9 (C-1'), 81.5 (C-4'), 73.3 (C-3'), 63.3 (C-5'), 35.7 (C-2'), 20.7 (CH_3), 20.5 (CH_3), 12.1 (CH_3).

MS ESI-QTof > 0: m/z 372.2 $(M+H)^+$.



2'-deoxy-3-nitrouridine 7

Compound **5** (5.6 g, 15.7 mmol) was treated with HCl/MeOH (0.17 M, 500 ml) for 48 h at room temperature, followed by evaporation of the solvent in *vacuo*, and co-evaporation with toluene. The residue was purified by silica gel column chromatography using CH_2Cl_2/ MeOH (9/1) as eluent to give **7** (2.87 g, 67 %) as a white foam.

Melting Point: (Acetone) 140–142 °C.

$[\alpha]_D^{20}$: +28.3 (c 1.06, DMSO).

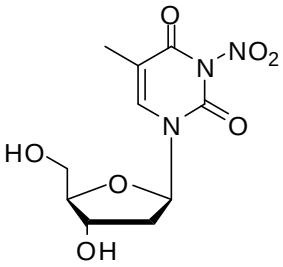
UV: (EtOH 95) λ_{max} 260 nm, (ε 6100).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 8.13 (d, J = 8.4 Hz, 1H, H-6), 6.1 (m, 2H, H-1′, H-5), 5.23 (br, 2H, 3′, 5′-OH), 4.26 (m, 1H, H-3′), 3.86 (m, 1H, H-4′), 3.60 (m, 2H, H-5′, H-5″), 2.32 (m, 2H, H-2′, H-2″).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 155.4 (C-4), 145.3 (C-2), 141.5 (C-6), 100.1 (C-5), 88.0 (C-1′), 86.2 (C-4′), 69.7 (C-3′), 60.7 (C-5′), 39.9 (C-2′).

MS ESI-QTof > 0: m/z 274.1$(M+H)^+$, 547.1$(2M+H)^+$.

Analysis: Calculated for $C_9H_{11}N_3O_7$: C, 39.57; H, 4.06; N, 15.38
Found: C, 39.33; H, 3.72; N, 14.98.



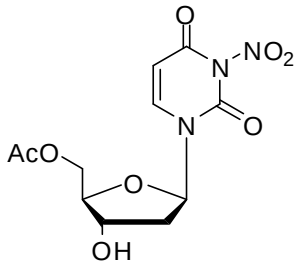
2'-deoxy-3-nitrothymidine[130] **8**

Compound **6** (6.48 g, 17.45 mmol) was treated with HCl/MeOH (0.17 M, 600 ml) for 48 h at room temperature, followed by evaporation of the solvent in *vacuo*, co-evaporation with toluene. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **8** (3.3 g, 66%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.98 (s, 1H, H-6), 6.11 (t, J = 6.4 Hz,1H, H-1'), 5.05-5.4 (br d, 2H, OH-3', 5'), 4.27 (m, 1H, H-3'), 3.82 (m, 1H, H-4'), 3.67-3.54 (m, 2H, H-5',5''), 2.29-2.12 (m, 2H, H-2',2''), 1.92 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 155.3 (C-4), 144.0 (C-2), 135.9 (C-6), 107.61 (C-5), 86.7 (C-1'), 84.7 (C-4'), 68.7 (C-3'), 59.7 (C-5'), 38.6 (C-2'), 11.2 (CH_3).

MS ESI-QTof > 0: m/z 288.1 $(M+H)^+$.



5'-*O*-acetyl-2'-deoxy-3-nitrouridine 9

Compound **7** (2.7 g, 9.9 mmol) was suspended in dioxane (100 ml). Triphenylphosphine (3.9 g, 14.9 mmol) and glacial acetic acid (5.7 ml, 98 mmol) were added. The resulting mixture was heated to 60 °C under stirring, and a solution of diethyl azodicaroboxylate (2.3 ml, 14.8 mmol) in dioxane (10 ml) was added dropwise. The solution was stirred at 60 °C for 1h, followed by cooling to room temperature and evaporation of the solvent. The oily residue was purified by silica gel chromatography using $CHCl_3$/Acetone (9/1) as eluent to give **9** (2.34 g, 75%) as a white foam.

$[\alpha]_D^{20}$: +35 (c 1.06, DMSO)

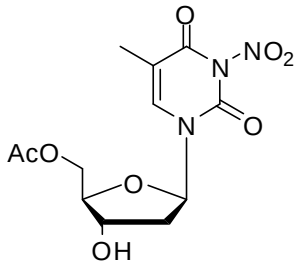
UV: (EtOH 95) λ_{max} 259 nm, (ε 7000).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.86 (d, 1H, J = 8.4 Hz, H-6), 6.08-6.15 (m, 2H, H-1′, H-5), 5.52 (s, 1H, OH-3′), 4.13-4.24 (m, 3H, H-3′, H-5′, H-5″), 4.01 (m, 1H, H-4′), 2.27-2.42 (m, 2H, H-2′), 2.04 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 (CO), 155.5 (C-4), 145.3 (C-2), 141.7 (C-6), 100.3 (C-5), 86.4 (C-1′), 84.2 (C-4′), 69.7 (C-3′), 63.5 (C-5′), 38.9 (C-2′), 20.6 (CH_3).

MS ESI-QTof > 0: 316.1$(M+H)^+$, 631.1$(2M+H)^+$.

Analysis: Calculated for $C_{11}H_{13}N_3O_8$: C, 41.91; H, 4.16; N, 13.33
Found: C, 41.66; H, 4.16; N, 13.08.



5'-*O*-acetyl-2'-deoxy-3-nitrothymidine 10

Compound **8** (2 g, 7 mmol) was suspended in dioxane (100 ml). Triphenylphosphine (2.75 g, 10.4 mmol) and glacial acetic acid (3.8 ml, 66 mmol) were added. The resulting mixture was heated to 60 °C under stirring, and a solution of diethyl azodicaroboxylate (1.62 ml, 10.3 mmol) in dioxane (10 ml) was added dropwise. The solution was stirred at 60 °C for 1h, followed by cooling to room temperature and evaporation of the solvent. The oily residue was purified by silica gel column chromatography using $CHCl_3$/Acetone (95/5) as eluent to provide **10** (1.7 g, 74%) as a white foam.

Melting Point: (Toluene) 116-117 °C.

$[\alpha]_D^{20}$: +17.5 (c 1.00, DMSO)

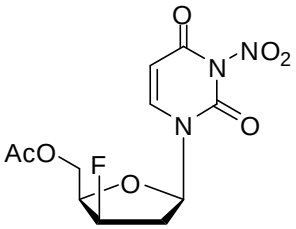
UV: (EtOH 95) λ_{max} 264 nm, (ε 9700).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.65 (s, 1H, H-6), 6.13 (t, J = 6.5 Hz, 1H, H-1′), 5.49 (d, 1H, J = 4.5 Hz, OH-3′), 4.28-4.16 (m, 3H, H-3′, H-5′, H-5″), 3.96 (m, 1H, H-4′), 2.39-2.12 (m, 2H, H-2′,H-2″), 2.05 (s, 3H, $COCH_3$), 1.92 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.2 (CO), 156.3 (C-4), 145.1 (C-2), 136.9 (C-6), 109.1 (C-5), 85.8 (C-1′), 84.1 (C-4′), 69.7 (C-3′), 63.6 (C-5′), 38.6 (C-2′), 20.6 (CH_3CO), 12.1 (CH_3).

MS ESI-QTof > 0: 352.1$(M+Na)^+$, 681.1$(2M+Na)^+$.

Analysis: Calculated for $C_{12}H_{15}N_3O_8$: C, 43.77; H, 4.59; N, 12.76.
Found: C, 44.15, H, 4.69, N, 12.47.



1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 11

To a stirred solution of compound **9** (2.25 g, 7.1 mmol) in anhydrous CH_2Cl_2 containing pyridine (50 ml, 4/1) was added DAST (4.37 ml, 35.7 mmol) at -67 °C under argon. The reaction mixture was allowed to warm up to room temperature and stirring was continued overnight. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ until gas evolution ceased, washed with H_2O and the organic phase was dried over Na_2SO_4, concentrated to dryness and purified by silica gel chromatography using ether as eluent to give **11** (1.02 g, 45%) as a white foam.

$[\alpha]_D^{20}$: +13 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 258 nm, (ε 9200).

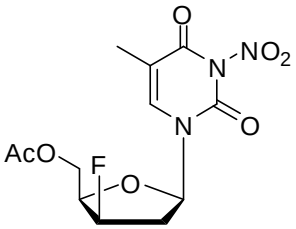
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.75 (d, *J* = 8.1, 1H, H-6), 6.16 (d, *J* = 6.9, 1H, H-1′), 6.09 (d, *J* = 8.4, 1H, H-5), 5.90 (d, *J* = 53.4, 1H, H-3′), 4.25-4.47 (m, 3H, H-4′, H-5′, H-5″), 2.87(m, 1H, H-2″), 2.46 (m, 1H, H-2′), 2.07(s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.0 (CO), 155.0 (C-4), 145.3 (C-2), 140.9 (C-6), 100.4 (C-5), 92.2 (d, *J* = 178.4 Hz, C-3′), 85.8 (C-1′), 81.2(d, *J* = 18.1 Hz, C-4′), 61.1 (C-5′), 38.3 (d, *J* = 20.2 HzC-2′), 20.5 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.7 (dddd, *J* = 81.7, *J* = 43.5, *J* = 31.3, *J* = 24.0 Hz).

MS ESI-QTof > 0: 318.1$(M+H)^+$, 635.2$(2M+H)^+$.

Analysis: Calculated for $C_{11}H_{12}FN_3O_7$: C, 41.65, H; 3.81; N, 13.25.
Found: C, 41.6; H, 3.89; N, 12.85.



1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 12

To a stirred solution of compound **10** (1.38 g, 4.2 mmol) in anhydrous CH_2Cl_2 containing pyridine (50 ml, 4/1) was added DAST (2.57 ml, 21.1 mmol) at -67 °C under argon. The reaction mixture was allowed to warm up to room temperature and stirring was continued overnight. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ until gas evolution ceased, washed with H_2O and the organic phase dried over Na_2SO_4, concentrated to dryness and purified by silica gel chromatography using ether as eluent to give **12** (0.6 g, 43%) as a white foam.

$[\alpha]_D^{20}$: +17 (c 1.00, DMSO)

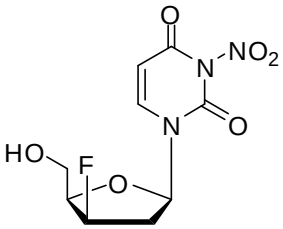
UV: (EtOH 95) λ_{max} 264 nm, (ε 6800).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.05 (s, 1H, H-6), 6.16 (dd, 1H, *J* = 8.0, 1.8 Hz, H-1′), 5.4 (ddd, 1H, *J* = 54.2, *J* = 4.3, *J* = 2.4 Hz, H-3′), 4.42 (m, 2H, H-5′, H-5″), 4.3 (m, 1H, H-4′), 2.74 (m, 1H, H-2″), 2.43 (m, 1H, H-2′), 2.07 (s, 3H, CH_3), 1.94 (s, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 (CO), 156.3 (C-4), 145.1 (C-2), 136.0 (d, *J* = 7.3, C-6), 109.0 (C-5), 92.2 (d, *J* =178.3, C-3′), 85.4 (C-1′), 81.0 (d, *J* = 18.2, C-4′), 61.1 (d, *J* = 10.9, C-5′), 38.1 (d, *J* = 20.2, C-2′), 20.5 (CH_3), 12.3 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.3 (dddd, *J* = 54.3, *J* = 42.2, *J* = 29.8, *J* = 24.9 Hz).

MS ESI-QTof > 0: 354 $(M+Na)^+$, 685.1 $(2M+Na)^+$.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrouracil 13

Compound **11** (0.15 g, 0.47 mmol) was treated with HCl/MeOH (0.17 M, 25 ml) overnight at room temperature, followed by evaporation of the solvent in vacuo, co-evaporated with toluene. The residue was purified by silica gel column chromatography using CH_2Cl_2/ Acetone (7/3) as eluent gave **13** (98 mg, 75 %) as a white foam.

Melting Point: (Acetone) 168-169 °C.

$[\alpha]_D^{20}$: +13.1 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 259 nm, (ε 8800).

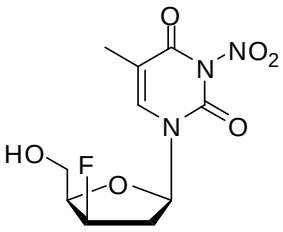
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.67 (d, *J* = 8.3 Hz, 1H, H-6), 6.13 (d, *J* = 7.9 Hz, 1H, H-1′), 6.09 (d, *J* = 8.4, 1H, H-5), 5.22 (ddd, *J* = 54.1, J = 4.0, *J* = 2.4 Hz, 1H, H-3′), 5.06 (t, *J* = 5.5 Hz, 1H, OH), 4.0 (m, 1H, H-4′), 3.79 (m, 2H, H-5′,5″), 2.79 (m, 1H, H-2″), 2.43 (m, 1H, H-2′).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 155.4 (C-4), 145.3 (C-2), 140.9 (C-6), 100.2 (C-5), 91.8 (d, *J* = 178.1 Hz, C-3′), 85.7 (C-1′), 84.7 (d, *J* = 18.8 Hz, C-4′), 57.9 (C-5′), 38.4 (d, *J* = 20.5 Hz, C-2′).

NMR ^{19}F (282 MHz, DMSO-d_6) δ – 191.0 (dddd, *J* = 54.2, *J* = 43.2, *J* = 31.1, *J* = 22.8 Hz).

MS ESI-QTof > 0: 276.1 $(M+H)^+$, 551.1 $(2M+H)^+$.

Analysis: Calculated for $C_9H_{10}FN_3O_6$: C, 39.28; H, 3.66; N, 15.27.
Found: C, 39.63; H, 4.01; N, 15.02.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-nitrothymine 14

Compound **12** (0.2 g, 0.6 mmol) was treated with HCl/MeOH (0.17 M, 50 ml) overnight at room temperature, followed by evaporation of the solvent in vacuo, co-evaporated with toluene. The residue was purified by silica gel column chromatography using ether/ acetone (9/1) as eluent gave **14** (124 mg, 71 %) as a white foam.

Melting Point: (Acetone) 178-180 °C.

$[\alpha]_D^{20}$: +3 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 264 nm, (ε 7000).

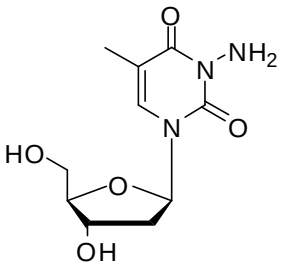
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.49 (s, 1H, H-6), 6.14 (dd, *J* = 7.9, *J* = 1.6 Hz, 1H, H-1′), 5.32 (ddd, *J* = 54.32, *J* = 4.2, *J* = 2.5 Hz, 1H, H-3′), 5.05 (t, *J* = 5.6 Hz, 1H, OH), 4.06 (m, 1H, H-4′), 3.71-3.84 (m, 2H, H-5′, H-5′′), 2.78 (m, 1H, H-2′′), 2.41 (ddd, *J* = 23.6, *J* = 16.0, *J* = 2.0 Hz, 1H, H-2′), 1.93 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 156.3 (C-4), 145.1 (C-2), 136.1 (C-6), 108.8 (C-5), 91.8 (d, *J* = 178.2 Hz, C-3′), 85.2 (C-1′), 84.4 (d, *J* = 18.6, C-4′), 58.0 (C-5′), 38.3 (d, *J* = 20.3 Hz, C-2′), 12.4 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.6 (dddd, *J* = 54.3, *J* = 42.5, *J* = 30.5, *J* = 24.1 Hz)

MS ESI-QTof > 0: 290.1 $(M+H)^+$, 579.1 $(2M+H)^+$.

Analysis: Calculated for $C_{10}H_{12}FN_3O_6$: C, 41.53; H, 4.18; N; 14.53.
Found: C, 41.57; H, 4.32; N, 14.82.



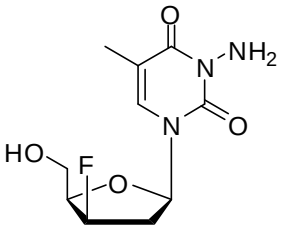
2'-deoxy-3-aminothymidine[134] **15**

A mixture of compound **8** (225 mg, 0.78 mmol) and hydrazine hydrate (4 ml) were stirred at room temperature for 30 minute, then MeOH (4 ml) was added. The solvent was evaporated and co-evaporated with MeOH and the residue was purified by silica gel column chromatography using ether/MeOH as eluent (9/1) to give **15** (95 mg, 47%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.75 (s, 1H, H-6), 6.21 (t, J = 6.8 Hz, 1H, H-1'), 5.49 (s, 2H, NH_2), 5.27 (d, 1H, J = 4.2 Hz, OH-3'), 5.06 (t, J = 5.1 Hz, 1H, OH-5'), 4.25 (m, 1H, H-3'), 3.79 (m, 1H, H-4'), 3.65-3.53 (m, 2H, H-5',5''), 2.12 (m, 2H, H-2',2''), 1.85 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 159.8 (C-4), 148.1 (C-2), 133.0 (C-6), 107.6 (C-5), 87.4 (C-1'), 85.0 (C-4'), 70.2 (C-3'), 61.1 (C-5'), 39.7 (C-2'), 12.8 (CH_3).

MS ESI-QTof > 0: 258.1 $(M+H)^+$.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)-3-aminothymine 16

A mixture of compound **14** (70 mg, 0.24 mmol) and hydrazine hydrate (1 ml) were stirred at room temperature for 15 min, 5 ml of acetone was added. The solvent was evaporated in *vacuo*, co evaporated with MeOH. The residue was purified by silica gel column chromatography using ether/MeOH (8/2) as eluent to give **16** (40 mg, 64 %) as a white foam.

Melting Point: (EtOH) 153-154 °C.

$[\alpha]_D^{20}$: +7.5 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 264 nm, (ε 9300).

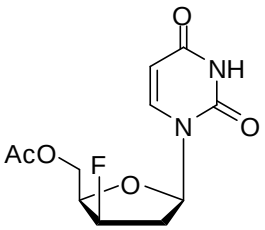
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.35 (s, 1H, H-6), 6.19 (dd, *J* = 8.0, *J* = 1.8 Hz, 1H, H-1′), 5.49 (s, 2H, NH_2), 5.30 (ddd, *J* = 54.4, *J* = 4.3, *J* = 2.4 Hz, 1H, H-3′), 5.02 (t, *J* = 5.6 Hz, 1H, OH), 4.03 (m, 1H, H-4′), 3.71-3.80 (m, 2H, H-5′, H-5″), 2.76 (m, 1H, H-2″), 2.26 (ddd, *J* = 24.4, *J* =16.0, *J* = 2.3 Hz, 1H, H-2′), 1.87 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 159.8 (C-4), 148.1 (C-2), 132.1 (C-6), 107.6 (C-5), 92.0 (d, *J* = 178.4 Hz, C-3′), 84.5 (C-1′), 83.9 (d, *J* = 18.6 Hz, C-4′), 58.1 (C-5′), 38.5 (d, *J* = 20.2, C-2′), 12.9 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.5 (dddd, *J* = 54.9, *J* = 42.5, *J* = 30.5, *J* = 24.9 Hz)

MS ESI-QTof > 0: 260.1 $(M+H)^+$.

Analysis: Calculated for $C_{10}H_{14}FN_3O_4$. 0.1M H_2O; C, 46.01; H, 5.48; N, 16.10. Found: C, 45.66; H, 5.85; N, 16.29.



1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil 17

A mixture of compound **11** (0.75 g, 2.37 mmol), Bu_3SnH (0.7 ml, 2.62 mmol) and AIBN (0.12 g, 0.72 mmol) in 50 ml anhydrous toluene were heated at 100 °C for 2h. The reaction mixture was cooled and evaporated the solvent. The residue was purified by silica gel column chromatography using CH_2Cl_2/acetone (8/2) as eluent to give **17** (0.43 g, 67%) as a white foam.

Melting Point: (Toluene) 167-168 °C.

$[\alpha]_D^{20}$: +4.5 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 260 nm, (ε 9100).

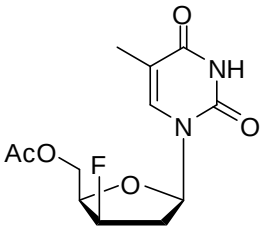
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.28 (s, 1H, NH), 7.44 (d, *J* = 8.1, 1H, H-6), 6.08 (d, *J* = 8.1 Hz, 1H, H-1′), 5.62 (d, *J* = 8.1, 1H, H-5), 5.29 (ddd, *J* = 54.6, *J* = 4.5, *J* = 2.4 Hz, 1H, H-3′), 4.23 (m, 3H, H-4′, H-5′,5′′), 2.6 (m, 1H, H-2′′), 2.24 (ddd, *J* = 27.4, *J* = 16.5, *J* = 2.5 Hz, 1H, H-2′), 2.01 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.0 (CO), 163.0 (C-4) 150.3 (C-2), 139.7 (C-6), 102.0 (C-5), 92.5 (d, *J* = 178.1 Hz, C-3′), 83.8 (C-1′), 80.3 (d, *J* = 18.0 Hz, C-4′), 61.2 (C-5′), 38.1 (d, *J* = 20.5 Hz, C-2′), 20.52 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.2 (dddd, *J* = 54.9, *J* = 41.3, *J* = 31.9, *J* = 25.3 Hz).

MS ESI-QTof > 0: 273.1 $(M+H)^+$, 545.3 $(2M+H)^+$.

Analysis: Calculated for $C_{11}H_{13}FN_2O_5$: C, 48.53; H, 4.81; N, 10.29.
Found: C, 48.33, H, 5.16, N, 9.99.



1-(5-*O*-acetyl-2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine 18

A mixture of compound **12** (0.3 g, 0.9 mmol), Bu_3SnH (0.29 ml, 1.1 mmol) and AIBN (45 mg, 0.27 mmol) in 30 ml anhydrous toluene were heated at 100 °C for 2h. The reaction mixture was cooled and the solvent was evaporated. The residue was purified by silica gel column chromatography using CH_2Cl_2/acetone as eluent (8/2) to give **18** (0.22 g, 85%) as a white foam.

Melting Point: (Toluene) 142-143 °C.

$[\alpha]_D^{20}$: +16.5 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 265 nm, (ε 6700).

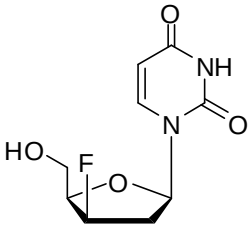
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.29 (s, 1H, NH), 7.25 (s, 1H, H-6), 6.11 (dd, *J* = 8.2, *J* = 2.4 Hz, 1H, H-1′), 5.29 (ddd, *J* = 54.6, *J* = 4.6, *J* = 2.4 Hz, 1H, H-3′), 4.36-4.22 (m, 2H, H-5′), 4.12 (m, 1H, H-4′), 2.74 (m, 1H, H-2″), 2.18 (ddd, J = 26.0, J = 16.1, J = 2.8 Hz, 1H, H-2′), 2.01(s, 3H, CH_3CO), 1.75 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.1 (CO), 163.6 (C-4), 150.3 (C-2), 135.1 (C-6), 109.7 (C-5), 92.5 (d, *J* = 178.2 Hz, C-3′), 83.3 (C-1′), 80.0 (d, *J* = 18.0 Hz, C-4′), 61.2 (C-5′), 38.0 (d, *J* = 20.1, C-2′), 20.5 (CH_3), 12.3 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -189.8 (dddd, *J* = 54.9, *J* = 41.4, *J* = 31.9, *J* = 26.3 Hz).

MS ESI-QTof > 0: 287.1 $(M+H)^+$, 573.2 $(2M+H)^+$.

Analysis: Calculated for $C_{12}H_{15}FN_2O_5$: C, 50.35; H, 5.28; N, 9.79.
Found: C, 50.28, H, 5.40, N, 9.44.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)uracil 19

Compound **19** (100 mg, 0.37 mmol) was treated in methanolic ammonia (10 ml) (saturated beforehand at -10 °C and stoppered tightly) was stirred in a stainless-steel bomb overnight at room temperature. The solution was evaporated to dryness under reduced pressure and co-evaporated several times with methanol. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **19** (63 mg, 74 %) as a white foam.

Melting Point: (EtOAc) 167-168 °C.

$[\alpha]_D^{20}$: –11.2 (c 0.8, DMSO).

UV: (EtOH 95) λ_{max} 261 nm, (ε 8800).

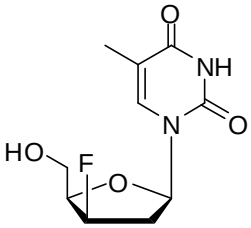
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.34 (s, 1H, NH), 7.48 (d, *J* = 8.1 Hz, 1H, H-6), 6.14 (d, *J* = 8.0 Hz, 1H, H-1′), 5.69 (d, *J* = 8.1 Hz, 1H, H-5), 5.29 (ddd, *J* = 54.3, *J* = 4.2, *J* = 2.4 Hz, 1H, H-3′), 5.01 (s, 1H, OH), 4.01 (m, 1H, H-4′), 3.65-3.78 (m, 2H, H-5′,5″), 2.76 (dddd, *J* = 42.8, *J* = 16.4, *J* = 8.2, *J* = 4.2, 1H, H-2″), 2.22 (ddd, *J* = 28.0, *J* = 16.4, *J* = 2.3 Hz, 1H, H-2′).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 163.1 (C-4), 150.4 (C-2), 139.7 (C-6), 101.9 (C-5), 92.0 (d, *J* = 178.2 Hz, C-3′), 84.0 (C-1′), 83.6 (d, *J* = 15.9 Hz, C-4′), 58.1 (C-5′), 38.4 (d, *J* = 20.0 Hz, C-2′).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.5 (dddd, *J* = 54.7, *J* = 43.0, *J* = 30.0, *J* = 25.4 Hz).

MS ESI-QTof > 0: 231.1 $(M+H)^+$, 461.1 $(2M+H)^+$.

Analysis: Calculated for $C_9H_{11}FN_2O_4$: C, 46.96; H; 4.82, N; 12.17.
Found: C, 47.02; H, 4.92; N, 11.99.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)thymine[56] **<u>20</u>**

A solution of compound **20** (0.15 g, 0.52 mmol) in methanolic ammonia (14 ml) (saturated beforehand at -10 °C and stoppered tightly) was stirred in a stainless-steel bomb overnight at room temperature. The solution was evaporated to dryness under reduced pressure and co-evaporated several times with methanol. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **20** (0.1 g, 78 %) as a white foam.

$[\alpha]_D^{20}$: -21.2 (c 1.00, DMSO).

UV: (EtOH 95) λ_{max} 266 nm, (ε 10500).

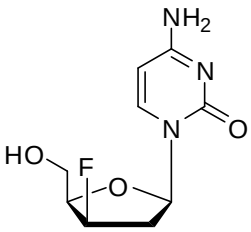
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.34 (s, 1H, NH), 7.31 (s, 7.31, H-6), 6.16 (dd, *J* = 8.2, 2.2 Hz, 1H, H-1′), 5.28 (ddd, *J* = 54.6, *J* = 4.5, *J* = 2.4 Hz, 1H, H-3′), 4.99 (s, 1H, OH), 3.95 (m, 1H, H-4′), 3.72 (m, 2H, H-5′,H-5″), 2.75 (m, 1H, H-2″), 2.22 (ddd, *J* = 25.2, *J* = 15.9, *J* = 2.7 Hz, 1H, H-2′), 1.78 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 162.2 (C-4), 148.9 (C-2), 133.8 (C-6), 108.0 (C-5), 90.6 (d, *J* = 178.4 Hz, C-3′), 82.1 (C-1′), 81.6 (d, *J* = 25.1 Hz, C-4′), 56.6 (C-5′), 38.19 (d, *J* = 20.6 Hz, C-2′), 12.4 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -190.1 (dddd, *J* = 54.5, *J* = 42.4, *J* = 30.8, *J* = 25.1 Hz)

MS ESI-QTof > 0: 245.1 $(M+H)^+$, 489.2 $(2M+H)^+$.

Analysis: Calculated $C_{12}H_{15}FN_2O_5$. 0.6 H_2O; C, 47.10, H, 5.61, N, 10.98.
Found; C, 46.96, H, 5.66, N, 10.63.



1-(2,3-dideoxy-3-fluoro-β-D-*threo*-pentofuranosyl)cytosine <u>22</u>

Lawesson's reagent (0.296 g, 0.73 mmol) was added under argon to a solution of compound **19** (0.2 g, 0.73 mmol) in anhydrous 1,2-dichloroethane (25 ml) and the reaction mixture was stirred under reflux for 6h. The solvent was then evaporated under reduced pressure and the residue was purified using silica gel column chromatography using CH_2Cl_2/Acetone (8/2) as

eluent to give 4-thio intermediate **21** 0.19 g (90 %) as a yellow foam. A solution of the 4-thio intermediate in methanolic ammonia (16 ml) (saturated beforehand at -10 °C and stoppered tightly) was heated at 100 °C in a stainless-steel bomb for 3h, and then cooled to room temperature. The solution was evaporated to dryness under reduced pressure and co-evaporated several times with mehanol. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (8/2) as eluent to give **22** (0.13 g, 86 %), overall yield 77 % as a white foam.

$[\alpha]_D^{20}$: +10.8 (c 0.6, DMSO).

UV: (EtOH 95) λ_{max} 272 nm, (ε 8100).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.51 (d, *J* = 7.4 Hz, 1H, H-6), 7.1 (br d, 2H, NH_2), 6.09 (d, *J* = 7.8 Hz, 1H, H-1′), 5.75 (d, *J* = 7.4 Hz, 1H, H-5), 5.25 (ddd, *J* = 54.3, *J* = 4.0, *J* = 2.4 Hz, 1H, H-3′), 4.99 (t, *J* = 5.4 Hz, 1H, OH), 4.00 (m, 1H, H-4′), 3.69-3.78 (m, 2H, H-5′,5″), 2.69 (m, 1H, H-2″), 2.09 (ddd, *J* = 26.4, *J* = 15.8, *J* = 2.1 Hz, 1H, H-2′).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 165.3 (C-4), 154.7 (C-2), 139.9 (C-6), 93.6 (C-5), 91.8 (d, *J* = 177.9, C-3′), 84.1 (C-1′), 83.62 (d, *J* = 18.8 Hz, C-4′), 57.91 (d, *J* = 9.3, C-5′), 39.16 (d, *J* = 19.9, C-2′).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -191.0 (dddd, *J* = 54.5, *J* = 43.2, *J* = 30.4, *J* = 24.0 Hz).

MS ESI-QTof > 0: 230.1 $(M+H)^+$, 459.2 $(2M+H)^+$.

Analysis: Calculated $C_9H_{12}FN_3O_3$. 0.9 M H_2O; C, 44.05, H, 5.67; N, 17.12.
Found: C, 44.24; H, 5.38; N, 16.87.

Chapter III

Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino), (*N*-methoxyimino) and (hydroxylamino)-pyrimidine nucleosides

I. Introduction and background of chapter III:

Introduction of hydroxyl amino and oximino derivatives at 2′- or 3′- position of nucleoside analogues has produced a variety of interesting antiviral[138,139] and antitumoral agents[140]. Federov *et al.*[138] reported that 3′-*N*-hydroxyimino and 3′-*N*-acetoxyimino derivatives are endowed with significant activity against HIV with EC_{50} values ranging from 0.02 and 0.4 µg/ml for both HIV-1 and HIV-2. Moreover, the 3′-*N*-hydroxyimino derivative also shows promising activity against HBV (EC_{50} = 0.25 µg/ml) and against HSV-1 and HSV-2 (Figure **22**).

Ogawa *et al.*[140] also reported that 2′-deoxy-2′-(hydroxylamino)cytidine (2′-DHAC) showed potent cytotoxicity against mouse leukemic L1210 and human epidermoid KB cells, with IC_{50} values of 1.58 and 1.99 µM, respectively. 3′-Deoxy-3′-(hydroxylamino)cytidine (3′-DHAC) and 2′,3′-dideoxy-3′-(hydroxylamino)cytidine (3′-dDHAC) were also cytotoxic against L1210 cells, with IC_{50} values of 4.03 and 1.84 µM, respectively, but not against KB cells (Figure **22**).

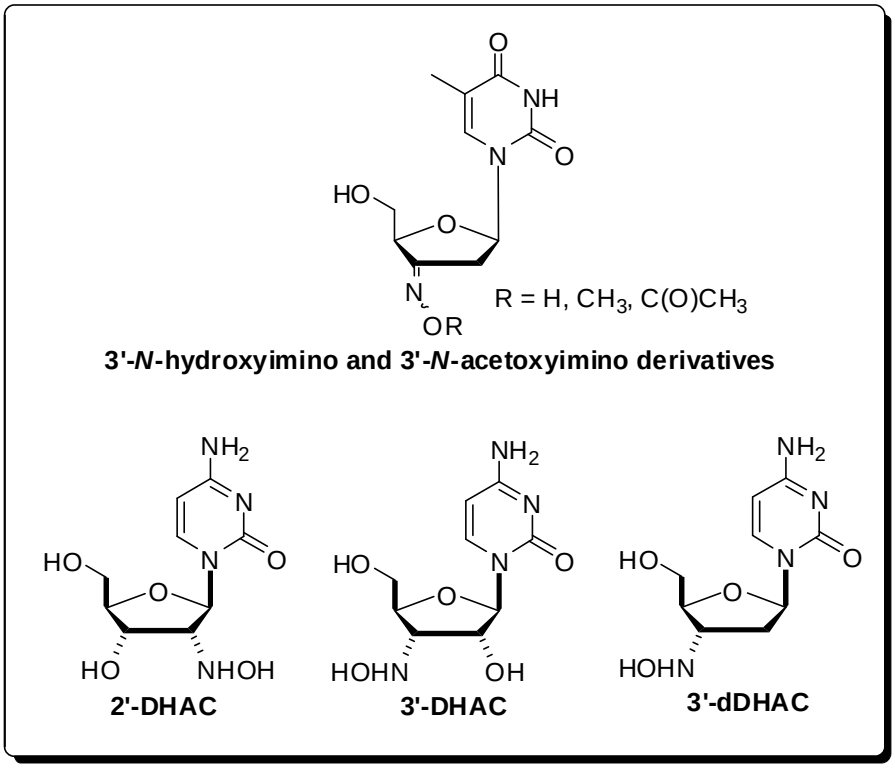
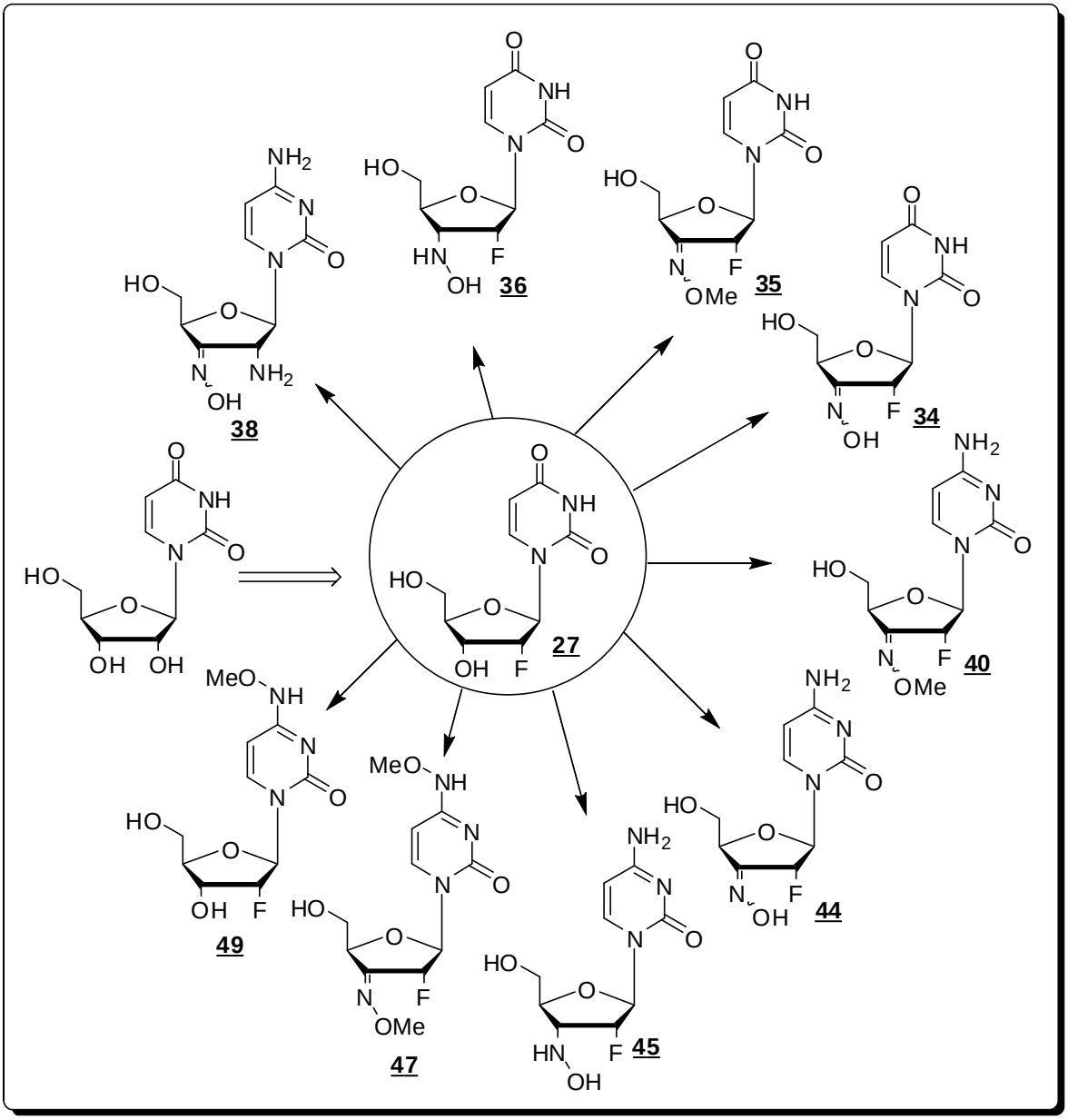


Figure **22**: 2′- hydroxyimino, 3′-hydroxylamino and 3′-hydroxyimino derivatives.

In this chapter, we describe the synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino) and 2′,3′-dideoxy-2′-fluoro-3′-(hydroxyimino)pyrimidine nucleosides. To the best of our knowledge, these compounds have not been previously synthesized or studied as antiviral agents. During this work, we synthesized nine novel nucleoside analogues starting from uridine to evaluate them as antiviral agents (Scheme **63**).

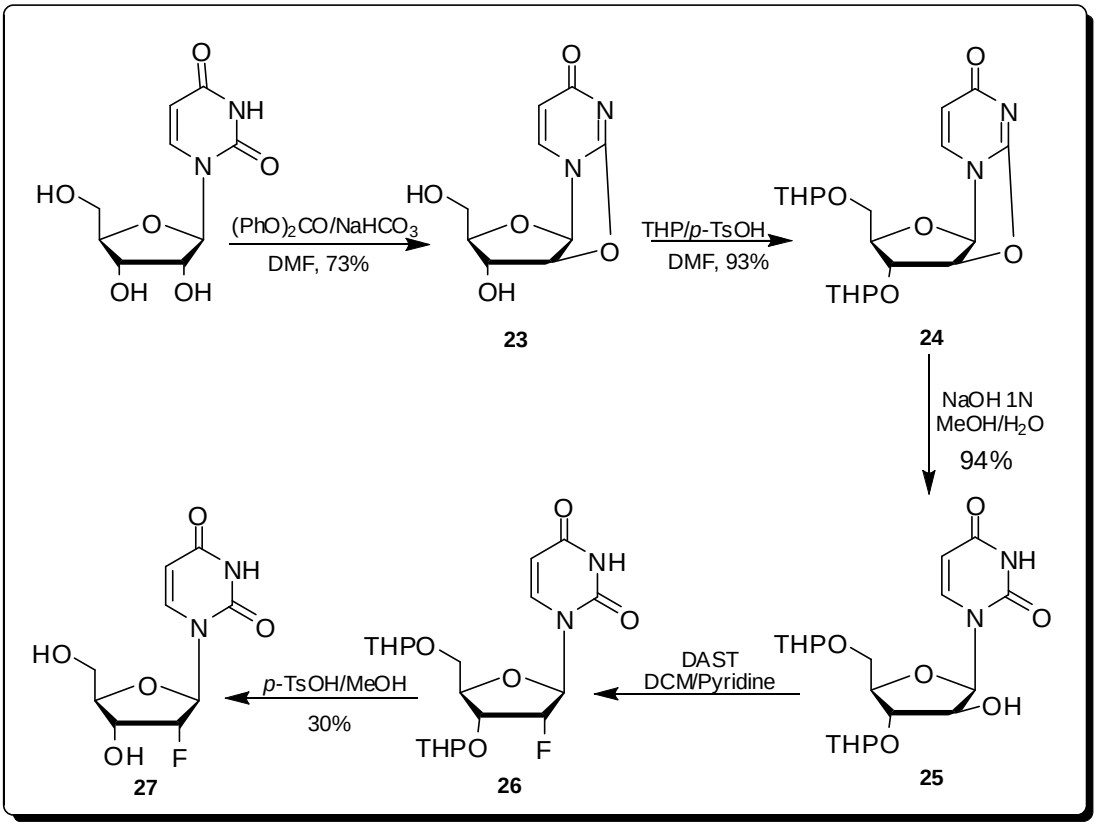


Scheme (**63**): The 2′-fluoro- 3′-hydroxylamino and 3′-hydroxyimino pyrimidine derivatives

II. Synthesis

II.1 Synthesis of 2′-deoxy-2′-fluorouridine 27 and 2′-deoxy-2′-fluorocytidine 30:

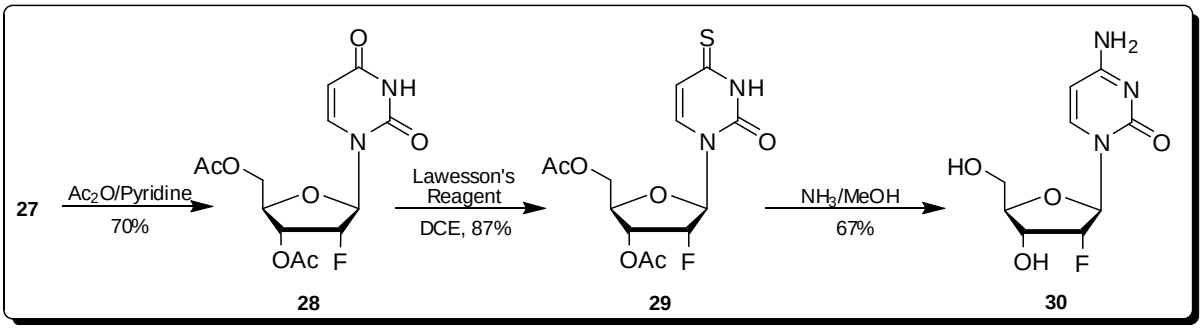
The synthesis began with the preparation 2′-deoxy-2′-fluorouridine **27** and 2′-deoxy-2′-fluorocytidine **30** which were obtained from commercially available uridine following a reported procedure by Shi *et al.*[51] 2,2′-Anhydrouridine **24** was obtained in 73% yield. The remaining hydroxyl groups at the 3′- and 5′-positions were protected with tetrahydropyranyl (THP) groups by treatment with 3,4-dihydropyran to give 2,2′-anhydro-3′,5′-di-*O*-tetrahydropyranyluridine **24** in 93% yield. The anhydro-ring opening reaction of compound **24** was carried out with a NaOH solution (1 N) to provide 3′,5′-di-*O*-tetrahydropyranylarabinouridine **25** in 94% yield. Fluorination with DAST followed by deprotection of **26** afforded 2′-deoxy-2′-fluorouridine **27** in 30% overall yield from **24** (Scheme **64**).



Scheme **64**: Synthesis of 2′-deoxy-2′-fluorouridine **28**

Next, acetylation of **27** with acetic anhydride in dry pyridine gave 3′,5′-di-*O*-acetyl-2′-deoxy-2′-fluorouridine **28**. Conversion of **28** to 2′-deoxy-2′-fluorocytidine **30** with overall yield 76%

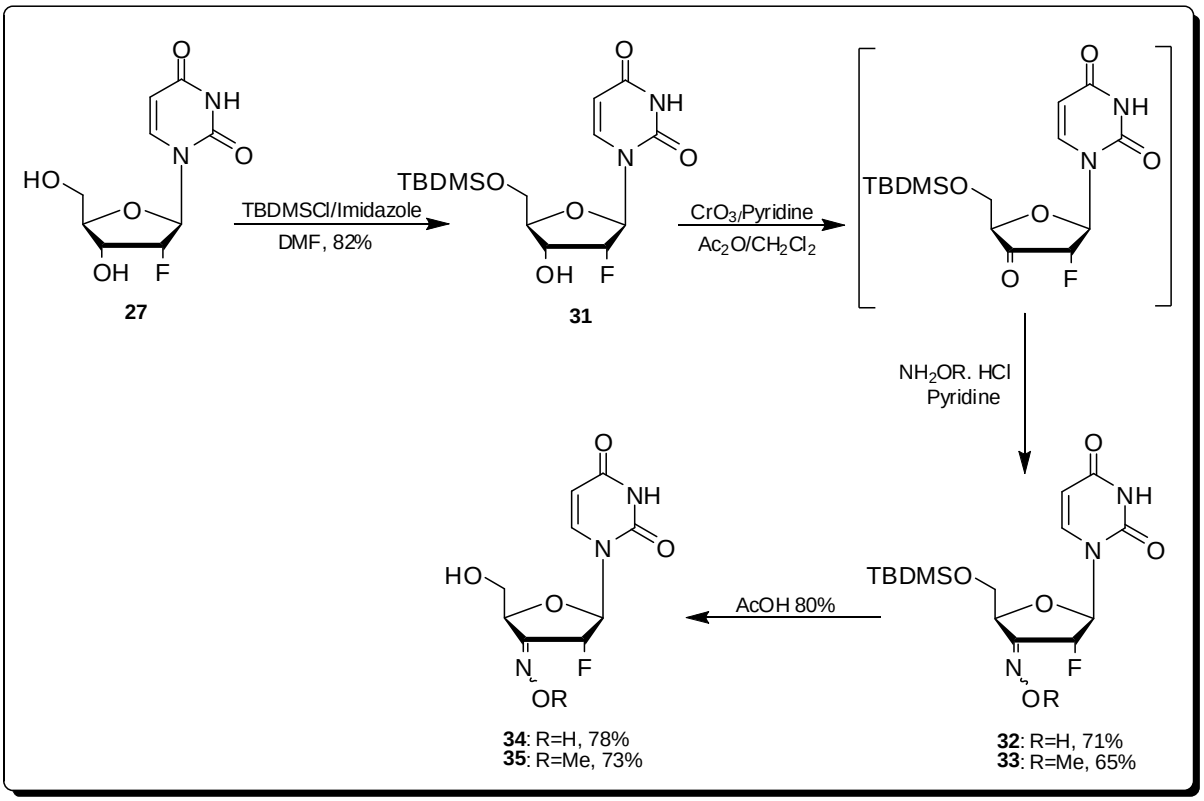
was carried out via a treatment with Lawesson's reagent[136,137], followed by the treatment of 4-thioamide intermediate **29** with methanolic ammonia at 100 °C in stainless-steel bomb (Scheme **65**).



Scheme (**65**): Synthesis of 2′-deoxy-2′-fluorouridine **30**

II.2 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 34 (E/Z) and 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pento-furanosyl] uracil 35 (E/Z):

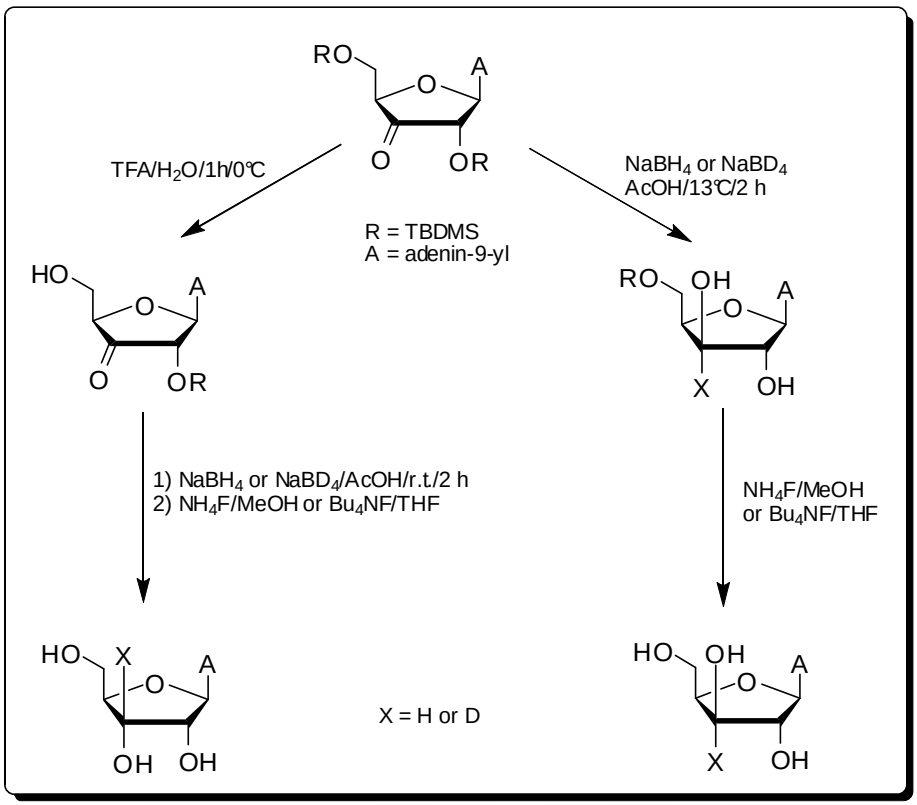
Ogilvie *et al.*[141] reported that selective silylation of uridine by treatment with *tert*-butyldimethylsilyl chloride (1.25 eq.) and imidazole (2.25 eq.) in DMF gave 5′-*O*-TBDMS-uridine in 80% yield. Thus, protection of the primary alcohol by selective silylation of **27** was carried out using *tert*-butyldimethylsilyl chloride and imidazole in DMF at room temperature for 3 h and led to 5′-*O*-TBDMS-2′-deoxy-2′-fluorouridine **31** in 82% yield. Subsequent oxidation under Jones conditions[142] afforded 3′-keto intermediate, which was reacted with hydroxylamine hydrochloride or with *O*-methylhydroxylamine hydrochloride to give, in quantitative yields, the mixture of isomeric protected nucleosides, 1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]uracil **32 (E/Z)** and 1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]uracil **33 (E/Z)** (ratio 1:1) in 71% and 65% yield, respectively. Deprotection of the isomeric mixture of **32 (E/Z)** and **33 (E/Z)** by 80% aqueous acetic acid gave of 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]uracil **34 (E/Z)** and 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil **35 (E/Z)** (ratio 1:2 and 1:1) in 78% and 73% yield, respectively (Scheme **66**).



Scheme (**66**): Synthesis of 3-(*N*-hydroxyimino) and 3-(*N*-methoxyimino) derivatives

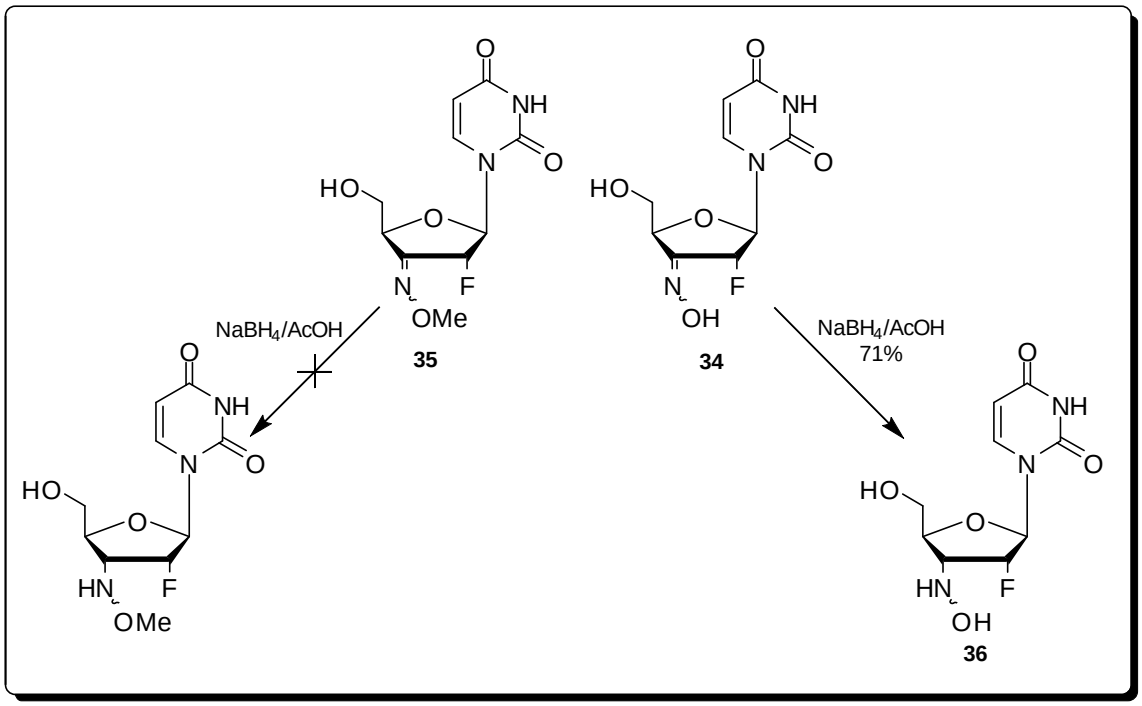
II.3 Stereoselective reduction of 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 34 (E/Z):

Robins *et al.*[143] reported that the treatment of protected 3′-keto and 2′-ketonucleosides with sodium triacetoxyborohydride in acetic acid resulted in hydride delivery on the α-face with high stereoselectivity but after selective removal of the 5′-*O*-TBDMS and a treatment of these 5′-hydroxy-(3′- and 2′-)-ketonucleosides with sodium triacetoxyborohydride provided selective delivery of hydride at the β-face gave ribo/xylo (~99:1) nucleosides in high yield (Scheme **67**).



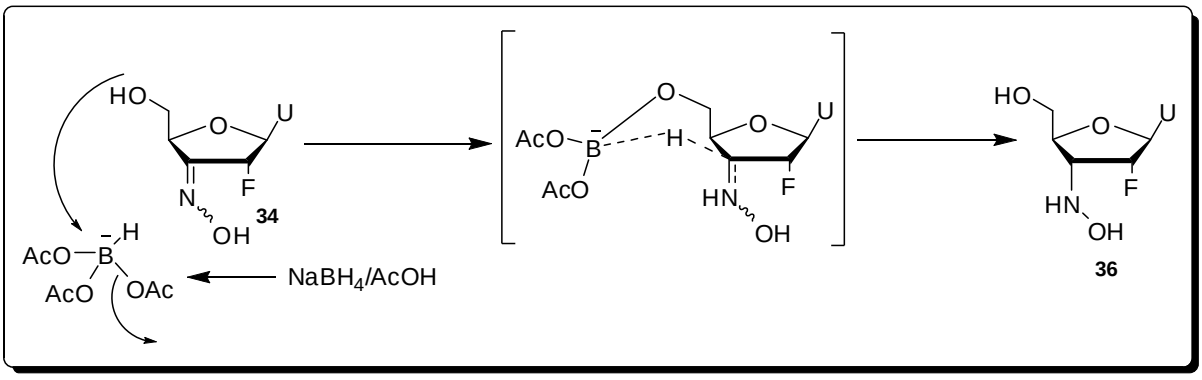
Scheme (**67**): Reduction with hydride delivery on α and β face according to Robins *et al*

Additionally, Ogawa *et al.*[140] reported that the desired 3′-hydroxylamino derivatives with a *ribo*-configuration can be obtained by hydride reduction of the corresponding 3′-oximes when the 5′-hydroxyl group is not protected. In our case, the oxime group in compound **34** was reduced using sodium triacetoxyborohydride (generated *in situ* from $NaBH_4$ and AcOH) in AcOH to give 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)uridine **36** in 71% yield. However, all our attempts to reduce the oxime group of compound **35** failed (Scheme **68**).



Scheme (**68**): Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)uridine **36**

Sodium triacetoxyborohydride is a mild reducing agent formed by addition of sodium borohydride to an excess of cold acetic acid. The stereochemical control was attributed to *in situ* formation of an alkoxydiacetoxyborohydride intermediate, by acid-catalyzed displacement of one acetoxy ligand on boron by a hydroxyl oxygen, followed by intramolecular delivery of hydride from the β-face to give the desired compound **36** (Scheme **69**).



Scheme (**69**): The proposed mechanism of the preparation of **36** and **45**

The structure assignment of **36** was based upon NOE experiments. Thus, when 3′*β*-proton and H-6 were irradiated, NOE enhancements were observed between 3′*β*-proton and H-5′, and between H-6 and H-5′, and H-6 and H-2′ (Figure **24**).

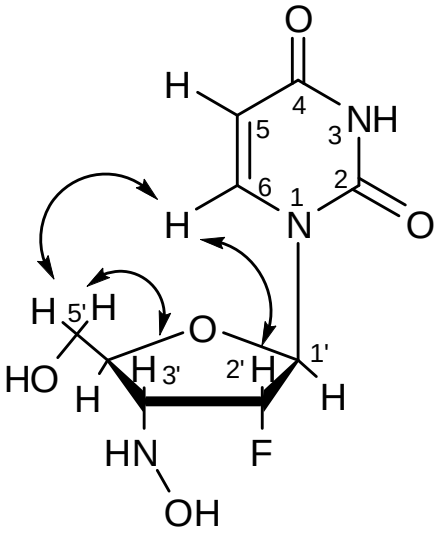
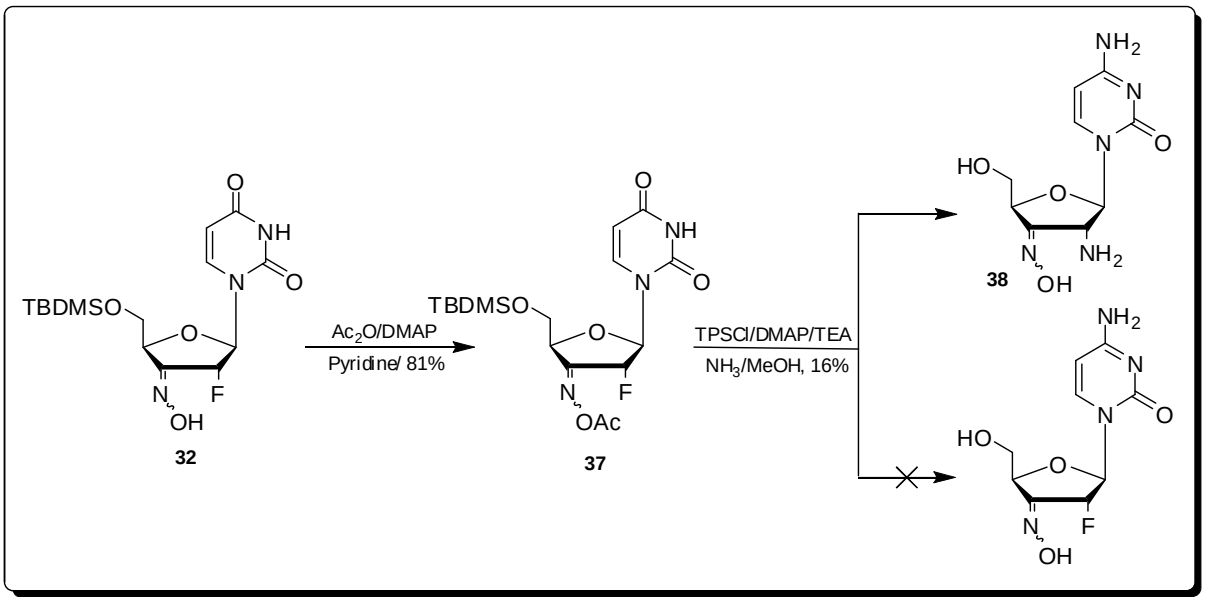


Figure (**24**): NOE correlations of **36**.

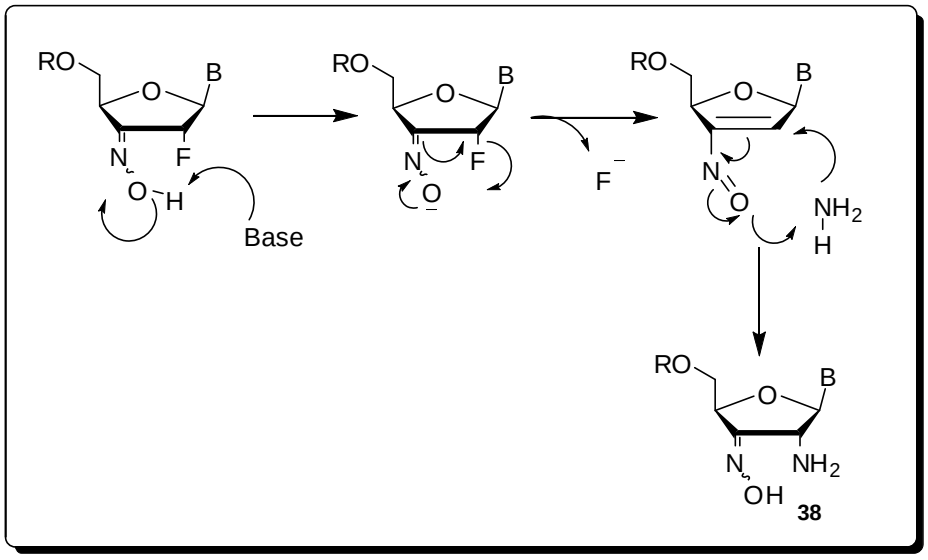
II.4 Synthesis of 1-[2-amino-2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] cytosine dihydrochloride 38:

The nucleoside **32** was converted into its acetate **37, (E/Z)** (ratio 1:1), with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine (DMAP), followed by activation of position C4 of the uracil moiety with triisopropylbenzenesulfonyl (TPS) chloride. The resulting 4-*O*-TPS derivative was further treated with methanolic ammonia at room temperature. Unfortunately, the desired cytosine derivative was not formed and the unexpected product 1-[2-amino-2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*pento-furanosyl]cytosine dihydrochloride **38** was obtained in 16% yield (Scheme **70**).



Scheme (**70**): Aminolysis of compound **37**

Regarding the formation of compound **38**, this reaction may be proceeded by abstraction of acidic proton of hydroxyimino group by ammonia, followed by intramolecular rearrangement to release the fluoride ion. Due to the high reactivity of nitroso-olefin, an 1,4 addition of ammonia was added to the olefin and gave compound **38** (Scheme **71**).

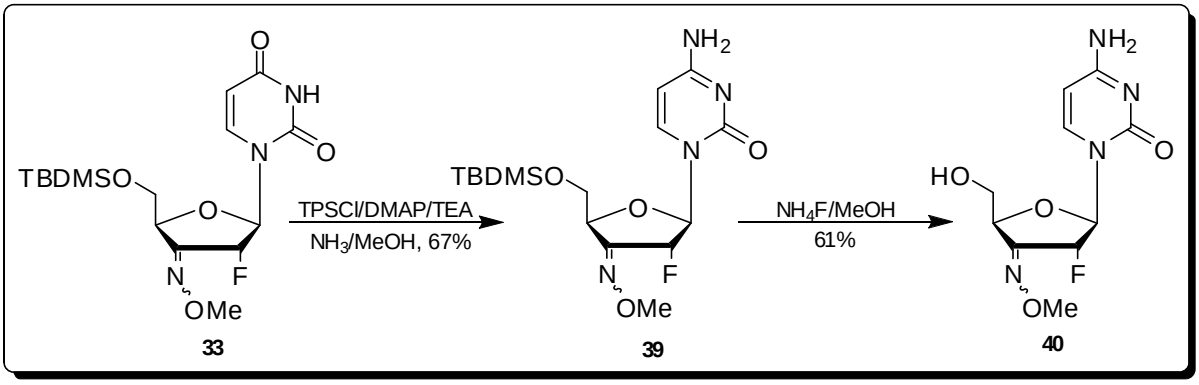


Scheme (**71**): The proposed mechanism of the synthesis of **38**

II.5 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] cytosine 40 (E/Z):

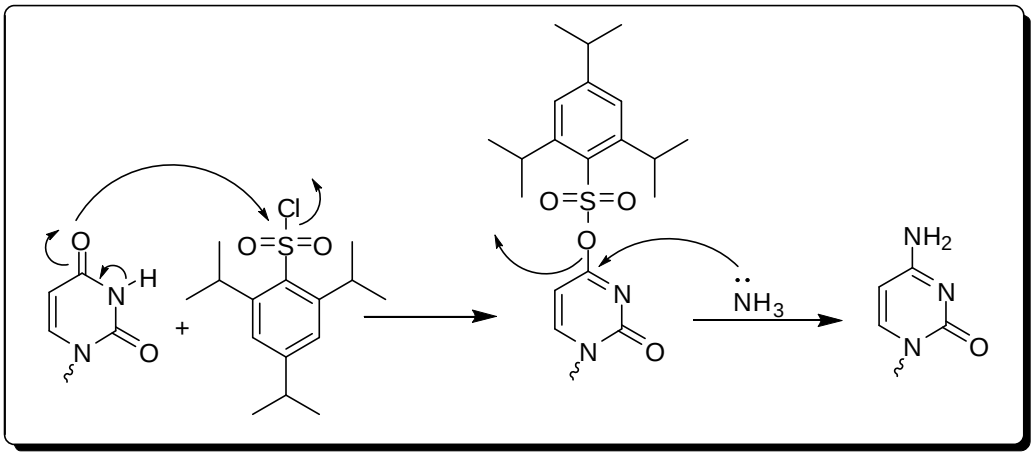
The ring transformation of **33** was accomplished by activation of position C4 of the uracil moiety with triisopropylbenzenesulfonyl (TPS) chloride followed by treatment with methanolic ammonia in a stainless steel bomb at room temperature for 12 h and gave 1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine **39 (E/Z) (**ratio 2:1.2) in 67% yield. The desilylation was carried out by refluxing with NH_4F in anhydrous MeOH for 3 h and gave 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine **40 (E/Z)** (ratio 2:1.8) in 61% yield (Scheme **72**).

In the case of *N*-OMe, the reaction of the ring transformation proceeded normally by activation with TPS followed by aminolysis, on the other hand, in the case of *N*-OH, the aminolysis reacted by another behaviour due to the presence of acidic proton of *N*-OH in the basic medium.



Scheme (**72**): Synthesis of 3-(N-methoxyimino) cytidine derivative

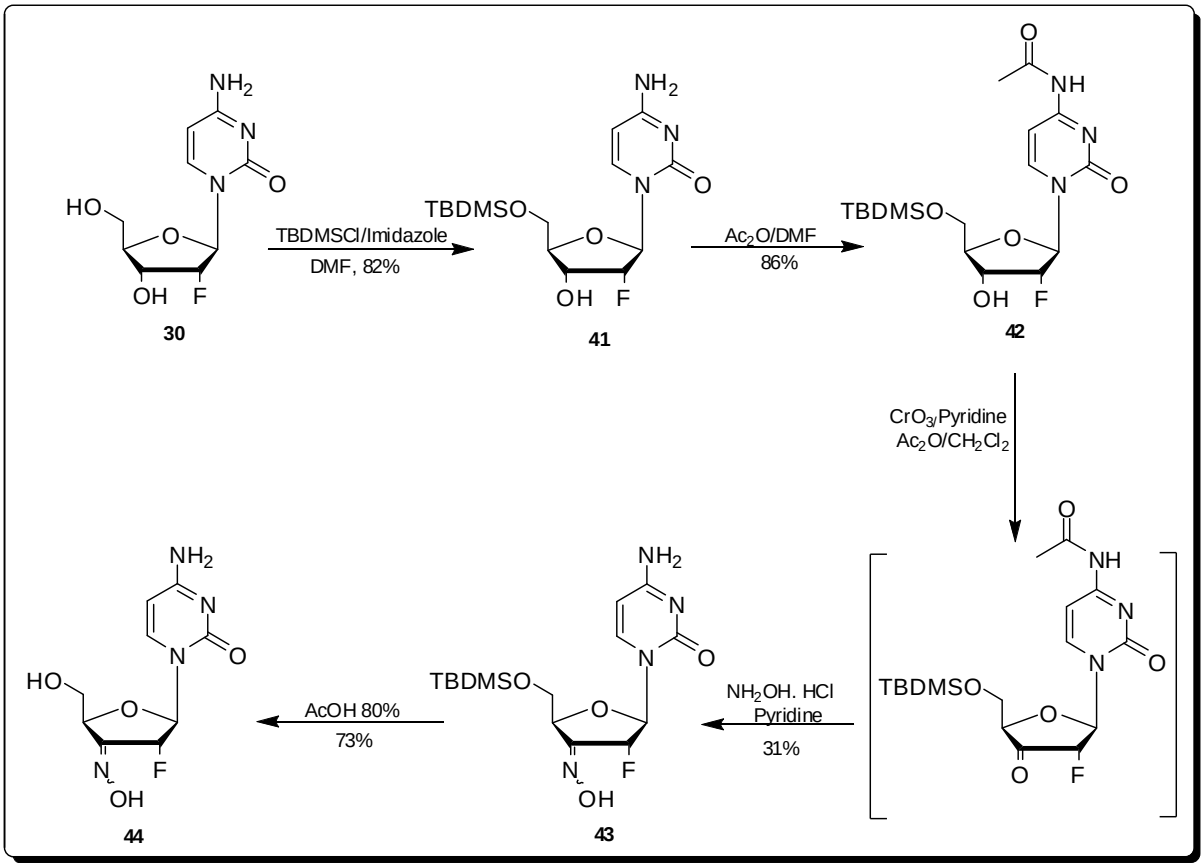
From a mechanistic point of view, the ring transformation was carried out through 4-(triisopropylsulfonyl) intermediate followed by aminolysis to afford cytosine moiety (Scheme **73**).



Scheme (**73**): ring transformation activated by TPS

II.6 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(N-hydroxyimino)-*β*-D-*glycero*-pento-furanosyl]cytosine 44 (E/Z):

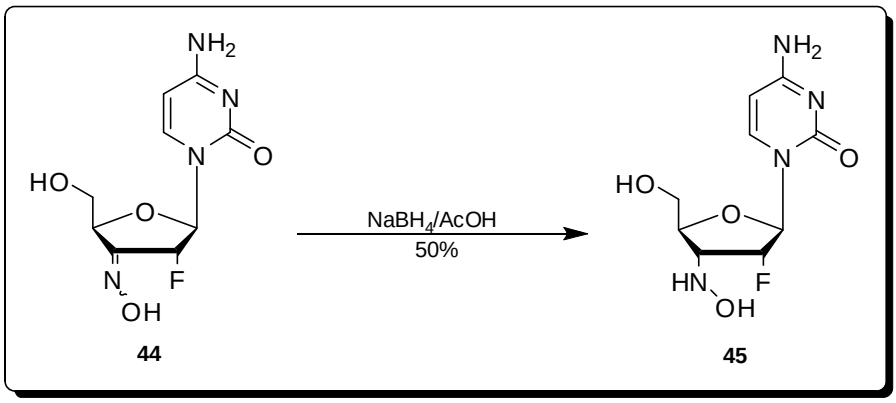
From our previous observations on the reaction of hydroxyimino group in compound **38** in basic medium, we suggested that 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine derivative could be directly prepared starting from cytidine. The synthesis began with regioselective silylation of 2′-deoxy-2′-fluorocytidine **30** with *tert*-butyldimethylsilyl chloride and imidazole in DMF at room temperature for 4 h and led to 5′-*O*-TBDMS-2′-deoxy-2′-fluorocytidine **41** in 77% yield, according to Ogilvie *et al.*.[144] A selective *N*-acylation of compound **41** by stirring with 1.6 equivalent of acetic anhydride in DMF at room temperature gave 5′-*O*-TBDMS-2′-deoxy-2′-fluoro-N^4-acetylcytidine **42** in 86% yield, according to Bhat *et al.*.[145] Oxidation of compound **42** under Jones conditions[142] gave the corresponding 3′-ketocytidine derivative which was treated with hydroxylamine and gave 1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(N-hydroxy-imino)-*β*-D-*glycero*-pentofuranosyl]cytosine **43 (E/Z)** (ratio 2:1.2) in 31% yield. It is to be noted that during this reaction, deprotection of *N*-acetyl group was observed. Desilylation of compound **43** was achieved by stirring with 80% AcOH to give 1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine **44 (E/Z)** (ratio 1.2: 2) in 73% yield (Scheme **74**).



Scheme (**74**): Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylimino)cytidine

II.7 Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine 45:

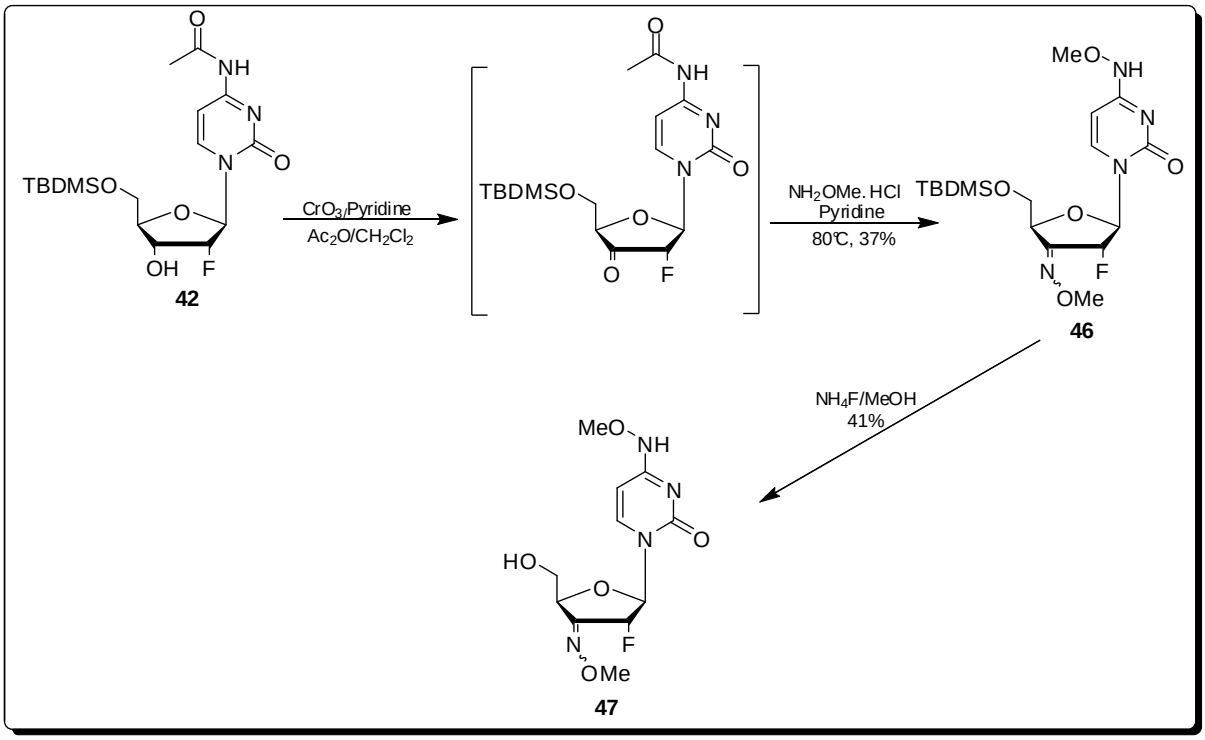
The desired 3′-hydroxylamino derivatives with a *ribo*-configuration was also obtained by hydride reduction of the corresponding 3′-oximes when the 5′-hydroxyl group is not protected.[140] Stereoselective reduction of the oxime group in compound **44** was accomplished using $NaBH_4$ in AcOH to give 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine **45** in 50% yield (Scheme **75**).



Scheme (**75**): Synthesis of 2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine

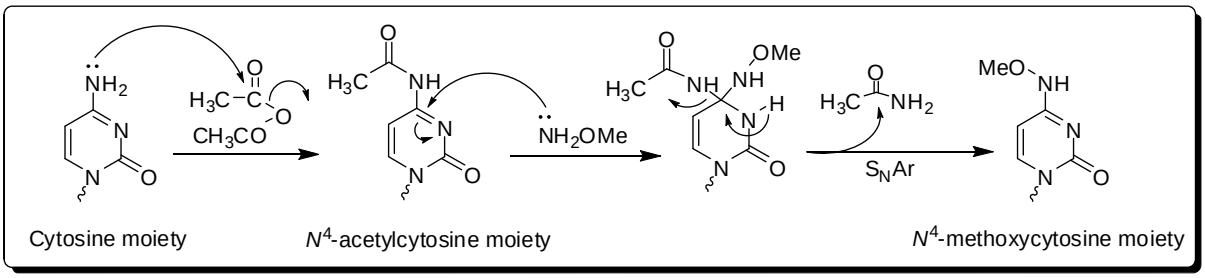
II.8 Synthesis of 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pento-furanosyl]-N^4-methoxycytosine 47 (E/Z):

On the other hand, when compound **42** was oxidized to 3′-ketocytidine followed by a treatment with *O*-methylhydroxylamine hydrochloride at room temperature, no reaction occurred. However, when the reaction mixture was refluxed at 80°C for 3 h, the 1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]-N^4-methoxycytosine **46 (E/Z)** (ratio 3:2) was obtained in 37% yield. Desilylation of compound **46** was carried out by refluxing with NH_4F in anhydrous methanol and gave 1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] N^4-methoxycytosine **47 (E/Z)** (ratio 2:1.4) in 41% yield (Scheme **76**).



Scheme (**76**): Synthesis of 3′-(*N*-methoxyimino)- N^4-methoxycytidine derivatives **47**

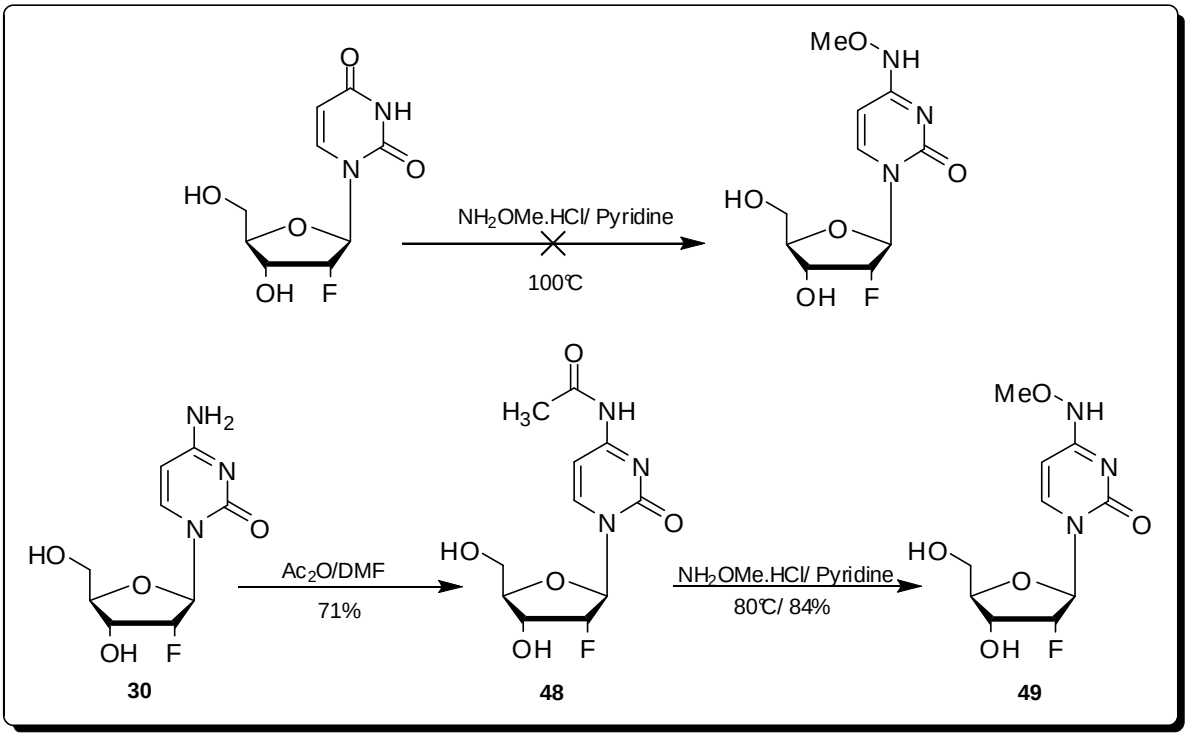
The ring transformation from cytosine moiety to N^4-methoxycytosine moiety can be envisioned by activation of position C4 of cytosine moiety by acetyl group followed by nucleophilic attack of *O*-methoxyhydroxylamine at position C4 to afford compound **46**, followed by desilylation to give compound **47** (Scheme **77**).



Scheme (**77**): Ring transformation from cytosine to N^4-methoxycytosine

II.9 Synthesis of 2′-deoxy-2′-fluoro-N^4-methoxycytidine 49:

From these results, we tried to apply the same procedure for the preparation of 2′-deoxy-2′-fluoro-N^4-methoxycytidine **49**. Ko *et al.*[146] reported direct method to prepare N^4-methoxyctidine derivative from uridine by the reaction with *O*-methylhydroxylamine hydrochloride in pyridine at 100°C. However, in our hands, no reaction occurred. So, we used our method by activation of the *N*-4 position of cytosine moiety by acetyl group followed by reaction with *O*-methylhydroxylamine. Selective *N*-acetylation of 2′-deoxy-2′-fluorocytidine **30** according to Bhat et *al.*[145] with acetic anhydride in DMF at room temperature for 24 h gave 2′-deoxy-2′-fluoro-N^4-acetylcytidine **48** in 71% yield, followed by refluxing with *O*-methylhydroxylamine in dry pyridine for 3 h at 80°C gave 2′-deoxy-2′-fluoro-N^4-methoxycytidine **49** in 84% yield (Scheme **78**).



Scheme (**78**): Synthesis of 2′-deoxy-2′-fluoro-N^4-methoxycytidine

II.10 ^{19}F NMR study of the nucleosides *N*-Oxime:

The coupling constants of 2′-deoxy-2′-fluorouridine **27** and 2′-deoxy-2′-fluorocytidine **30** are $J_{F\text{-}2'}$ = 53.3, $J_{F\text{-}3'}$ = 20.8, $J_{F\text{-}1'}$ = 17.9 and $J_{F\text{-}2'}$ = 53.5, $J_{F\text{-}3'}$ = 22.4, $J_{F\text{-}1'}$ = 18.4 Hz, respectively, with splitting pattern doublet of doublets of doublets (Figure **28**). When the 3′-keto derivatives was treated with *O*-hydroxylamine or *O*-methylhydroxylamine to give oxime derivatives, we expected the disappearance of the coupling constant $J_{F\text{-}3'}$ and the splitting pattern would be appear as doublet of doublets. Nevertheless, we found that a new coupling constant was generated and ranged from 3-5 Hz with splitting pattern as doublet of doublets of doublets in sole isomer configuration. While, for the other isomer configuration, the splitting pattern appeared as a doublet of doublets.

In this regard, Mallory *et al.*[147] reported that on the basis of the lone-pair orbital overlap theory which was developed to account for the phenomenon of "through-space" F-F nuclear spin-spin coupling between intramolecular crowded fluorine atoms (Figure **25**), the existence of "through -space" N-F coupling was predicted for molecules having spatially proximate nitrogen (oxime) and fluorine atoms with lone-pair orbitals oriented for overlap as illustrated in (Figure **26**).

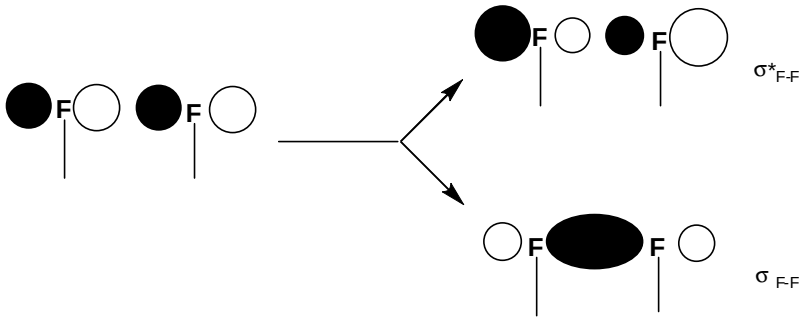


Figure (**25**): Bonding (σ_{FF}) and antibonding (σ^*_{FF}) orbitals generated by overlap of lone-pair orbitals ($2P_F$) on intramoleculary crowded fluorine atoms

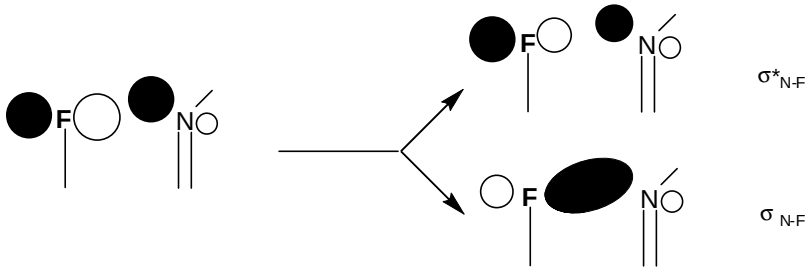


Figure (**26**): Bonding (σ_{NF}) and antibonding (σ^*_{NF}) orbitals generated by overlap of lone-pair orbitals ($2P_F$ and $2sp^2_N$) on intramoleculary crowded fluorine atoms

Mallory *et al.*[148] also reported that the phenomenon of through-space nuclear spin-spin coupling between intramoleculary crowded ^{15}N and ^{19}F nuclei shows the same sort of striking dependence on molecular structure, and the magnitude of the coupling between intramoleculary crowded atoms that each bear a lone-pair depends on the effectiveness of the overlap interactions between the two lone-pair basis orbitals. This leads to the expectation that for a given type of through-space coupling, e.g., F-F or N-F, the coupling constants should rise with the increase of the overlap between the appropriate lone pair orbitals.

From their work, they also found that oxime **A** has the exceptionally large J_{NF} value of 22.4 Hz, whereas oxime **B,** which possesses the same bond connectivity as **A** but lacks the close spatial proximity between the nitrogen and fluorine atoms that is enforced in **A**, and has a J_{NF} value of only 3.2 Hz (Figure **27**).

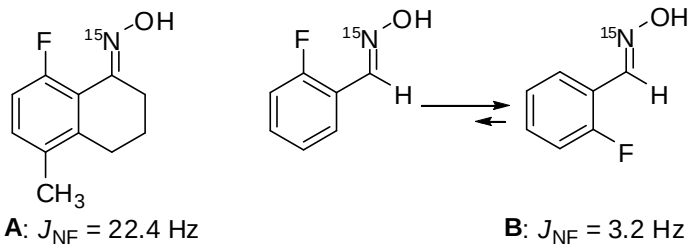


Figure (**27**): the *J* value depending on molecular structure according to Mallory et *al*[30]

The resulting chemical shifts, $J_{H\text{-}F}$ coupling and the $J_{N\text{-}F}$ coupling constants in *N*-oxime nucleoside derivatives **32-47** are listed in table **15**, which reveal a comparison of coupling pattern between **27** and **30** versus *N*-Oxime (E/Z) nucleoside derivatives **32-47**.

Different coupling patterns appeared in the synthesized nucleosides:

- In compounds **27** and **30**, doublet of doublet of doublet due to $J_{F\text{-}2'}$, $J_{F\text{-}3'}$ and $J_{F\text{-}1'}$.
- In oxime (E configuration), doublet of doublet of doublet due to $J_{F\text{-}2'}$, $J_{F\text{-}1'}$ and $J_{N\text{-}F}$.
- In oxime (Z configuration), doublet of doublet due to $J_{F\text{-}2'}$and $J_{F\text{-}1'}$.

Table (**15**): The ^{19}F-H and ^{19}F-N coupling constants of compounds **27**, **30** and oxime derivatives

Compounds	^{19}F NMR Chemical shift (δ) ppm, ddd The coupling constants (*J*) Hz
27	-202.3 $J_{F\text{-}2'}$ = 53.3, $J_{F\text{-}3'}$ = 20.8, $J_{F\text{-}1'}$ = 17.9
30	-200.9 $J_{F\text{-}2'}$ = 53.5, $J_{F\text{-}3'}$ = 22.4, $J_{F\text{-}1'}$ = 18.4

Compounds	^{19}F NMR Chemical shift (δ) ppm The coupling constants (*J*) Hz, ddd (E) isomer	^{19}F NMR Chemical shift (δ) ppm The coupling constants (*J*) Hz, dd (Z) isomer
32	-191.2 $J_{F\text{-}2'}$ = 54.0, $J_{F\text{-}1'}$ = 15.0, $J_{N\text{-}F}$ = 5.3	-184.0 $J_{F\text{-}2'}$ =50.8, $J_{F\text{-}1'}$ =19.2
33	-180.4 $J_{F\text{-}2'}$ = 50.7, $J_{F\text{-}1'}$ = 22.4, $J_{N\text{-}F}$ = 3.7	-181.2 $J_{F\text{-}2'}$ = 52.9, $J_{F\text{-}1'}$ = 18.3
34	-181.1 $J_{F\text{-}2'}$ =51.7, $J_{F\text{-}1'}$ = 21.9, $J_{N\text{-}F}$ = 4.2	-184.5 $J_{F\text{-}2'}$ =55.3, $J_{F\text{-}1'}$ =18.5
35	-181.1 $J_{F\text{-}2'}$ = 50.6, $J_{F\text{-}1'}$ = 22.2, $J_{N\text{-}F}$ = 3.7	-185.5 $J_{F\text{-}2'}$ = 53.5, $J_{F\text{-}1'}$ = 16.4
39	-177.9 $J_{F\text{-}2'}$ = 52.1, $J_{F\text{-}1'}$ = 23.3, $J_{N\text{-}F}$ = 3.7	176.6 $J_{F\text{-}2'}$ = 55.1, $J_{F\text{-}1'}$ = 19.0
40	-178.6 $J_{F\text{-}2'}$ = 52.1, $J_{F\text{-}1'}$ = 23.5, $J_{N\text{-}F}$ = 3.7	-181.3 $J_{F\text{-}2'}$ = 54.1, $J_{F\text{-}1'}$ = 18.6
43	-178.0 $J_{F\text{-}2'}$ = 52.2, $J_{F\text{-}1'}$ = 23.4, $J_{N\text{-}F}$ = 3.9	-176.7 $J_{F\text{-}2'}$ = 55.3, $J_{F\text{-}1'}$ = 17.0
44	-178.9 $J_{F\text{-}2'}$ =52.2, $J_{F\text{-}1'}$ = 23.1, $J_{N\text{-}F}$ = 3.9	-181.3 $J_{F\text{-}2'}$ = 56.0, $J_{F\text{-}1'}$ = 19.3
46	-181.2 $J_{F\text{-}2'}$ = 50.5, $J_{F\text{-}1'}$ = 20.6, $J_{N\text{-}F}$ = 2.8	-184.9 $J_{F\text{-}2'}$ = 54.4, $J_{F\text{-}1'}$ = 18.2
47	-182.4 $J_{F\text{-}2'}$ = 51.9, $J_{F\text{-}1'}$ = 21.7, $J_{N\text{-}F}$ = 2.9	-189.2 $J_{F\text{-}2'}$ = 53.6, $J_{F\text{-}1'}$ = 16.6

The observed magnitudes of J_{NF} appeared in just one isomer configuration demonstrates unequivocally that the C=N double bond in these oximes has the **E** configuration and these magnitudes appeared as a doublet of doublet of doublet owing to the coupling constant $J_{H\text{-}F}$ and the through space coupling between the lone pair orbital in oxime (E configuration) and fluorine atom $J_{N\text{-}F.}$ While, the new coupling constant, which appeared in E configuration, did not appeared in Z configuration due to the absence of overlapping interaction between lone pair orbital in oxime (Z) and fluorine atom (Figure **28**).

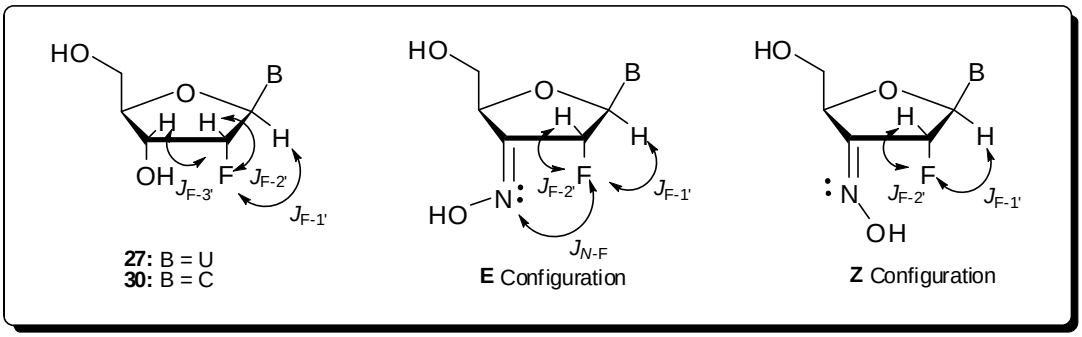


Figure (**28**): Coupling pattern between **27** and **30** versus *N*-Oxime **(E/Z)** nucleoside derivatives

III. Biological Evaluation:

The nucleoside analogues **34, 35, 36, 38, 40, 44, 45, 47** and **49**, which were obtained during this chapter, were evaluated in the laboratory:

- **IDENIX (Cambridge, USA).**

The activity and cytotoxicity were evaluated against:

- HCV (Luciferase Assay).

Also, the nucleoside analogues **34, 35, 36, 38,** and **40**, which were obtained during this chapter, were evaluated in the laboratory:

- **Jan Balzarini, Rega Institute, Leuven, Belgium.**

The activity and cytotoxicity were evaluated against the following viruses in cell cultures:

- Varicella-zoster virus (VZV) in human embryonic lung (HEL) cells.
- Herpes simplex, Vaccinia and Vesicular stomatitis viruses in HEL cell culture.
- Cytomegalovirus in human embryonic lung (HEL) cells.
- Feline Corona Virus (FIPV) and Feline Herpes in CRFK cell culture.
- Anti-influenza virus activity and cytotoxicity in MDCK cell cultures.
- Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus in HeLa cell cultures.
- Para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures.

The results obtained are shown in tables (**16**), (**17**), (**18**), (**19**), (**20**), (**21**), (**22**) and (**23**).

The nucleoside analogues **44**, **45**, **47** and **49** are still under evaluation as antiviral agents.

Table (**16**): Anti-HCV (Luciferase Assay).

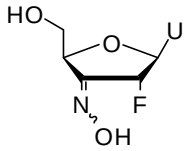
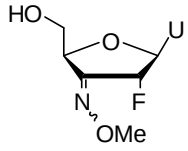
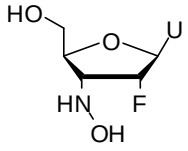
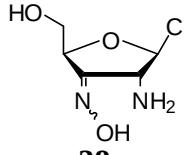
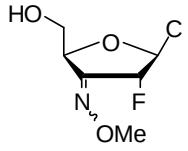
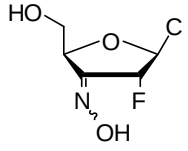
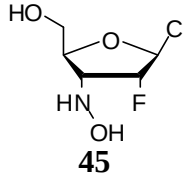
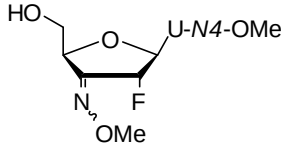
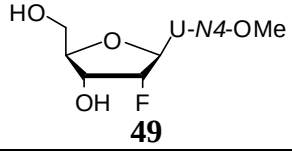
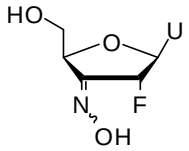
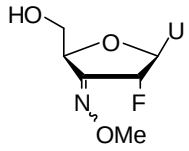
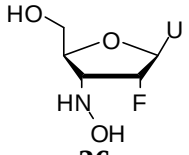
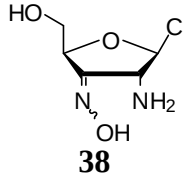
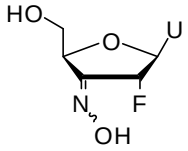
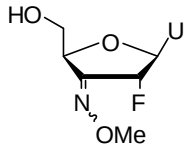
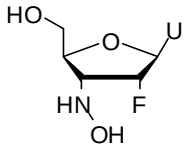
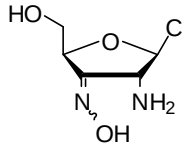
	HCV Replicon	
	CC_{50} (μM)	EC_{50} (μM)
 **34**	>100	>10
 **35**	>100	>10
 **36**	>100	>10
 **38**	>100	>10
 **40**	>100	>10
 **44**	>100	>10
 **45**	>100	>10
 **47**	>100	>10
 **49**	>100	>10

Table (**17**): Activity against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells

	Varicella-Zoster virus (VZV) in HEL cells			
	Cytotoxicity (μM)		[a]EC_{50} (μM)	
	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]	TK^+ VZV Strain OKA	TK^- VZV Strain 07-1
 **34**	>100	>100	>100	>100
 **35**	>100	>100	>100	>100
 **36**	>100	>100	>100	>100
 **38**	>100	>100	>100	>100

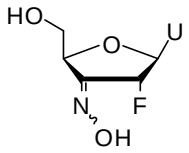
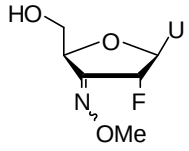
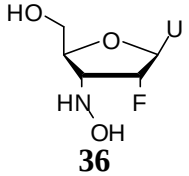
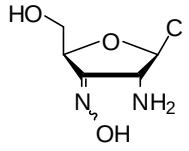
[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

Table (**18**): Cytotoxicity and antiviral activity against Herpes simplex, vaccinia and Vesicular stomatitis viruses in HEL cell culture.

	Herpes simplex, Vaccinia and Vesicular stomatitis viruses in HEL cell cultures.					
	EC_{50} [b] (μM)					
	Minimum Cytotoxic conc. [a] (μM)	Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
 **34**	>100	>100	>100	>100	>100	>100
 **35**	>100	>100	>100	>100	>100	>100
 **36**	>100	>100	>100	>100	>100	>100
 **38**	>100	>100	>100	>100	>100	>100

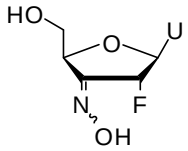
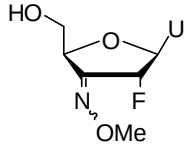
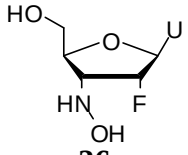
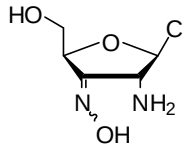
[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table (**19**): Activity against cytomegalovirus in human embryonic lung (HEL) cells.

	Cytomegalovirus HEL cells			
	Cytotoxicity (μM)		[a]EC_{50} (μM)	
	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]	AD-169 strain	Davis strain
 **34**	>100	>100	>100	>100
 **35**	>100	>100	>100	>100
 **36**	>100	>100	>100	>100
 **38**	100	>100	>100	>100

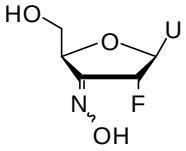
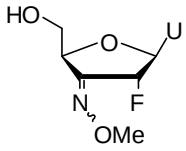
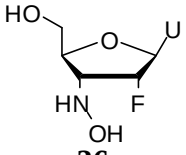
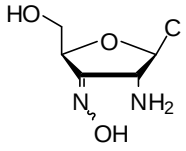
[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

Table (**20**): Virus activity and Cytotoxicity in CRFK cell culture against Feline Corona Virus (FIPV) and Feline Herpes.

	Feline viruses in CRFK cell cultures		
	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
 **34**	>100	>100	>100
 **35**	>100	>100	>100
 **36**	>100	>100	>100
 **38**	>100	>100	>100

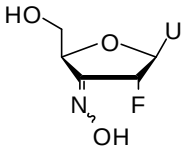
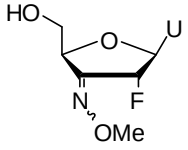
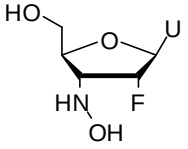
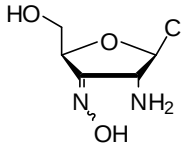
[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
CRFK cells: Crandell-Rees Feline Kidney cells.

Table (**21**): Activity as anti-influenza virus activity and cytotoxicity in MDCK cell cultures.

	Anti-influenza virus activity and cytotoxicity in MDCK cell cultures.							
			Antiviral EC_{50} [c]					
			Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
 **34**	CC_{50} [a] (μM)	Minimum cytotoxic conc.[b] (μM)	Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
 **35**	>100	>100	>100	>100	>100	>100	>100	>100
 **36**	>100	>100	>100	>100	>100	>100	>100	>100
 **38**	>100	>100	>100	>100	>100	>100	>100	>100

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
MDCK cells: Madin Darby canine kidney cells.

Table (**22**): Cytotoxicity and antiviral activity against Vesicular stomatitis virus, Coxsackie virus B4 and Respiratory syncytial virus in HeLa cell cultures.

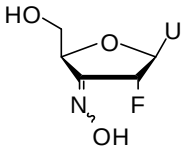
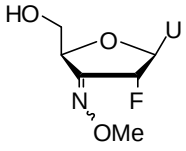
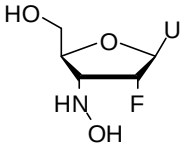
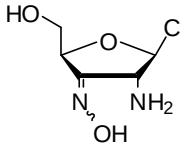
	Vesicular Stomatis, Coxasackie and Respiratory synctial viruses in HeLa cell cultures.							
	CC_{50} [a] (μM)	Minimum cytotoxic conc.[b] (μM)	Antiviral EC_{50} [c]					
			Vesicula stomatitis virus		Coxsackie virus B4		Raspiratory syncytical virus	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
 **34**	>100	>100	>100	>100	>100	>100	>100	>100
 **35**	>100	>100	>100	>100	>100	>100	>100	>100
 **36**	>100	>100	>100	>100	>100	>100	>100	>100
 **38**	>100	>100	>100	>100	>100	>100	>100	>100

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table (**23**): Cytotoxicity and antiviral activity against Para-influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures.

	Viruses in Vero cell cultures					
	EC_{50} [b] (μM)					
	Minimum Cytotoxic conc. [a] (μM)	Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
 **34**	>100	>100	>100	>100	>100	>100
 **35**	>100	>100	>100	>100	>100	>100
 **36**	>100	>100	>100	>100	>100	>100
 **38**	>100	>100	>100	>100	>100	>100

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

IV. Conclusion of chapter III:

In the third chapter, we designed and synthesized several 2′-fluoronucleosides with *N*-hydroxyimino as well as *N*-methoxyimino and *N*-hydroxylamino groups at the sugar moiety bearing uracil and cytosine moieties in order to discover new nucleosides derivatives as potential antiviral agents. Moreover, we observed new spin-spin coupling constant ranged from 3-5 Hz in **E** configuration of oximes derivatives. This spin-spin coupling is related to "Through-space" coupling between the lone pair on nitrogen and fluorine atoms. However, when the nucleosides **34, 35, 36, 38, 40, 44, 45, 47** and **49** were evaluated against several DNA and RNA viruses in cell culture experiments, none of the nucleoside derivatives showed any activity nor cytoxicity at the highest concentration tested (usually 100 µM). The nucleosides **44**, **45**, **47** and **49** are still under evaluation against several DNA and RNA viruses.

General Conclusion

The work in this manuscript has been focused on the synthesis of new nucleoside analogues bearing fluorine atom in positions 3′-β or 2′-α in the sugar moiety, hoping to discover new fluorinated nucleoside analogues as potential antiviral drugs.

In the first chapter, we presented the human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), as well as the therapies used to treat the diseases associated. In a second part, we argument the importance of the incorporation of fluorine atom into nucleoside analogues, and in a third part of this chapter, we presented the recent literature sources of the synthesis and biological activity of the fluorinated nucleosides.

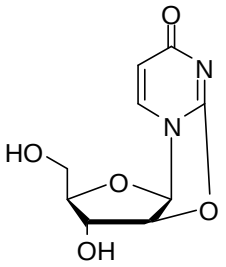
In the second chapter, we designed and synthesized a series of 2′,3′-dideoxy-3′-fluoro-*threo*-pyrimidine nucleosides by direct and rapid methodology and evaluated them for their inhibitory effects on a number of RNA and DNA viruses in cell culture experiments. None of their nucleoside derivatives showed any antiretroviral activity nor cytotoxicity.

In the third chapter of this manuscript, we synthesized a new series of 2′,3′-dideoxy-2′-fluoro-3′-(*N*-hydroxyimino),(N-methoxyimino) and (hydroxylamino)-pyrimidine nucleosides and also evaluated for their inhibitory effects on a number of RNA and DNA viruses, without finding any activity or cytotoxicity, and still some of these nucleoside are under evaluation.

Although, the results obtained in this work don’t give satisfactory activity as antiviral agents, the synthesis and study of new series of nucleosides analogues is still one of the promising researches in the development of new structures that may interfere selectively with viral proliferation.

Lack of biological activities of the synthesized coumpounds may be related to the impossibility of kinases to phosphorylate them or the impossibility of the triphosphate to interact with viral polymerases. These results promote us in the future to synthesize and evaluate the triphosphate form or the prodrug of these compounds to avoid this problem.

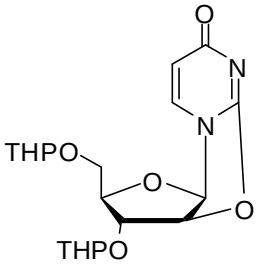
Experimental Part



2,2′-anhydrouridine[149] **23**

A mixture of uridine (25 g, 0.1 mole), diphenylcarbonate (24.12 g, 0.11 mole), in DMF (25 ml) was heated to 90°C, then $NaHCO_3$ (0.25 g) was added. The reaction was held at 110°C for 2.5 h (suspension-solution-suspension) during which time gas evolved (CO_2). The reaction suspension was cooled and the solid isolated by filtration, washed with diethyl ether (50 ml), and then cold methanol (100 ml). The crude product was boiled in methanol (50 ml) for 3 h, cooled to room temperature and isolated by filtration. The resulting white solid was dried to afford **23** (17 g, 73%).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.84 (d, 1H, *J* = 7.4 Hz, H-6), 6.3 (d, 1H, *J* = 5.7 Hz, H-1′), 5.88 (d, 1H, *J* = 4.2 Hz, OH-3′), 5.85 (d, 1H, *J* = 7.4 Hz, H-5), 5.19 (d, 1H, *J* = 5.7 Hz, H-2′), 4.98 (t, *J* = 5.2 Hz,1H, OH-5′), 4.39 (s, 1H, H-3′), 4.07 (q, 1H, H-4′), 3.22 (m, 2H, H-5′,5″).

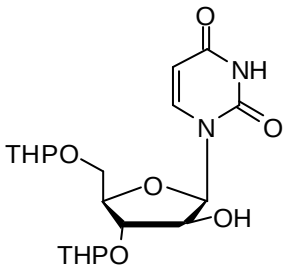


2,2′-anhydro-3′,5′-di-*O*-tetrahydropyranyluridine[150] **24**

To a suspension of 2,2′-anhydrouridine **24** in DMF (300 ml) and 3,4-dihydropyran (180 ml) at 0 °C was added p-toluenesulfonic acid (14 g) and the mixture was stirred at 0 °C for 4 h when a clear solution was obtained. It was neutralized with Et_3N (30 ml) and then evaporated to dryness. The residue was redissolved in EtOAc, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. Removal of solvent gave a residue, which was triturated with hexanes and filtered. The filter cake was washed with hexanes and dried to give **24** as a white solid (27.5 g, 93 %), which was pure enough for the next reaction.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.85 (d, 1H, H-6), 6.33 (d, 1H, H-1′), 5.84 (dt, 1H, H-5), 5.45 (t, 1H, H-2′), 4.76-4.91 (m, 2H, 2 CH), 4.37-4.55 (m, 2H, H-3′,4′), 3.36-3.88 (m, 6H, H-5′,5″, 2 CH_2), 1.2-1.81 (m, 12H, 6 CH_2).

MS ESI-QTof > 0 : m/z 395.2$(M+H)^+$.

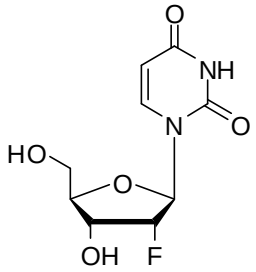


3′,5′-di-*O*-tetrahydropyranylarabinouridine[150] **25**

A suspension of **24** (23 g, 58.5 mmol) in MeOH (300 ml) and 1 N NaOH (100 ml) was stirred at room temperature for 2 h, then neutralized with dilute acetic acid. The mixture was evaporated to dryness and the residue was loaded to a silica gel pad and eluted with EtOAc to give **25** as a white solid (22.6, 94%).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.29 (s, 1H, NH), 7.57 (dd, 1H, H-6), 6.01 (d, 1H, H-1′), 5.78 (d, 1H, H-5), 5.56 (d, 1H, 2-OH), 4.64-4.84 (m, 2H, 2 CH), 3.39-4.23 (m, 9H, H-2′,3′,4′,5′,5″, 2 CH_2), 1.31-1.8 (m, 12H, 6 CH_2).

MS ESI-QTof > 0 : m/z 395.2$(M+H)^+$.



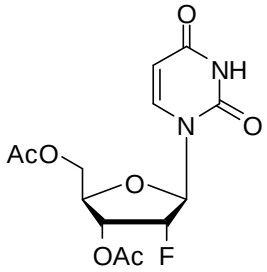
2′-deoxy-2′-fluorouridine[151] **<u>27</u>**

To a stirred mixture of **25** (20.6 g, 50 mmol) in anhydrous CH_2Cl_2 (300 ml) and anhydrous pyridine (50 ml), at -60 °C was added DAST (25 g, 155 mmol) under argon. It was slowly warmed up to room temperature and then refluxed for 4 h. The reaction was quenched by saturated $NaHCO_3$ and ice-water, then extracted with CH_2Cl_2 (100 ml x 3), washed with saturated $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvent gave dark-brown syrup (18.9 g) **26**, which was redissolved in MeOH (300 ml). To this solution was added p-toluenesulfonic acid (6 g) and the mixture was stirred at room temperature for 3 h, it was neutralized with (50ml) pyridine. The solvent was evaporated under reduced pressure and co-evaporated with toluene and the residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give 2′-deoxy-2′-fluorouridine **27** (3.7 g, 30%) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.38 (s, 1H, NH), 7.92 (d, 1H, *J* = 8.1 Hz, H-6), 5.91 (d, 1H, *J* = 17.5 Hz, H-1′), 5.63 (d, 1H, H-5), 5.61 (d, 1H, 3′-OH), 5.21 (t, 1H, 5′-OH), 5.03 (d, 1H, *J* = 53.4 Hz, H-2′), 4.11-4.35 (m, 1H, H-3′), 3.87 (m, 1H, H-4′), 3.67 (m, 2H, H-5′,5″).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -202.3 (ddd, *J* = 53.3, *J* = 20.8, *J* = 17.9 Hz).

MS ESI-QTof > 0: 247.2 $(M+H)^+$.

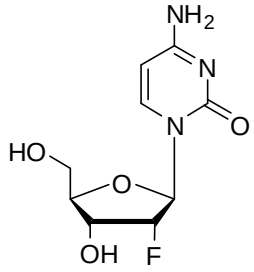


3′,5′-di-*O*-acetyl-2′-deoxy-2′-fluorouridine[51] **<u>28</u>**

A mixture of **27** (4.5 g, 18.28 mmol) and acetic anhydride (8.64 ml, 91.51 mmol) in dry pyridine were stirred at room temperature overnight. The solvent was removed under reduced pressure. The residue was purified on a silica gel column chromatography using MeOH/CH_2Cl_2 (95/5) as eluent and recrystalization from ethanol gave **28** (4.3 g, 71 %).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 8.93 (s, 1H, NH), 7.33 (d, 1H, *J* = 8.1 Hz, H-6), 5.73 (m, 2H, H-5, H-1'), 5.31 (ddd, 1H, *J* = 52.1, *J* = 6.6, *J* = 1.7 Hz, H-2'), 5.09 (m, 1H, H-3'), 4.21-4.41 (m, 3H, H-4',5',5''), 2.07 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.2 and 169.7 (2xCO), 162.7 (C-4), 149.5 (C-2), 140.6 (C-6), 102.9 (C-5), 91.3 (C-1'), 90.9 (C-2', *J* = 192.4 Hz), 78.6 (C-4'), 69.6 (C-3'), 62.2 (C-5), 20.5 (CH_3).



2′-deoxy-2′-fluorocytidine[51] **<u>30</u>**

Lawesson's reagent (3.48 g, 9.49 mmol) was added under argon to a solution of compound **28** (4.05 g, 12.26 mmol) in anhydrous 1,2-dichloroethane (250 ml) and the reaction mixture was stirred under reflux for 3 h. The solvent was then evaporated under reduced pressure and the residue was purified using silica gel column chromatography using MeOH/CH_2Cl_2 (98/2) as eluent to give 4-thio intermediate 3.7 g **29** in 87% yield. A solution of the 4-thio intermediate

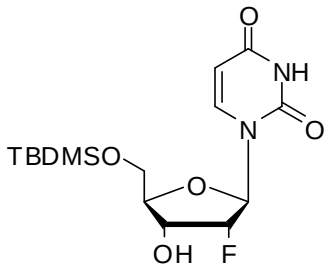
in methanolic ammonia (200 ml) (saturated beforehand at -10 °C and stoppered tightly) was heated at 100°C in a stainless-steel bomb for 3 h, and then cooled to room temperature. The solution was evaporated to dryness under reduced pressure and co-evaporated several times in methanol. The residue was purified by silica gel column chromatography using MeOH/CH_2Cl_2 (7/3) as eluent to give **30** (2.3 g, 88%) as a white foam, overall yield 76 %.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.89 (d, 1H, *J* = 7.4 Hz, H-6), 7.22 (br s, 2H, NH_2), 5.88 (d, 1H, *J* = 17.3 Hz, H-1'), 5.71 (d, 1H, *J* = 7.4 Hz, H-5), 5.55 (d, 1H, *J* = 6.1 Hz, OH-3'), 5.16 (t, 1H, *J* = 5.1 Hz, OH-5'), 4.87 (ddd, 1H, *J* = 53.3, *J* = 5.5, *J* = 1.34 Hz, H-2'), 4.11 (m, 1H, H-3'), 3.86 (m, 1H, H-4'), 3.78 (m, 1H, H-5'), 3.58 (m, 1H, H-5'').

NMR ^{13}C (75 MHz, DMSO-d_6) δ 165.7 (C-4), 154.8 (C-2), 141.0 (C-6), 94.0 (C-2', *J* = 185.2 Hz), 93.9 (C-5), 88.1 (C-1'), 82.7 (C-4'), 67.2 (C-3'), 59.3 (C5').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -200.9 (ddd, *J* = 53.4, *J* = 22.4, *J* = 18.4 Hz).

MS ESI-QTof > 0: 246.1 $(M+H)^+$, 491.2 $(2M+H)^+$.



5′-*O*-TBDMS-2′-deoxy-2′-fluorouridine[152] **31**

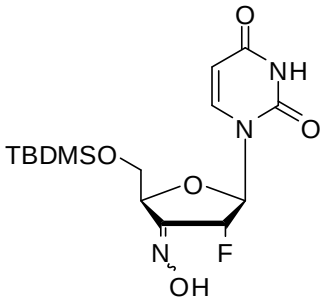
TBDMSCl (0.76 g, 5mmol) was added to an ice-cooled solution of **27** (1 g, 4.1 mmol) and imidazole (0.7 g, 10.28 mmol) in DMF (7 ml). The mixture was allowed to warm slowly to room temperature and stirred 3 h. After being diluted with EtOAc (100 ml), the reaction mixture was washed with water (50 ml) and then brine (50 ml). The organic layer separated was dried over anhydrous $MgSO_4$, evaporated, and purified on a silica gel column

chromatography using CH_2Cl_2/MeOH (95/5) as eluent to give **31** (1.19 g, 82 %) as a white foam.

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.34 (s, 1H, NH), 7.71 (d, 1H, *J* = 8.1, H-6), 5.79 (d, 1H, *J* = 17.2 Hz, H-1′), 5.57 (d, 1H, 3′-OH), 5.44 (d, 1H, *J* = 8.1 Hz, H-5),4.94 (d, 1H, *J* = 53.0, H-2′), 4.05 (m, 1H, H-3′), 3.86 (m, 2H, H-5′,5′′), 3.7 (m, 1H, H-4′), 0.8 (s, 9H, 3 CH_3), 0.01 (s, 6H, 2 CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 163.1 (C-4), 150.1 (C-2), 139.9 (C-6), 101.3 (C-5), 93.5 (C-2′, *J* = 184.8 Hz), 87.4 (C-1′), 82.3 (C-4′), 66.9 (C-3′), 61.0 (C-5′), 25.7 (3xCH_3), 18.0 (C), -5.6 (2xCH_3).

NMR ^{19}F (376 MHz, DMSO-d_6) δ -201.19 (ddd, *J* = 53.0, *J* = 22.8, *J* = 18.4 Hz).



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 32 (E/Z)

Anhydrous pyridine (0.18 ml) and acetic anhydride (0.14 ml) were added successively to an ice-cold suspension of chromium (VI) oxide (0.14 g, 1.4 mmol) in anhydrous CH_2Cl_2 (10 ml), while stirring at room temperature under argon atmosphere was continued until a homogeneous solution was obtained. Compound **31** (0.2 g, 0.55 mmol) was added and stirring was continued for 2 h. The reaction mixture was poured into cold ethyl acetate and the resulting precipitate was filtered off. The filtrate was concentrated, and the residue was used in the next step without purification. To its solution in methanol (4 ml) and pyridine (1 ml) was added hydroxylamine hydrochloride (0.19 g, 2.77 mmol). The reaction mixture was

stirred at room temperature for 1 h, the solvent was evaporated under reduced pressure. The residue was extracted with EtOAc, washed with water and brine. The organic layer separated was dried over anhydrous $MgSO_4$, evaporated the solvent and purified on a silica gel column chromatography using CH_2Cl_2/EtOAc (1/1) as eluent to give **33** (0.15 g, 71%) as a white foam.

UV: (EtOH 95) λ_{max} 257 nm, (ε 9000).

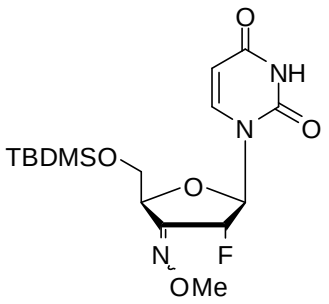
NMR ^{1}H (300 MHz, DMSO-d_6) δ 9.13 (br d, 2H, N**OH**, NH), 7.83 and 7.62 (each d, 1H, *J* = 8.1, *J* = 8.1 Hz, H-6), 6.27 and 6.06 (each d, *J* = 15.9, *J* = 16.6, 1H, H-1′), 5.7 and 5.32 (each d, 1H, *J* = 54.0, *J* = 57.0 Hz, H-2′), 5.72 and 5.69 (each d, 1H, *J* = 8.9, *J* = 8.4 Hz, H-5), 5.07, 4.83 (each s, 1H, H-4′), 3.87-4.13 (m, 2H, H-5′,5″), 0.08 (s, 9H, 3xCH_3), 0.01 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 163.1 (C4), 154.1, 152.8 (C3′), 150.1 (C2), 140.9, 139.8 (C6), 103.1, 102.9 (C5), 90.4, 89.2 (C2′, *J* =197.1, *J* = 175.7 Hz), 89.5, 86.6 (C1′), 80.5, 78.9 (C4′), 63.9, 62.3 (C5′), 25.8 (3xCH_3), 18.3 (C), -5.4 (2xCH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -184.0 (dd, *J* = 50.8, *J* = 19.2 Hz), -191.2 (ddd, *J* = 54.0, *J* = 15.0, *J* = 5.3 Hz).

MS ESI-QTof > 0: 374.2 $(M+H)^+$.

Analysis: Calculated for $C_{15}H_{24}FN_3O_5Si$: C, 48.24; H, 6.48; N 11.25.
Found : C, 48.11; H, 6.25; N, 11.26.



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 33 (E/Z)

Anhydrous pyridine (0.45 ml) and acetic anhydride (0.34 ml) were added successively to an ice-cold suspension of chromium (VI) oxide (0.35 g, 3.5 mmol) in anhydrous CH_2Cl_2 (20 ml), while stirring at room temperature under argon atmosphere was continued until a homogeneous solution was obtained. Compound **31** (0.5 g, 1.38 mmol) was added and stirring was continued for 2 h. The reaction mixture was poured into cold ethyl acetate and the resulting precipitate was filtered off. The filtrate was concentrated, and the residue was used in the next step without purification. To its solution in pyridine (10 ml) was added *O*-methylhydroxylamine hydrochloride (0.58 g, 6.9 mmol). The reaction mixture was refluxed at 80°C for 4 h, cool the mixture, the solvent was evaporated under reduced pressure. The residue was extracted with EtOAc, washed with water and brine. The organic layer separated was dried over $MgSO_4$, evaporated the solvent and purified on a silica gel column chromatography using CH_2Cl_2/EtOAc (7/3) as eluent to give **33** (0.35 g, 65%) as a white foam.

UV: (EtOH 95) λ_{max} 257 nm, (ε 10300).

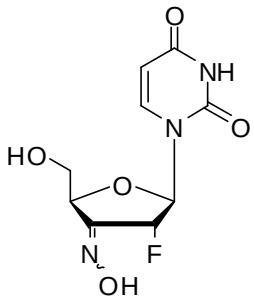
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.5 (s, 1H, NH), 7.78 and 7.74 (each d, 1H, *J* = 7.9, *J* = 7.0, H-6), 5.92 and 6.19 (m, 2H, H-1′, H-2′), 5.7 and 5.6 (each d, 1H, *J* = 8.1, *J* = 9.1, H-5), 5.0 and 4.8 (each s, 1H, H-4′), 3.8- 3.99 (m, 2H, H-5′,5′′), 3.94 (s, 3H, CH_3), 0.89 (s, 9H, 3 CH_3), 0.01 (s, 6H, 2 CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 163.1 (C-6), 153.6 (C-3′), 150.4 (C-2), 143.1 and 141.1 (C-6), 102.3 and 101.8 (C-5), 91.4 and 87.8 (C-1′), 86.2 (C-2', *J* = 184.9 Hz) 79.7 (C-4′), 64.7 (CH_3), 62.4 (C-5′), 25.7 (CH_3), 18.0 (C), -5.5 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -180.4 (ddd, J = 50.7, J = 22.4, J = 3.7 Hz), -181.2 (dd, J = 52.9, J = 18.3 Hz).

MS ESI-QTof > 0: 388.2 $(M+H)^+$.

Analysis: Calculated for $C_{16}H_{26}FN_3O_5Si$: C, 49.6; H, 6.76; N, 10.84.
Found: C, 49.21; H, 6.99; N, 11.16.



1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*pentofuranosyl] uracil 34 (E/Z)

Desilylation of **32** (0.13 g, 0.35 mmol) in 80% acetic acid was stirred overnight at room temperature, then the reaction mixture was concentrated and the last traces of acetic acid removed by co-evaporation with toluene. The residue was purified a silica gel column chromatography using EtOAc or CH_2Cl_2/MeOH (9/1) as eluent to give **34** (70 mg, 78%) as a white foam.

UV: (EtOH 95) λ_{max} 257 nm, (ε 9400).

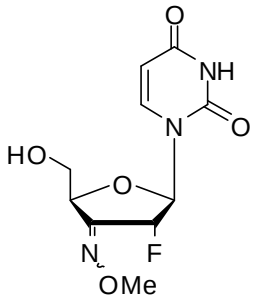
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.81 and 11.74 (each s, 1H, NOH), 11.49 (s, 1H, NH), 7.88 and 7.8 (each d, 1H, J = 8.1, J = 8.0 Hz, H-6), 6.11 and 5.98 (each d, 1H, J = 22.0 Hz, H-1′), 5.77 (d, 1H, J = 51.9 Hz, H-2′), 5.72 and 5.65 (each d, 1H, J = 8.1 Hz, H-5), 4.96 and 4.76 (each s, 1H, H-4′), 5.15 and 5.01 (each t, 1H, 5′-OH), 3.59 (m, 2H, H-5′,5″).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 161.2 (C-4), 151.3 and 150.4 (C-3′), 148.5 (C-2), 141.1 and 139.5 (C-6), 100.5 and 99.9 (C-5), 88.9 and 85.2 (C-1′), 88.4 and 84.1 (C-2′, J = 187.37, J = 179.12 Hz), 78.2 and 77.6 (C-4′), 60.7 and 57.8 (C-5′).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -181.1 (ddd, J = 51.7, J = 21.9, J = 4.2 Hz), -184.5 (dd, J = 55.3, J = 18.5 Hz).

MS ESI-QTof > 0: 260.1 $(M+H)^+$.

Analysis: Calculated for $C_9H_{10}FN_3O_5$ +0.4 H_2O: C, 40.58; H, 4.09; N, 15.77.
Found : C, 40.95; H, 4.25; N, 15.38.



1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]uracil 35 (E/Z)

A mixture of **33** (150 mg, 0.39 mmol) and ammonium fluoride (0.19 g, 3.8 mmol) were refluxed in anhydrous MeOH for 3 h, then the solvent was evaporated under reduced pressure. The crude mixture was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **35** (77 mg, 73%) as a white foam.

UV: (EtOH 95) λ_{max} 257 nm, (ε 7700).

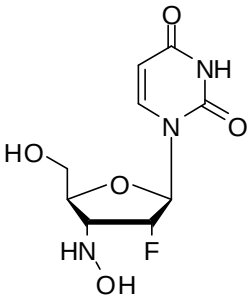
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.52 (s, 1H, NH), 7.90 and 7.85 (each d, 1H, J = 8.0 Hz, J = 7.5 Hz, H-6), 5.9-6.22 (m, 2H, H-1′, H-2′), 5.77 and 5.69 (each d, 1H, J = 8.3 Hz, J = 8 Hz, H-5), 5.25 and 5.09 (each br s, 1H, 5′-OH), 4.99 and 4.8 (each s, 1H, H-4′), 3.91 (s, 3H, CH_3), 3.71 (dm, 2H, H-5′,5″).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 163.7 (C-4), 154.3 (C-3′), 150.8 (C-2), 143.7 and 141.9 (C-6), 102.9 and 102.3 (C-5), 88.5 and 86.7 (C-2′, J = 189.1, J = 172.0 Hz), 91.4 and 87.2 (C-1′), 80.2 (C-4′), 63.1 (CH_3), 60.2 (C-5′).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -181.1 (ddd, J = 50.6, J = 22.2, J = 3.7 Hz), -185.5 (dd, J = 53.5, J = 16.4 Hz).

MS ESI-QTof > 0: 274$(M+H)^+$.

Analysis: Calculated for $C_{10}H_{12}FN_3O_5$ +0.6MeOH: C, 43.53; H, 4.96; N, 14.75. Found: C, 43.72; H, 5.31; N, 14.75.



2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)uridine 36

A solution of **34** (83 mg, 0.32 mmol) in acetic acid (1 ml) was added dropwise to a solution of acetic acid (2 ml) containing $NaBH_4$ (36 mg, 0.95 mmol) at 0°C. After the mixture was stirred at room temperature for 2 h, the solvent was removed in *vacuo*. The residue was co-evaporated with toluene two times and purified on a silica gel column chromatography using CH_2Cl_2/MeOH (8/2) as eluent and recrystallized from ethanol to provide **36** (60 mg, 71%) as a white foam.

$[\alpha]_D^{20}$: +53.3 (c 0.3, MeOH).

UV: (EtOH 95) λ_{max} 262 nm, (ε 11900).

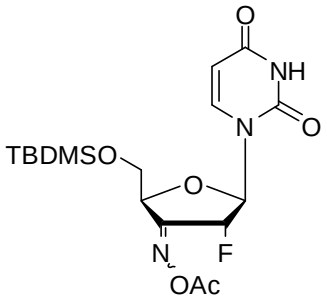
NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.37 (s, 1H, NH), 7.98 (d, 1H, J = 8.1 Hz, H-6), 7.67 (s, 1H, **NH**OH), 5.97 (d, 1H, J = 17.5 Hz, H-1′), 5.98 (s, 1H, NH**OH**), 5.63 (d, 1H, J = 8.0 Hz, H-5), 5.27 (s, 1H, OH-5'), 5.18 (d, 1H, J = 51.5, H-2'), 3.95 (d, 1H, J = 8.1 Hz, H-4'), 3.78 (d, 1H, J = 12.0 Hz, H-3'), 3.61 (m, 2H, H-5',5'').

NMR ^{13}C (75 MHz, DMSO-d_6) δ 162.6 (C-4), 149.7 (C-2), 139.8 (C-6), 101.0 (C-5), 92.7 (C-2', J = 184.0 Hz), 87.4 (C-1'), 80.7 (C-4'), 60.5 (C-5'), 59.9 (C-3').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -201.1 (ddd, J = 52.2, J = 20.3, J = 18.4 Hz)

MS ESI-QTof > 0: 261.1 $(M+H)^+$, 523.3 $(2M+H)^+$.

Analysis: Calculated for $C_9H_{12}FN_3O_5$ +0.2 EtOH: C, 41.75; H, 4.92; N, 15.54.
Found: C, 41.36; H, 5.24; N, 15.83.



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-acetoxyimino)-*β*-D-*glycero*-pentofuranosyl] uracil 37

Acetic anhydride (0.19 ml, 2.05 mmol) was added to a mixture of **32** (0.5 g, 1.34 mmol) and DMAP (6 mg) in anhydrous pyridine (10 ml). The reaction mixture was stirred for 2.5 h at room temperature, and then ice-water was added to the mixture. The mixture was diluted with EtOAc (100 ml) and the whole was washed with H_2O (50 ml), aqueous saturated $NaHCO_3$, then H_2O (50 ml). The separated organic phase was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by a silica gel column chromatography using CH_2Cl_2/MeOH (97/3) as eluent to give **37** (0.45 g, 81%) as a white foam.

UV: (EtOH 95) λ_{max} 256 nm, (ε 11500).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.53 (br s, 1H, NH), 7.81 and 7.72 (each d, J = 8.0, J = 8.1 Hz, H-6), 6.24 and 6.06 (each d, J = 17.1, J = 21.0 Hz, 1H, H-1′), 6.26 and 5.99 (each d, J = 51.0 Hz, H-2′), 5.72 and 5.67 (d, J = 8.0, 7.9 Hz, 1H, H-5), 5.21 and 5.06 (each s, 1H, H-4′), 4.02 and 3.88 (each m,

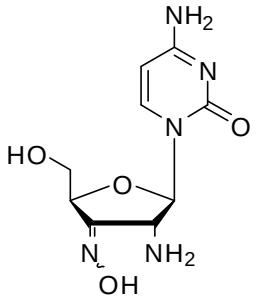
2H, H-5′,5″), 0.83 (s, 9H, 3xCH_3), 0.01 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 167.1 (CO), 163.0 (C4), 162.2 (C3′), 150.6 (C2), 143.3 and 140.9 (C6), 102.5 and 101.9 (C5), 89.2 (C2′, *J* = 174.4 Hz), 87.0 (C1′), 79.6 (C4′), 64.9 and 62.3 (C5′), 25.6 (3XCH_3), 19.0 (CH_3), 18.0 (C), -5.5 (2xCH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -180.5 (dd, *J* = 49.6, J= 22.1 Hz), 186.4 (dd, *J* = 51.8, *J* = 17.6 Hz).

MS ESI-QTof > 0: 416.2 $(M+H)^+$.

Analysis: Calculated for $C_{17}H_{26}FN_3O_6Si$: C, 49.14; H, 6.31; N, 10.11.
Found: C, 49.1; N, H, 6.55; N, 9.91.



1-[2-amino-2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine dihydrochloride <u>38</u>

Triethylamine (0.52 ml, 3.72 mmol) was added to a mixture of **37** (0.52 g, 1.24 mmol), TPSCl (1.13 g, 3.73 mmol), and DMAP (25 mg) in CH_3CN (10 ml) at 0°C under argon. The reaction mixture was stirred for 2 h at room temperature then the solvent was removed in *vacuo.* The residue was treated with saturated NH_3/MeOH (20 ml) for 13 h at room temperature, after concentration of the mixture, the resulting solid was purified on a silica gel column eluted MeOH (0-50%) in CH_2Cl_2. This compound was transformed into its hydrochloride after three coevaporations with a mixture of 2M hydrochloride acid (3 ml) and ethanol (6 ml), and precipitated to afford **38** (50 mg, 16%) as a white powder.

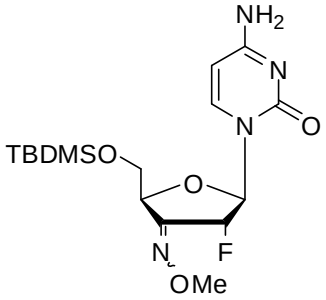
UV: (EtOH 95) λ_{max} 268 nm, (ε 6200).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.73 (s, 1H, NOH), 9.75 and 8.75 (each br s, 3H, NH_3^+), 9.75 and 8.68 (each br s, 2H, NH_3), 7.98 (d, 1H, *J* = 7.6 Hz, H-6), 6.06 (d, 1H, J = 7.6 Hz, H-1′), 5.99 (d, 1H, *J* = 7.5 Hz, H-5), 4.67 (s, 1H, H-2′), 4.19 (s, 1H, H-4′), 3.48 (m, 2H, H-5′,5″).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 160.0 (C-4), 152.4 (C-3′), 148.6 (C-2), 144.3 (C-6), 95.6 (C-5), 84.2 (C-1′), 79.0 (C-4′), 59.9 (C-5′), 53.6 (C-2′).

MS ESI-QTof > 0: 255.1(M-2Cl) $^{+}$.

Analysis: Calculated for $C_9H_{13}N_5O_4.2\ HCl.1.1\ H_2O$: C, 31.07; H, 4.98; N, 20.13. Found : C, 31.4; H, 5.38; N, 20.05.



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] cytosine 39

Triethylamine (0.43 ml, 3.08 mmol) was added to a mixture of compound **33** (0.3 g, 0.77 mmol), triisopropylbenzenesulfonyl chloride (0.47 g, 1.55 mmol), and DMAP (94 mg, 0.77 mmol) in CH_3CN (10 ml) at 0°C under argon. The reaction mixture was stirred for 2 h at room temperature, and then the solvent was removed in *vacuo*. The residue was treated with NH_3/MeOH for 12 h at room temperature. After concentration of the mixture, the resulting solid was purified on a silica gel column chromatography using MeOH/CH_2Cl_2 (9/1) as eluent to afford **39** (0.2 g, 67%) as a white foam.

UV: (EtOH 95) λ_{max} 264 nm, (ε 8200).

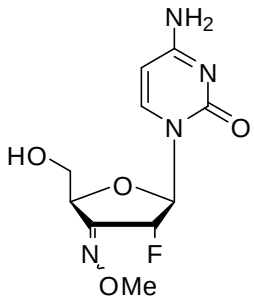
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.74 and 7.73 (each d, 1H, *J* = 7.4, *J* = 7.4 Hz, H-6), 7.38 (s, 2H, NH_2), 5.77 and 5.73 (d, 1H, *J* = 7.4, *J* =7.3 Hz, H-5), 5.04-6.62 (m, 2H, H-1′, H-2′), 4.82 and 5.02 (s, 1H, H-4′), 3.9 (s, 3H, CH_3), 3.81-3.99 (m, 2H, H-5′,5″), 0.85 (s, 9H, 3xCH_3), 0.01 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 166.0 (C-4), 155.4 and 154.5 (C-3′), 155.0 (C-2), 144.1 and 142.6 (C-6), 94.6 and 94.3 (C-5), 93.0 and 89.7 (C-1′), 91.2 and 86.6 (C-2′, *J* = 163.7, *J* = 176.8 Hz), 80.0 (C-4′), 65.3 (C-5′), 62.3 (OCH_3), 25.7 (3xCH_3), 18.0 (C), -5.4 (2xCH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -177.9 (ddd, *J* = 52.1, *J*= 23.3, *J* = 3.7 Hz), 176.6 (dd, *J* = 55.1, *J* = 19.0 Hz).

MS ESI-QTof > 0: 387.2 $(M+H)^+$.

Analysis: Calculated for $C_{16}H_{27}FN_4O_4Si$: C, 49.72, H, 7.04, N14.5,
Found: C, 49.57; H, 7.42; N, 14.81.



1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine <u>40</u>

A mixture of **39** (140 mg, 0.36 mmol) and ammonium fluoride (0.14 g, 3.8 mmol) were refluxed in anhydrous MeOH for 3 h, then the solvent was evaporated under reduced pressure.

The crude mixture was purified by silica gel column chromatography using CH_2Cl_2/MeOH (8/2) as eluent to provide **40** (60 mg, 61%) as a white foam.

UV: (EtOH 95) λ_{max} 267 nm, (ε 10600).

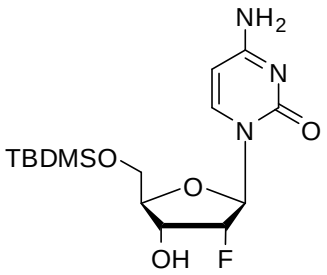
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.79 and 7.75 (each d, 1H, *J* = 7.5, *J* =7.4 Hz, H-6), 7.39 (br s, 2H, NH_2), 5.69-6.07 (m, 2H, H-1′, H-2′), 5.79 and 5.71 (each d, *J* = 7.5, *J* = 7.8 Hz, H-5), 5.08 and 4.92 (t, *J* = 5.4, *J* = 5.6 Hz, OH-5′), 4.94 and 4.76 (s, 1H, H-4′), 3.91 and 3.88 (each s, 3H, CH_3), 3.75 and 3.66 (each m, 2H, H-5′,5″).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 166.1 (C-4), 155.2 and 154.5 (C-3′), 155.0 (C-2), 144.4 and 143.0 (C-6), 94.8 and 94.3 (C-5), 92.8 and 89.0 (C-1′), 90.5 and 86.5 (C-2′, *J* = 170.6, *J* = 177.3 Hz), 79.9 (C-4′), 63.1 and 60.2 (C-5′), 62.4 (OCH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -178.6 (ddd, *J* = 52.1, *J* = 23.5, *J* = 3.7 Hz), -181.3 (dd, *J* = 54.1, *J* = 18.6 Hz).

MS ESI-QTof > 0: 273.1 $(M+H)^+$.

Analysis: Calculated for $C_{10}H_{13}FN_4O_4$ +0.5H_2O: C, 42.71. H, 5.02, N, 19.92. Found: C, 42.69; H, 5.13; N, 19.79.



5′-*O*-TBDMS-2′-deoxy-2′-fluorocytidine <u>41</u>

TBDMSCl (1.46 g, 9.6 mmol) was added to a solution of a compound **30** (2 g, 8.16 mmol) and imidazole (1.34 g, 19.6 mmol) in dry DMF (10 ml). The mixture was stirred at room temperature for 4 h. After being diluted with EtOAc (50 ml), the reaction mixture was washed with water and brine. The organic layer separated was dried over anhydrous $MgSO_4$, evaporated the solvent. The residue was purified on a silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent gave **41** (2.26 g, 77%) as a white foam.

$[\alpha]_D^{20}$: +57 (c 1.00, MeOH)

UV: (EtOH 95) λ_{max} 271 nm, (ε 8400).

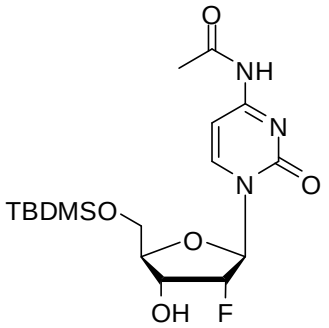
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.7 (d, 1H, *J* = 7.4 Hz, H-6), 7.13 (s, 1H, NH_2), 5.79 (d, 1H, *J* = 17.8 Hz, H-1'), 5.59 (d, 1H, *J* = 7.4 Hz), 5.49 (d, 1H, *J* = 6.4 Hz, OH-3'), 4.74 (d, 1H, *J* = 57.0 Hz, H-2'), 4.02 (m, 1H, H-3'), 3.91(d, 1H, *J* = 10.1 Hz, H-4'), 3.81 (m, 1H, H-5'), 3.7 (m, 1H, H-5''), 0.81 (s, 9H, 3xCH_3), 0.01 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 165.7 (C-4), 154.6 (C-2), 140.3 (C-6), 94.1 (C-2', *J* = 185.0 Hz), 93.7 (C-5), 87.8 (C-1'), 81.8 (C-4'), 66.7 (C-3'), 60.8 (C-5'), 25.7 (CH_3), 18.0 (C), -5.5 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -200.2 (ddd, *J* = 53.3, *J* = 24.4, *J* = 17.9 Hz).

MS ESI-QTof > 0: 360.1 $(M+H)^+$.

Analysis: Calculated for $C_{15}H_{26}FN_3O_4Si$: C, 50.12; H, 7.29; N, 11.69.
Found: C, 49.74; H, 7.65; N, 11.45.



5′-*O*-TBDMS-2′-deoxy-2′-fluoro-*N*^{4}-acetylcytidine 42

To a solution of compound **41** (1 g, 2.8 mmol) in DMF (15 ml), acetic anhydride (0.43 ml, 4.5 mmol) was added, the reaction mixture was stirred at room temperature for 24 h, then the solvent was removed under reduced pressure. The product was extracted with EtOAc, washed with water and brine. The organic layer separated was dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was purified on a silica gel column chromatography using CH_2Cl_2/MeOH (9:1) as eluent to afford **42** (0.95 g, 86%) as a white foam.

$[\alpha]_D^{20}$: +52 (c 1.00, MeOH)

UV: (EtOH 95) λ_{max} 297 nm, (ε 6600), λ_{max} 248 nm, (13200), λ_{min} 273, (ε 3400).

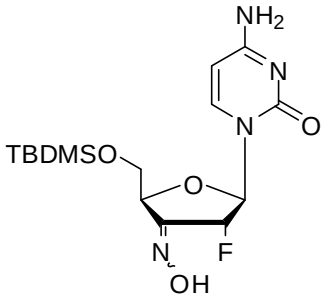
NMR ^{1}H (300 MHz, DMSO-d_6) δ 10.84 (s, 1H, NH), 8.19 (d, 1H, *J* = 7.5 Hz, H-6), 7.1 (d, 1H, *J* = 7.5 Hz, H-5), 5.8 (d, 1H, *J* = 17.3, H-1'), 5.54 (s, 1H, OH-3'), 4.84 (d, 1H, *J* = 54.0, H-2'), 3.93 (m, 3H, H-3',H-4', H-5'), 3.72 (m, 1H, H-5''), 0.79 (s, 9H, 3xCH_3), 0.0 (s, 6H, 2xCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 170.8 (CO), 162.3 (C-4), 153.8 (C-2), 143.8 (C-6), 94.8 (C-5), 93.6 (C-2', *J* = 182.7 Hz), 88.2 (C-1'), 81.9 (C-4'), 66.1 (C-3'), 60.3 (C-5'), 25.5 (CH_3), 24.1 (CH_3), 17.8 (C), -5.8 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -200.7 (ddd, *J* = 53.1, *J* = 26.0, *J* = 17.5 Hz).

MS ESI-QTof > 0: 402.2 $(M+H)^+$.

Analysis: Calculated for $C_{17}H_{28}FN_3O_5Si$: C, 50.85; H, 7.03; N, 10.47.
Found: C, 50.53; H, 7.19; N, 10.52.



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-hydroxy-imino)-*β*-D-*glycero*-pentofuranosyl] cytosine 43 (E/Z)

Anhydrous pyridine (0.59 ml) and acetic anhydride (0.43 ml) were added successively to an ice-cold suspension of chromium (VI) oxide (0.46 g, 4.6 mmol) in anhydrous CH_2Cl_2 (40 ml), while stirring at room temperature under argon atmosphere was continued until a homogeneous solution was obtained. Compound **42** (0.74 g, 1.84 mmol) was added and stirring was continued for 2 h. The reaction mixture was poured into cold ethyl acetate and the resulting precipitate was filtered off. The filtrate was concentrated, and the residue was used in the next step without purification. To its solution in pyridine (15 ml) was added hydroxylamine hydrochloride (0.64 g, 9.22 mmol). The reaction mixture was stirred at room temperature for 1 h, the solvent was evaporated under reduced pressure. The residue was extracted with EtOAc, washed with water and brine. The organic layer separated was dried over anhydrous $MgSO_4$, evaporated the solvent and purified on a silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to give **43** (0.21 g, 31%) as a white foam.

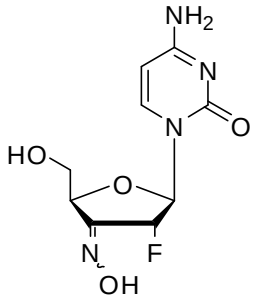
UV: (EtOH 95) λ_{max} 266 nm, (ε 7600).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.83 and 11.66 (each s, 1H, NOH), 7.43 and 7.39 (each d, 1H, *J* = 7.4, 7.3 Hz, H-6), 7.35 (s, 2H, NH_2), 6.01-5.56 (m, 1H, H-1', H-2'), 5.76 and 5.72 (each d, *J* = 7.4, *J* =7.3, H-5), 4.8 and 5.02 (each s, 1H, H-4'), 3.8-4.1 (m, 2H, H-5',5''), 0.87 (s, 9H, 3xCH_3), 0.1 (s, 6H, 2xCH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -178.0 (ddd, J = 52.2, J = 23.4, J = 3.9 Hz), -176.7 (dd, J = 55.3, J = 17.0 Hz).

MS ESI-QTof > 0: 373.2 $(M+H)^+$.

Analysis: Calculated for $C_{15}H_{25}FN_4O_4Si$: C, 48.37; H, 6.77; N, 15.04.
Found: C, 48.32; H, 6.91, N, 14.99.



1-[2,3-dideoxy-2-fluoro-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]cytosine <u>44 (E/Z)</u>

Desilylation of **43** (0.17 g, 0.46 mmol) in 80% acetic acid was stirred for 3 days at room temperature, then the reaction mixture was concentrated and the last traces of acetic acid removed by coevaporation with toluene. The residue was purified on a silica gel column chromatography using CH_2Cl_2/MeOH as eluent (9/1) to give **44** (85 mg, 73%) as a white foam.

UV: (EtOH 95) λ_{max} 264 nm, (ε 7000).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 11.74 and 11.62 (each s, 1H, NOH), 7.8 and 7.75 (each d, J = 7.4, J = 7.3 Hz, H-6), 7.38 (br s, 2H, NH_2), 5.6-6.05 (m, 2H, H-1', H-2'), 5.78 and 5.73 (each d, 1H, J = 7.5 Hz, H-5), 5.02 and 4.89 (each t, 1H, OH-5'), 4.93 and 4.74 (each s, 1H, H-4'), 3.79 and 3.68 (each m, 2H, H-5',5'').

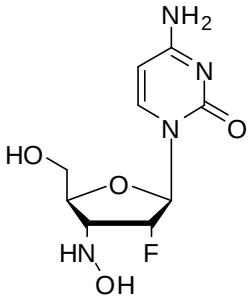
NMR ^{13}C (75 MHz, DMSO-d_6) δ 165.9 (C-4), 155.0 (C-2), 154.0 and 153.1 (C-3'),

144.1 and 142.9 (C-6), 94.0 and 94.8 (C-5), 92.6 and 89.0 (C-1'), 90.6 and 86.2 (C-2', *J* = 181.1, *J* = 177.2 Hz), 79.86 (C-4'), 62.95 and 60.06 (C-5').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -181.3 (dd, *J* = 56.0, *J* = 19.3 Hz), -178.9 (ddd, 52.2, *J* = 23.1, *J* = 3.9 Hz).

MS ESI-QTof > 0: 259.1 $(M+H)^+$, 517.2 $(2M+H)^+$.

Analysis: Calculated for $C_9H_{11}FN_4O_4$ +0.6 H_2O: C, 40.18; H, 4.57; N, 20.83. Found: C, 40.11; H, 4.82; N, 20.52.



2′,3′-dideoxy-2′-fluoro-3′-(hydroxylamino)cytidine 45

A solution of **44** (60 mg, 0.23 mmol) in acetic acid (1 ml) was added dropwise to a solution of acetic acid (2 ml) containing $NaBH_4$ (60 mg, 1.58 mmol) at 0°C. After the mixture was stirred at room temperature for 2 h, the solvent was removed in *vacuo*. The residue was co-evaporated with toluene two times and purified on a silica gel column chromatography, which using CH_2Cl_2/MeOH (7/3) as eluent to provide **45** (30 mg, 50%) as a white foam.

$[\alpha]_D^{20}$: +16.66 (c 0.3, MeOH)

UV: (EtOH 95) λ_{max} 264 nm, (ε 7600).

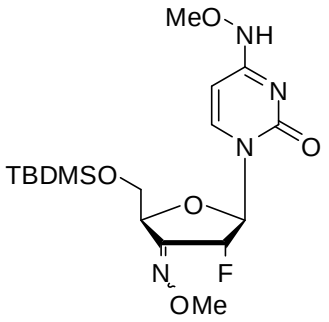
NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.96 (d, 1H, *J* = 7.4 Hz, H-6), 7.22 (br d, 3H, NH_2, NH), 5.91 (d, 1H, *J* = 16.9 Hz, H-1'), 5.72 (d, 1H, *J* = 7.4 Hz, H-6), 5.04 (ddd, 1H, *J* = 53.0, *J* = 3.8, J=1.0 Hz, H-2'), 3.8 (d, 1H, *J* = 12.2, H-4'), 3.65 (d, 1H, *J* = 15.0 Hz, H-3'), 3.5 (m, 2H, H-5',5'').

NMR ^{13}C (75 MHz, DMSO-d_6) δ 164.9 (C-4), 154.5 (C-2), 140.4 (C-6), 93.5 (C-5), 93.0 (C-2', J = 181.1 Hz), 88.3 (C-1'), 80.1 (C-4'), 60.0 (C-5'), 59.5 (C-3').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -199.5 (ddd, J = 52.6, J = 23.1, J = 18.0 Hz).

MS ESI-QTof > 0: 261.1 $(M+H)^+$, 521.2 $(2M+H)^+$.

Analysis: Calculated for $C_9H_{13}FN_4O_4$: C, 41.54; H, 5.04; N, 21.53.
Found: C, 41.23; H, 4.92; N, 21.36.



1-[5-*O*-TBDMS-2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]-N^4-methoxycytosine 46 (E/Z)

Anhydrous pyridine (0.24 ml) and acetic anhydride (0.18 ml) were added successively to an ice-cold suspension of chromium (VI) oxide (186 mg, 1.86 mmol) in anhydrous CH_2Cl_2 (15 ml), while stirring at room temperature under argon atmosphere was continued until a homogeneous solution was obtained. Compound **42** (0.3 g, 0.75 mmol) was added and stirring was continued for 2 h. The reaction mixture was poured into cold ethyl acetate and the resulting precipitate was filtered off. The filtrate was concentrated, and the residue was used in the next step without purification. To its solution in pyridine (15 ml) was added *O*-methylhydroxylamine hydrochloride (0.3 g, 3.59 mmol). The reaction mixture was refluxed at 80°C for 3 h, the solvent was evaporated under reduced pressure. The residue was extracted with EtOAc, washed with water and brine. The organic layer separated was dried over anhydrous $MgSO_4$, evaporated the solvent and purified on a silica gel column

chromatography using CH_2Cl_2/EtOAc (95/5) as eluent to give **46** (114 mg, 37%) as a white foam.

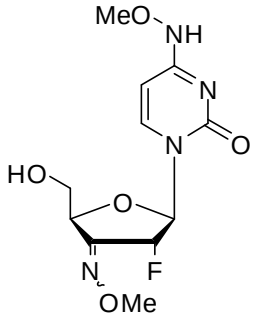
UV: (EtOH 95) λ_{max} 275 nm, (ε 7800).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 10.19 and 10.14 (each s, 1H, NH), 7.05 and 7.04 (each d, 1H, *J* = 8.1, *J* = 8.2 Hz, H-6), 5.51-6 (m, 3H, H-1', H-5, H-2'), 4.81 and 4.98 (each s, 1H, H-4'), 3.88 (s, 3H, OCH_3), 3.8 (m, 2H, H-5',5''), 3.66 (s, 3H, OCH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 153.8 (C-3'), 149.0 (C-4), 143.4 (C-2), 133.4 and 131.2 (C-6), 98.2 and 97.6 (C-5), 90.2 and 86.6 (C-1'), 89.5 and 86.2 (C-2', *J* = 183.1, *J* = 179.1 Hz), 79.0 (C-4'), 64.8 (OCH_3), 62.6 (OCH_3), 62.0 and 61.0 (C-5'), 25.7 (CH_3), 18.0 (C), -5.4 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -181.2 (ddd, *J* = 50.5, *J* = 20.6, *J* = 2.8 Hz), -184.9 (dd, *J* = 54.4, *J* = 18.2 Hz).

MS ESI-QTof > 0: 417.1 $(M+H)^+$.



1-[2,3-dideoxy-2-fluoro-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl] N^4-methoxy-cytosine 47 (E/Z)

A mixture of **46** (70 mg, 0.168 mmol) and ammonium fluoride (68 mg, 1.8 mmol) were refluxed in anhydrous MeOH for 3 h, then the solvent was evaporated under reduced pressure.

The crude mixture was purified by silica gel column chromatography using Et_2O/EtOH (98/2) as eluent to provide **47** (40 mg, 41%) as a white foam.

UV: (EtOH 95) λ_{max} 275 nm, (ε 7900).

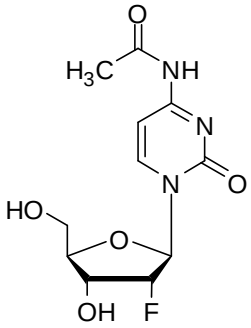
NMR ^{1}H (300 MHz, DMSO-d_6) δ 10.28 (s, 1H, NH), 7.23 and 7.17 (each d, 1H, *J* = 8.2, *J* = 8.1 Hz, H-6), 5.82-6.08 (m, 1H, H-1' H-2'), 5.72 and 5.64 (each d, 1H, *J* = 8.1 Hz, H-5), 5.28 and 5.09 (each t, 1H, OH-5'), 4.81 and 4.98 (each s, 1H, H-4'), 3.95 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.69 (m, 2H, H-5',5'').

NMR ^{13}C (75 MHz, DMSO-d_6) δ 153.7 (C-3'), 149.0 (C-4), 143.5 (C-2), 133.4 and 131.3 (C-6), 98.3 and 97.7 (C-5), 90.2 and 85.4 (C-1'), 88.5 and 86.7 (C-2', *J* = 179.5, *J* = 178.1 Hz), 79.1 (C-4'), 62.3 (OCH_3), 61.0 (OCH_3), 62.6 and 59.8 (C-5').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -182.4 (ddd, *J* = 51.9, *J* = 21.7, *J* = 2.9 Hz), -189.2 (dd, *J* = 53.6, *J* = 16.6 Hz).

MS ESI-QTof > 0: 303.1 $(M+H)^+$, 605..3 $(2M+H)^+$.

Analysis: Calculated for $C_{11}H_{15}FN_4O_5$ +0.2 EtOH: C, 43.96; H, 5.24; N, 17.99. Found: C, 43.68; H, 5.46; N, 17.83.



2′-deoxy-2′-fluoro-*N*^{4}-acetylcytidine <u>48</u>

To a solution of compound **30** (0.3 g, 1.22 mmol) in DMF (4 ml), acetic anhydride (0.15 ml, 1.58 mmol) was added, the reaction mixture was stirred at room temperature for 24 h, then the solvent was removed under reduced pressure. The residue was purified on a silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent to afford **48** (0.25 g, 71%) as a white foam.

$[\alpha]_D^{20}$: +76 (c 0.5, MeOH).

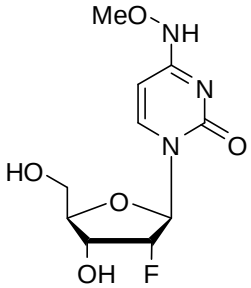
UV: (EtOH 95) λ_{max} 298 nm, (ε 7000), λ_{max} 248 nm, (14700), λ_{min} 273, (ε 3800).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 10.93 (s, 1H, NH), 8.43 (d, 1H, *J* = 7.5 Hz, H-6), 7.19 (d, 1H, *J* = 7.5 Hz, H-5), 5.91 (d, 1H, *J* = 17.1 Hz, H-1'), 5.61 (d, 1H, *J* = 6.3 Hz, OH-3'), 5.28 (t, 1H, *J* = 4.9 Hz, OH-5'), 4.96 (dd, 1H, *J* = 52.8, *J* = 4.0 Hz, H-2'), 4.13 (m, 1H, H-3'), 3.94 (m, 1H, H-4'), 3.86 (m, 1H, H-5'), 3.63 (m, 1H, H-5''), 2.1 (s, 3H, CH_3).

NMR ^{13}C (75 MHz, DMSO-d_6) δ 171.0 (CO), 162.5 (C-4), 154.2 (C-2), 144.9 (C-6), 95.2 (C-5), 93.9 (C-2', *J* = 185.7 Hz), 88.5 (C-1'), 82.9 (C-4'), 66.7 (C-3'), 58.7 (C-5'), 24.3 (CH_3).

NMR ^{19}F (282 MHz, DMSO-d_6) δ -201.2 (ddd, *J* = 53.0, *J* = 25.0, *J* = 17.3 Hz)

MS ESI-QTof > 0: 288.1 $(M+H)^+$, 575.2 $(2M+H)^+$.



2′-deoxy-2′-fluoro-*N*^{4}-methoxycytidine 49

A mixture of compound **48** (0.1 g, 0.35 mmol) and *O*-methylhydroxylamine hydrochloride (73 mg, 0.87 mmol) were refluxed in dry pyridine (4 ml) for 3 h. The solvent was evaporated

and co-evaporated with toluene. The residue was purified on a silica gel column chromatography using CH_2Cl_2/MeOH (9/1) as eluent, and recrystalization from EtOAc to afford **49** (80 mg, 84%) as a white foam.

$[\alpha]_D^{20}$: +14 (c 0.5, MeOH).

UV: (EtOH 95) λ_{max} 279 nm, (ε 7700), λ_{max} 237 nm, (12300), λ_{min} 262, (ε 6500).

NMR ^{1}H (300 MHz, DMSO-d_6) δ 7.16 (d, 1H, *J* = 8.2 Hz, H-6), 5.87 (d, 1H, *J* = 17.7 Hz, H-1'), 5.6 (d, 1H, *J* = 5.4 Hz, OH-3'), 5.13 (t, 1H, *J* = 4.8 Hz, OH-5'), 5.56 (d, 1H, *J* = 8.2 Hz, H-5), 4.97 (ddd, 1H, *J* = 53.3, *J* = 7.7, *J* = 3.1 Hz, H-2'), 4.13 (m, 1H, H-4'), 3.82 (m, 1H, H-3'), 3.67 (s, 3H, CH_3), 3.52 (m, 2H, H-5',5'').

NMR ^{13}C (75 MHz, DMSO-d_6) δ 148.8 (C-4), 143.7 (C-2), 131.0 (C-6), 97.5 (C-5), 92.9 (C-2', *J* = 186.2 Hz), 86.3 (C-1'), 83.2 (C-4'), 67.9 (C-3'), 60.9 (CH_3), 59.9 (C-5').

NMR ^{19}F (282 MHz, DMSO-d_6) δ -203.3 (ddd, *J* = 53.5, *J* = 17.6 Hz).

MS ESI-QTof > 0: 276.1 $(M+H)^+$, 551.2 $(2M+H)^+$.

Analysis: Calculated for $C_{10}H_{14}FN_3O_5$: C, 43.64; H, 5.13; N, 15.27.
Found: C, 43.75; H, 5.44, N, 15.19.

Résumé

Résumé

Ce mémoire de thèse est constitué de 3 chapitres qui s'articulent ainsi:

Chapitre I: Analogues nucléosidiques et chimiothérapie antivirale.

Chapitre II: Synthèse de 2′,3′-didésoxy-3′-fluoro-*thréo*- nucléosides pyrimidiques.

Chapitre III: Synthèse de 2′,3′-didésoxy-2′-fluoro-3′-(*N*-hydroxyimino), (*N*-méthoxyimino) et (hydroxylamino) nucléosides pyrimidiques.

Chapitre I

Partie I: La chimiothérapie et infections virales

Nous avons illustré la nécessité d'accroître l'arsenal thérapeutique pour le traitement de trois infections virales majeures que sont les infections causées par le VIH et les virus des hépatites B et C. De nos jours, l'organisation Mondiale de la santé (OMS) estime en effet à près de 600 millions le nombre total de personne infectées par ces trois virus. Ces pathologies constituent donc des problèmes majeurs de santé publique et la recherche de nouvelles molécules susceptibles d'interférer spécifiquement avec leur réplication reste un défi à relever.

I.1 Le virus de l'immunodéficience humaine (VIH):

Dans ce paragraphe, nous avons présenté le VIH, l'histoire de ce virus et les composés thérapeutique approuvés. Après avoir présenté la structure du VIH (Figure **1**) et son génome (Figure **2**), nous avons discuté par la suite du cycle de réplication. Ce dernier commence à partir du moment où la gp 120 de l'enveloppe virale se lie aux récepteurs CD4. Les étapes suivantes sont la fusion, la décapsidation, la transcription inverse, le transport dans le noyau et l'intégration dans l'ADN hôte. L'ADN est ensuite transcrit par la polymérase cellulaire pour générer après épissage les ARNm. Les ARNm viraux sont ensuite traduits dans le cytoplasme et les protéines virales nouvellement produites sont assemblées en virions immatures. Les particules virales une fois libérées deviennent infectieuses après une étape de maturation par la protéase du VIH. (Figure **3**).

Le métabolisme des NRTIs, y compris la perméabilité cellulaire, la phosphorylation, et le catabolisme a également été discuté (Figure **4**). L'inhibition de la transcriptase inverse, la liaison des nucléotides dans le site actif de la transcriptase inverse du VIH a également été montré (Figure **5**). Enfin, nous avons discuté des structures approuvées par les organismes de

réglementation (Figure **6**) ainsi que les structures en cours d'évaluation (Figure **7**) des NRTIs pour le traitement du sida.

I.2 Le Virus de l'hépatite B (VHB):

Le VHB touche également une large population. Nous avons d'abord abordé sa transmission et ses principaux symptômes, puis nous avons présenté son génome (Figure **8**) et son cycle de réplication (Figure **9**). Le cycle de réplication du VHB est un processus complexe qui comporte de nombreuses étapes et met notamment en jeu un processus de rétrotranscription. Enfin, nous avons discuté du traitement de l'hépatite B par les inhibiteurs de la polymérase du VHB (Figure **10**).

I.3 Le virus de l'hépatite C (VHC):

Le cycle de réplication du VHC a été présenté en détail de l'attachement à la production des nouvelles particules virales (Figure **12**). Bien que le cycle infectieux du VHC ne soit pas encore totalement élucidé, il serait proche du cycle de réplication des autres virus de la famille *Flaviviridae*. Pour terminer, nous avons exposé le traitement de l'hépatite C chronique par l'interféron (IFN) et la ribavirin (Figure **13**), ainsi que les structures en cours d'évaluation (Figure **14**) pour le traitement de l'hépatite C.

Partie II: L'incorporation du fluor dans des analogues nucléosidiques.

II.1 Propriétés uniques du fluor et de leurs pertinences dans la chimie médicinale.

Dans cette partie, nous avons expliqué le succès thérapeutique de médicaments contenant du fluor, et nous avons présenté quelques exemples des médicaments utilisés pour traiter certaines maladies graves (Figure **16**), comme par exemple, l'efavirenz (VIH antiviral), l'acides shikimique fluorés (antibactérien), l'epothilone B (anticancéreux), le torcetrapib (un puissant inhibiteur de la protéine de transfert des esters de cholestérol) et la sitagliptine (antidiabétique de type 2 diabète).

Nous avons abordé par la suite les motivations pour l'introduction d'atomes de fluor dans des composés thérapeutiques.

II.1.1 Mimer l’effet du fluor:

Nous avons résumé certaines propriétés physico-chimiques du fluor (Tableau **2**) en comparaison avec d’autres éléments choisis, par exemple, le rayon de van der Waals et de longueur de la liaison entre le fluor et l'hydrogène. En raison de sa similitude de taille avec l'hydrogène, il a été montré que les micro-organismes ou des enzymes sont à méme de reconnaître un substrat naturel et son analogue fluoré.

II.2.1 La lipophile du fluor et des groupements contenant du fluor:

Nous avons présenté l’effet du fluor sur l’augmentation de la lipophilic au traverse de l’étude du parametre π de Hansch avec comme modèle d’étude des benzènes monosubstitués (Tableau **3**).

Partie III: Synthèse et activité biologique des nucléosides fluorés.

Dans cette partie, nous avons introduit l'importance des nucléosides fluorés, et nous avons donné quelques exemples de nucléosides fluorés utilisés en chimiotherapie antivirale et antitumorale (Figure **15**). Après cela, nous avons décrit les principales méthodes de synthèses de ces composés:

- **Synthèse des nucléosides fluorés:**

Dans ce paragraphe, nous avons décrit la synthèse des analogues nucléosidiques fluorés sur la partie osidique:

1. Synthèse des 1'-fluoronucléosides.
2. Synthèse des 2'-fluoronucléosides.
   - Synthèse des 2′α-fluoronucléosides.
   - Synthèse des 2′β-fluoronucléosides.
3. Synthèse des 3'-fluoronucléosides.
   - Synthèse des 3′α-fluoronucléosides.
   - Synthèse des 3′β-fluoronucléosides.
4. Synthèse des 4'-fluoronucléosides.
5. Synthèse des 5'-fluoronucléosides.
6. Synthèse des 6'-fluoronucléosides.

Chapitre II

Synthèse de 2′,3′-didésoxy-3′-fluoro-*thréo*-nucléosides pyrimidiques

I. Introduction:

Jusqu'à présent, les 3′-β-fluoro nucléosides ont été synthétisés par réaction de glycosylation entre un 3'-fluoro-D-xylo-furanoside avec les bases hétérocycliques ou par introduction d'un atome de fluor sur un 2',3'-anhydro-β-D-ribo-furanonucléoside. Les 3-désoxy-3-fluoro-D-xylo-nucléosides peuvent ensuite être soumis à une désoxygénation de Barton-type réducteur pour aboutir aux analogues 2',3'-didésoxy-3'-β-fluoro nucléosidiques correspondants. Ces méthodologies ont été utilisées pour la synthèse des dérivés de nucléosides portant les bases adénine, guanine et thymine. Afin d'avoir un accès rapide aux produits dérivés inconnus jusqu'à présent inedits de l'uracile et la cytosine afin d'évaluer leurs propriétés antivirales, nous avons envisagé leur préparation à partir des dérivés 2'-désoxynucléosides correspondants (Schéma **48**). Toutefois, après protection sélective en position 5', une réaction de fluoration nucléophile directe, en utilisant un agent de fluoration tels que le trifluorure de diéthylaminosulfure (DAST) ne conduit pas au nucléoside fluoré 3′-β mais au dérivé 2,3′-*O*-anhydro correspondant.

La cyclisation des composés anhydro peut être favorisée par la présence de l'ion fluorure, qui peut jouer le role de base pour faire abstraction du proton porté par l'atome d'azote N-3, facilitant ainsi la fermeture de cycle (Schéma **50**).

À cet égard, Serra *et al.* ont décrit l'utilisation du groupement nitro comme groupement protection pour la position *N*-3. L'utilisation de groupement électro-attracteur empêche la formation de l'anhydronucléoside lors de la substitution nucléophile sur la 2'-*O*-triflyluridine ainsi que sur des analogues 2',3'- sulfate cyclique pyrimidiques (Figure **17**).

II. Synthèse:

II.1 Synthèse des 2'-désoxy-3-nitrouridine 7 et 2'-désoxy-3-nitrothymidine 8:

Les composés 5'-*O*-acétyl-2'-désoxy-3-nitrouridine **9** et 5'-*O*-acétyl-2'-désoxy-3-nitrothymidine **10** ont été choisis comme intermédiaires clés et ont été préparés en quatre étapes à partir de la 2'-désoxyuridine **1** et 2'-désoxythymidine **2,** respectivement. L'acétylation de la 2'-désoxyuridine **1** et de la 2'-désoxythymidine **2** avec l'anhydride acétique dans la pyridine à température ambiante pendant 12 h a donné la 3',5'-di-*O*-acétyl-2'-désoxyuridine **3** et la 3',5'-di-*O*-acétyl-2'-désoxythymidine **4** avec un rendement de 90% et 96%, respectivement (Schéma **51**).

Il est connu que par un traitement avec HNO_3, les nucléosides peuvent faire l'objet d'une *O*-nitration, d'oxydation des alcools primaires en acides, et/ou à la nitration en C5 (dans le cas de l'uridine), en fonction des conditions de réaction et du type de la protection. Pour parvenir à une réaction de *N*-nitration, en évitant l'hydrolyse du nucléosides et la perte des groupements protecteurs, des expériences préliminaires sur la 2',3',5'-tri-*O*-acétyluridine, en utilisant soit (i) benzoate de nitronium (préparé à partir de $AgNO_3$/PhCOCl dans CH_3CN et filtration du précipité de AgCl), (ii) $Cu(NO_3)_2$ / Ac_2O ou (iii) NO_2BF_4 dans CH_3CN, à des températures différentes, avec ou sans 2,6-di-*tert*-butyle-4-méthyle pyridine, ont été infructueuses.

La réaction de *N*-nitration de la 3',5'-di-*O*-acétyl-2'-désoxyuridine **3** et de la 3',5'-di-*O*-acétyl-2'-désoxythymidine **4** a été effectuée avec le nitronium de trifluoroacétate généré *in situ* à partir du NH_4NO_3 (4 eq.) et $(CF_3CO)_2O$ (8 eq.), dans le dichlorométhane à froid. Les composés désirés 3',5'-di-*O*-acétyl-2'-désoxy-3-nitrouridine **5** et 3',5'-di-*O*-acétyl-2'-désoxy-3-nitrothymidine **6** ont ainsi été obtenus avec de bons rendement 80% et 75%, respectivement.

Le groupe *N*-NO_2 est stable en milieu acide, et la deprotection des groupements protecteurs a été effectuée par un traitement en présence de MeOH / HCl à température ambiante. Les dérivés nitrés, **5** et **6**, ont été traités avec HCl (0.17M) dans le méthanol à température ambiante pendant 48 h pour conduire à la 2'-désoxy-3-nitrouridine **7** et la 2'-désoxy-3-nitrothymidine **8** avec un rendement de 67% et 66%, respectivement (Schéma **5**). La disparition du proton NH, des déplacements chimiques ver les champs faible des protons H5 et H6, et des déplacements importants ver les champs fort du carbone C2 ($\Delta\delta \approx -5$ ppm) et C4 ($\Delta\delta \approx -8$ ppm) sont les principales caractéristiques des spectres RMN des dérivés nitrés par

rapport à leurs composés parents. Dans le cas de l'uridine, Ariza *et al* ont observé ces caractéristiques similaires de dérivés *N*-nitro uridine par rapport à l'uridine.

II.2 Synthèse des 5'-*O*-acétyl-2'-désoxy-3-nitrouridine 9 et 5'-*O*-acétyl-2'-désoxy-3-nitrothymidine 10:

Mitsunobu a décrit que l'acétylation sélective du groupe 5'-hydroxyle de la thymidine peut être atteinte avec 55% de rendement en ajoutant une solution d'acide acétique (1 eq.) et azodicarboxylate de diéthyle (1 eq.) à la thymidine et de la triphénylphosphine (1 eq.) en suspension dans le dioxane. He et Bischofberger ont rapporté une modification de la procédure pour cette synthèse. Ainsi, quand une solution de dioxane azodicarboxylate de diéthyle (1.5 eq.) a été ajoutée à une suspension contenant les désoxyribonucléosides, la triphénylphosphine (1.5 eq.) dans le dioxane, les produits désirés 5'-acétyl-2′-désoxyribonucléosides ont été obtenus avec de bons rendements (Schéma **52**).

L'acétylation sélective de la 2'-désoxy-3-nitrouridine **7** et de la 2'-désoxy-3-nitrothymidine **8** a été réalisée par la procédure modifiée de Mitsunobu. Nous avons ainsi obtenu les intermédiaires clefs 5'-*O*-acétyl-2'-désoxy-3-nitrouridine **9** et 5'-*O*-acétyl-2'-désoxy-3-nitrothymidine **10** avec un rendement de 75% et 74% respectivement, après purification par chromatographie sur gel de silice (Schéma **53**).

II.3 Synthèse des 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrouracile 13 et 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine 14:

La transformation souhaitée des composés **9** et **10** en 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrouracile **11** and 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine **12** a été réalisée par traitement avec DAST dans le dichlorométhane/pyridine avec un rendement de 45% et 40%, respectivement, après purification par chromatographie sur gel de silice (Schéma **54**).

D'un point de vue méchanistique, la réaction de fluorodéoxygenation des 5'-*O*-acétyl-2'-désoxy-3-nitronucléosides pyrimidiques avec le DAST commençe par le déplacement du fluorure par une attaque nucléophile par le groupement hydroxyle, la fonction hydroxy est ensuite transformé en un bon groupement partant alcoxy (diéthylamino) difluorure de soufre intermédiaires ($CO\text{-}SF_2NEt_2$) qui est déplacé par une attaque nucléophile (réaction de type

S_N^2) par l'ion fluorure libéré. Le fait que la réaction se produit avec inversion de configuration permet l'introduction de la liaison C-F avec un contrôle stéréochimique et le groupement nitro évite la formation d'*O*-anhydronucleosides en raison de l'instabilité de l'intermédiaire chargé (Schéma **55**).

La déprotection des composés **11** et **12** à l'aide d'une solution HCl (0.17 M) dans le méthanol a conduit à la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrouracile **13** et la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine **14** avec un rendement de 75% et 71%, respectivement (Schéma **54**).

II.4 Tentative d'amélioration du rendement des 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrouracile 11 et 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine 12:

Lorsque nous avons essayé d'améliorer le rendement de la réaction de fluoration à l'aide de réactif Deoxo-Fluor, les rendements ont diminué de 5% pour les deux produits. Après réaction des composés **9** et **10** avec le Deoxo-Fluor (5 eq.) dans dichlorométhane/pyridine, les composés **11** et **12** sont obtenus avec un rendement de 40% et 35%, respectivement (Schéma **56**).

II.5 Modification de la base hétérocyclique des 2'-désoxy-3-nitrothymidine 8 et 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine 14 aux dérivés *N*-NH_2 15 and 16:

Ayant dans nos mains le groupement *N*-NO_2, nous avons décidé de modifier la base par introduction du groupe NH_2 en position *N*-3. Selon le mécanisme d'ouverture et fermeture du cycle, la 2'-désoxy-3-nitrothymidine **8** et la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine **14** ont réagit rapidement avec de l'hydrazine pour donner la 2'-désoxy-3-aminothymidine **15** et la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-aminothymine **16** avec un rendement de 47% et 64%, respectivement (Schéma **57**).

Apparemment, une fois que l'ouverture du cycle a lieu, la cyclisation vers à un cycle six chaînons est favorisée en dépit du fait que l'azote (N_a) dans la partie CO-N_aH-$N_b$$H_2$ devrait être mieux nucléophile que l'azote (N_b). En effet, aucune activation n'est nécessaire pour faciliter l'étape de fermeture. Il est également plausible que le l'azote (N_b) de l'intermédiaire

soit protoné et / ou que les espèces transitoires participent à l'étape de cyclisation (Schéma **58**).

II.6 Synthèse des 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)uracile 19 et 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)thymine 20:

La dénitration de *N*-NO_2 en accord avec la méthode de Serra *et al*. en utilisant NaI/acétone a échouée. Toutefois, Imrie a décrit que la dénitration de *N*-nitrosamines peut être réalisé avec l'hydrure de tributylétain dans le benzène ou le toluène en présence d'un initiateur de radicaux (par exemple, AIBN). Ainsi, la 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrouracile **11** et la 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-3-nitrothymine **12** ont réagit avec Bu_3SnH/AIBN dans toluène à 100 ° C pendant 1 h pour donner la 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)-uracile **17** et la 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)thymine **18** avec un rendement de 67% et 84% respectivement. La déprotection du groupe acétyle dans NH_3/MeOH a conduit à la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)uracile **19** et la 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)thymine **20** avec un rendement de 80% et 79%, respectivement (Schéma **59**).

La première réaction des radicaux du tributylétain avec les composés **11** et **12** est susceptible d'impliquer la formation du radical nitroxyde. Une décomposition ultérieure du radical nitroxyde en radicaux aminyl suivie par une élimination de l'hydrogène d'hydrure de tributylétain conduit à la dénitration de la base hétérocyclique (Schéma **60**).

Elucidation de la structure des composés 19 & 20:

La structure du composé **19** a été étudiée par les spectres NOESY et NOE. Cependant ces études n'ont pas permis d'attribuer sans ambiguïté la structure de **19** en particular orientation de l'atom de fluor.

La structure du composé **19** a été confirmé sans ambiguïté par cristallographie aux rayons X et a révélé la position attendue 3'-β-fluoro, 2'-exo/3'-endo (nord) plissement (Figure **21**). En particulier, les propriétés physico-chimiques du composé **20** ont été identiques à ceux précédemment rapportés par Gosselin *et al*.. Dans le tableau (**5**), nous montron que la

constante de couplage (*J*) entre H et F en ^{1}H RMN et ^{19}F RMN du composé **20** que nous avons synthétisé par cette méthode et le même que celle du composé préparé auparavant par Gosselin *et al.*

Synthèse de 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)cytosine 22:

La conversion de la 1-(5-*O*-acétyl-2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)uracile **17** en 1-(2,3-didésoxy-3-fluoro-β-D-*thréo*-pentofuranosyl)cytosine **22** a été réalisée par un traitement avec le réactif de Lawesson, suivie par le traitement du dérivé 4-thioamide intermédiaire avec une solution de méthanol saturé d'ammoniac à 100 °C permettant l'obtention du dérivé de la cytidine (Schéma **61**).

D'un point de vue méchanistique, le réactif se dissocie en un ylure d'aryldithiophosphine qui va s'additionner sur le carbonyle pour conduire à la formation d'une thiobétaine. Celle-ci se décompose alors, comme dans la réaction de Wittig, ce qui entraîne la formation du thioamide attendu. Une fois cet intermédiaire soufré obtenu, un traitement à 100 °C par une solution de méthanol saturé d'ammoniac permet l'obtention du dérivé de la cytidine (Schéma **62**).

III. Évaluations biologiques:

Les analogues nucléosidiques **7**, **8**, **13**, **14**, **15**, **16**, **19**, **20** et **22**, obtenu au cours de ce chapitre, ont été évalués dans laboratoire:

- **Jan Balzarini, Institut Rega, Leuven, Belgique.**

Pour mesurer l'activité et la cytotoxicité contre les virus dans les cultures cellulaires suivants:

- Anti-VIH-1 et VIH-2 chez l'homme des lymphocytes T (CEM) des cellules.
- Virus varicelle-zona (VZV) dans les poumons embryonnaires humains (HEL) des cellules.
- Herpes simplex, la vaccine et vésiculeuse stomatite virus en culture cellulaire HEL.
- Cytomégalovirus dans le poumon embryonnaire humain (HEL) des cellules.
- Feline Corona Virus (FIPV) et Feline Herpes en culture cellulaire CRFK.
- l'activité du virus anti-grippe et la cytotoxicité des cultures de cellules MDCK.
- La stomatite vésiculeuse, le virus Coxsackie B4 et le virus respiratoire syncytial dans les cultures de cellules HeLa.

- Para-influenza-3 virus, réovirus-1, le virus Sindbis, virus Coxsackie B4 et Punta Toro virus dans des cultures de cellules Vero.

Les résultats obtenus sont indiqués dans les tableaux (**7**), (**8**), (**9**), (**10**), (**11**), (**12**), (**13**) et (**14**).

IV. Conclusion:

Une synthèse rapide de 2',3'-didésoxy-3'-fluoro-β-D-*thréo*-nucléosides portant les bases pyrimidiques des acides nucléiques et N^3-nitro ou N^3-amino a été développée afin de mettre au point de nouveaux dérivés nucléosidiques afin d'evaluer leurs activités antivirales. La synthèse a été réalisée par substitution du proton-imide par le groupement nitro comme groupement électro-attracteur pour éviter la formation de l'*O*-anhydronucleoside. La désoxyfluoration directe à l'aide au DAST a conduit aux 2',3'-didésoxy-3'-fluoro-β-D-*thréo*-3-nitro-nucléosides pyrimidiques. Enfin, la déprotection des groupements protecteurs conduit aux composés désirés. Les nucléosides **7**, **8**, **13**, **14**, **15**, **16**, **19**, **20** et **22** ont été évalués contre divers virus à ADN et ARN (y compris le VIH) dans des expériences de cultures cellulaires. Aucun des dérivés synthétisés n'a montré une activité antirétrovirale ni cytotoxicité à la plus forte concentration testée (généralement 100 μM).

Chapitre III

Synthèse de 2′,3′-didésoxy-2′-fluoro-3′-(*N*-hydroxyimino), (*N*-methoxyimino) et (hydroxylamino) nucléosides pyrimidiques

I. Introduction:

L'introduction d'une fonction hydroxyle aminé ou oximino en position 2'- ou 3'- sur des analogues nucléosidiques a conduit à un certain nombre d'agents antiviraux et antitumoraux. Federov *et al* ont décrit que les dérivés 3'-*N*-hydroxyimino et 3'-*N*-acétoxyimino sont dotés d'une activité significative contre le VIH avec des valeurs EC_{50} allant de 0.02 à 0.4 μg/ml pour le VIH-1 et VIH-2. Par ailleurs, les dérivés 3'-*N*-hydroxyimino montrent également une activité prometteuse contre le VHB (EC_{50} = 0.25 μg/ml) et contre le HSV-1 et HSV-2 (Figure **22**).

Ogawa *et al.* ont également décrit que la 2'-désoxy-2'-(hydroxylamino) cytidine (2'-DHAC) a montré une forte cytotoxicité contre des cellules murines L1210 et des cellules humaines KB, avec des valeurs IC_{50} de 1.58 et 1.99 μM, respectivement. Les composés 3'-désoxy-3'-(hydroxylamino)cytidine (3'-DHAC) et 2',3'-didésoxy-3'-(hydroxylamino)cytidine (3'-dDHAC) sont également cytotoxique contre les cellules L1210, avec IC_{50} de 4.03 et 1.84 μM, respectivement, mais pas contre les cellules KB (Figure **22**).

Dans ce chapitre, nous avons décrit la synthèse de 2',3'-didésoxy-2'-fluoro-3'-(hydroxylamino) et 2',3'-didésoxy-2'-fluoro-3'-(hydroxyimino) nucléosides pyrimidiques. D'après la littérature, ces composés n'ont pas encore été synthétisés ou étudiés comme agents antiviraux. Au cours de ce travail, nous avons synthétisé neuf nouveaux analogues nucléosidiques à partir de l'uridine, ces composés ont été évalués pour leur propriétés antivirales (Schéma **63**).

II. Synthèse:

II.1 Synthèse des 2'-désoxy-2'-fluorouridine 27 et 2'-désoxy-2'-fluorocytidine 30:

La synthèse a commencé avec la préparation de la 2'-désoxy-2'-fluorouridine et de la 2'-désoxy-2'-fluorocytidine qui ont été obtenus à partir de l'uridine selon une procédure rapporté par Shi *et al.*. La 2,2'-anhydrouridine **24** a été obtenue avec un rendement de 73% après la protection du groupe hydroxyle en position 2'. Les autres groupes hydroxyles en position 3' et 5' ont été protégés avec le groupement tétrahydropyranyle (THP) pour donner la 2,2'-anhydro-

3',5'-di-*O*-tetrahydropyranyluridine **24** avec un rendement de 93%. La réaction d'ouverture du composé **24** a été réalisée dans solution 1 N NaOH pour fournir la 3',5'-di-*O*-tetrahydropyranylarabinouridine **25** avec un rendement de 94%. Une fluoration avec le DAST suivie par la déprotection du composé **26** a conduit à la 2'-désoxy-2'-fluorouridine **27** avec un rendement global de 30% à partir de **24** (Schéma **64**).

L'acétylation du composé **27** avec de l'anhydride acétique dans la pyridine anhydre a donné la 3',5'-di-*O*-acétyl-2'-désoxy-2'-fluorouridine **28**. La conversion du composé **28** en 2'-désoxy-2'-fluorocytidine **30**, a été effectuée par un traitement avec le réactif de Lawesson, suivie par un traitement de la 4-thioamide intermédiaire **29** avec une solution de méthanol saturé d'ammoniac à 100 °C avec un rendement global de 76% (Schéma **65**).

II.2 Synthèse des 1-[2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]uracile 34 (E/Z) et 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]uracile 35 (E/Z):

La protection de l'alcool primaire par silylation sélective de **27** a été réalisée à l'aide du chlorure de *tert*-butyldiméthylsilyle et d'imidazole dans le DMF à température ambiante pendant 3 h pour conduire à la 5'-*O*-TBDMS 2'-désoxy-2'-fluorouridine **31** avec un rendement de 82 %. Une oxydation dans les conditions de Jones aboutit l'intermédiaire 2'-céto, qui a été mis en réaction avec le chlorhydrate de l'hydroxylamine et le chlorhydrate de *O*-méthylhydroxylamine pour conduire à le mélange d'isomères oximes protégées (avec des rendements quantitatifs), 1-[5-*O*-TBDMS-2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]uracile **32** (E/Z) et 1-[5-*O*-TBDMS-2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl] uracile **33** (E/Z) dans un rapport 1:1, respectivement. La déprotection du mélange d'isomères de **32** (E/Z) et **33** (E/Z) dans une solution d'acide acétique aqueux 80% a conduit à la 1-[2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]uracile **34** (E/Z) et la 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]uracile **35** (E/Z) dans un rapport 1:2 et 1:1, respectivement (Schéma **66**).

II.3 Réduction stéréosélective de la 1-[2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]uracile 34 (E/Z):

Robins *et al* ont décrit que le traitement des 3'-ceto et 2'-cetonucleosides protégées en position 5′ avec le triacétoxyborohydrure de sodium dans l'acide acétique aboutit à la délivrance de l'hydrure sur la face α avec une stéréosélectivité élevée. Toutefois, une élimination du groupe 5′-TBDMS et le traitement de ces 5'-hydroxy-(3'- et 2'-)-cétonucleosides avec le triacétoxyborohydrure de sodium permet d'effectuer une délivrance sélective de l'hydrure sur la face β pour aboutir au ribo (ribo/xylo ; 99:1) nucléoside avec un bon rendement (Schéma **67**).

Par ailleurs, Ogawa *et al.* ont également décrit que les dérivés 3'-hydroxylamino avec un configuration ribo peuvent être obtenus par réduction des 3'-oximes correspondantes quand l'alcool en position 5′ n'est pas protégé. Le groupe oxime dans le composé **32** a été réduit à l'aide du triacétoxyborohydrure de sodium (généré à partir de $NaBH_4$/AcOH) dans l'acide acétique pour conduire à la 2',3'-didésoxy-2'-fluoro-3'-(hydroxylamino) uridine **36** avec un rendement de 71% (Schéma **68**).

D'un point de vue méchanitique, le triacétoxyborohydrure de sodium est un agent réducteur doux qui se forme par addition de borohydrure de sodium en présence d'acide acétique à froid. Le contrôle stéréochimique peut être attribué à la formation d'un alkoxydiacétoxyborohydrure intermédiaire qui permet le déplacement d'un ligand acétoxy du bore par un hydroxyle, suivi par la délivrance intramoléculaire de l'hydrure sur la face β pour donner le composé désiré **36** (Schéma **69**). La stéréochimie du composé **36** a été confirmée par des expériences NOE (Figure **24**).

II.4 Synthèse de la 1-[2-amino-2,3-didésoxy-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]cytosine 38:

Le composé **32** a été transformé en acétate **37**, (E/Z) (rapport 1:1) avec de l'anhydride acétique dans la pyridine en présence de 4-diméthylaminopyridine (DMAP), suivie par l'activation de la position 4 de l'uracile avec le chlorure de triisopropylbenzenesulfonyl (TPS). Le dérivé 4-*O*-TPS a été traité avec une solution de méthanol saturée d'ammoniac à température ambiante. Malheureusement, le dérivé de la cytosine désiré n'a pas été formé, mais un produit inattendu, la 1-[2-amino-2,3-didésoxy-3-(*N*-hydroxyimino)-β-D-glycéro-

pentofuranosyl]cytosine dihydro chlorure **38** a été obtenue avec un rendement de 16% (Schéma **70**).

En ce qui concerne le méchanisme, cette réaction peut se dérouler par une étape d'élimination-addition par abstraction du proton acide du groupe hydroxyimino par l'ammoniac, suivie d'un réarrangement intramoléculaire pour libérer l'ion fluorure. Par la suite, en raison de forte réactivité du motif nitroso-oléfine, ce dernier subit une addition de type 1,4 de l'ammoniac (Schéma **71**).

II.5 Synthèse de la 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]cytosine 40:

La transformation du composé **33** est effectuée par l'activation préalable de la position 4 par le chlorure de triisopropylbenzenesulfonyl (TPS). Le composé obtenu est ensuite traité par une solution de méthanol saturée d'ammoniac à température ambiante pendant 12 h pour conduire à la 1-[5-*O*-TBDMS-2,3-didéoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl] cytosine **39** avec un rendement de 67% (E/Z) (rapport 2:1.2). La désilylation a été réalisée au reflux dans MeOH anhydre en présence de NH_4F pendant 3 h pour donner la 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]cytosine **40** avec un rendement de 41% (E/Z) (rapport 2 : 1.8) (Schéma **72**).

Notons que dans le cas du dérivé 3′-*N*-méthoxyimino, contrairement au cas du composé 3′-N-hydroxyimino, l'obtention du derivé de la cytosine s'effectue sans réaction secondaire.

II.6 Synthèse de la 1-[2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]cytosine 44 (E/Z):

En raison de nos observations précédentes sur la réactivité du groupe hydroxyimino en milieu basique, nous avons envisagé de préparer la 2',3'-didésoxy-2'-fluoro-3'-(hydroxylamino)cytidine dérivés à partir de son composé parent. La synthèse débute par la silylation régiosélective de la 2'-désoxy-2'-fluorocytidine **30** en présence de chlorure de *tert*-butyldiméthylsilyle et d'imidazole dans le DMF à température ambiante pendant 4 h pour mener à la 5'-*O*-TBDMS-2'-désoxy-2'-fluorocytidine **41** avec un rendement de 77%. Cette étape est suivie par une *N*-acylation sélective du composé **41** en présence d'anhydride acétique dans le DMF à température ambiante pour conduire à la 5'-*O*-TBDMS-2'-désoxy-2'-fluoro-N^4-

acetylcytidine **42** avec un rendement de 86%. L'oxydation du composé **42** dans les conditions de Jones donne le dérivé correspondant 3'-cétocytidine qui réagit avec le chlorhydrate d'hydroxylamine pour aboutir à un mélange d'isomères (E/Z). En outre, l'hydroxylamine libérée dans le milieu réactionnel permet de déprotéger le groupe *N*-acétyl pour donner la 1-[5-O-TBDMS-2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]-cytosine **43** (E/Z) avec un rendement de 31% (rapport 2:1.2). La désilylation du composé **43** a été réalisée en présence d'AcOH à 80% pour donner la 1-[2,3-didésoxy-2-fluoro-3-(*N*-hydroxyimino)-β-D-glycéro-pentofuranosyl]cytosine **44** (E/Z) (rapport 1.2:2) avec un rendement de 73% (Schéma **74**).

II.7 Synthèse de la 2',3'-didésoxy-2'-fluoro-3'-(hydroxylamino)cytidine 45:

Les dérivés 3'-hydroxylamino souhaités avec une configuration ribo peuvent également être obtenus par réduction en présence d'hydrures des dérives 3'-oxime correspondants lorsque le groupe 5'-hydroxyle n'est pas protégé. La réduction stéréosélective du groupe oxime du composé **44** a été réalisé à l'aide de $NaBH_4$ dans AcOH pour donner la 2',3'-didésoxy-2'-fluoro-3'-(hydroxylamino)cytidine **45** avec un rendement de 50% (Schéma **75**).

II.8 Synthèse de la 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]-N^4-methoxycytosine 47 (E/Z) :

Le composé **42** une fois oxydé en position 3' est mis à réagir avec le chlorhydrate d'*O*-méthylhydroxylamine à température ambiante. Dans ces conditions aucune réaction n'est observée. Le mélange réactionnel a été alors porté à reflux à 80 °C pendant 3 h, pour finalement conduire à la 1-[5-*O*-TBDMS-2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycero-pentofuranosyl]-N^4-methoxycytosine **46** (E/Z) désirée (rapport 3:2) avec un rendement de 37%. L'étape de désilylation du composé **46** s'est faite en présence de NH_4F au reflux du méthanol anhydre pour mener à la 1-[2,3-didésoxy-2-fluoro-3-(*N*-méthoxyimino)-β-D-glycéro-pentofuranosyl]-N^4-methoxycytosine **47** (E/Z) (rapport 2:1.4) avec un rendement de 41% (Schéma **76**).

La transformation de la cytosine en N^4-méthoxycytosine s'est déroulée *via* activation de la position C4 par un groupement acétyle suivie d'une attaque nucléophile de l'*O*-méthoxyhydroxylamine en position C4 pour former le composé **46**. Une étape de désilylation permet alors d'obtenir le composé **47** (Schéma **77**).

II.9 Synthèse de la 2'-désoxy-2'-fluoro-*N*^{4}-methoxycytidine 49:

En raison des résultants obtenus dans la synthése du composé **47**, nous avons envisagé la preparation de la 2'-désoxy-2'-fluoro-*N*^{4}-méthoxycytidine **49**. Ko *et al*. ont rapporté une méthode directe de préparation du dérivé *N*^{4}-méthoxyctidine de dérivés de l'uridine par la réaction avec le chlorhydrate de *O*-méthylhydroxylamine dans la pyridine à 100 ° C. Cependant, cette méthode n'a pas donné de résultat dans notre cas. Nous avons donc appliqué la même méthode que précédemment pour la préparation de la 2'-désoxy-2'-fluoro-*N*^{4}-méthoxycytidine. De ce fait, nous avons utilisé notre méthode par l'activation de la position C4 de la cytosine par un groupe acétyle suivie par une réaction avec l'*O*-méthylhydroxylamine. Une acétylation sélective de la 2'-désoxy-2'-fluorocytidine **30** selon Bhat *et al*. par agitation avec de l'anhydride acétique dans le DMF à température ambiante pendant 24 h a conduit à la 2'-désoxy-2'-fluoro-*N*^{4}-acétylcytidine **48** désirée avec un rendement de 71%. Le composé **48** en présence d'*O*-méthylhydroxylamine dans la pyridine anhydre à reflux pendant 3 h à 80 °C conduit à la formation de la 2'-désoxy-2'-fluoro-*N*^{4}-methoxycytidine **49** avec un rendement de 84% (Schéma **78**).

II.10 Etude par RMN du fluor des nucléosides 3′-*N*-hydroxyimino et 3′-*N*-méthoxyimino:

Les constantes de couplage de la 2'-désoxy-2'-fluorouridine **28** et de la 2'-désoxy-2'-fluorocytidine **30** sont respectivement $J_{F\text{-}2'}$ = 53.3, $J_{F\text{-}3'}$ = 20,8, $J_{F\text{-}1'}$ = 17,9 et $J_{F\text{-}2'}$ = 53,5, $J_{F\text{-}3'}$ = 22,4, $J_{F\text{-}1'}$ = 18,4 Hz, avec une multiplicité de doublet de doublets de doublets. Lors de la transformation des dérivés 3'-ceto en dérivés oximes en présence d'*O*-hydroxylamine ou d'*O*-méthylhydroxylamine pour aboutir des dérivés oxime, nous nous attendions à la disparition de la constante de couplage $J_{F\text{-}3'}$ ainsi qu'un changement de la multiplicité en doublet de doublets. De manière surprenante, nous avons plutôt observé la formation d'une nouvelle constante de couplage de 3 à 5 Hz avec une multiplicité de doublet de doublet de doublet pour un des deux isomère (E ou Z). Dans le cas de l'autre isomère, nous avons observé une multiplicité de doublet de doublet.

À ce titre, Mallory *et al*. ont décrit que sur la base de la théorie du recouvrement orbitalaire de paires d'électrons libre, un couplage F-F pour des atomes de fluor vicinaux pouvait être observé. Par ailleurs, l'existence à travers l'espace de couplage N-F a été également prédit

pour des molécules ayant une fonction oxime à proximité d'atomes de fluor au travers du recouvrement orbitalaire des paires non liantes entre ces 2 hétéro-atomes (Figure **25** et **26**).

Dans notre cas, une etude par RMN du fluor a permis de déterminer sans ambiguïte la valeur du couplage N-F et la multiplicité (ddd) des oximes de configuration E par rapport aux oximes de configuration Z (dd, pas de couplage N-F)

III. Évaluations biologiques:

Les analogues nucléosidiques **34**, **35**, **36**, **38**, **40**, **44**, **45**, **47**, et **49**, obtenus au cours de ce chapitre, ont été évalués par le laboratoire:

- **Idenix (Cambridge, USA).**

Pour mesurer l'activité et la cytotoxicité contre

- Le VHC.

Ces mêmes analogues nucléosidiques **34**, **35**, **36**, **38** and **40**, ont été également évalués par le laboratoire:

- **Prof. Jan Balzarini, Rega Institut, Leuven, Belgium.**

Pour mesurer l'activité et la cytotoxicité contre les virus dans les cultures cellulaires suivants:

- Virus varicelle-zona (VZV) dans les poumons embryonnaires humains (HEL) des cellules.
- Herpes simplex, la vaccine et vésiculeuse stomatite virus en culture cellulaire HEL.
- Cytomégalovirus dans le poumon embryonnaire humain (HEL) des cellules.
- Feline Corona Virus (FIPV) et Feline Herpes en culture cellulaire CRFK.
- l'activité du virus anti-grippe et la cytotoxicité des cultures de cellules MDCK.
- La stomatite vésiculeuse, le virus Coxsackie B4 et le virus respiratoire syncytial dans les cultures de cellules HeLa.
- Para-influenza-3 virus, réovirus-1, le virus Sindbis, virus Coxsackie B4 et Punta Toro virus dans des cultures de cellules Vero.

Les résultats obtenus sont indiqués dans les tableaux (**16**), (**17**), (**18**), (**19**), (**20**), (**21**), (**22**) et (**23**).

IV. Conclusion:

Dans ce troisième chapitre, nous avons synthétisé plusieurs 2'-fluoronucléosides avec les groupements *N*-hydroxyimino ainsi que *N*-méthoxyimino et *N*-hydroxylamino portant les bases hétérocycliques uracile et cytosine afin de découvrir des nouveaux nucléosides comme agents antiviraux. Par ailleurs, nous avons mis évidence les constantes de couplages spin-spin (3-5) Hz de configuration E de dérivés oximes.

- **Conclusion Generale:**

Le travail présenté dans ce manuscrit a été axé sur la synthèse et l'étude d'analogues de nucléosides fluorés, dans l'espoir de découvrir de nouvelles drogues à visée antivirale. Dans le premier chapitre, nous nous sommes plus particulièrement intéressés aux virus de l'immunodéficince humaine et des hépatites B et C ainsi qu'aux thérapies utilisées dans le traitement de ces affections. Nous avons introduit l'importance des nucléosides fluorés, et nous avons donné quelques exemples de nucléosides fluorés utilisés en chimiothérapie antivirale et antitumorale. Dans le second chapitre, nous avons présenté une synthèse rapide de 2',3'-didésoxy-3'-fluoro-β-D-*thréo*-nucléosides portant les bases pyrimidiques des acides nucléiques et N^3-nitro ou N^3-amino et les composés obtenus ont été évalués contre divers virus à ADN et ARN (y compris le VIH) dans des expériences de cultures cellulaires. Dans le troisième chapitre, nous nous somme intéressés à la synthèse de différents 2'-fluoronucléosides avec les groupements *N*-hydroxyimino ainsi que *N*-méthoxyimino et *N*-hydroxylamino portant les bases hétérocycliques et les composés obtenus ont été évaluées pour leurs activités antivirales en cultures cellulaires. Bien que les résultants obtenus dans ce travail n'aient pas conduit à la découverte de composés actifs, la synthèse et l'étude de nouvelle séries d'analogues de nucléosides demeure encore aujord'hui une voie de recherche prometteuse dans l'élaboration de structures susceptible d'interférer sélectivement avec la réplication virale.

References

1. Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L., Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **1983,** *220* (4599), 868-71.

2. Furman, P. A.; Fyfe, J. A.; St Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; et al., Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Nat. Acad. Sci. U S A* **1986,** *83* (21), 8333-7.

3. Gallo, R. C.; Salahuddin, S. Z.; Popovic, M.; Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B.; et al., Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **1984,** *224* (4648), 500-3.

4. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St Clair, M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S., 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc. Nat. Acad. Sci. U S A* **1985,** *82* (20), 7096-100.

5. Cihlar, T.; Ray, A. S., Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. *Antiviral. Res.* **2010,** *85* (1), 39-58.

6. Sierra, S.; Kupfer, B.; Kaiser, R., Basics of the virology of HIV-1 and its replication. *J. Clin. Virol.* **2005,** *34* (4), 233-44.

7. Greene, W. C.; Debyser, Z.; Ikeda, Y.; Freed, E. O.; Stephens, E.; Yonemoto, W.; Buckheit, R. W.; Este, J. A.; Cihlar, T., Novel targets for HIV therapy. *Antiviral. Res.* **2008,** *80* (3), 251-65.

8. La Femina, R. L.; Editor, *Antiviral Research: Strategies in Antiviral Drug Discovery*. 2009; p 373 pp.

9. Hunsucker, S. A.; Mitchell, B. S.; Spychala, J., The 5'-nucleotidases as regulators of nucleotide and drug metabolism. *Pharmacol. Ther.* **2005,** *107* (1), 1-30.

10. Pruvost, A.; Negredo, E.; Benech, H.; Theodoro, F.; Puig, J.; Grau, E.; Garcia, E.; Molto, J.; Grassi, J.; Clotet, B., Measurement of intracellular didanosine and tenofovir phosphorylated metabolites and possible interaction of the two drugs in human

immunodeficiency virus-infected patients. *Antimicrob. Agents Chemother.* **2005,** *49* (5), 1907-14.

11. Cretton, E. M.; Sommadossi, J. P., Reduction of 3'-azido-2',3'-dideoxynucleosides to their 3'-amino metabolite is mediated by cytochrome P-450 and NADPH-cytochrome P-450 reductase in rat liver microsomes. *Drug Metab. Dispos.* **1993,** *21* (5), 946-50.

12. Resetar, A.; Spector, T., Glucuronidation of 3'-azido-3'-deoxythymidine: human and rat enzyme specificity. *Biochem. Pharmacol.* **1989,** *38* (9), 1389-93.

13. McDowell, J. A.; Chittick, G. E.; Ravitch, J. R.; Polk, R. E.; Kerkering, T. M.; Stein, D. S., Pharmacokinetics of [(14)C]abacavir, a human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitor, administered in a single oral dose to HIV-1-infected adults: a mass balance study. *Antimicrob. Agents Chemother.* **1999,** *43* (12), 2855-61.

14. Sarafianos, S. G.; Marchand, B.; Das, K.; Himmel, D. M.; Parniak, M. A.; Hughes, S. H.; Arnold, E., Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. *J. Mol. Biol.* **2009,** *385* (3), 693-713.

15. World Health Organization, Hepatitis B Virus, Fact Sheet. *http://www.who.int* **2009**.

16. Summers, J.; Mason, W. S., Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. *Cell* **1982,** *29* (2), 403-15.

17. Soussan, P.; Garreau, F.; Zylberberg, H.; Ferray, C.; Brechot, C.; Kremsdorf, D., In vivo expression of a new hepatitis B virus protein encoded by a spliced RNA. *J. Clin. Invest.* **2000,** *105* (1), 55-60.

18. Breiner, K. M.; Schaller, H.; Knolle, P. A., Endothelial cell-mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms. *Hepatology* **2001,** *34* (4 Pt 1), 803-8.

19. Neuveut, C.; Wei, Y.; Buendia, M. A., Mechanisms of HBV-related hepatocarcinogenesis. *J. Hepatol.* **2010,** *52* (4), 594-604.

20. Ferir, G.; Kaptein, S.; Neyts, J.; De Clercq, E., Antiviral treatment of chronic hepatitis B virus infections: the past, the present and the future. *Rev. Med. Virol.* **2008,** *18* (1), 19-34.

21. Lauer, G. M.; Walker, B. D., Hepatitis C virus infection. *N. Engl. J. Med.* **2001,** *345* (1), 41-52.

22. Robertson, B.; Myers, G.; Howard, C.; Brettin, T.; Bukh, J.; Gaschen, B.; Gojobori, T.; Maertens, G.; Mizokami, M.; Nainan, O.; Netesov, S.; Nishioka, K.; Shin i, T.; Simmonds, P.; Smith, D.; Stuyver, L.; Weiner, A., Classification, nomenclature, and database

development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. *Arch. Virol.* **1998,** *143* (12), 2493-503.

23. Roingeard, P.; Hourioux, C.; Blanchard, E.; Brand, D.; Ait-Goughoulte, M., Hepatitis C virus ultrastructure and morphogenesis. *Biol. Cell.* **2004,** *96* (2), 103-8.

24. Pawlotsky, J. M.; Chevaliez, S.; McHutchison, J. G., The hepatitis C virus life cycle as a target for new antiviral therapies. *Gastroenterology* **2007,** *132* (5), 1979-98.

25. McHutchison, J. G.; Gordon, S. C.; Schiff, E. R.; Shiffman, M. L.; Lee, W. M.; Rustgi, V. K.; Goodman, Z. D.; Ling, M.-H.; Cort, S.; Albrecht, J. K., Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N. Engl. J. Med.* **1998,** *339* (21), 1485-92.

26. Feld, J. J.; Hoofnagle, J. H., Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* **2005,** *436* (7053), 967-72.

27. Harris, J. M.; Martin, N. E.; Modi, M., Pegylation: a novel process for modifying pharmacokinetics. *Clin. Pharmacokinet.* **2001,** *40* (7), 539-51.

28. Sulkowski, M.; Lawitz, E.; Shiffman, M. L.; Muir, A. J.; Galler, G.; McCone, J.; Nyberg, L.; Lee, W. M.; Ghalib, R.; Schiff, E.; Galati, J.; Bacon, B.; Davis, M.; Mukhopadhyay, P.; Noviello, S.; Pedicone, L.; Albrecht, J.; McHutchison, J., Final results of the ideal (individualized dosing efficacy versus flat dosing to assess optimal pegylated interferon therapy) phase IIIB study. *J. Hepatol.* **2008,** *48*, S370-S371.

29. Franciscus, A., Hepatitis C treatments in current clinical development. *HCV project manager* *www.hcvadvocate.org*, **2010**.

30. Fried, J.; Sabo, E. F., Synthesis of 17α-hydroxycorticosterone and its 9α-halo derivatives from ll-epi-17α-hydroxycorticosterone. *J. Am. Chem. Soc.* **1953,** *75*, 2273-4.

31. Heidelberger, C.; Chaudhuri, N. K.; Danneberg, P.; Mooren, D.; Griesbach, L.; Duschinsky, R.; Schnitzer, R. J.; Pleven, E.; Scheiner, J., Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* **1957,** *179* (4561), 663-6.

32. MedAdNews 200-World's Best-Selling Medicines, MedAdNews. *July 2007 (http://en.wikipedia.org/wiki/List_of_top_selling_drugs).*

33. Tan, L.; Chen, C.-y.; Tillyer, R. D.; Grabowski, E. J. J.; Reider, P. J., A novel, highly enantioselective ketone alkynylation reaction mediated by chiral zinc aminoalkoxides. *Angew. Chem. Int. Ed.* **1999,** *38* (5), 711-13.

34. Humphreys, J. L.; Lowes, D. J.; Wesson, K. A.; Whitehead, R. C., Arene cis-dihydrodiols-useful precursors for the preparation of antimetabolites of the shikimic acid

pathway: application to the synthesis of 6,6-difluoroshikimic acid and (6S)-6-fluoroshikimic acid. *Tetrahedron* **2006,** *62* (21), 5099-108.

35. Chou, T.-C.; Dong, H.; Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Tong, W. P.; Danishefsky, S. J., Design and total synthesis of a superior family of epothilone analogues, which eliminate xenograft tumors to a nonrelapsable state. *Angew. Chem. Int. Ed.* **2003,** *42* (39), 4762-67.

36. Damon, D. B.; Dugger, R. W.; Magnus-Aryitey, G.; Ruggeri, R. B.; Wester, R. T.; Tu, M.; Abramov, Y., Synthesis of the CETR inhibitor Torcetrapib: The resolution route and origin of stereoselectivity in the iminium ion cyclization. *Org. Process Res. Dev.* **2006,** *10* (3), 464-71.

37. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E., (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin -7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2005,** *48* (1), 141-51.

38. Yamazaki, T.; Taguchi, T.; Ojima, I., Unique properties of fluorine and their relevance to medicinal chemistry and chemical biology. *Fluorine in Medicinal Chemistry and Chemical Biology, Blackwell Publishing, Ltd.* **2009**, 3-46.

39. Anon, *Exploring QSAR Fundamentals and Applications in Chemistry and Biology, Volume 1. Hydrophobic, Electronic and Steric Constants, Volume 2. Edited by Corwin Hansch, Albert Leo, and David Hoekman (Pomona College).* 1995; Vol. 117, p 9782.

40. Barchi, J. J.; Jeong, L. S.; Siddiqui, M. A.; Marquez, V. E., Conformational analysis of the complete series of 2' and 3' monofluorinated dideoxyuridines. *J. Biochem. Biophys. Methods* **1997,** *34* (1), 11-29.

41. Morton, G. O.; Lancaster, J. E.; Van Lear, G. E.; Fulmor, W.; Meyer, W. E., Structure of nucleocidin. III. revised structure. *J. Amer. Chem. Soc.* **1969,** *91* (6), 1535-7.

42. Hertel, L. W.; Kroin, J. S.; Misner, J. W.; Tustin, J. M., Synthesis of 2-deoxy-2,2-difluoro-D-ribose and 2-deoxy-2,2'-difluoro-D-ribofuranosyl nucleosides. *J. Org. Chem.* **1988,** *53* (11), 2406-9.

43. Bonate, P. L.; Arthaud, L.; Cantrell, W. R., Jr.; Stephenson, K.; Secrist, J. A., 3rd; Weitman, S., Discovery and development of clofarabine: a nucleoside analogue for treating cancer. *Nat. Rev. Drug Discov.* **2006,** *5* (10), 855-63.

44. Korba, B. E.; Furman, P. A.; Otto, M. J., Clevudine: a potent inhibitor of hepatitis B virus in vitro and in vivo. *Expert Rev. Anti Infect. Ther.* **2006,** *4* (4), 549-61.

45. Billich, A., Lodenosine National Institutes of Health. *Curr. Opin. Invest. Drugs* **1999,** *1* (2), 179-85.

46. De Clercq, E., Emerging anti-HIV drugs. *Expert Opin. Emerging Drugs* **2005,** *10* (2), 241-74.

47. Rusconi, S., Alovudine(Medivir). *Curr. Opin. Invest. Drugs* **2003,** *4* (2), 219-23.

48. Kodama, T.; Shuto, S.; Matsuda, A., Synthesis of pyrimidine 1'-fluoro nucleosides. *Tetrahedron Lett.* **2006,** *47* (26), 4429-32.

49. Kodama, T.; Matsuda, A.; Shuto, S., Synthesis of 1'-fluorouracil nucleosides as potential antimetabolites. *Tetrahedron* **2006,** *62* (42), 10011-17.

50. Codington, J. F.; Doerr, I. L.; Fox, J. J., Nucleosides. XVIII. Synthesis of 2'-fluorothymidine, 2'fluorodeoxyuridine, and other 2'-halo-2'-deoxy nucleosides. *J. Org. Chem.* **1964,** *29* (3), 558-64.

51. Shi, J.; Du, J.; Ma, T.; Pankiewicz, K. W.; Patterson, S. E.; Tharnish, P. M.; McBrayer, T. R.; Stuyver, L. J.; Otto, M. J.; Chu, C. K.; Schinazi, R. F.; Watanabe, K. A., Synthesis and anti-viral activity of a series of D- and L-2'-deoxy-2'-fluororibonucleosides in the subgenomic HCV replicon system. *Bioorg. Med. Chem.* **2005,** *13* (5), 1641-52.

52. Robins, M. J.; Mullah, K. B.; Wnuk, S. F.; Dalley, N. K., Nucleic acid related compounds. 73. Fluorination of uridine 2'-thioethers with xenon difluoride or (diethylamino)sulfur trifluoride. Synthesis of stable 2'-[alkyl(or aryl)sulfonyl]-2'-deoxy-2'-fluorouridines. *J. Org. Chem.* **1992,** *57* (8), 2357-64.

53. Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W., Design, Synthesis, and Antiviral Activity of 2'-Deoxy-2'-fluoro-2'-C-methyl-cytidine, a Potent Inhibitor of Hepatitis C Virus Replication. *J. Med. Chem.* **2005,** *48* (17), 5504-8.

54. Clark, J. L.; Mason, J. C.; Hollecker, L.; Stuyver, L. J.; Tharnish, P. M.; McBrayer, T. R.; Otto, M. J.; Furman, P. A.; Schinazi, R. F.; Watanabe, K. A., Synthesis and antiviral

activity of 2'-deoxy-2'-fluoro-2'-C-methyl purine nucleosides as inhibitors of hepatitis C virus RNA replication. *Bioorg. Med. Chem. Lett.* **2006,** *16* (6), 1712-5.

55. Huang, J. T.; Chen, L. C.; Wang, L.; Kim, M. H.; Warshaw, J. A.; Armstrong, D.; Zhu, Q. Y.; Chou, T. C.; Watanabe, K. A.; et al., Fluorinated sugar analogues of potential anti-HIV-1 nucleosides. *J. Med. Chem.* **1991,** *34* (5), 1640-6.

56. Gosselin, G.; Puech, F.; Genu-Dellac, C.; Imbach, J. L., 1,2-Di-O-acetyl-5-O-benzoyl-3-deoxy-3-fluoro-D-xylofuranose. A versatile precursor for the synthesis of 3-deoxy-3-fluoro-beta-D-xylofuranosyl nucleosides as potential antiviral agents. *Carbohydr. Res.* **1993,** *249* (1), 1-17.

57. McAtee, J. J.; Schinazi, R. F.; Liotta, D. C., A Completely Diastereoselective Electrophilic Fluorination of a Chiral, Noncarbohydrate Sugar Ring Precursor: Application to the Synthesis of Several Novel 2'-Fluoronucleosides. *J. Org. Chem.* **1998,** *63* (7), 2161-67.

58. Smith, D. B.; Kalayanov, G.; Sund, C.; Winqvist, A.; Maltseva, T.; Leveque, V. J. P.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Benkestock, K.; Zhou, X.-X.; Kaiser, A. C.; Maag, H.; Cammack, N.; Martin, J. A.; Swallow, S.; Johansson, N. G.; Klumpp, K.; Smith, M., The Design, Synthesis, and Antiviral Activity of Monofluoro and Difluoro Analogues of 4'-Azidocytidine against Hepatitis C Virus Replication: The Discovery of 4'-Azido-2'-deoxy-2'-fluorocytidine and 4'-Azido-2'-dideoxy-2',2'-difluorocytidine. *J. Med. Chem.* **2009,** *52* (9), 2971-78.

59. Pfundheller, H. M.; Wengel, J., Oligonucleotides containing 4'-C-aminomethyl-2'-modified thymidines show increased binding affinity towards DNA and RNA. *Bioorg. Med. Chem. Lett.* **1999,** *9* (18), 2667-72.

60. Prhavc, M.; Dyatkina, N.; Keicher, J.; Liehr, S.; Koo-McCoy, S.; Latour, D.; Fung, K.; Dunlop, K.; Pouliot, J.; Wang, T.; Li, W.; Lou, L.; Roberts, C.; Griffith, R., Synthesis and biological activity of 7-deaza-7-ethynyl-2'-deoxy-2'-fluoro-2'-C-methyladenosine and its 2'-C-methyl-ribo analogue. *Nucleic Acids Symp. Ser.* **2008,** *52* (1), 643-44.

61. Wright, J. A.; Taylor, N. F.; Fox, J. J., Nucleosides. LX. Fluorocarbohydrates. XXII. Synthesis of 2-deoxy-2-fluoro-D-arabinose and 9-(2-deoxy-2-fluoro-alpha- and -beta-D-arabinofuranosyl)adenines. *J. Org. Chem.* **1969,** *34* (9), 2632-6.

62. Reichman, U.; Watanabe, K. A.; Fox, J. J., A practical synthesis of 2-deoxy-2-fluoro-D-arabinofuranose derivatives. *Carbohydr. Res.* **1975,** *42* (2), 233-40.

63. Watanabe, K. A.; Su, T. L.; Klein, R. S.; Chu, C. K.; Matsuda, A.; Chun, M. W.; Lopez, C.; Fox, J. J., Nucleosides. 123. Synthesis of antiviral nucleosides: 5-substituted 1-(2-

deoxy-2-halogeno-β-D-arabinofuranosyl)cytosines and -uracils. Some structure-activity relationships. *J. Med. Chem.* **1983,** *26* (2), 152-6.

64. Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, C., Jr.; Howell, H. G., Fluorocarbohydrates in synthesis. An efficient synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (β-FIAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (β-FMAU). *J. Org. Chem.* **1985,** *50* (19), 3644-7.

65. Juaristi, E.; Cuevas, G., Recent studies on the anomeric effect. *Tetrahedron* **1992,** *48* (24), 5019-87.

66. Montgomery, J. A.; Shortnacy, A. T.; Carson, D. A.; Secrist, J. A., 3rd, 9-(2-Deoxy-2-fluoro-beta-D-arabinofuranosyl)guanine: a metabolically stable cytotoxic analogue of 2'-deoxyguanosine. *J. Med. Chem.* **1986,** *29* (11), 2389-92.

67. Montgomery, J. A.; Shortnacy-Fowler, A. T.; Clayton, S. D.; Riordan, J. M.; Secrist, J. A., 3rd, Synthesis and biological activity of 2'-fluoro-2-halo derivatives of 9-beta-D-arabinofuranosyladenine. *J. Med. Chem.* **1992,** *35* (2), 397-401.

68. Bauta, W. E.; Schulmeier, B. E.; Burke, B.; Puente, J. F.; Cantrell, W. R., Jr.; Lovett, D.; Goebel, J.; Anderson, B.; Ionescu, D.; Guo, R., A New Process for Antineoplastic Agent Clofarabine. *Org. Process Res. Dev.* **2004,** *8* (6), 889-96.

69. Pankiewicz, K. W., Fluorinated nucleosides. *Carbohydr. Res.* **2000,** *327* (1-2), 87-105.

70. Sterzycki, R. Z.; Ghazzouli, I.; Brankovan, V.; Martin, J. C.; Mansuri, M. M., Synthesis and anti-HIV activity of several 2'-fluoro-containing pyrimidine nucleosides. *J. Med. Chem.* **1990,** *33* (8), 2150-7.

71. Marquez, V. E.; Tseng, C. K.; Mitsuya, H.; Aoki, S.; Kelley, J. A.; Ford, H., Jr.; Roth, J. S.; Broder, S.; Johns, D. G.; Driscoll, J. S., Acid-stable 2'-fluoro purine dideoxynucleosides as active agents against HIV. *J. Med. Chem.* **1990,** *33* (3), 978-85.

72. Ford, H., Jr.; Driscoll, J. S.; Siddiqui, M.; Kelley, J. A.; Mitsuya, H.; Shirasaka, T.; Johns, D. G.; Marquez, V. E., Chemistry and anti-HIV activity of 2'-β-fluoro-2',3'-dideoxyguanosine. *Nucleosides & Nucleotides* **1994,** *13* (1-3), 213-34.

73. Takamatsu, S.; Maruyama, T.; Katayama, S.; Hirose, N.; Naito, M.; Izawa, K., Practical synthesis of 9-(2,3-dideoxy-2-fluoro-β-d-threo-pentofuranosyl)adenine (FddA) via a purine 3'-deoxynucleoside. *Tetrahedron Lett.* **2001,** *42* (12), 2325-28.

74. Takamatsu, S.; Maruyama, T.; Katayama, S.; Hirose, N.; Naito, M.; Izawa, K., Synthesis of 9-(2,3-Dideoxy-2-fluoro-β-D-threo-pentofuranosyl)adenine (FddA) via a Purine 3'-Deoxynucleoside. *J. Org. Chem.* **2001,** *66* (22), 7469-77.

75. Choudhury, A.; Jin, F.; Wang, D.; Wang, Z.; Xu, G.; Nguyen, D.; Castoro, J.; Pierce, M. E.; Confalone, P. N., A concise synthesis of anti-viral agent F-ddA, starting from (S)-dihydro-5-(hydroxymethyl)-2(3H)-furanone. *Tetrahedron Lett.* **2003,** *44* (2), 247-50.

76. Qiu, X.-L.; Xu, X.-H.; Qing, F.-L., Recent advances in the synthesis of fluorinated nucleosides. *Tetrahedron* **2010,** *66* (4), 789-843.

77. Wang, S.; Chang, J.; Pan, S.; Zhao, K., Synthesis of potentially antiviral 2',3'-dideoxy-2'-fluoro-3'-(hydroxyamino)nucleosides. *Helv. Chim. Acta* **2004,** *87* (2), 327-39.

78. Moukha-chafiq, O.; Tiwari, K. N.; Secrist, J. A., III, Synthesis of some 2'-fluoro-2'-deoxy-3'-C-ethynyl and 3'-C-vinyl-β-D-lyxofuranosyl nucleosides. *Nucleosides, Nucleotides & Nucleic Acids* **2005,** *24* (5-7), 713-15.

79. Shortnacy-Fowler, A. T.; Tiwari, K. N.; Montgomery, J. A.; Secrist, J. A., III, Synthesis and biological activity of 4'-C-hydroxymethyl-2'-fluoro-D-arabinofuranosylpurine nucleosides. *Nucleosides, Nucleotides & Nucleic Acids* **2001,** *20* (4-7), 747-50.

80. Mikhailopulo, I. A.; Pricota, T. I.; Poopeiko, N. E.; Sivets, G. G.; Kvasyuk, E. I.; Sviryaeva, T. V.; Savochkina, L. P.; Beabealashvilli, R. S., 3'-Fluoro-3'-deoxyribonucleoside 5'-triphosphates: synthesis and use as terminators of RNA biosynthesis. *FEBS Lett.* **1989,** *250* (2), 139-41.

81. Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; De Clercq, E., Synthesis and antiviral and cytostatic properties of 3'-deoxy-3'-fluoro- and 2'-azido-3'-fluoro-2',3'-dideoxy-D-ribofuranosides of natural heterocyclic bases. *J. Med. Chem.* **1991,** *34* (7), 2195-202.

82. Battistini, C.; Giordani, A.; Ermoli, A.; Franceschi, G., Synthesis of 3'-fluoro-3'-deoxyadenosine starting from adenosine. *Synthesis* **1990,** (10), 900-5.

83. Puech, F.; Gosselin, G.; Imbach, J. L., Synthesis of 9-(3-deoxy-3-fluoro-β-D-ribofuranosyl)guanine, a new potent antiviral agent. *J. Chem. Soc., Chem. Commun.* **1989,** (14), 955-7.

84. Pankiewicz, K. W.; Krzeminski, J.; Watanabe, K. A., Nucleosides. 164. Studies directed toward the synthesis of 2'-deoxy-2'-substituted arabino nucleosides. 10. Synthesis of 2'-β-fluoro- and 3'-α-fluoro-substituted guanine nucleosides. Effect of sugar conformational shifts on nucleophilic displacement of the 2'-hydroxy and 3'-hydroxy group with DAST. *J. Org. Chem.* **1992,** *57* (26), 7315-21.

85. Etzold, G.; Hintsche, R.; Kowollik, G.; Langen, P., Nucleosides of fluoro sugars. VI. Synthesis and reactivity of 3'-fluoro- and 3'-chloro-3'-deoxythymidine. *Tetrahedron* **1971,** *27* (12), 2463-72.

86. Kowollik, G.; Etzold, G.; Von Janta-Lipinski, M.; Gaertner, K.; Langen, P., Nucleosides of fluorinated sugars. XII. Synthesis of 1-(2,3-dideoxy-3-fluoro-β-D-ribofuranosyl)pyrimidines. *J. Prakt. Chem.* **1973,** *315* (5), 895-900.

87. Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H., 3'-substituted 2',3'-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. *J. Med. Chem.* **1987,** *30* (8), 1270-8.

88. Robins, M. J.; Nowak, I.; Wnuk, S. F.; Hansske, F.; Madej, D., Deoxygenative [1,2]-hydride shift rearrangements in nucleoside and sugar chemistry: analoguey with the [1,2]-electron shift in the deoxygenation of ribonucleotides by ribonucleotide reductases. *J. Org. Chem.* **2007,** *72* (22), 8216-21.

89. Katayama, S.; Takamatsu, S.; Naito, M.; Tanji, S.; Ineyama, T.; Izawa, K., A synthesis of 3'-α-fluoro-2',3'-dideoxyadenosine via a bromine rearrangement during fluorination with MOST reagent. *J. Fluorine Chem.* **2006,** *127* (4-5), 524-28.

90. Maag, H.; Rydzewski, R. M.; McRoberts, M. J.; Crawford-Ruth, D.; Verheyden, J. P.; Prisbe, E. J., Synthesis and anti-HIV activity of 4'-azido- and 4'-methoxynucleosides. *J. Med. Chem.* **1992,** *35* (8), 1440-51.

91. Nguyen, C.; Kasinathan, G.; Leal-Cortijo, I.; Musso-Buendia, A.; Kaiser, M.; Brun, R.; Ruiz-Perez, L. M.; Johansson, N. G.; Gonzalez-Pacanowska, D.; Gilbert, I. H., Deoxyuridine triphosphate nucleotidohydrolase as a potential antiparasitic drug target. *J. Med. Chem.* **2005,** *48* (19), 5942-54.

92. Wright, J. A.; Wilson, D. P.; Fox, J. J., Nucleosides. LXIV. Fluoro sugar analogues of arabinosyl- and xylosylcytosines. *J. Med. Chem.* **1970,** *13* (2), 269-72.

93. Puech, F.; Gosselin, G.; Imbach, J. L., Synthesis of 9-(3-deoxy- and 2,3-dideoxy-3-fluoro-β-D-xylofuranosyl)guanines as potential antiviral agents. *Tetrahedron Lett.* **1989,** *30* (24), 3171-4.

94. Hayakawa, H.; Takai, F.; Tanaka, H.; Miyasaka, T.; Yamaguchi, K., Diethylaminosulfur trifluoride (DAST) as a fluorinating agent of pyrimidine nucleosides having a 2',3'-vicinal diol system. *Chem. Pharm. Bull.* **1990,** *38* (5), 1136-9.

95. Robins, M. J.; Fouron, Y.; Mengel, R., Nucleic acid related compounds. II. Adenosine 2',3'-ribo-epoxide. Synthesis, intramolecular degradation, and transformation into 3'-

substituted xylofuranosyl nucleosides and the lyxo-epoxide. *J. Org. Chem.* **1974,** *39* (11), 1564-70.

96. Fuentes, J.; Angulo, M.; Pradera, M. A., Fluoronucleosides, Isothiocyanato C-Nucleosides, and Thioureylene Di-C-nucleosides via Cyclic Sulfates. *J. Org. Chem.* **2002,** *67* (8), 2577-87.

97. Tsoukala, E.; Agelis, G.; Dolinsek, J.; Botic, T.; Cencic, A.; Komiotis, D., An efficient synthesis of 3-fluoro-5-thio-xylofuranosyl nucleosides of thymine, uracil, and 5-fluorouracil as potential antitumor or/and antiviral agents. *Bioorg. Med. Chem.* **2007,** *15* (9), 3241-7.

98. Brink, A. J.; De Villiers, O. G.; Jordaan, A., Nucleosides from fluorinated branched-chain sugars: some reactions of 3-deoxy-3-fluoro-3-C-hydroxymethyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose. *Carbohydr. Res.* **1977,** *54* (2), 285-91.

99. Zhao, B.; Chang, J.; Wang, Q.; Du, Y.; Zhao, K., Design and synthesis of 3'-deoxy-3'-fluoro-2'-O,3'-C-vinylene-linked bicyclic purine nucleoside. *Synlett* **2008,** (19), 2993-96.

100. Florini, J. R.; Bird, H. H.; Bell, P. H., Inhibition of protein synthesis in vitro and in vivo by nucleocidin, an antitrypanosomal antibiotic. *J. Biol. Chem.* **1966,** *241* (5), 1091-8.

101. Jenkins, I. D.; Verheyden, J. P. H.; Moffatt, J. G., 4'-Substituted nucleosides. 2. Synthesis of the nucleoside antibiotic nucleocidin. *J. Am. Chem. Soc.* **1976,** *98* (11), 3346-57.

102. Owen, G. R.; Verheyden, J. P. H.; Moffatt, J. G., 4'-Substituted nucleosides. 3. Synthesis of some 4'-fluorouridine derivatives. *J. Org. Chem.* **1976,** *41* (18), 3010-17.

103. Maguire, A. R.; Meng, W. D.; Roberts, S. M.; Willetts, A. J., Synthetic approaches towards nucleocidin and selected analogues; anti-HIV activity in 4'-fluorinated nucleoside derivatives. *J. Chem. Soc., Perkin Trans. 1* **1993,** (15), 1795-808.

104. Ajmera, S.; Bapat, A. R.; Stephanian, E.; Danenberg, P. V., Synthesis and interaction with uridine phosphorylase of 5'-deoxy-4',5-difluorouridine, a new prodrug of 5-fluorouracil. *J. Med. Chem.* **1988,** *31* (6), 1094-8.

105. Jung, M. E.; Toyota, A., Preparation of 4'-Substituted Thymidines by Substitution of the Thymidine 5'-Esters. *J. Org. Chem.* **2001,** *66* (8), 2624-35.

106. Lee, S.; Uttamapinant, C.; Verdine, G. L., A Concise Synthesis of 4'-Fluoro Nucleosides. *Organic Lett.* **2007,** *9* (24), 5007-9.

107. Kissman, H. M.; Weiss, M. J., 5-Deoxy-5-fluoro-D-ribofuranosyl derivatives of certain purines, pyrimidines, and 5,6-dimethylbenzimidazole. *J. Am. Chem. Soc.* **1958,** *80*, 5559-64.

108. O'Hagan, D.; Schaffrath, C.; Cobb, S. L.; Hamilton, J. T.; Murphy, C. D., Biochemistry: biosynthesis of an organofluorine molecule. *Nature* **2002,** *416* (6878), 279.

109. Schaffrath, C.; Cobb, S. L.; O'Hagan, D., Cell-free biosynthesis of fluoroacetate and 4-fluorothreonine in Streptomyces cattleya. *Angew. Chem. Int. Ed.* **2002,** *41* (20), 3913-15.

110. Cadicamo, C. D.; Courtieu, J.; Deng, H.; Meddour, A.; O'Hagan, D., Enzymatic fluorination in Streptomyces cattleya takes place with an inversion of configuration consistent with an SN2 reaction mechanism. *ChemBioChem* **2004,** *5* (5), 685-90.

111. Cobb, S. L.; Deng, H.; Hamilton, J. T. G.; McGlinchey, R. P.; O'Hagan, D., Identification of 5-fluoro-5-deoxy-D-ribose-1-phosphate as an intermediate in fluorometabolite biosynthesis in Streptomyces cattleya. *Chem. Commun.* **2004,** (5), 592-593.

112. Ashton, T. D.; Scammells, P. J., An improved synthesis of 5'-fluoro-5'-deoxyadenosines. *Bioorg. Med. Chem. Lett.* **2005,** *15* (14), 3361-3.

113. Le, H. P.; Muller, C. E., Rapid microwave-assisted fluorination yielding novel 5'-deoxy-5'-fluorouridine derivatives. *Bioorg. Med. Chem. Lett.* **2006,** *16* (23), 6139-42.

114. Kowollik, G.; Gaertner, K.; Etzold, G.; Langen, P., Nucleosides of fluorinated sugars. III. Synthesis of 5'-fluoro-substituted deoxyribonucleosides. *Carbohyd. Res.* **1970,** *12* (3), 301-11.

115. Ye, S.; Rezende, M. M.; Deng, W.-P.; Herbert, B.; Daly, J. W.; Johnson, R. A.; Kirk, K. L., Synthesis of 2',5'-Dideoxy-2-fluoroadenosine and 2',5'-Dideoxy-2,5'-difluoroadenosine: Potent P-Site Inhibitors of Adenylyl Cyclase. *J. Med. Chem.* **2004,** *47* (5), 1207-13.

116. Sufrin, J. R.; Spiess, A. J.; Kramer, D. L.; Libby, P. R.; Porter, C. W., Synthesis and antiproliferative effects of novel 5'-fluorinated analogues of 5'-deoxy-5'-(methylthio)adenosine. *J. Med. Chem.* **1989,** *32* (5), 997-1001.

117. Robins, M. J.; Wnuk, S. F.; Mullah, K. B.; Dalley, N. K.; Yuan, C.-S.; Lee, Y.; Borchardt, R. T., Nucleic Acid Related Compounds. 80. Synthesis of 5'-S-(Alkyl and aryl)-5'-fluoro-5'-thioadenosines with Xenon Difluoride or (Diethylamido)sulfur Trifluoride, Hydrolysis in Aqueous Buffer, and Inhibition of S-Adenosyl-L-homocysteine hydrolase by derived "Adenosine 5'-Aldehyde" Species. *J. Org. Chem.* **1994,** *59* (3), 544-55.

118. Robins, M. J.; Wnuk, S. F.; Mullah, K. B.; Dalley, N. K., Nucleic acid-related compounds. 68. Fluorination at C5' of nucleoside 5'-thioethers with DAST/antimony(III) chloride or xenon difluoride to give 5'-S-aryl-5'-fluoro-5'-thiouridines. *J. Org. Chem.* **1991,** *56* (24), 6878-84.

119. McCarthy, J. R.; Jarvi, E. T.; Matthews, D. P.; Edwards, M. L.; Prakash, N. J.; Bowlin, T. L.; Mehdi, S.; Sunkara, P. S.; Bey, P., 4',5'-Unsaturated-5'-fluoro adenosine nucleosides: potent mechanism-based inhibitors of S-adenosyl-L-homocysteine hydrolase. *J. Am. Chem. Soc.* **1989,** *111* (3), 1127-8.

120. Wnuk, S. F.; Yuan, C.-S.; Borchardt, R. T.; Balzarini, J.; De Clercq, E.; Robins, M. J., Nucleic Acid-Related Compounds. 84. Synthesis of 6'-(E and Z)-Halohomovinyl Derivatives of Adenosine, Inactivation of S-Adenosyl-L-homocysteine Hydrolase, and Correlation of Anticancer and Antiviral Potencies with Enzyme Inhibition. *J. Med. Chem.* **1994,** *37* (21), 3579-87.

121. Wnuk, S. F.; Mao, Y.; Yuan, C. S.; Borchardt, R. T.; Andrei, G.; Balzarini, J.; De Clercq, E.; Robins, M. J., Discovery of type II (covalent) inactivation of S-adenosyl-L-homocysteine hydrolase involving its "hydrolytic activity": synthesis and evaluation of dihalohomovinyl nucleoside analogues derived from adenosine. *J. Med. Chem.* **1998,** *41* (16), 3078-83.

122. Wnuk, S. F.; Robins, M. J., Stannyl Radical Mediated Cleavage of π-Deficient Heterocyclic Sulfones. Synthesis of α-Fluoro Esters and the First Homonucleoside α-Fluoromethylene Phosphonate. *J. Am. Chem. Soc.* **1996,** *118* (10), 2519-20.

123. Liu, P.; Sharon, A.; Chu, C. K., Fluorinated nucleosides: Synthesis and biological implication. *J. Fluorine Chem.* **2008,** *129* (9), 743-66.

124. Herdewijn, P.; Pauwels, R.; Baba, M.; Balzarini, J.; De Clercq, E., Synthesis and anti-HIV activity of various 2'- and 3'-substituted 2',3'-dideoxyadenosines: a structure-activity analysis. *J. Med. Chem.* **1987,** *30* (11), 2131-7.

125. Agyei-Aye, K.; Yan, S.; Hebbler, A. K.; Baker, D. C., Preparation of 2,3'-anhydropyrimidine nucleosides using N,N-diethylaminosulfur trifluoride (DAST). *Nucleosides & Nucleotides* **1989,** *8* (3), 327-37.

126. Serra, C.; Aragones, C.; Bessa, J.; Farras, J.; Vilarrasa, J., Stabilization of pyrimidine nucleoside triflates by N-nitro groups. *Tetrahedron Lett.* **1998,** *39* (41), 7575-78.

127. Serra, C.; Farras, J.; Vilarrasa, J., Cyclic sulfates as synthetic equivalents of α-epoxynucleosides. *Tetrahedron Lett.* **1999,** *40* (51), 9111-13.

128. Lin, T. S.; Gao, Y. S., Synthesis and biological activity of 5-(trifluoromethyl)- and 5-(pentafluoroethyl)pyrimidine nucleoside analogues. *J. Med. Chem.* **1983,** *26* (4), 598-601.

129. Ariza, X.; Bou, V.; Vilarrasa, J., A New Route to 15N-Labeled, N-Alkyl, and N-Amino Nucleosides via N-Nitration of Uridines and Inosines. *J. Am. Chem. Soc.* **1995,** *117* (13), 3665-73.

130. Gorchs, O.; Hernandez, M.; Garriga, L.; Pedroso, E.; Grandas, A.; Farras, J., A New Method for the Preparation of Modified Oligonucleotides. *Organic Lett.* **2002,** *4* (11), 1827-30.

131. Mitsunobu, O.; Kimura, J.; Fujisawa, Y., Nucleosides and nucleotides. II. Selective acylation of 5'-hydroxyl group of thymidine. *Bull. Chem. Soc. Jpn.* **1972,** *45* (1), 245-7.

132. He, G.-X.; Bischofberger, N., Selective 5'-O-acetylation of 2'-deoxynucleosides and nucleosides by a modified Mitsunobu procedure. *Nucleosides & Nucleotides* **1997,** *16* (3), 257-63.

133. Lal, G. S.; Pez, G. P.; Pesaresi, R. J.; Prozonic, F. M.; Cheng, H., Bis(2-methoxyethyl)aminosulfur trifluoride: A new broad-spectrum deoxofluorinating agent with enhanced thermal stability. *J. Org. Chem.* **1999,** *64* (19), 7048-54.

134. Kohda, K.; Kobayashi, I.; Itano, K.; Asano, S.; Kawazoe, Y., Syntheses and properties of N-aminopyrimidines. *Tetrahedron* **1993,** *49* (19), 3947-58.

135. Imrie, C., Reactions of secondary nitramines with tributyltin hydride and tris(trimethylsilyl)silane. *J. Chem. Res., Synop.* **1995,** (8), 328-9.

136. Cava, M. P.; Levinson, M. I., Thionation reactions of Lawesson's reagents. *Tetrahedron* **1985,** *41* (22), 5061-87.

137. Ozturk, T.; Ertas, E.; Mert, O., Use of Lawesson's Reagent in Organic Syntheses. *Chem. Rev.* **2007,** *107* (11), 5210-78.

138. Fedorov, II; Kazmina, E. M.; Gurskaya, G. V.; Jasko, M. V.; Zavodnic, V. E.; Balzarini, J.; De Clercq, E.; Faraj, A.; Sommadossi, J. P.; Imbach, J. L.; Gosselin, G., Novel 3'-C/N-substituted 2',3'-beta-D-dideoxynucleosides as potential chemotherapeutic agents. 1. Thymidine derivatives: synthesis, structure, and broad spectrum antiviral properties. *J. Med. Chem.* **1997,** *40* (4), 486-94.

139. Meerbach, A.; Holy, A.; Wutzler, P.; De Clercq, E.; Neyts, J., Inhibitory effects of novel nucleoside and nucleotide analogues on Epstein-Barr virus replication. *Antiviral Chem. Chemother.* **1998,** *9* (3), 275-82.

140. Ogawa, A.; Tanaka, M.; Sasaki, T.; Matsuda, A., Nucleosides and nucleotides. 180. Synthesis and antitumor activity of nucleosides that have a hydroxylamino group instead of a

hydroxyl group at the 2'- or 3'-position of the sugar moiety. *J. Med. Chem.* **1998,** *41* (25), 5094-107.

141. Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L., The synthesis of oligoribonucleotides. II. The use of silyl protecting groups in nucleoside and nucleotide chemistry. VII. *Can. J. Chem.* **1978,** *56* (21), 2768-80.

142. Pierra, C.; Amador, A.; Badaroux, E.; Storer, R.; Gosselin, G., Synthesis of 2'-C-methylcytidine and 2'-C-methyluridine derivatives modified in the 3'-position as potential antiviral agents. *Collect. Czech. Chem. Commun.* **2006,** *71* (7), 991-1010.

143. Robins, M. J.; Sarker, S.; Samano, V.; Wnuk, S. F., Nucleic acid related compounds. 94. Remarkably high stereoselective reductions of 2'- and 3'-keto nucleosides to give arabino, ribo, and xylofuranosyl nucleosides with hydrogen isotopes at C2' and C3'. *Tetrahedron* **1997,** *53* (2), 447-56.

144. Ogilvie, K. K.; Schifman, A. L.; Penney, C. L., The synthesis of oligoribonucleotides. III. The use of silyl protecting groups in nucleoside and nucleotide chemistry. VIII. *Can. J. Chem.* **1979,** *57* (17), 2230-8.

145. Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E., A simple and convenient method for the selective N-acylations of cytosine nucleosides. *Nucleosides & Nucleotides* **1989,** *8* (2), 179-83.

146. Ko, H.; Das, A.; Carter, R. L.; Fricks, I. P.; Zhou, Y.; Ivanov, A. A.; Melman, A.; Joshi, B. V.; Kovac, P.; Hajduch, J.; Kirk, K. L.; Harden, T. K.; Jacobson, K. A., Molecular recognition in the P2Y(14) receptor: Probing the structurally permissive terminal sugar moiety of uridine-5'-diphosphoglucose. *Bioorg. Med. Chem.* **2009,** *17* (14), 5298-311.

147. Mallory, F. B.; Mallory, C. W., Nuclear spin-spin coupling via nonbonded interactions. 5. Nitrogen-fluorine coupling. *J. Am. Chem. Soc.* **1985,** *107* (17), 4816-19.

148. Mallory, F. B.; Luzik, E. D., Jr.; Mallory, C. W.; Carroll, P. J., Nuclear spin-spin coupling via nonbonded interactions. 7. Effects of molecular structure on nitrogen-fluorine coupling. *J. Org. Chem.* **1992,** *57* (1), 366-70.

149. Schinazi, R. F.; Chen, M. S.; Prusoff, W. H., Antiviral and antineoplastic activities of pyrimidine arabinosyl nucleosides and their 5'-amino derivatives. *J. Med. Chem.* **1979,** *22* (10), 1273-7.

150. Kang, S. H.; Oh, S. J.; Yoon, M. K.; Ryu, J. S.; Lee, W. K.; Choi, S. J.; Park, K. P.; Moon, D. H., Simple and High radiochemical yield synthesis of 2'-Deoxy-2'-

[18F]fluorouridine via a new nosylate precursor. *J. Labelled Compd. Radiopharm.* **2006,** *49* (14), 1237-46.

151. Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C.; Cook, P. D., Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets. *J. Med. Chem.* **1993,** *36* (7), 831-41.

152. Kittaka, A.; Tanaka, H.; Miyasaka, T.; Yamaguchi, K., Reactions of 2'-deoxy-2'-halouridines and O2,2'-cyclouridine with lithium dialkylamides: formation of 1',2'-unsaturated derivatives. *Nucleosides & Nucleotides* **1992,** *11* (1), 37-47.