Univerzita Karlova

Přírodovědecká fakulta

Studijní program: Chemie

Studijní obor: Medicinální chemie

Syntéza fluorovaných nukleosidů

Synthesis of fluorinated nucleosides

Van Hai Nguyen

Diplomová práce

Školitel: prof. Ing. Michal Hocek, DSc., CSc.

Praha 2020

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval/a samostatně a že jsem uvedl/a všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 15. 06. 2020

Podpis

Acknowledgement

Before anything else I would like to thank my supervisor prof. Michal Hocek for giving me the opportunity to work on this interesting project in his research group. I also want to thank him for his advices and encouragement, which lead me to finish this thesis. My great gratitude also belongs to Dr. Michael Allan Downey from whom I've learned a lot about organic chemistry. I'd like to express my special thanks to Dr. Michal Tichý for his helpful advices and suggestions during the synthesis as well as detailed reading and correcting this thesis. Next, I would like to thank Alessandro Panattoni and Nemanja Milisavljevic for all useful advices, helps and funs during my work. I could not forget to thank all people in the Hocek research group for friendly working environment. I would like to thank team of mass spectrometry at IOCB AS CR for measurements of mass spectra. To all my friends I'm very grateful for all those great moments and endless funs. To my parents and my brother, I wish to express my deep gratitude for always being there for me and support me in every decision that I've made.

All the synthetic experiments were performed by me. Measurement and interpretation of NMR spectra of some compounds were done by Dr. Radek Pohl.

Abstract

The key intermediate 6-amino-7-iodo-7-deazapurine 3′-deoxy-3′-fluororibonucleoside was synthesized using multistep sequence of several reactions, which started from the commercially available D-xylose and 6-chloro-7-deazapurine. The synthetic strategy was based on fluorination of sugar and glycosylation with corresponding nucleobase afterwards. The fluorination of 5-protected-1,2-isopropylidine xylose with different protecting groups at position 5 always led to elimination. It was later discovered that isopropylidine forces the conformation, which is unfavorable for substitution. During the extensive optimization it was also found out that DAST appears to be an optimal fluorinating agent. Fluorination was performed on 2,3-unprotected xylose, which was subsequently used for glycosylation. After several unsuccessful attempts on "protection group free" glycosylation, Vorbrüggen glycosylation was successful and gave desired 3′-fluoro nucleoside in good yield. However, benzoyl group had to be introduced into position 2′. The protected nucleoside was then aminated and simultaneously deproctected with solution of aqueous NH_3 and 1,4-dioxane. The obtained key intermediate was used for synthesis of a small series of desired 6-amino-7-hetaryl nucleoside using Pd-catalyzed Suzuki reaction under aqueous conditions. The series of 6-amino-7-hetaryl-7-deazapurine-3-fluororibonucleosides were synthesized and tested for their biological activities.

Keywords

Nucleosides, 7-deazapurines, fluorinated nucleosides.

Abstrakt

Klíčový intermediát 6-amino-7-jodo-7-deazapurin 3´-deoxy-3´-fluororibonukleosid byl syntetizován přes vícekrokových sekvenčních reakcích, které byly zahájeny z komerčně dostupné D-xylózy a 6-chloro-7-deazapurinu. Syntetická strategie byla založena na fluorace cukru a následná glykosylace s odpovídající nukleobází. Fluorace 5-chráněného-1,2-isopropylidin xylózy s odlišnými chránicími skupinami v pozici 5 vždy vedla k eliminaci produktu. Později bylo zjištěno, že isopropylidin vnucuje konformaci, která nepreferuje substituci. Během rozsáhlé optimalizace bylo zjištěno, že DAST je optimálním fluoračním činidlem. Fluorace byla prováděna na 2,3-nechráněné xylózy, která následně byla použitá na glykosylace. Po několika neúspěšných pokusů o "protection group free" glykosylace, Vorbrüggenova glykosylace byla úspěšná a bylo získáno 3´-fluoro nukleosid s dobrým výtěžkem. Při použití Vorbrüggenovy glykosylace je potřeba ochránit OH skupinu na C2 benzoylem. Ochráněný nukleosid následně byl aminován a odchráněn pomocí roztoku NH_3 a 1,4-dioxanu. Získaný klíčový intermediát byl použit na syntézu malou série 6-amino-7-hetaryl nuklesosid pomocí Pd-katalyzované Suzuki reakce ve vodném prostředí. Série 6-amino-7-hetaryl-7-deazapurin-3-fluororibonukleosidy byla nasytetizována a otestována pro její biologické účinky.

Klíčová slova

Nukleosidy, 7-deazapuriny, fluorované nukleosidy,

Abbreviations

Ac_2O	acetic anhydride
AcOH	acetic acid
ADDP	1,1′-(azodicarbonyl)dipiperidine
ADK	adenosine kinase
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
DAST	diethylaminosulfur trifluoride
DCM	dichloromethane
DMF	dimethylformamide
DP	diphosphate
EtOAc	ethyl acetate
hCNT	human concentrative nucleoside transporter
hENT	human equilibrative nucleoside transporter
HIV-1 RT	HIV-1 reverse transcription
IC_{50}	inhibitory concentration
MeCN	acetonitrile
MeOH	methanol
MIC	minimum inhibitory concentration
Mtb	mycobacterium tuberculosis
NRTIs	Nucleoside/Nucleotide reverse transcriptase inhibitors.
$P_n(Bu)_3$	tri-*N*-butylphosphine
TBAF	tetrabutylammonium fluoride
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TP	triphosphate
TPPTS	3,3′,3″-phosphanetriyltris(benzenesulfonic acid) trisodium salt
UMP-CMP	uridine monophosphate-cytidine monophosphate

Contents

1 Introduction

Nucleosides and nucleotides, as building blocks of nucleic acids, have an underlying importance in all living systems. Their biological importance is far from being restricted to DNA and RNA. They can also act as energy carriers (ATP, GTP) or as precursors for the synthesis of nucleotide cofactors (NAD, FADH, SAM).[1] Even a small change in their structure can lead to significant impact on metabolic pathways. The five natural nucleosides or NADH and NADPH can serve as a great example.[2] Those significant roles of nucleotides and nucleosides open many possibilities for design and development of their analogues, which can lead to discovery of potential drugs, hence it has been of great interest for the field of medicinal chemistry.

One of the most widely distributed *N*-heterocycle in nature is purine. However, unsubstituted purine itself does not exists there.[3] The simplest form is the *β*-D-ribonucleoside nebularine (**I**) isolated from poisonous mushroom *Agaricus nebularis*. It has antibacterial effects and strong cytotoxicity due to its inhibition of adenosine deaminase.[4] The major impediments for the use of natural compounds as a drugs is their high toxicity, limited availability and structural complexity.[5] However, they can serve as an inspiration to design and development of biologically active compounds. Currently there are over 25 approved nucleoside and nucleotide analogues for treatment of AIDS, hepatitis and herpes viruses, with many more in clinical and preclinical trials.[6]

1.1 Deazapurines nucleosides

Pyrrolo[2,3-*d*]pyrimidines also known as 7-deazapurines have been explored by medicinal chemists due to their wide pharmacological profile.[7] Pyrrolo[2,3-*d*]pyrimidine scaffold resembles purine thus its nucleoside analogues can replace purine nucleosides in DNA and RNA.[8] Some naturally occurring 7-deazapurine nucleosides with biological activity have been reported. Tubercidin, toyocamycin and sangivamycin were obtained from Streptomyces culture.[9] All of them have antibacterial properties, moreover they show potent cytotoxicity against cancer cell lines.[10] Despite their structural similarities they do not share the same mechanism of action inside the cell (**Figure 1**). Inside the cell they are all phosphorylated by cellular kinases,[11] however, tubercidin (**II)** shows ability to impair many cellular processes such as mitochondrial respiration, methylation of tRNA and purine synthesis.[12] On the other hand, toyocamycin (**III)** acts as inhibitor of phosphatidylinositol kinase and X-box binding protein 1[13] and sangivamycin (**IV)** inhibits protein kinase C.[14] Despite the facts that they possess wide range of promising biological activities none of them proceeded to clinical use because of their high toxicity.[10]

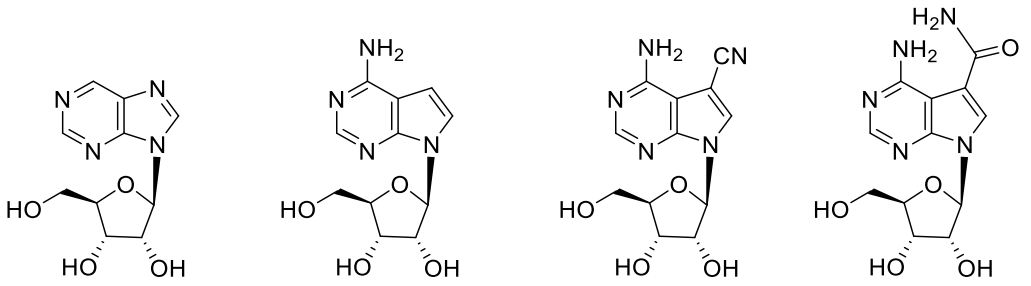
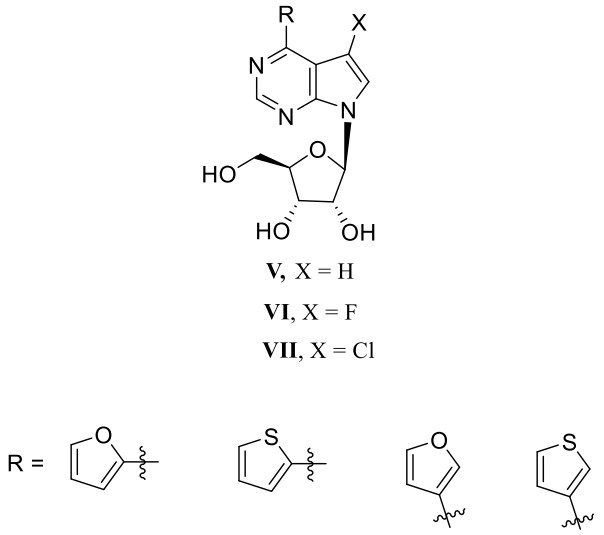


***Figure 1**: Natural purine and deazapurine nucleosides*

1.1.1 7-Deazapurine nucleosides developed in our group

Replacement of N7 nitrogen by carbon open many possibilities for introduction of various substituents and functional groups allowing synthesis of many potentially biologically active compounds. In our laboratory, we developed several active compounds by introducing heterocycle to C-6 and C-7 position of 7-deazapurine nucleosides. Recently we found that 6-hetaryl-7-deazapurines bearing small five-membered rings (i.e. furan or thiophene) (**Figure 2**) display potent nanomolar in vitro anti-proliferative activities against a panel of solid tumor and leukemia cell lines with potency similar to the clinically used nucleoside clofarabine.[15] Preliminary metabolism studies indicate that nucleosides are phosphorylated to their active form. The phosphorylation may be an obstruction of compounds activation thus cycloSal-phosphate was inserted to the compounds as masked phosphate. Unfortunately, the cytotoxic effects remain similar to the parent free nucleosides. Interestingly, significant inhibition of human ADK was observed in cycloSal-phosphate derivatives.[16]



V, X = H

VI, X = F

VII, X = Cl

***Figure 2**: 6-hetaryl-7-deazapurine ribonucleosides*

In order to expand the series of 7-deazapurine nucleosides, a series of C7 modified analogues was prepared. Modifications at C7 position also show nanomolar in vitro cytotoxicity to broad panel of cancer cells (**Figure 3**).[17] Interestingly phenyl derivatives were inactive. Addition of thiophene to C7 position led to **AB61**, a very promising anticancer agent.[18] Metabolism studies show that **AB61** is effectively phosphorylated in cancer cells but not in fibroblasts that explains a selectivity of this compound toward cancer cells.[18] The triphosphate form can incorporate as a ribonucleotide into DNA so RNA, where it blocks the translation and damages DNA. **AB61** is a substrate for mitochondrial DNA polymerase γ which means it can affect mitochondrial function as well as interfere with mtDNA replication.[18] However, not only modification with five-membered ring led to biologically active compounds, also bulky substituents like dibenzofuran are interesting. 7-Dibenzofurane-7-deazaadenosine **IX** shows antimycobacterial properties with a submicromolar Mtb-ADK-specific inhibition and was active against Mtb strains with a MIC of 4 μmol/L. This compound is a promising lead structure for further drug development.[19]

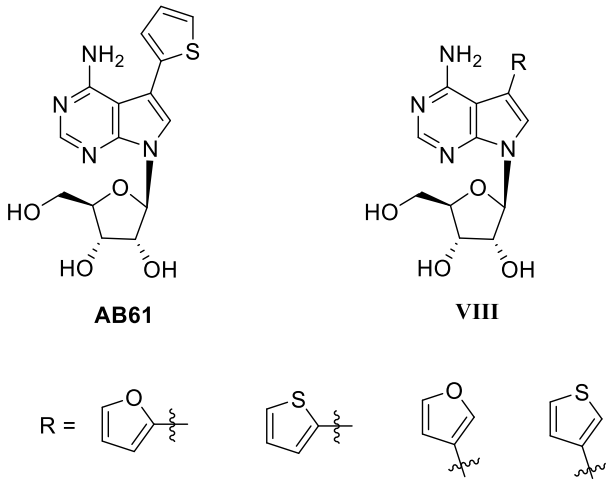


Figure 3: *7-hetaryl-7-deazapurine ribonucleosides*

We have discovered that not only substitution with heterocycles in 7-deazapurines analogues can provide biologically active compounds. Also 7-ethynyl-7-deazaadenosine **X**, structurally resembles toyocamycin, has nanomolar cytotoxic effect on cancer cell even greater than clofarabine or tubercidin[17]. However, substitution of 6-amino group in that compound leads to decreased cytotoxic activity.[17] Modification of a sugar moiety of 7-deazapurine nucleosides also affect its cytotoxic activities. Unfortunately, modifications in 2′-position of 6-hetaryl-7-deazapurine led to inactive or less active compounds.[7] Micromolar cytotoxicity was observed in 2′-fluorinated-7-hetaryl-7-deazapurine analogues.[20] Inactivity of those compounds show, that 2′-OH group in sugar moiety is crucial for their biological activity.

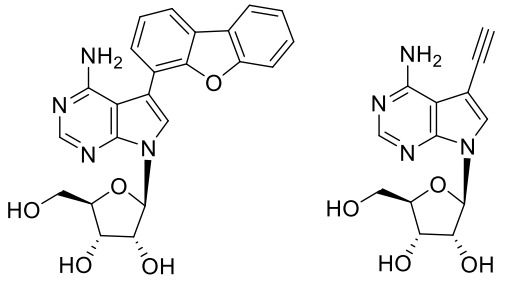


***Figure 4**: Biologically active 7-modified-7-deazaadenosines*

1.2 Fluorinated nucleosides

Introduction of fluorine atom(s) into drug-candidate compounds may profoundly alter their potent pharmacological properties. Fluorine atom is a good bioisostere of H or OH group. As the second smallest atom fluorine can perfectly mimic hydrogen without much distortion of molecular structure.[21] It can also serve as isosteric mimics of a hydroxyl group thanks to its high electronegativity and bond length. The C-F bond length (1.35 Å) is close to the C-O bond length (1.43 Å), moreover fluorine can also act as hydrogen-bond acceptor.[22] Due to the strong electronegativity of fluorine the carbon center of C-F bond is resistant to oxidation which decreases the formation of undesired metabolites during the metabolism.[23] C-F bond is one of the strongest in organic chemistry resulting in increasing of thermal and chemical compound stability.[22] Fluorine enhances the lipophilicity of the compound which influences the membrane permeability and therefore can be a huge advantage in the optimization of drug candidate's oral bioavailability.[21] Currently there are eight fluorinated nucleoside derivatives being used for treatment of cancers and viral infections.[24]

Fluorinated nucleosides can serve in treating cancer and viruses either as chain terminators preventing the replication of cancer cells or virus, or as selective inhibitors of important enzymes for cancer or viral replication.[24] Generally, nucleoside analogues can interact with and inhibit essential enzymes such as human and viral polymerases, kinases, ribonucleotide reductase, DNA methyltransferases, purine and pyrimidine nucleoside phosphorylase and thymidylate synthase.[25] Although they generally enter the cell through specific nucleoside transporters, certain analogues can enter through anion, cation or peptide's transporters as well.[6,26] After entering cell, the analogues are subsequently phosphorylated to triphosphates, which are their active forms. The phosphorylation is catalyzed by a nucleoside kinase and a nucleoside monophosphate kinase, and then a nucleoside diphosphate kinase.[26] The last step is phosphorylation by creatine kinase or 3- phosphoglycerate kinase.[26] They act either as certain enzyme's inhibitors or can be incorporated into newly synthesized DNA and RNA as described above. The incorporation can also accumulate mutations in viral progeny or induce an apoptosis. [25]

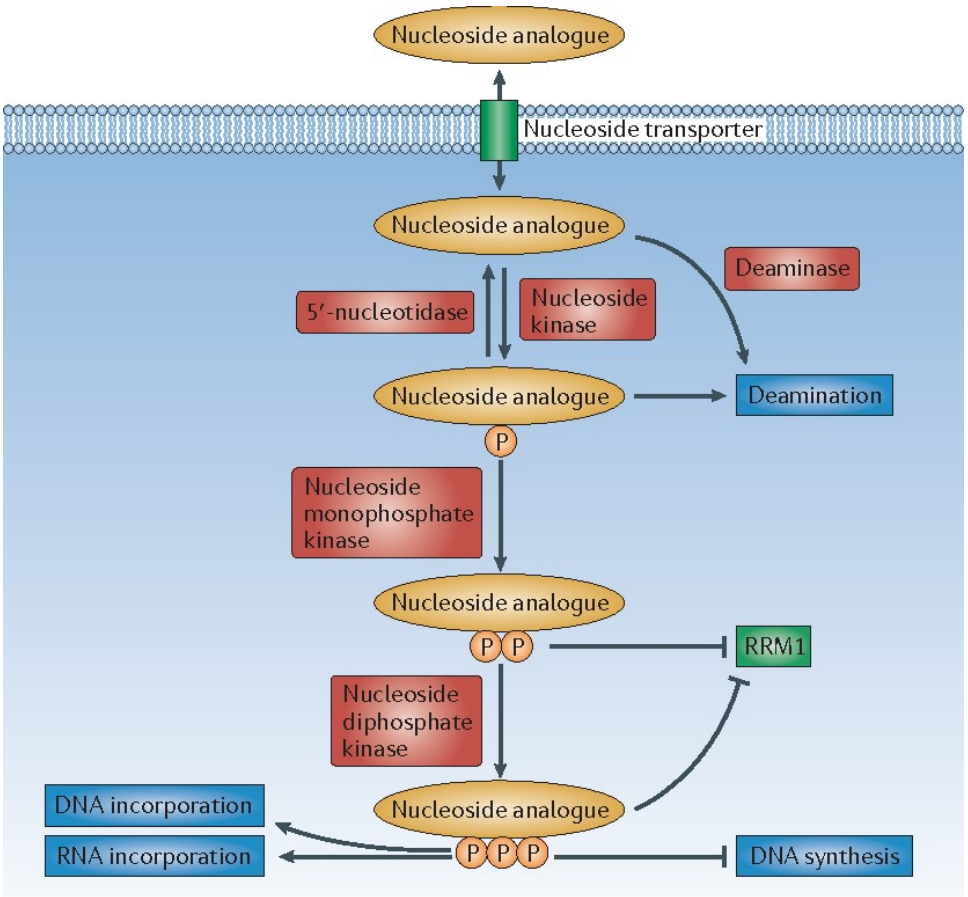
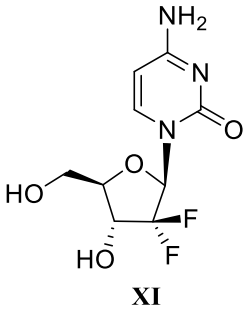


***Figure 5**: Nucleoside mechanism of action (picture taken from ref. 25)*

1.2.1 Fluorinated anticancer nucleosides

Cancer is a term for a collection of related diseases. In all types of cancer, certain body cells begin to divide without control and spread into surrounding tissues. The aim of cancer drugs is to suppress the cancer cell growth and division or induce apoptosis. The basis for the selectivity of anticancer agents is much less clear than antiviral ones because the enzymes involved in activation and activity of cancer cells are identical to those in normal cells.[25] A lot of pyrimidine- and purine-based fluorinated nucleosides have been used in cancer treatment.

Gemcitabine **XI** (2′,2′-difluoro-2′-deoxycytidine) is a very prominent and widely used anticancer drug approved for the treatment of pancreatic, breast, non-small-cell lung and ovarian cancers.[27] Gemcitabine enters the cell by nucleoside transporters hENT-1, and to a lesser extent by hCNT-1 and hCNT- 3.[28] Once the agent gets into cytoplasm, it is phosphorylated to active metabolites: gemcitabine-5′-triphosphate and gemcitabine-5′-diphosphate. The important triphosphate active form causes the mask chain termination of DNA synthesis by incorporating into it during S-phase which results in cell death.[27,28] Interestingly, the diphosphate form also contributes to anticancer activity of triphosphate. It decreases the amount of natural deoxynucleotide pool by inhibiting ribonucleotide reductase therefore the incorporation of triphosphate as a substrate for DNA polymerase is enhanced.[27] Gemcitabine is inactivated via deamination by cytidine deaminase and by deoxycytidylate deaminase in the monophosphate form.[27]



XI

Gemcitabine

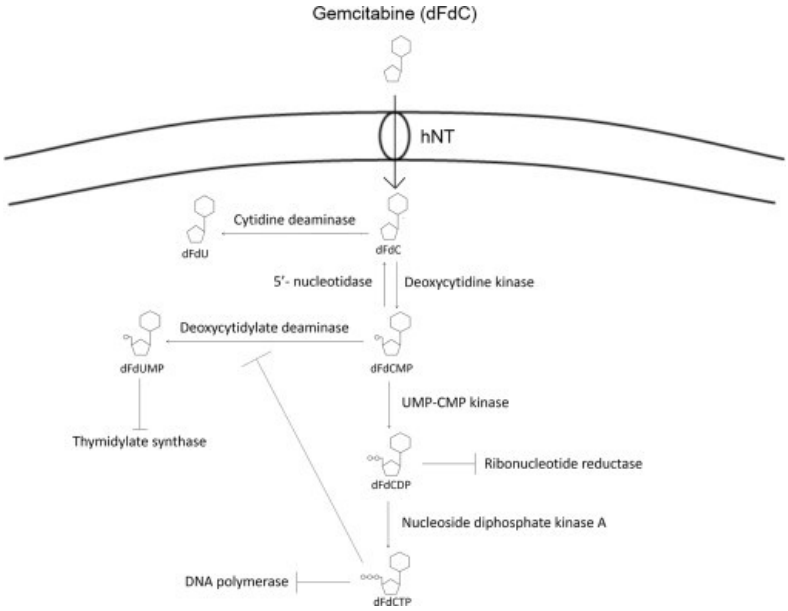


***Figure 6**: Gemcitabine's mechanism of action (picture taken from ref. 27)*

Other well-known anticancer drug is clofarabine **XII** (2-chloro-2′-fluoro-2′-deoxyarabinosyladenine) used in treatment of acute lymphocytic leukemia. Clofarabine's mechanism of action is similar to that of gemcitabine. The importation into the cell can be either done via nucleoside transporters (hENT1, hENT2 and hCNT2) or by passive diffusion through the lipid membranes in higher concentration and longer exposure.[29] 5′-Monophosphate form is generated by 2′-deoxycytidine kinase but in contrast to many other nucleoside prodrugs this is not a rate-limiting step.[29] The rate-limiting step is the generation of 5′-diphosphate by purine nucleotide monophosphate kinase. Clofarabine-5′-diphosphate serves as an intracellular reservoir for its active triphosphate form due to its longer retention in the cell. Active clofarabine-5′-triphosphate has several major anticancer activity mechanisms: inhibition of DNA synthesis and induction of apoptosis.[29,30] Like gemcitabineDP, clofarabine can inhibit ribonucleotide reductase. It also inhibits DNA polymerases α and ε, which are two important enzymes involving in the chromosomal DNA replication.[31] Apoptosome complex is responsible for apoptosis induction. ClofarabineTP can replace dATP affecting cytosolic apoptotic protease-activation factor 1 (APAF-1), therefore causing caspase activation.[30,32] ClofarabineTP can also affects mitochondria by altering transmembrane potential thus causes releasing cytochrome c and apoptosis-inducting factor (AIF). The formation of apoptosome complex is combined by actions of cytochrome c, AIF, APAF-1 and caspase-9. [30,32]

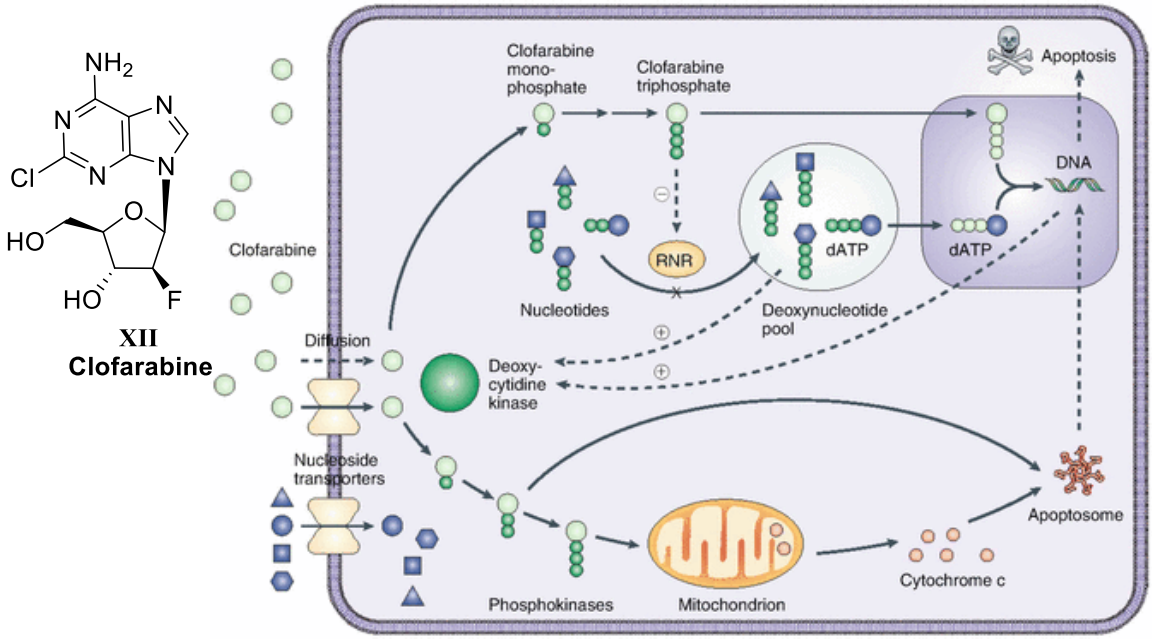


***Figure 7**: Clofarabine's mechanism of action (picture taken from ref. 25).*

1.2.2 Fluorinated antiviral nucleosides

Nucleosides are not only potent cytostatics, they also represent a very promising class of antiviral agents. Antiviral nucleosides are structurally more diverse. They are often modified at sugar moiety and they include even acyclic nucleosides. On top of that they also show a better tolerance profile due to a low level of activity on mammalian enzymes.[33] The most common targets of nucleosides in viral therapy are reverse transcriptase inhibitors (NRTIs).[24] Many purine- and pyrimidine-based fluorinated nucleosides have shown significant antiviral activity.

Sofosbuvir (**XIII**), the pyrimidine-based fluorinated nucleotide prodrug of *β*-D-2′-deoxy-2′-*α*-fluoro-2′-*β*-C-methyluridine, is a drug for hepatitis C treatment sold under the brand name Sovaldi. Sofosbuvir acts as an inhibitor of NS5B. NS5B, an RNA-dependent RNA polymerase having essential function during viral RNA replication.[34] After absorption, sofosbuvir undergoes first-pass metabolism and it is metabolized directly at the desired site of action – the liver to its active nucleoside triphosphate form.[34] The metabolic pathway for activation of prodrug starts with hydrolysis of the carboxyl ester catalyzed by cathepsin A (CatA) and carboxylesterase 1 (CES1).[33,34] The next step is non-enzymatic nucleophilic attack to form phosphoamide intermediate. Then the deamination catalyzed by histidine triad nucleotide-binding protein 1 (Hint1) proceeds to yield an inactive 5′-monophosphate form which is further phosphorylated to corresponding 5′-diphospate by UMP-CMP kinase.[33,34] The active 5′-triphospate form is achieved by nucleoside diphosphate kinase. Sofosbuvir triphosphate mimics the natural uridine and NS5B incorporates it into elongating RNA resulting in chain termination.[35] The catalytic site of NS5B is relatively well conserved across all the HCV genotypes, yielding pan-genotypic efficacy of sofosbuvir.

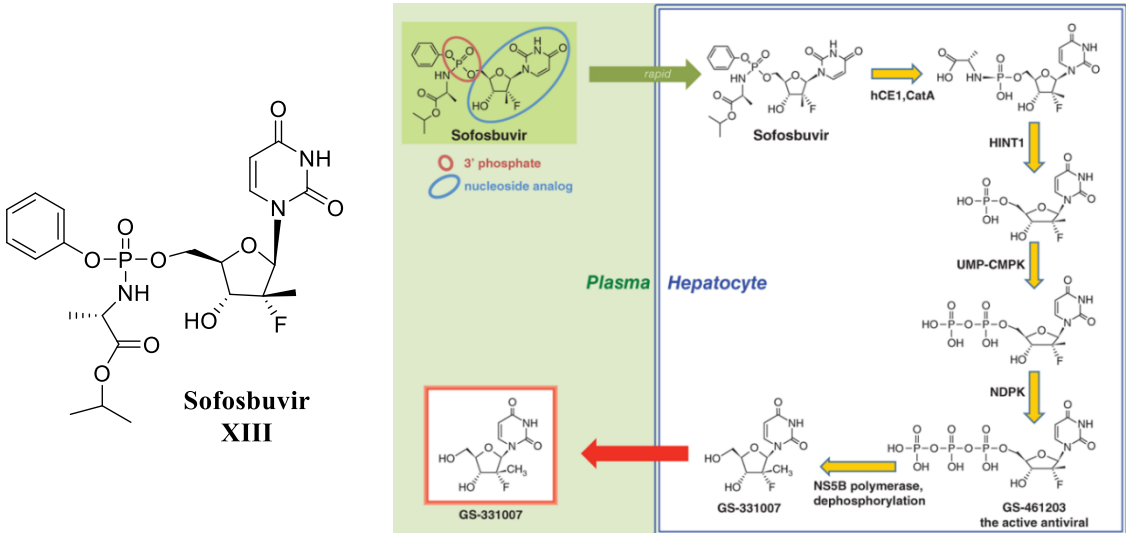


***Figure** 8: Sofosbuvir's mechanism of action (picture taken from ref. 34).*

Clevudine (**XIV**), a prodrug of *β*-L-2′-fluoro-thymidine, was a very promising antiviral agent for the treatment of HBV.[36] Like many other nucleoside drugs, for manifesting Clevudine's pharmacological activities, biotransformation to its triphosphate form is needed. In the cell it is monophosphorylated by thymidine and deoxycytidine kinase, followed by phosphorylation by thymidine kinase and 3-phosphoglycerate to trisphosphate.[33] In 2006 Clevudine was approved in South Korea but recently the Phase III clinical trial was stopped due to a drug-induced myopathy during long-term treatment.[37] Later it was revoked from South Korean market.[24]

There are several examples of purine-base fluorinated nucleosides which show an effective in vitro antiviral activity. Among them it is possible to highlight PSI-353661 (**XV**) and PSI-352938 (**XVI**)[38] which are both prodrugs of *β*-D-2′-deoxy-2′-*α*-fluoro2′-*β*-C-methylguanosine-5′-monophosphate and has great anti HCV activity[39]. Another 2′- fluoro-purine based phosphonamidate GS9131 (**XVII**) is capable of inhibiting HIV-1 RT.[40] All mentioned compounds are currently under clinical evaluation.[38–40]

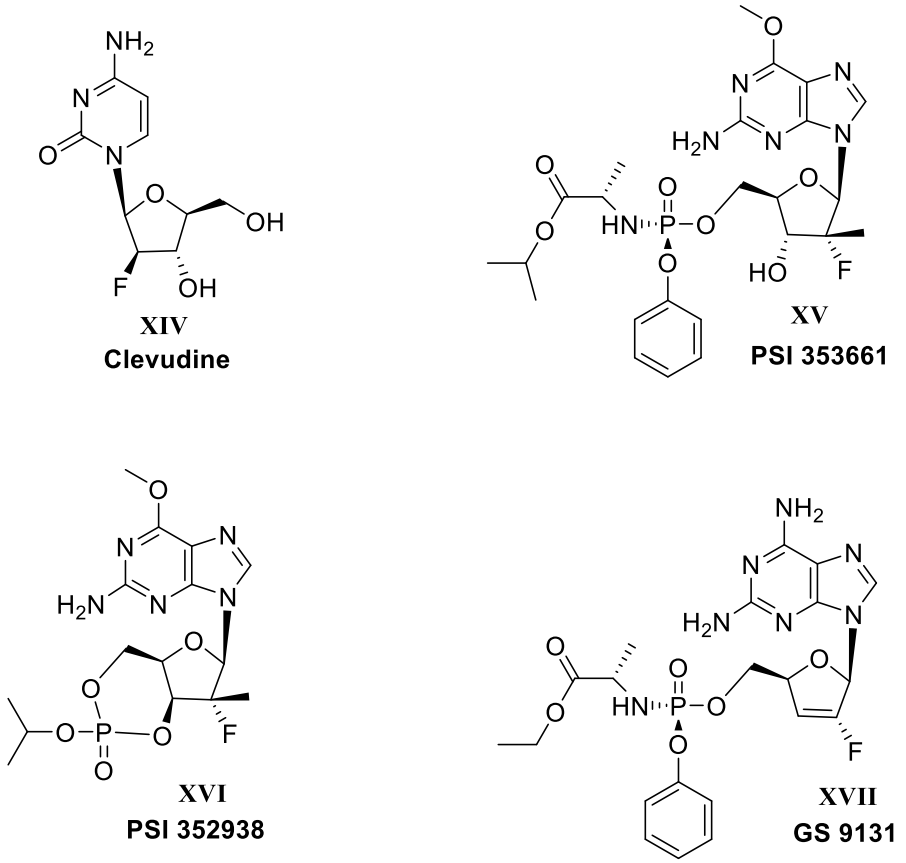
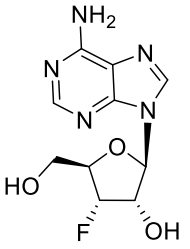


***Figure 9**: Fluorinated antiviral nucleoside.*

1.2.3 3′-Deoxy-3′-fluoroadenosine

3′-Deoxy-3′-fluoroadenosine (3′F3′dAdo) (**XVIII**) has been established as effective inhibitor against broad-spectrum of viruses and proliferation of different cancer cell lines. Its potent efficacy in certain cases are even greater than that achieved by some well-known reported compounds. 3′F3′dAdo shows better activity against Colorado tick fever virus than ribavirin and 3-deazaguanine. In case of banzi, San Angelo and Venezuelan equine encephalitis viruses its activity is superior to ribavirin and tiazofurin.[41] Among five 3′- fluorinated ribonucleosides 3′F3′dAdo displays the best antiviral activity against DNA viruses [pox (vaccinia)], dsRNA viruses (reo), (+)ssRNA viruses [picorna (polio, Coxsackie B)], toga (Sindbis, Semliki Forest).[42] Against Semliki Forest viruses 3′F3′dAdo shows comparable potencies to ribavirin 5′-sulfate and EICAR analog, however those two mentioned compounds are more cytotoxic.[41] 3′F3′dAdo's mechanism of antiviral action remain unsolved.[43] Plagermann et al.[44] claim that 3′F3′dAdo uses the nucleoside transport for entering the cell due to observation that in the presence of nitrobenzylthioinosine, a nucleoside transport inhibitor, the antiviral activity of 3′F3′dAdo decreases. Poor ability of 3′F3′dAdo to block the uptake of adenosine and 2′-deoxyadenosine into cells arouses hypothesis that 3′F3′dAdo enters cells by non-facilitated diffusion like 3′- azido-3′- deoxythymidine.[41] Cytostatic activities of 3′- deoxy- 3′- fluoroadenosine were reported by Mikhailopulo et al.[45] It shows that 3′F3′dAdo compared to other 3′-fluorinated ribonucleosides has much better cytostatic properties against tested tumor cell lines.



XVIII

3′-Deoxy-3′-fluoroadenosine

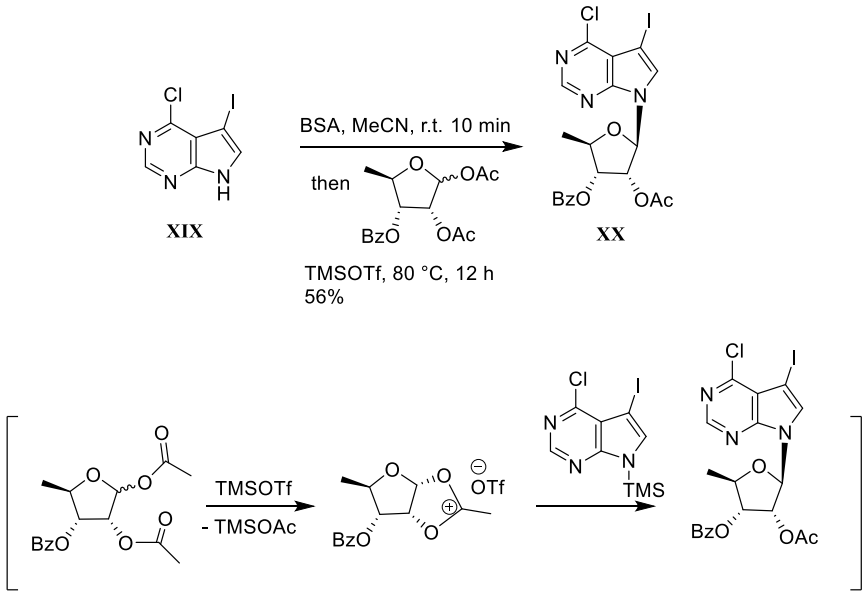
***Figure 10**: 3′-Deoxy-3′-fluoroadenosin 3′F3′dAdo.*

1.3 Synthetic approaches towards nucleosides

The most important step in synthesis of nucleosides is formation of the glycosidic bond. Usually only *β*-anomers are biologically active thus the formation has to be stereoselective. The most used methods for synthesis of glycosidic bond in organic chemistry is glycosylation. There are many types of glycosylation reactions. Every type has its own advantages and disadvantages.

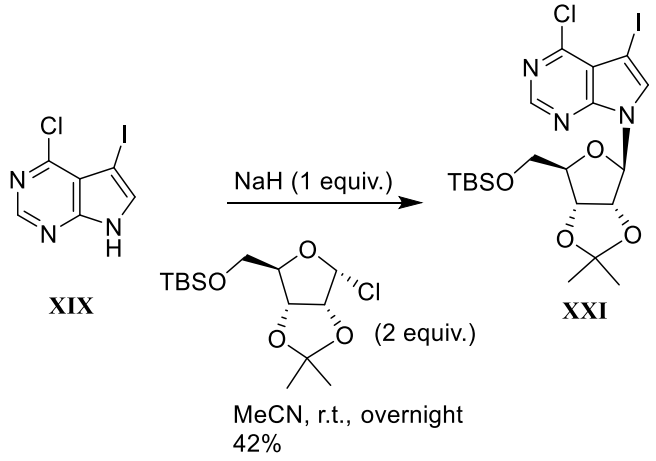
1.3.1 Glycosylation

Glycosylation is a coupling of nucleobase and sugar derivative to form the *N*-glycosidic bond. One of commonly used reactions in the synthesis of nucleosides is the Vorbrüggen glycosylation also called sillyl-Hilbert-Johnson reaction.[46] The reaction is usually carried out in MeCN and TMSOTf as a Lewis acid. It is necessarily to emphasize that participation of neighboring group is very important in this reaction. It was reported that the coupling can be performed with unprotected as well as 1-*O*-methyl-protected sugar.[47] However, with acetyl or benzoyl protected sugar the reaction yields exclusively *β*-nucleoside. The Vorbrüggen procedure was used in the synthesis of 6-amino-9-(5′-deoxy-*β*-D-xylofuranosyl)-7-iodo-7-deazapurine (**XX**). In this procedure the nucleobase is silylated by BSA in MeCN. Afterwards the nucleobase reacts with oxonium intermediate from sugar formed in the presence of TMSOTf. Nucleophilic attack of the nucleobase comes from the opposite site of the 2- *O*- protected group of the sugar in compliance with Baker's rule (**Scheme 1**).[48]



***Scheme 1**: Glycosylation based on Vorbrüggen conditions.*[48]

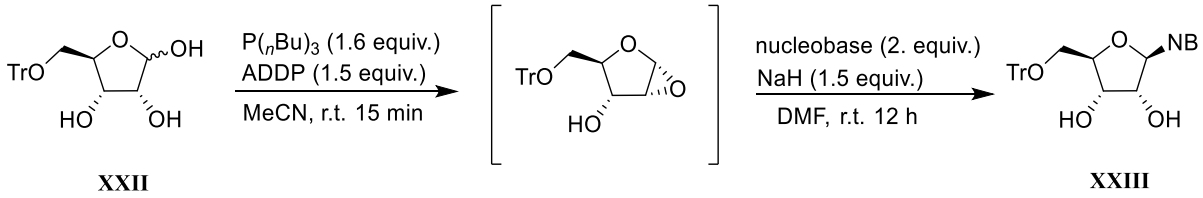
Another means to synthesize a nucleoside is through a metal salt glycosylation. This method is mainly used for synthesis of 2′-deoxynucleosides where there is no option to create an oxonium intermediate and control the stereoselectivity of the product. The disadvantages of this method are stability and accessibility of the glycosyl halide. Moreover, strong basic condition can be limiting for sensitive substituents. Synthesis of 5-iodoturbercidin analogues using this method was done by Ugarkar and coworkers.[49] The sodium salt of 7-deazapurine was generated by NaH in anhydrous MeCN. The salt then reacts with α-chloro sugar to give a desired nucleoside via S_N2 displacement (**Scheme 2**).



***Scheme 2**: Metal salt glycosylation.*[49]

Recently, Downey A. M. et al reported a new method of nucleoside synthesis through direct glycosylation with 5-*O*-monoprotected or 5-modified sugar. The advantage of this

method is no protection of anomeric hydroxyl group needed and like Vorbrüggen this method also gives exclusively β-anomer. The reaction is carried out under modified Mitsunobu conditions, where sugar reacts with ADDP and PBu_3 to form an epoxide. Thereafter the nucleophilic epoxide opening with sodium salt of nucleobase takes place to give corresponding nucleoside (**Scheme 3**).[50,51]



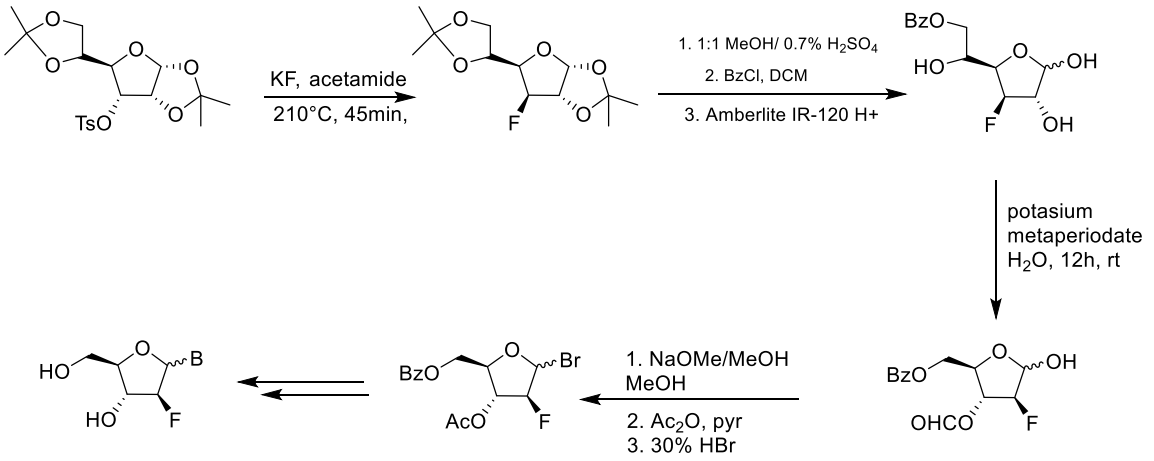
XXII **XXIII**

***Scheme 3**: Direct glycosylation from 5-O-protected sugar.*[50]

1.4 Synthesis of fluorinated nucleosides

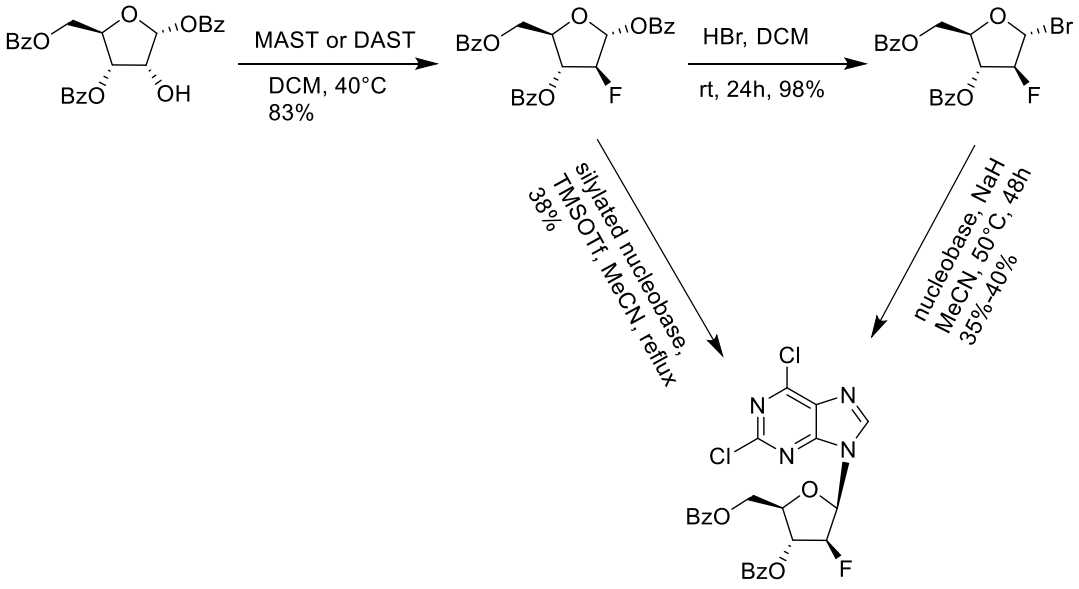
There are two alternatives toward the fluorinated nucleosides. Firstly, direct fluorination of the sugar moiety of *β*-nucleoside. In this case the stereoselective formation of *N*-glycosidic bond does not have to be taken into consideration. The separation of anomers is often difficult. However, the main disadvantage of this method is to choose a selective protection and deprotection for functional groups and suitable conditions for the reaction. The second alternative is condensation of fluorinated sugar with required nucleobase. This thesis will focus on the second approach because there are several glycosylation methods which exclusively yield the *β*-nucleoside. Furthermore, this method allows to couple the fluorinated sugar with various nucleobases.

As was mention earlier fluorinated nucleosides showed promising therapeutic potentials. There are over 300 structures containing a fluorine atom(s) at sugar moiety of nucleosides. However, 77% of fluorinated nucleosides contain fluorine atom(s) at C´-2 of the sugar.[52] Modification of the sugar moiety for condensation with the nucleobases are very tricky and interesting at the same time. A very interesting synthesis of fluorinated nucleosides through the glycosylation of nucleobase with fluoro-sugar derivatives was reported by Watanabe and co-workers[53] (**Scheme 4**). They synthesize the 2-deoxy-2-fluoro-D-arabinose from 3-deoxy-3-fluoro-D-glucose. The key step of this procedure is the oxidation of the fluorinated glucose with sodium metaperiodate to afford exclusively 2-fluoroarabinofuranose.



***Scheme 4**:Synthesis of 2´-deoxy-2´-fluoro-D-arabinofuranose nucleosides.*

Other approach towards protected 2-fluoroarabinonucleoside was prepared by Elzagheid, M. I et al.[54] The synthesis includes fluorination of 1,3,5-tri-*O*-benzoyl-*α*-D-ribofuranose followed by conversion into bromide derivatives and glycosylation with desired nucleobase. This procedure has higher yield than the one mention above however it starts with protected *α*-D-ribofuranose.



***Scheme 5**: Synthesis of 2´-fluoroarabinose nucleoside.*

1.4.1 Synthesis of fluorinated sugars

There are two classes of fluorinating agents:

i) Nucleophilic reagents with a fluoride ion as a donor (e.g. DAST, Deoxo-Fluor);
ii) Electrophilic source of fluorine which usually features N–F bond (e.g. Selectfluor).

The nucleophilic fluorination source has also two classes: deoxofluorinating agents and the metal/pyridinium/tetraalkyl ammonium fluorides. The metal/pyridinium/tetraalkyl ammonium fluorides are relatively cheap. However, metal fluorides have many liabilities such as toxicity, high volatility, poor solubility in organic solvents and fluorination usually has to be carried out at high temperature. Deoxofluorinating reagents are used for nucleophilic fluorination which usually in one step converts alcohols/ketones into fluoroalkanes.[55] The electrophilic sources of fluorine are represented by a group of inorganic fluorides with elements of high electronegativity. Among these are $FClO_3$ and various fluorides containing N–F, O–F bonds (e.g *N*-fluorosulfoamides and *N*-fluorosulfoimides) [56].

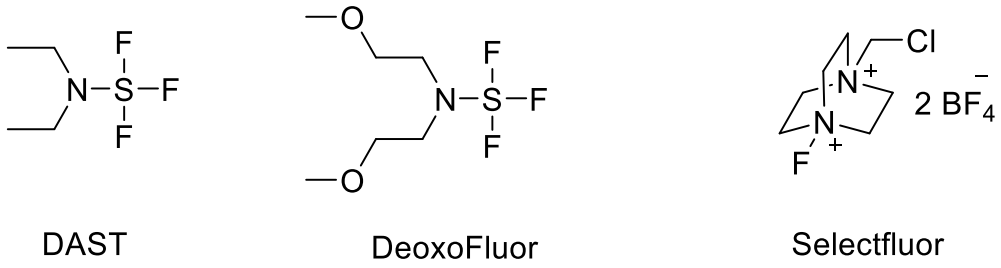
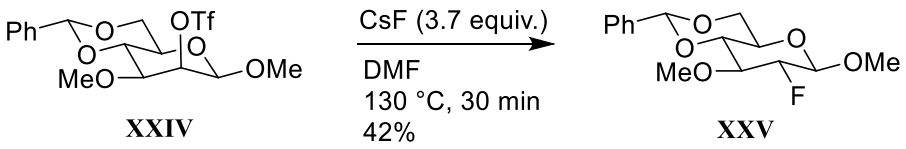


***Figure 11**: Examples of fluorinating agents*

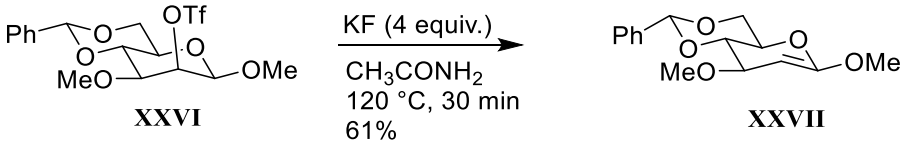
One way to synthesize fluorinated sugars is via substitution reactions. This way highly depends on the source of fluoride ion and the nature of leaving group. The other important thing in carbohydrate chemistry is neighboring group effects. Participation of neighboring group profoundly affects the course of the reaction. Also 1,3-diaxial or 1,2-steric effects can modify the course of reaction.[57] Various protocols have been employed and optimized through the years. One of them is the synthesis of 2-deoxy-2-fluoropyranose **XXV** using CsF in DMF. The displacement requires a good leaving group in C2 position of a protected mannose. Triflate was chosen and the displacement proceeded easily (**Scheme 6**).[58]



XXIV **XXV**

***Scheme 6**: Fluorination via displacement reaction using CsF* [55]

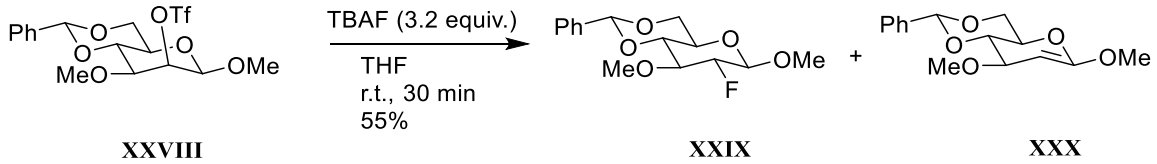
The results from previously mentioned study were proved by Haradahira *et al.* However, it was found that with some fluorinating agents only elimination product **XXVII** was observed (**Scheme 7**).[59]



XXVI **XXVII**

***Scheme 7**: Fluorination via displacement using KF.* [56]

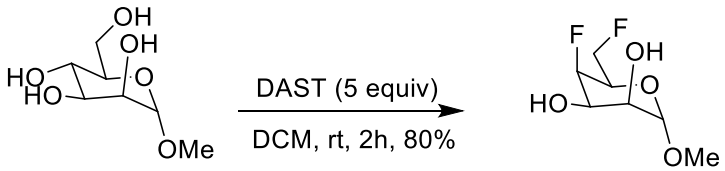
On the contrary TBAF can greatly improve the yield of the reaction at much lower temperature. The elimination product was only obtained as a side-product (**Scheme 8**).[59]



XXVIII **XXIX** **XXX**

Scheme 8: Fluorination via displacement using TBAF.[56]

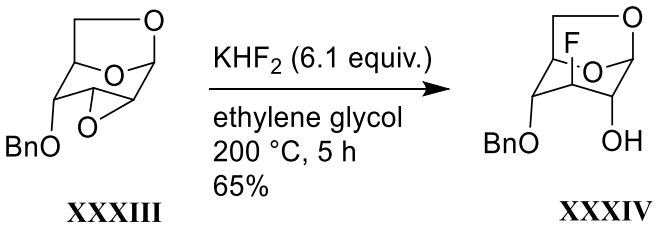
The most widely used fluorinating agents are animosulfur fluoride analogues. Among them very famous are DAST and deoxofluor. Synthesis of methyl 4,6-dideoxy-4,6-difluoro-α-D-talopyranoside reported by Peter J. Card is an example. The fluorination of hydroxyl group proceeds in one step with inversion of configuration (**Scheme 9**).[60]



XXXI **XXXII**

***Scheme 9**: Fluorination using DAST.*[60]

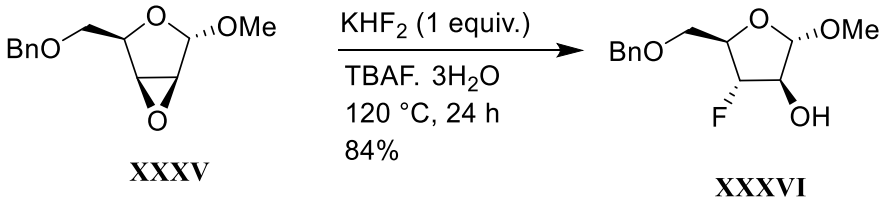
Other approach toward fluorinated sugars is epoxide-opening reaction. Using a fluoride to open epoxides has been a successful method for the replacement of a secondary hydroxyl group with fluorine.[57] The tendency of transdiaxially epoxide opening is useful in predicting the product stereochemistry. Most common fluorinating reagent for epoxide opening are hydrogen fluoride and potassium bifluoride. Denavit V. *et al*. reported preparation of 3-deoxy-3-fluoro-glucopyranose by epoxide opening with potassium bifluoride (**Scheme 10**).[61]



XXXIII **XXXIV**

***Scheme 10**: Fluorination via epoxide opening reaction.*[58]

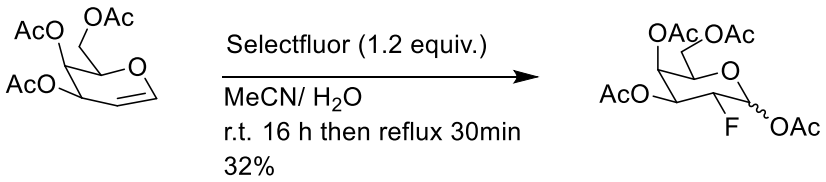
Different way to open an epoxide is using TBAF/KHF_2 reported by Yan, N. and coworkers (**Scheme 11**).[62]



XXXV **XXXVI**

***Scheme 11**: Fluorination via epoxide opening using KHF_2.*[59]

Another way to fluorinate a sugar is by addition to unsaturated sugar center. Noticeable example is synthesis of 2-deoxy-2-fluoro-galactopyranoside from tri-*O*-acetyl-D-galactal using Selectfluor (**Scheme 12**).[63]

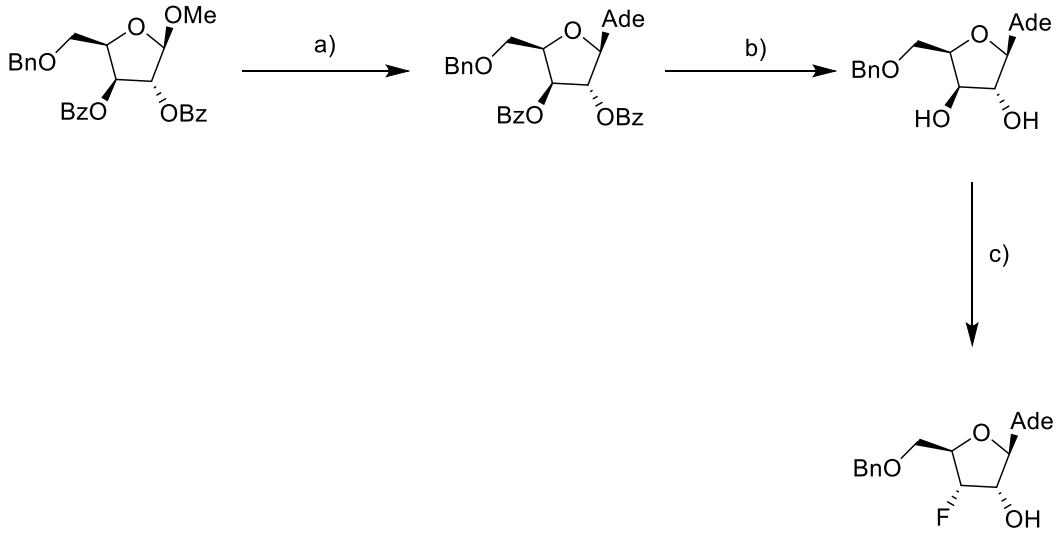


XXXVII **XXXVIII**

***Scheme 12**: Fluorination by addition to double bond.*[60]

1.5 Synthesis of 3′-deoxy-3′-fluororibonucleosides

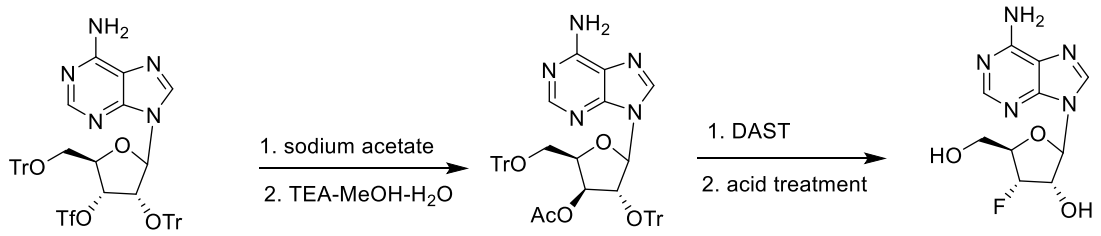
As was mentioned earlier there are two possibilities to synthesize the 3′-fluororibonucleoside. The direct fluorination on nucleoside was performed with protected nucleoside to yield 3-fluoroadenosine (**Scheme 13**).[64]



a) persilylated N^6-benzoyladenine, $SnCl_4$, MeCN, rt, 30min, 63%
b) NH_3/MeOH, rt, 24h, 70%
c) DAST, DCM/pyr, rt, 5h, 48%

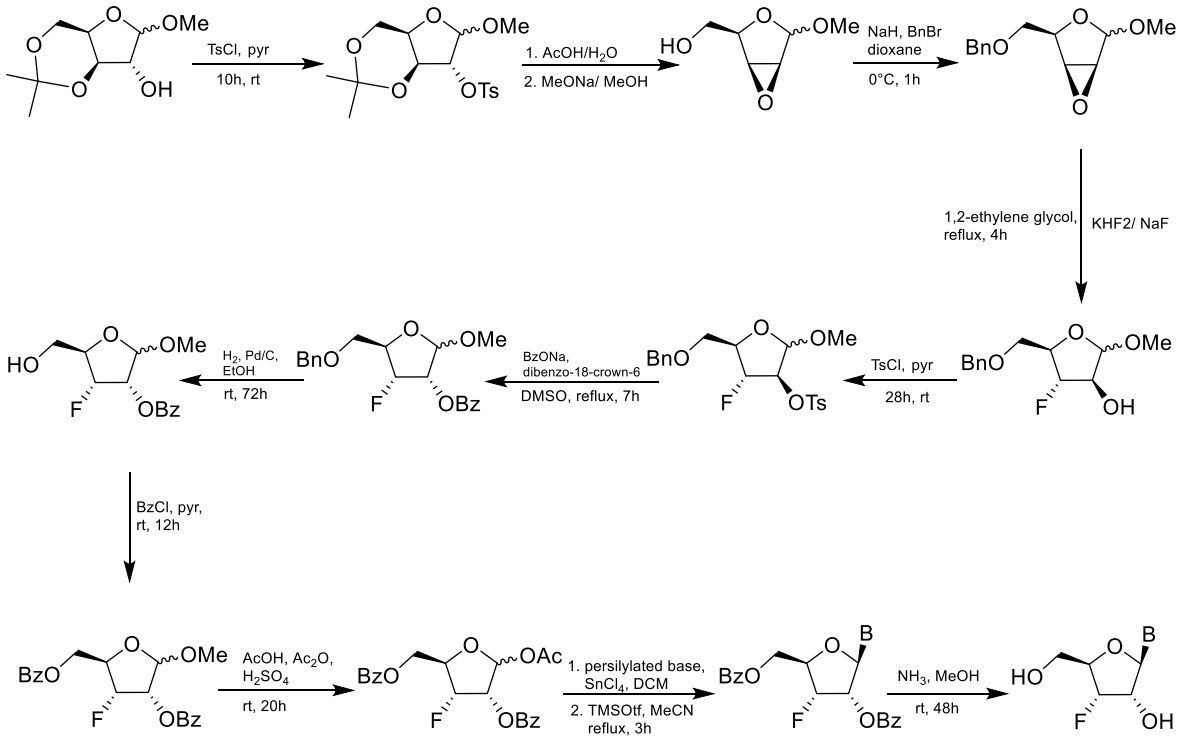
Scheme 13: *Synthesis of 3′-deoxy-3′-fluoroadenosine via fluorination of nucleoside.*

Hansske et all[65] reported the direct fluorination on the nucleosides (**Scheme 14**). The procedure involves the treatment of nucleoside triflate with sodium acetate. The key step of the approach is selective deprotection of 3′-acetyl using triethylamine-methanol-water solution. The next step is fluorination using DAST followed by deprotection of trityl protecting groups to yield the fluorinated nucleoside.



Scheme 14*:Synthesis of 3′-deoxy-3′-fluoroadenosine via direct fluorination of adenosine.*

An example of the synthetic pathway toward 3′-deoxy-3′-fluoronucleoside via glycosylation with modified sugar moiety was done by Mikhaipulo at al.[43] The synthesis starts with methyl 3,5-*O*-isopropylidine-D-xylofuranose followed by conversion of the starting material into corresponding epoxide and opening of epoxide using KHF_2 or NaF. Obtained fluorinated sugar was later glycosylated with various nucleobase to afford the 3′-fluororibonucleoside (**Scheme 15**). However, this approach is laborious, it requires 12 steps to get the fluorinated nucleoside, some of them with low yield. In this procedure they chose to fluorinate the sugar via epoxide opening which raises a major disadvantage of this method. First, to synthesize the epoxide, the 2-OH group has to be converted into a good leaving group, in this case it is tosylation. Second, epoxide opening always yields a transdiaxial product. In this case the fluorinated xylose then has to be converted into ribose using nucleophilic substitution with sodium benzoate in DMSO by reflux. Not to mention that 2-OH group has to be again transformed into a good leaving group. Moreover, refluxing in DMSO requires a high temperature which might lead to degradation of the sugar. The last disadvantage is the most crucial. We can see that they firstly protected a primary 5-OH group with benzyl ether then deprotected it and protected it again with benzoyl ester after fluorination. This is for avoiding the nucleophilic attack of the fluorinating agent to the carbonyl group.



***Scheme 15**: Synthetic approach towards 3′-deoxy-3′-fluororibonucleoside.[43]*

2 Specific aims of the thesis

1. Development and optimization of ribose fluorination in position 3.
2. Optimization of glycosylation of 6-chloro-7-iodo-7-deazapurine with synthesized 3-deoxy-3-fluororibose
3. Synthesis of 6-amino-7-iodo-7-deazapurine 3′-deoxy-3′fluororibonucleoside as a key-intermediate for synthesis of 6-amino-7-hetaryl-7-deazapurine-3′-fluororibonucleosides for biological activity study.
4. Synthesis of small series of 6-amino-7-hetaryl-7-deazapurine-3′-fluororibonucleoside.

2.1 Rationale of the specific aims

Two classes of 7-deazapurine nucleosides with strong biological activities were discovered during the long-term project of modified nucleobase and nucleoside analogues in Hocek research group. One of the most potent compounds **AB61** (7-(2-thienyl)-7-deazaadenosine) shows nanomolar cytostatic activities. In order to improve its biological activities, various modifications on C2 of sugar moiety were prepared. Unfortunately, most of them were inactive or less active than parent compound AB61 which indicates the importance of hydroxyl group at C2 position.

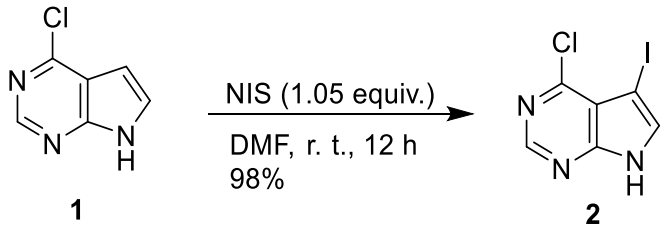
Other class are 7-deazaadenosines bearing bulky hetaryl group in position 7. Among them the 7-dibenzofuran-7-deazaadenosine displays antimycobacterial properties with a submicromolar Mtb-ADK-specific inhibition which can be a promising lead structure for further drug development.

My task is to prepare the 3-fluoro-analog of compound **AB61** and other related 7- hetaryl-7-deazaadenosines to investigate the effect of 3′-fluoromodification on their biological activities.

3 Results and discussion

3.1 Synthesis of 6-chloro-7-iodo-7-deazapurine

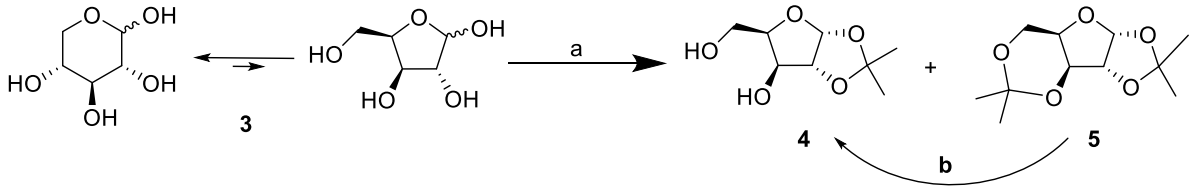
The first starting material needed for glycosylation was 6-chloro-7-iodo-7-deazapurine (**2**) which was synthesized from commercially available 6-chloro-7-deazapurine (**1**) using well established procedure.[66] Iodination was carried out in the presence of NIS in DMF to yield the iodinated nucleobase in 98% **(Scheme 16)**.



***Scheme 16**: Synthesis of 6-chloro-7-iodo-7-deazapurine.*

3.2 Synthesis of 3-deoxy-3-fluororibofuranose

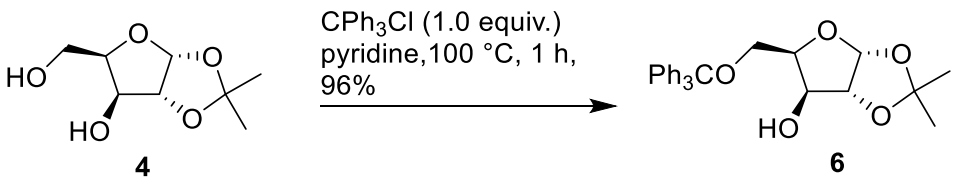
The synthetic pathway to 3-deoxy-3- fluororibofuranose was started from D-xylose **(3)** because the fluorination is assumed to proceed as S_N2 with conversion of configuration. Choosing the right protecting group is very important in sugar chemistry. The major isomer of pentoses in solution is the pyranose form. Isopropylidine protecting group is well known for forcing the furanose conformation of pentoses besides it protects both hydroxyl groups at C1 and C2 therefore 1,2-*O*-isopropylidine-α-D-xylofuranose (**4)** was a convenient starting material for our synthesis. Anhydrous $CuSO_4$ and H_2SO_4 was used for the conversion of D-xylose into **4** yielding a mixture of **4** and 1,2:3,5-di-*O*-isopropylidine-α-D-xylofuranose (**5**) (**Scheme 17**). The mixture was then partially hydrolyzed with 0.1 M HCl in MeOH to afford a pure product **4** with 97% yield.



Conditions: **a**: $CuSO_4$ (1.88 equiv.), H_2SO_4, acetone, 40 h, r. t.; **b**: 0.1 M HCl, MeOH, 3 h, 40 °C, 97%

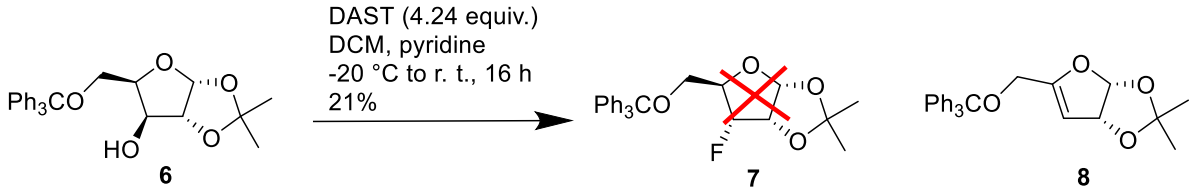
***Scheme 17**: Synthesis of 1,2-O-isopropylidine-α-D-xylofuranose.*

With starting material **4** in hands, the second step was protection of primary OH group at C5 position. The trityl group was chosen as logical option to protect mentioned OH group. Due to trityl's bulkiness it is known that it reacts mainly with primary OH. Moreover, both trityl and isopropylidine are unstable in acidic conditions and after fluorination both can be cleaved in one step and the obtained product can be used to synthesize nucleoside using Downey's conditions.[51] The tritylation was performed with **4** and trityl chloride in dry pyridine to give a product **6** in 96% yield **(Scheme 18)**.



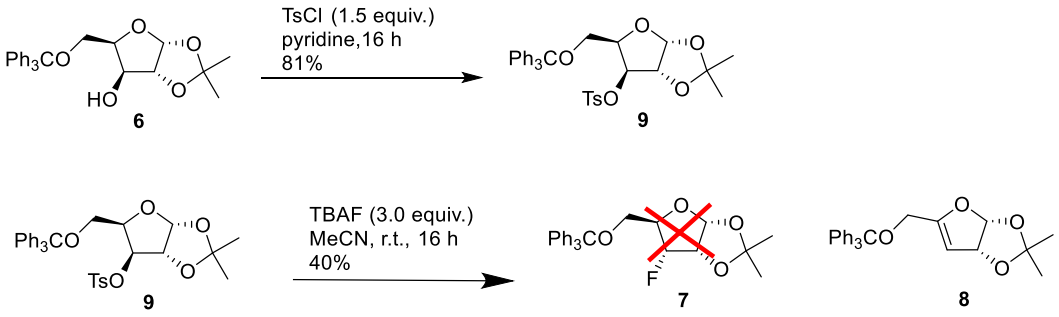
***Scheme 18**: The protection with trityl group.*

TBAF and DAST, two typical fluorinating agents, were tried for fluorination. The first fluorination of compound **6** was attempted with DAST in dry DCM and pyridine because DAST can fluorinate OH group in one step. Unfortunately, no fluorinated sugar was observed (**Scheme 19)**. In the case of TBAF a free hydroxyl group had to be converted into a good leaving group. Tosyl was chosen as a leaving group. The tosylated intermediate **9** was prepared in 81% yield **(Scheme 20)**.



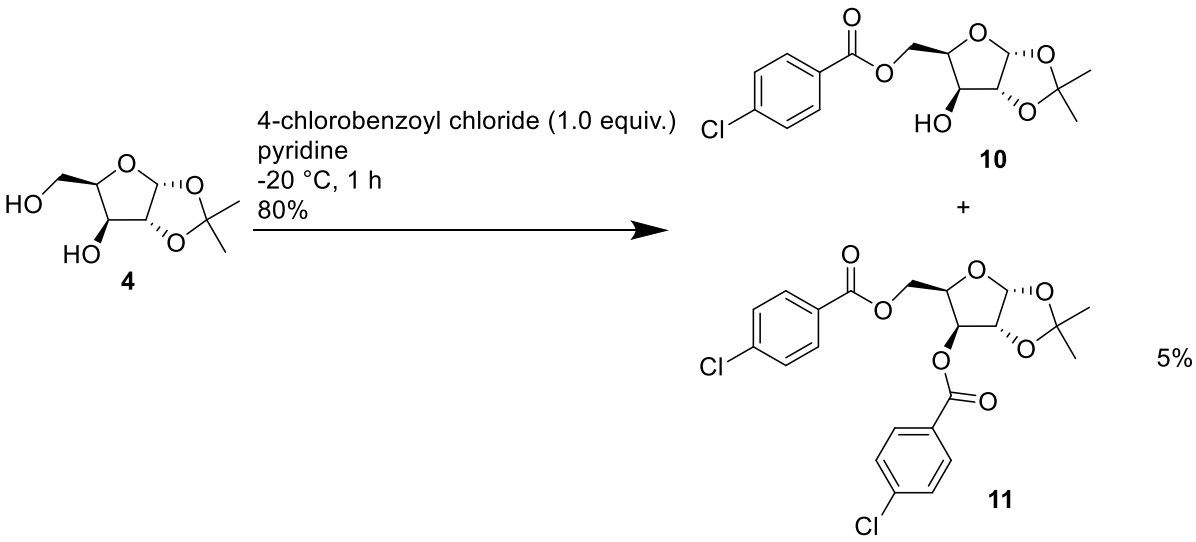
***Scheme 19**: Tosylation of protected sugar.*

As for compound **9** reaction with TBAF was carried out in dry MeCN (**Scheme 20**). Same as DAST, no fluorinated sugar was obsereved. In both cases, only an elimination product **8** was obtained.



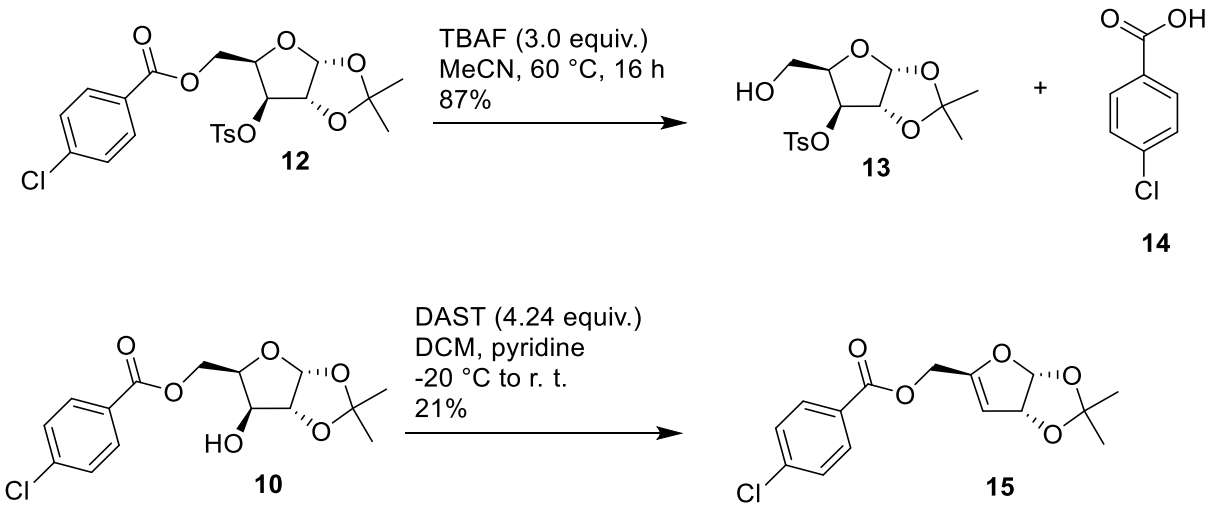
***Scheme 20**: Fluorination with TBAF and DAST.*

My first hypothesis was that trityl group is too bulky which caused a steric hindrance, so elimination proceeded instead of nucleophilic substitution, so we decided to use less bulky protecting group. Commonly used less bulky are benzoyl and benzyl protecting groups. The benzoylation of **4** was performed in dry pyridine to yield 80% of desired product **10** and 5% of dibenzoylated side product **11** (**Scheme 21)**.



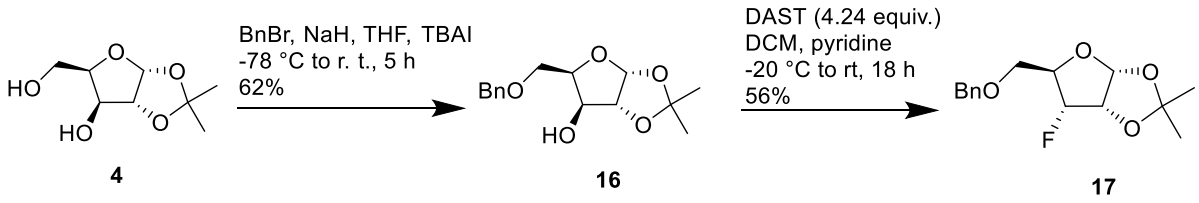
Scheme 21: *Benzoylation of compound* **4.**

The compound **10, 12** were reacted with DAST and TBAF in the same manners as described above. In the case of TBAF the cleavage of *p*-chlorobenzoyl **14** occurred (**Scheme 22)**. TBAF is quite basic so the cleavage is understandable but even with 3 equiv. of TBAF no desired product was acquired. Unfortunately, reaction with DAST proceeded in the same manner as with the tritylated compound and gave only an elimination product **15** (**Scheme 22**).



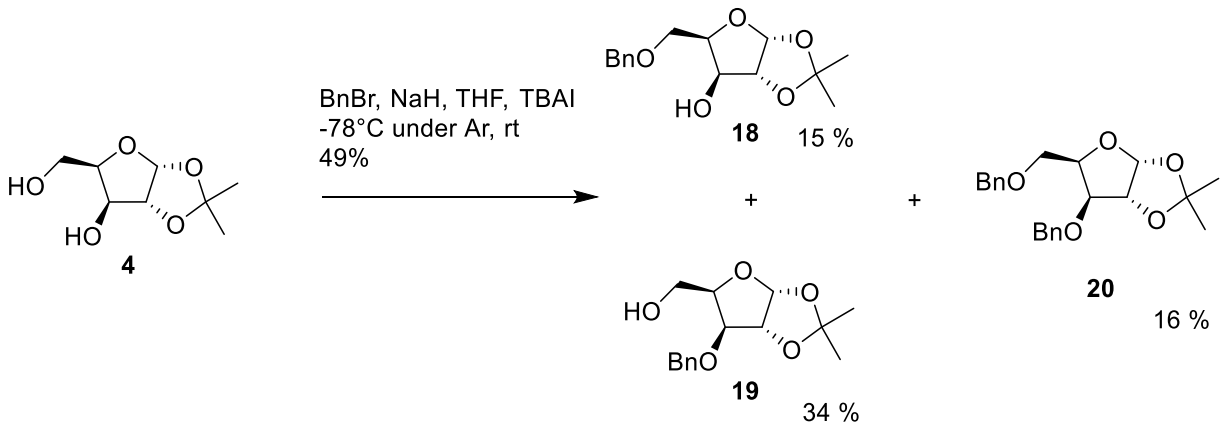
Scheme 22*: Fluorination of compound* ***12*** *with TBAF.*

Another option is to protect the primary hydroxyl group as benzyl ether. De Swarup et al reported the reaction of 5-*O*-(benzyl)-1,2-*O*-isopropylidene-D-xylofuranose (**16**) with DAST to yield corresponding 5-*O*-(benzyl)-1,2-*O*-isopropylidene-3-fluoro-α-D-ribofuranose (**17**) (**Scheme 23**).[67]



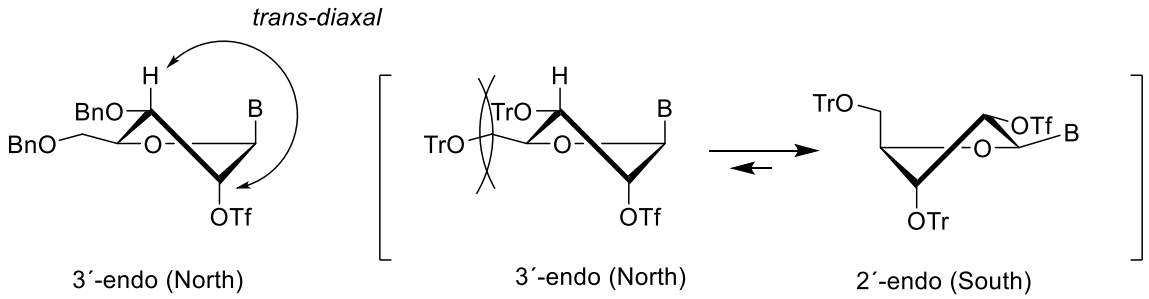
***Scheme 23**: Synthesis of fluorinated sugar by De Swarup et al.*[63]

However, when the procedure was repeated surprising results were observed. Fluorination proceeded on the C5 instead on the desired C3. The reaction was repeated with various fluorinating agents but ended up with the same result. After careful examination it was found out that the problem lay in the benzylation reaction. The benzylation gave a side product (di-benzylated sugar **20**) and mixture of mono-benzylated sugar **18** and **19** (**Scheme 24)**. The mixture is inseparable, however NMR analysis resulted in a ratio of 5:2 in favor of 3-*O*-(benzyl)-1,2-*O*-isopropylidene-D-xylofuranose **19** which explained the formation of 5-fluoro compound.



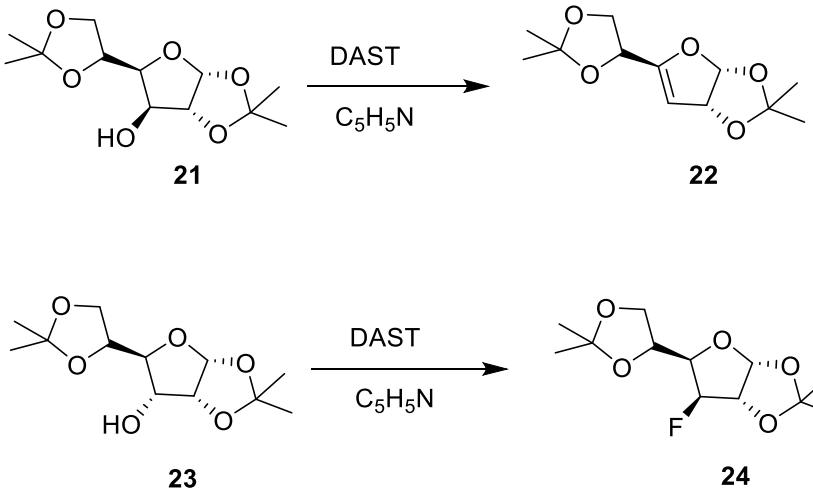
***Scheme 24**: Attempt of benzylation using condition as reported by De Swarup et al.*

With those results arose a thought that isopropylidine might be the problem causing the elimination. J. Krzeminski et al[68] reported the issue with elimination during the synthesis of 2-deoxy-2-β-fluoroarabinoside. They assumed that the problem is caused by the 3′-endo conformation of the sugar moiety. The 3′-hydrogen and 2′-leaving group are almost in trans-diaxial orientation which favors elimination. To prevent the elimination W. Pankiewicz et al[69] introduced two trityl groups to induce a steric hindrance and force the 2′-endo conformation, which is unfavorable for elimination (**Scheme 25**).



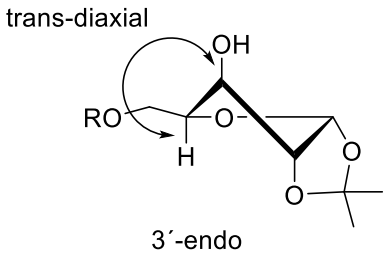
***Scheme 25**: Conformation of sugar moiety.*

Another DAST induced elimination was reported by T. J. Tewson and M. J. Welch.[70] Fluorination of two diastereomeric saccharides furnished different results 1,2:5,6-di-*O*-isopropylidine-α-D-allofuranose (**23**) gave the corresponding fluorodeoxysugar but 1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose (**21**) underwent the elimination (**Scheme 26**).[70]



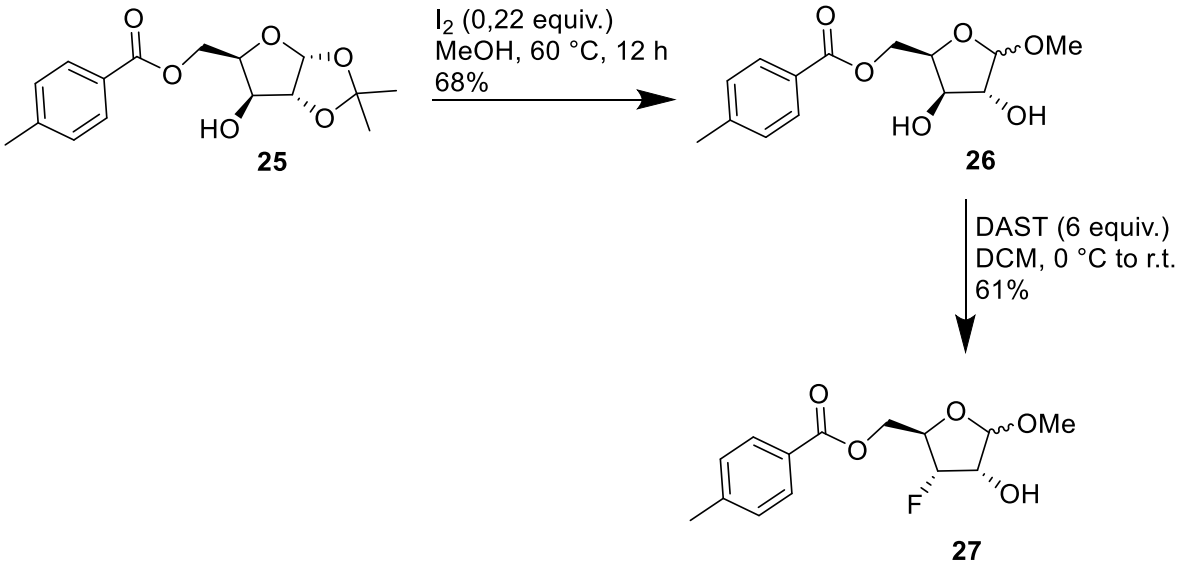
***Scheme 26**: Reaction of DAST with diastereomeric saccharides.[64]*

As it seems, the isopropylidine protecting group forces the undesired 3´-endo conformation (**Scheme 27**), which leads to elimination product, so we decided to change the strategy and try fluorination of 2,3-unprotected sugar **25** with DAST.[64]



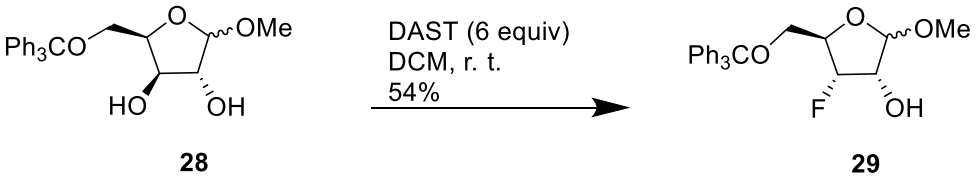
***Scheme 27**: 3´-endo conformation of protected xylose.*

In order to get compound **27**, isopropylidine derivative **25** was hydrolysed with I_2 in MeOH. 2,3-Unprotected sugar **26** then was treated with DAST to give the desired 3-fluoro-sugar **27** in 61% of yield (**Scheme 28**) and neglected amount of side product which I was unable to characterize by NMR.



***Scheme 28**: Reaction of compound* **25**.

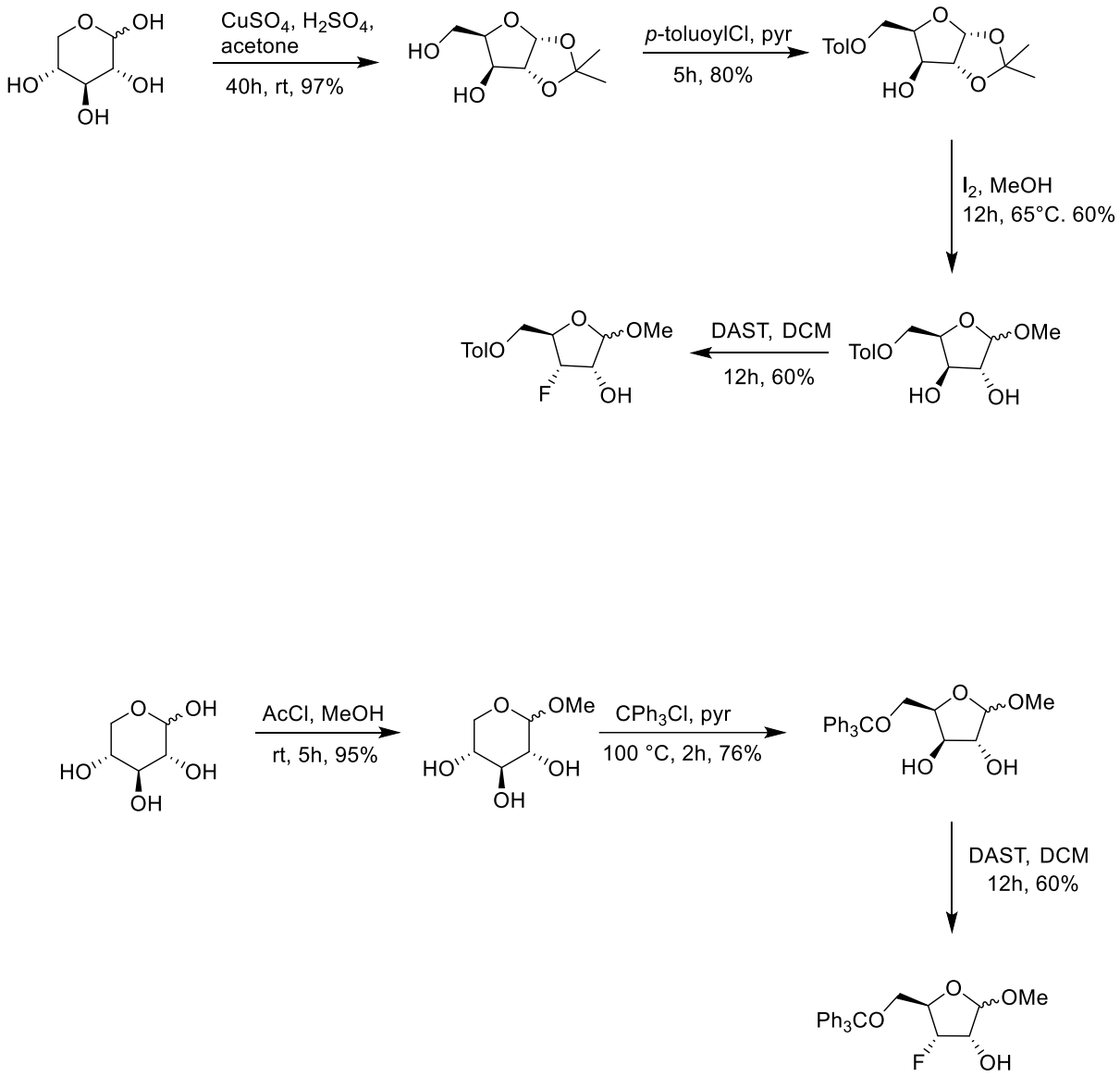
In the paper there is no explanation or proposed mechanism for cleavage of isopropylidine with I_2 in MeOH and fluorination mainly at C3. It was discovered that the cleavage of isopropylidine does not work with dry MeOH. My proposed mechanism for the cleavage is a creation of HI with I_2 and MeOH and hydrolysis of isopropylidine afterwards. It was discovered that adding the DAST at 0° C and leaving it slowly warm up to room temperature gave better yield. Also fluorination worked better in DCM compared to MeCN. Another important finding is that the reaction is not sensitive to protecting group in position 5. It works nicely also with 5-*O*-trityl derivative **28** (**Scheme 29**).



28 **29**

***Scheme 29**: Fluorination of DAST with 5-O-trityl derivatives.*

The synthetic approach towards protected 3-deoxy-3-fluororibose is summarized in **Scheme 30**. It is possible to synthesize the protected 3-deoxy-3-fluororibose from commercially available D-xylose in 3 or 4 steps depends on protection of primary OH group at C5.

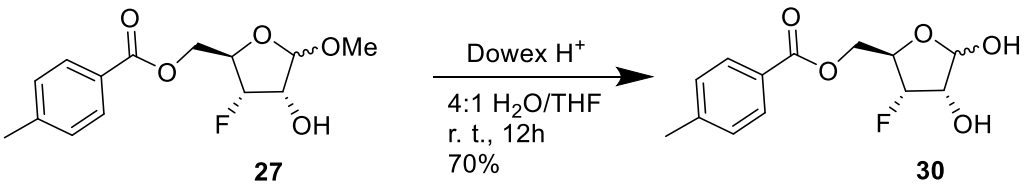


***Scheme 30**: Synthetic approaches towards 3-fluororibose.*

3.3 Synthesis of 3´-deoxy-3´-fluororibonucleoside

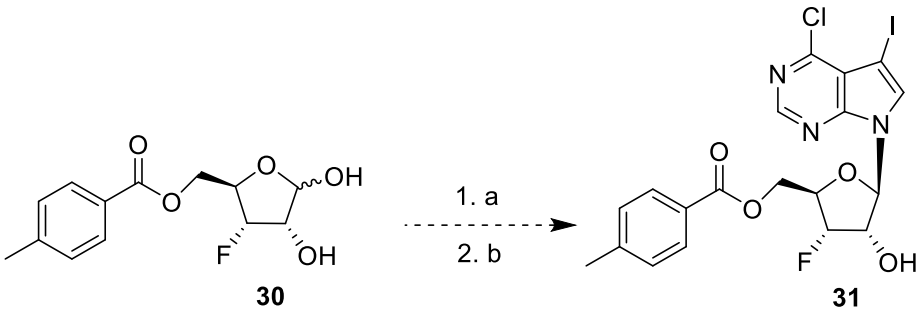
With fluorinated sugar and nucleobase in our hands we moved to glycosylation. This work will focus on synthesis of nucleosides via Vorbrüggen and recently developed glycosylation under modified Mitsunobu conditions which will be referred here as "Downey glycosylation".[51] The metal salt glycosylation was excluded due to its poor stereoselectivity control.

Firstly, Downey glycosylation was attempted, as it minimalizes using of protection groups. Fluorinated sugar **27** was first deprotected by using Dowex hydrogen form in 4:1 solution of H_2O/THF to give a compound **30** in 70% of yield (**Scheme 31**).



Scheme 31: *Deprotection of methoxy group using Dowex hydrogen form.*

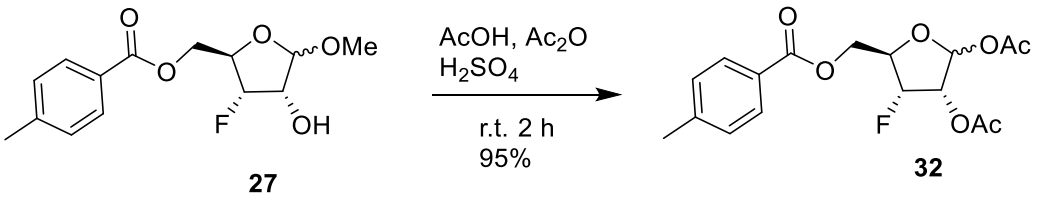
The glycosylation was tried under the best published conditions using 1.5 equiv. of ADDP, 1.5 equiv. of $P_n(Bu)_3$, 1 equiv. of nucleobase and 1.5 equiv. of NaH, all with respect to the sugar. Unfortunately, it led to a complex mixture of products which contained also small amount of desired 3-fluoro nucleoside **31** which was identified in a reaction mixture by TLC MS. The mixture was purified by flash column chromatography on silica as well as by reverse phase, even using a sepax HP-Diol column with various mobile phases. However, all attempts to isolate and purify the product were unsuccessful. As the separation was too difficult, the mixture was directly used for amination with hope that it might be better afterward. Unfortunately, the result was not better. Although a desired aminonucleoside was observed in reaction mixture by TLC-MS, all attempts on isolation failed.



Conditions: ***a)*** *$Pn(Bu)_3$ (1.5 equiv.), ADDP (1.5 equiv.), MeCN, RT until became homogenous;*
b) **3** *(1.0 equiv.) NaH (1.5 equiv.), DMF, RT, 15 min, after that 12h*

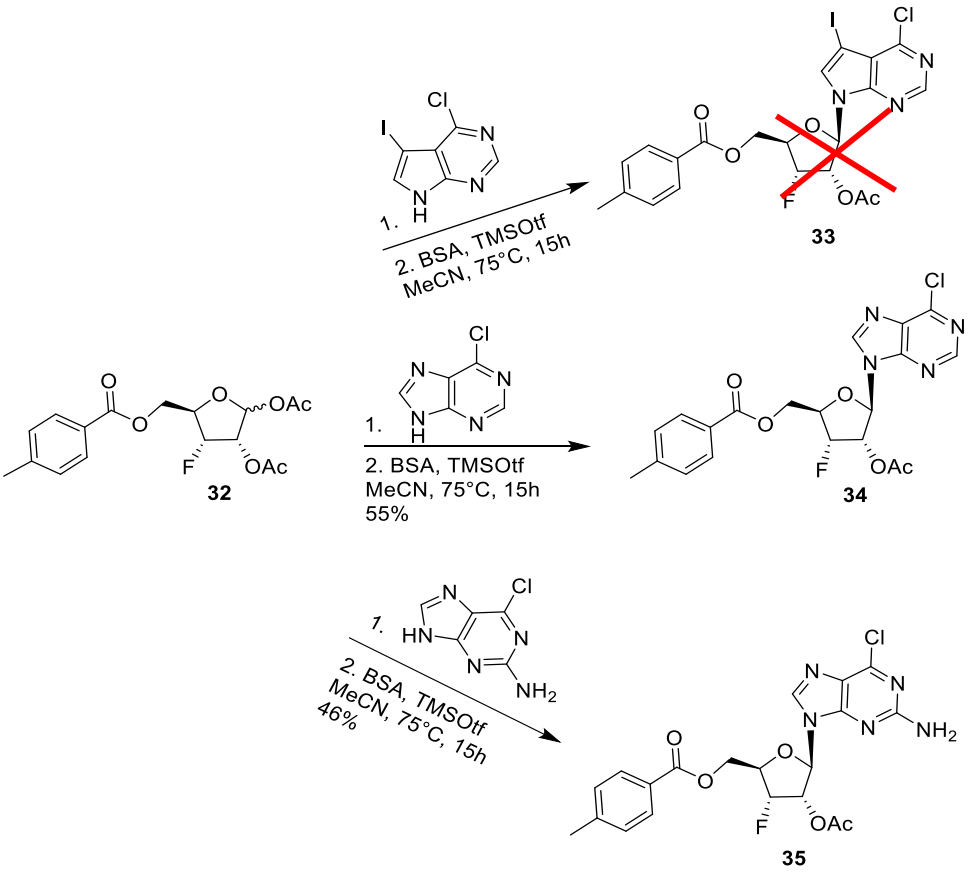
Scheme 32: *Glycosylation using Downey's conditions.*

For the Vorbrüggen glycosylation, it is necessary to convert methoxy group to acetyl, which means that one more step is needed to proceed with the glycosylation. Acetolysis of **27** was carried out in a mixture of AcOH, Ac_2O and H_2SO_4 to provide the corresponding 1,2-di-*O*-acetyl-D-ribofuranose derivative **32** in 95% yield **(Scheme 33)**.



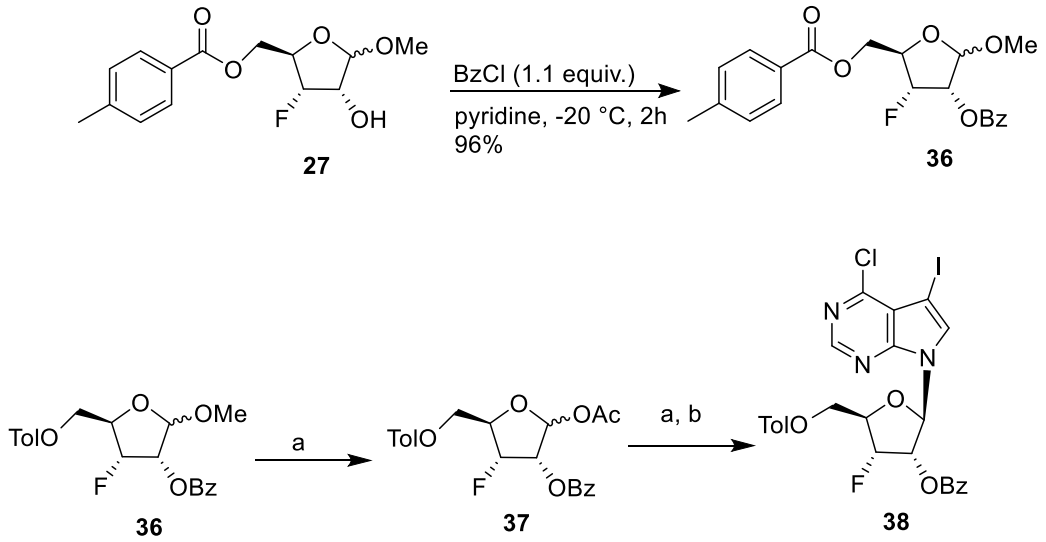
Scheme 33*: Acetolysis of compound* **27**.

Unfortunately, the glycosylation of nucleobase **2** with sugar derivative **32** under the Vorbüggen conditions did not give any product according to the TLC and the MS. However, the same sugar was successfully coupled with 6-chloropurine and 6-chloro-2-aminopurine to give the corresponding nucleoside derivatives **34** and **35** in 55% of yield for **34** and 46 % for **35**. **(Scheme 34).**



***Scheme 34**: Attempts on Vorbrüggen glycosylation.*

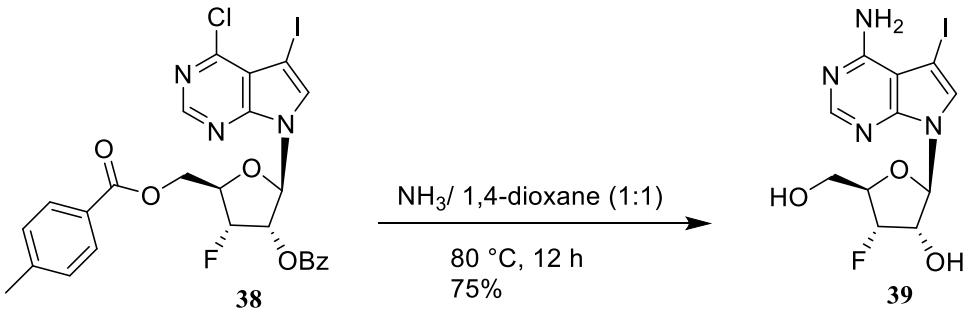
Glycosylation did not work with acetyl protected sugar, so we proceeded with benzoylation. The oxonium intermediate is more stable with benzoyl group due to aromatic stabilization. However, our first intention was not to benzoylate the hydroxyl group because it means extra step in the synthesis. Also compound **32** works with purine but not with deazapurine indicating that deazapurines are less nucleophilic than purines. Protection proceeded well with 96 % yield. Finally, benzoylated intermediate **37** reacted with deazapurine **2** under Vorbrüggen conditions and furnished desire 3′-fluoro nucleoside **38** in 32 % yield (**Scheme 35**).



Conditions: ***a)*** *AcOH (0.6 ml), Ac_2O (1.1 ml), H_2SO_4 (7 ul)*
b) 2 *(1.0 equiv.), BSA (1.2 equiv.), MeCN, RT, 15 min ; TMSOtf (1.0 equiv.), RT, 15 min, then 80 °C, 12 h, 32%*

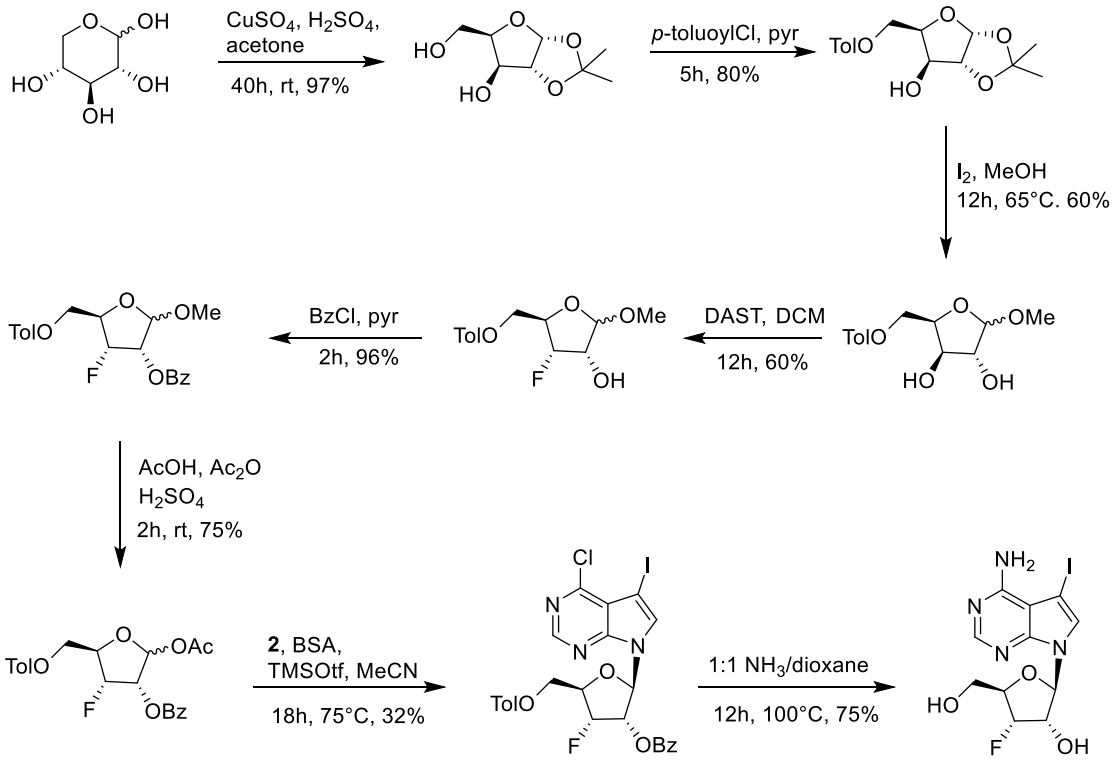
Scheme 35*: Synthesis of compound* **38**.

Amination and deprotection of protecting groups can be done in one step using aqueous NH_3 and 1,4-dioxane in 1:1 (**Scheme 36)**. The reaction worked nicely and after purification by reverse phase it furnished desire amino nucleoside **39** as a key intermediate in good 75% yield.



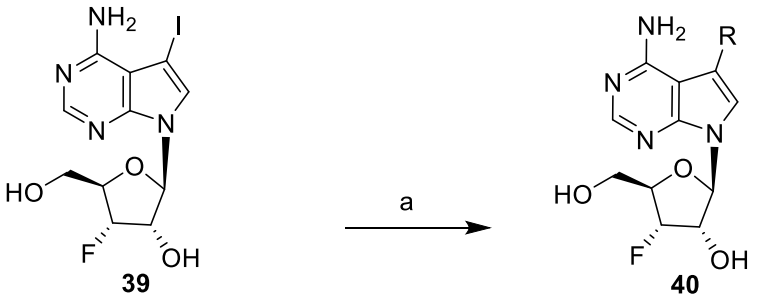
Scheme 36*: Amination and deprotection leading to desired key-intermediate* **39**.

The whole synthetic pathway towards the key intermediate 6-amino-7-iodo-7-deazapurine- 3′- fluororibonucleoside **39** is summarized in **Scheme 37**. This procedure, compares with the one of Mikhaipulo at al.[43] mentioned earlier **Scheme 15,** is shorter starting from very cheap commercially available D-xylose **1**. The synthesis of protected 3-fluororibose **37** from D-xylose **1** was done in 6 steps with overall yield of 20%. Reactions used in this procedure are well established with very high yields. Only the glycosylation yielded quite low because of the deazapurine's nucleophilic properties as it was explained earlier. On the other hand, the reference's approach to protected 3′-fluororibonucleoside was done in 10 steps with overall yield of only 7.2%. Using DAST as a fluorinating agent we can avoid the reference's procedure disadvantages. First, DAST can directly fluorinate the OH group, therefore we do not have to synthesize the epoxide, so the tosylation and conversion of xylose into ribose can be avoided. Second, DAST does not react with ester protecting groups which means laborious protection and deprotection of 5′-OH group can be also avoided. However, in both procedures the most interesting step is fluorination. To open an epoxide using fluoride ion in the case of *β*-anomer we might expect also 2-deoxy-2-fluoroxylofuranose. Surprisingly, they reported that no 2-deoxy-2-fluoroxylofuranose was isolated. In our case fluorination of 2,3-unprotected sugar gave 3-fluororibose in very good yield.



***Scheme 37**: Synthetic pathway towards key intermediate **40**.*

The last step towards our desired 6-amino-7-hetaryl-7-deazapurine-3′-fluororibonucleoside is coupling of commercially available organoboronic acid with our obtained nucleoside **39**. The coupling was performed via Pd-catalyzed Suzuki reaction under aqueous conditions and gave the desired final products**.** Coupling reactions and their purifications proceeded without any problems with moderate yields (**Scheme 38**). Now, the synthetic strategy towards 7-hetaryl-7-deazaadenosine 3′-fluororibonucleosides is developed and optimized and can be used for preparation of series of target nucleosides.

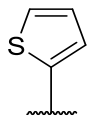
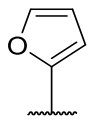
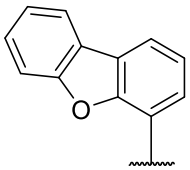
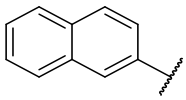


a) organoboronic acid (1.35 equiv.), $Pd(OAc)_2$ (0.05 equiv.), TPPTS (0.15 equiv.),Na_2CO_3 (3.0 equiv.), H_2O/MeCN (2:1) 85°C, 3h, under Ar

Scheme 38*: Coupling via Suzuki reaction in aqueous conditions.*

Generally, cross-coupling reactions were the easiest step in the whole synthetic pathway. For preparation of final compounds were applied Suzuki cross-coupling of organoboronic acids with unprotected nucleoside **39** in aqueous solution. As a ligand was used water-soluble TPPTS. Purification by reverse phase chromatography were obtained pure products in very good yield (**Table 1**), even though some heteroarylboronic acids are prone to protodeboronation in polar protic solvents.[71]

Table 1*: Aqueous Suzuki reactions*

	Substituent R			
Compound	**41**	**42**	**43**	**44**
				
Yield	77%	64%	84%	79%

The synthesized final products were tested for their biological activities. In vitro cytotoxic activity of final compounds was evaluated against human solid tumor cell lines including osteosarcoma (U2OS), colon carcinomas (HCT116 and HCT116p53-/-), lung cells (A549) and leukemia cell lines (CCRF-CEM, CEMD-DNR, K562 and K562-TAX) and two non-malignant BJ and MRC5- fibroblasts (**Table 2**). The IC_{50} values are given in μM. The IC_{50} values higher than 50 are taken as inactive. Among all final products the compound **39** displays very good results with low IC_{50} values on leukemia cell lines.

Table 2*: Cytotoxic activities of final compounds on cancer cell lines*.

		Cell lines									
		CEM-DNR	CCRF-CEM	K562	K562-TAX	A549	HCT116	HCT116P53$^{-/-}$	U2OS	BJ	MRC-5
Compounds	**39**	0.71	2.86	9.53	0.82	8.53	4.62	5.41	9.21	12.93	32.95
	40	15.14	21.89	38.23	14.58	44.07	30.11	>50	38.20	50.00	>50
	41	29.38	34.55	>50	21.63	>50	43.85	>50	>50	>50	>50
	42	26.71	21.32	>50	18.58	>50	>50	>50	>50	>50	>50
	43	6.40	14.59	9.81	5.82	>50	21.84	28.64	10.40	25.02	43.61

4 Conclusion

Fluorination of ribose in position 3 was developed and optimized. First attempts on fluorination of 5-protected-1,2-*O*-isopropylidinexylofuranose always led to elimination, which is caused by isopropylidine forced 3´-endo conformation of the sugar. Removal of isopropylidine protection group and simultaneous introduction of methyl group into position 1 with I_2 in MeOH gave 1-methyl-5-*O*-toluoylxylofuranose, which was successfully fluorinated with DAST selectively to position 3.

Obtained protected 3-fluororibose was used for optimization of glycosylation of 6-chloro-7-iodo-7-deazapurine. All attempts on glycosylation of 5-protected 3-fluororibose under Downey conditions were unsuccessful, only negligible amount of desired nucleoside was formed and it was impossible to isolate and purify.

Vorbrüggen glycosylation with 5-*O*-toluoyl-3-fluoro-1,2-*O*-diacetylribose worked well with 6-chloropurine and 2-amino-6-chloropurine, however, it failed with desired 6-chloro-7- iodo-7-deazapurine. 2-Acetyl protecting group had to be replaced with benzoyl, which afterwards furnished desired nucleoside in good yield. Reaction of obtained nucleoside with aqueous ammonia gave 6-amino-7-iodo-7-deazapurine 3´-fluororibonucleoside as a key-intermediate for synthesis of final nucleosides. The whole reaction sequence was optimized for gram scale with 5% of overall yield by 8-steps synthesis.

Different hetaryl groups were introduced into position 7 of synthesized key intermediate **39** using Suzuki cross-coupling in good yields. Final compounds were tested against eight human cancer cell lines including osteosarcoma (U2OS), colon carcinomas (HCT116 and HCT116p53-/-), lung (A549) and leukemia (CCRF-CEM, CEMD-DNR, K562 and K562-TAX) and two non-malignant BJ and MRC5- fibroblasts. Among all tested compounds the compound **39** showed the best result.

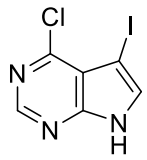
5 Experimental part

5.1 General remarks

All the solvents, starting materials and reagents were purchased from commercial suppliers and used as received. Reaction were monitored by thin layer chromatography (TLC) on TLC Silica gel 60 F_{254} (Merck) and detected by UV (254 nm) or by solution of 4-anisaldehyde in ethanol. In reasonable cases, reactions were monitored by Advion expression Compact Mass Spectrometer using electrospray ionization (ESI). Melting point were determined on a Stuart SMP40 melting point apparatus and are uncorrected. Optical rotations were measured in DMSO on Autopol IV polarimeter (Rudolph Research Analytical), $[\alpha]_D^{20}$ values are given in 10^{-1} deg·cm^2·g^{-1}. NMR spectra were measured on Bruker Avance 400 MHz spectrometer (400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C) or Bruker Avance 500 MHz spectrometer (499.8 MHz for ^{1}H and 125.7 MHz for ^{13}C and ^{31}P at 202.3 MHz) or Bruker Avance 600 MHz spectrometer (600.1 MHz for ^{1}H and 150.9 MHz for ^{13}C) in DMSO-*d6* (referenced to the residual solvent signal, [δ (^{1}H) = 2.50 ppm, δ (^{13}C) = 39.7 ppm]), in D_2O (dioxane used as external standard, [δ (^{1}H) = 3.75 ppm, δ (^{13}C) = 67.19 ppm]) or in $CDCl_3$ (referenced to the residual solvent signal, [δ (^{1}H) = 7.26 ppm, δ (^{13}C) = 77.0 ppm]). Chemical shifts are given in ppm (δ-scale), coupling constants (J) in Hz. Complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,H-ROESY, H,C-HSQC and H,C-HMBC experiments. Low resolution mass spectra were measured on LCQ Fleet (Thermo Fisher Scientific) using electrospray ionization (ESI). High resolution mass spectra were measured on LTQ Orbitrap XL (Thermo Fisher Scientific). All mass spectra were acquired by the MS service at IOCB. High performance flash chromatography (HPFC) were performed with ISCO Combiflash Rf system on RediSep Rf Gold Silica Gel Disposable columns or Reverse Phase (C18) RediSep Rf column. Purity of all final compounds (>95%) was determined by analytical HPLC or by elemental analysis and by clean NMR spectra.

5.2 Synthesis of key intermediates

4-Chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin (2)[66]

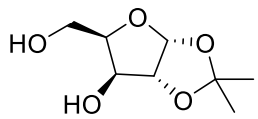


To a solution of 6-chloro-7-deazapurine **1** (10 g, 65.12 mmol) in DMF (98 ml) was added NIS (15.38 g, 68.38 mmol). The mixture was stirred 12 h at r. t. The mixture was then poured into ice water (320 ml) and the precipitate was filtered off, washed with H_2O (500 ml). The precipitate was dried in exicator for 2 days to give **2** (17.9 g, 98 %) as an orange solid.

R_f = 0.30 (SiO_2; cHex/EtOAc 2:1)

^{1}H NMR is in agreement with literature.[66]

1,2-*O*-Isopropylidine-*α*-D-xylofuranose (4)[72]

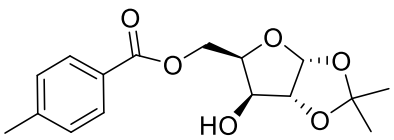


$CuSO_4$ (100 g, 0.63 mol) was suspended in acetone (875 ml), H_2SO_4 (5 ml) and D-xylose (50 g, 0.33 mol) were added and the mixture was stirred at r. t. for 40 h. After 40 h the mixture was filtered off and neutralized with aq. NH_3 (20 ml) and filtered again. The filtrate was concentrated in vacuo to produce a dark oil. The oil was dissolved in MeOH (375 ml) and treated with HCl (0.1 M, 110 ml) and the mixture was stirred at 40 °C for 3 h. The mixture then was neutralized with $NaHCO_3$ (15.75 g) and filtered with active carbon. The filtrate was evaporated and coevaporated with EtOH and toluene to yield **4** (56.38 g, 89 %) as a brown oil.

R_f = 0.25 (SiO_2; cHex/EtOAc 1:1)

^{1}H NMR is in agreement with literature.[73]

5-*O*-Toluoyl-1,2-*O*-isopropylidin-α-D-xylofuranose (25)[74]



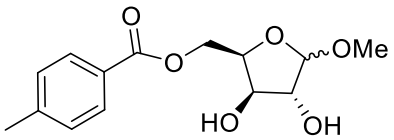
A solution of compound **4** (17 g, 89.43 mmol) in dry pyridine (85 ml) was treated with a solution of *p*-toluoyl chloride (12 ml, 89.43 mmol) in dry pyridine (20 ml) was added dropwise with rapid stirring at –20 °C under Ar. The reaction was left stirring at –20 °C for 2 h. The mixture was diluted with DCM and washed with NH_4Cl and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The residue was co-evaporated with toluene to remove traces of pyridine. The residue was purified by column chromatography on silica gel (cHex/EtOAc 3:1) to give product **25** (20g, 79%) as a colorless oil.

R_f = 0.80 (SiO_2; DCM/MeOH 19:1)

HR MS (ESI) for $C_{16}H_{20}O_6Na$ [*M* + Na]$^+$: calcd 331.11567; found 331.11521.

^{1}H NMR is in agreement with literature.[74]

Methyl-5-*O*-toluoyl-D-xylofuranose (26)



The compound **25** (20 g, 64. 9 mmol) was dissolved in MeOH (200 ml) and treated with I_2 (3.66 g, 14.42 mmol). The mixture was stirred at 60 °C for 12 h under Ar. After cooling down the mixture was poured into sat. $Na_2S_2O_3$ and extracted with $CHCl_3$ three times. The combined organic layer was washed with brine and dried over Na_2SO_4. The mixture was evaporated till dryness. The residue was purified by column chromatography on silica gel (cHex/ EtOAc 4:1 → 2:1) to give product **26**, mixture of anomers (12 g, 65%) as a white solid anomeric mixture.

R_f = 0.20 (SiO_2; DCM/MeOH 19:1)

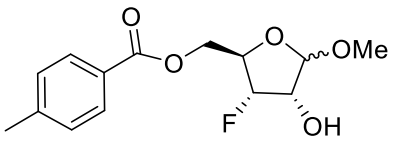
^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.40 (d, *J* = 2.6 Hz, 8H, CH_3) 3.41–3.51 (s, 7H, OMe), 4.25 (t, *J* = 2.6, 2.6 Hz, 2H, H-2), 4.31–4.45 (m, 2H, H-3), 4.45–4.54 (m, 1H, H-4), 4.59 – 4.76 (m, 4H, H-5), 5.05 (d, *J* = 4.4 Hz, 1H, H-1), 7.26 (s, 6H, H-aromatic), 7.68–8.17 (m, 5H, H-aromatic).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 21.70 (Me), 55.72 (OMe), 64.14 (C-4), 75.10 – 78.11 (C-3), 79.58 (C-2), 80.88 (C-4), 102.05 (C-1), 128.23 – 131.45 (C-aromatic), 144.00 (C-tol), 166.86 (C=O).

HR MS (ESI) for $C_{14}H_{18}O_6$ [*M* + H]$^+$: calcd 283.11728; found 283.11761.

HR MS (ESI) for $C_{14}H_{18}O_6Na$ [*M* + Na]$^+$: calcd 305.09924; found 305.09956.

Methyl-5-*O*-toluoyl-3-deoxy-3-fluoro-D-ribofuranose (27)



To a solution of compound **26** (6 g, 21.44 mmol) in dry DCM (20 ml) DAST (17 ml, 128.67 mmol) was added at 0 °C. The reaction was slowly warmed up to r. t. and left stirring for 12 h. The mixture was then cooled to 0 °C and poured into cold sat. $NaHCO_3$. The aqueous layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4 and evaporated. Purification by HPFC (SiO_2, 120 g, cHex/ EtOAc 10 → 33%) gave product **27** (3.65 g, 60%) as a yellow oil.

R_f = 0.50 (SiO_2; cHex/EtOAc 2:1)

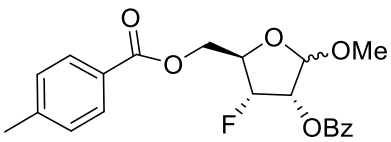
^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.40 (d, *J* = 1.8 Hz, 5H, CH_3-tol), 3.34 (s, 3H, OMe), 3.49 (s, 2H, OMe´), 4.21 – 4.27 (m, 1H, H-2), 4.45 – 4.53 (m, 2H, H-5), 4.54 – 4.62 (m, 1H, H-4), 4.90 (t, *J* = 1.8, 1.8 Hz, 1H, H-1), 4.96 (dd, *J* = 4.4, 1.2 Hz, 1H, H-1´), 5.18 (dt, *J* = 53.8, 4.7, 4.7 Hz, 1H, H-3), 7.18 – 7.26 (m, 3H, H-aromatic), 7.87 (d, *J* = 8.2 Hz, 1H, H-aromatic), 7.94 (d, *J* = 8.2 Hz, 2H, H-aromatic).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 21.69 (CH_3-tol), 55.55 (OMe), 55.79 (OMe), 74.43 (d, $J_{C,F}$ = 15.2 Hz, C-2), 78.47 (d, *J* = 25.3 Hz, C-5), 80.58 (d, $J_{C,F}$ = 25.1 Hz, C-4), 92.34 (d, $J_{C,F}$ = 186.5 Hz, C-3), 102.29 (C-1´), 107.96 (d, *J* = 3.3 Hz, C-1), 129.16 (C-aromat), 129.28 (C-aromat), 129.62 (C-aromat), 129.75 (C-aromat), 166.18 (d, *J* = 18.4 Hz, C=O).

^{19}F NMR (377 MHz, Chloroform-*d*) δ –216.37 to –209.52 (m, α-anomer), –197.94 to –193.28 (m, β-anomer).

HR MS (ESI) for $C_{14}H_{17}O_5F$ Na $[M + Na]^+$: calcd 307.0950; found 307.0952.

Methyl-5-*O*-toluoyl-2-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose (36)



To a solution of compound **25** (1.42 g, 5 mmol) in dry pyridine (17 ml) was dropwise added solution of BzCl (640 µml, 5.5 mmol) in dry pyridine (3 ml) at –20 °C. After addition, the reaction was left stirring for 2 h under Ar. The mixture was diluted with DCM, washed with NH_4Cl and H_2O. The combined organic layers were dried over Na_2SO_4, evaporated and co-evaporated with toluene. Purification by HPFC (SiO_2, 80 g, cHex/ EtOAc 20%) gave product **36** (1.86 g, 96%) as a colorless oil.

R_f = 0.40 (SiO_2; cHex/EtOAc 4:1)

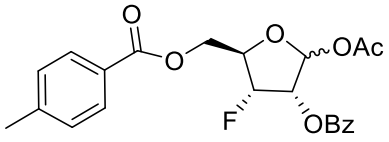
^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.44 (s, 5H, CH_3-tol), 3.42 (s, 3H, OMe), 3.53 (s, 1H, Ome´), 4.46 – 4.56 (m, 1H, H-4), 4.57–4.66 (m, 2H, H-5), 4.67 – 4.72 (m, 1H,H-2), 5.15–5.17 (m, 1H, H-1), 5.33–5.37 (m, 1H, H-1´), 5.38–5.52 (td, *J* = 53.3, 4.9 Hz, 1H, H-3), 7.24–7.34 (m, 3H, H-tol), 7.49 (td, *J* = 7.8, 7.5, 1.6 Hz, 3H, H-Bz), 7.58–7.67 (m, 1H, H-Bz), 7.93–8.04 (m, 3H, H-tol), 8.14 (ddd, *J* = 16.5, 8.3, 1.4 Hz, 3H, H-Bz).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 21.71 (CH_3-tol), 55.80 (d, *J* = 18.7 Hz, CH_3-ac), 63.76 (d, *J* = 4.6 Hz, C-4), 79.32 (d, *J* = 25.3 Hz, C-5), 80.25 (d, *J* = 25.2 Hz, C-2), 89.27 (d, *J* = 196.9 Hz, C-3), 91.20 (d, *J* = 194.5 Hz, C-3), 101.71 (C-1´), 106.09 (d, *J* = 3.2 Hz, C-1), 128.50 (d, *J* = 2.0 Hz, C-Bz), 129.21 (C-tol), 129.33 (C-Bz), 129.71 (C-Bz), 129.78 (C-tol), 129.94 (C-Bz), 130.08 (C-Bz), 165.59 (d, *J* = 39.7 Hz, C=O_{Bz}), 166.17 (d, *J* = 9.6 Hz, C=O_{Tol}).

^{19}F NMR (377 MHz, Chloroform-*d*) δ –211.29 (ddd, *J* = 53.3, 19.0, 5.2 Hz), –193.94 (ddd, *J* = 55.6, 25.9, 22.7 Hz).

HR MS (ESI) for $C_{21}H_{21}O_6F$ Na $[M + Na]^+$: calcd 411.12079; found 411.12144.

1-*O*-Acetyl-5-*O*-toluoyl-2-*O*-benzoyl-3-deoxy-3-fluoro-D-ribofuranose (37)



To the compound **36** (1.86 g, 4.79 mmol) was added Ac_2O (3.7 ml) followed by AcOH (2 ml) and H_2SO_4 (23 µl). The mixture was stirred for 2 h at r.t. The mixture then treated with H_2O, neutralized with $NaHCO_3$ and extracted with DCM. The extracts were dried over Na_2SO_4, evaporated till dryness. Purification by HPFC (SiO_2, 40 g, cHex/ EtOAc 20%) gave product **37** (1.50 g, 75%) as a colorless oil.

R_f = 0.60 (SiO_2; cHex/EtOAc 2:1)

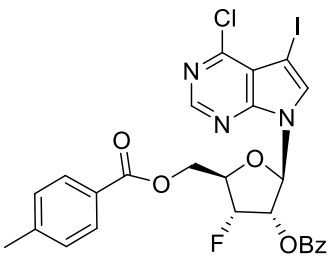
^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.99 – 2.15 (m, *J* = 7.8 Hz, 7H, Ac), 2.42 (d, *J* = 1.2 Hz, 6H, CH_3), 4.44 – 4.60 (m, 3H,), 4.62 – 4.78 (m, 2H), 4.85 (dtd, *J* = 25.8, 3.7, 3.7, 1.4 Hz, 1H), 5.30 – 5.41 (m, 2H,), 5.40 – 5.55 (m, 1H, H-2), 5.60 (ddd, *J* = 8.7, 4.8, 2.3 Hz, 1H, H-3), 6.41 (t, *J* = 1.9, 1.9 Hz, 1H, H-1 anomer), 6.65 (d, *J* = 4.5 Hz, 1H, H-1), 7.26 (s, 5H, H-Bz), 7.40 – 7.54 (m, 6H, H-tol), 7.56 – 7.67 (m, 3H, H-Bz), 7.86 – 8.19 (m, 10H, H-Bz).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 21.00 (C-Ac), 21.72 (C-CH_3-tol), 62.28 – 63.90 (C-5), 80.95 (d, *J* = 24.9 Hz, C-4), 82.48 (d, *J* = 25.1 Hz, C-2), 87.55 – 89.46 (d, *J* = 191.7 Hz, C-3), 129.26 (C-Bz), 129.38 (C-Bz), 129.71 (C-tol), 129.79 (C-tol), 129.94 (C-tol), 130.00 (C-Bz), 133.77 (C-Tol), 164.82 – 166.30 (C=O), 169.56 (C=O).

^{19}F NMR (377 MHz, Chloroform-*d*) δ -208.82 (ddd, *J* = 52.2, 19.1, 8.5 Hz), -195.27 (ddd, *J* = 56.1, 25.8, 21.7 Hz).

HR MS (ESI) for $C_{22}H_{21}O_7F$ Na $[M + Na]^+$: calcd 439.11670; found 439.11635.

4-Chloro-5-iodo-7-(5-*O*-toluoyl-2′-*O*-benzoyl-3′-deoxy-3′-fluoro-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (38)



To the solution of **2** (1.0 g, 3.60 mmol) in dry MeCN (8 ml) BSA (1.1 ml, 4.32 mmol) was added. The mixture was stirred for 15 min then the solution of sugar **37** in MeCN (20 ml) was added follow by TMSOTf (650 µl). The mixture was stirred for 15 min at r.t. then at 80 °C under Ar for 12 h. The mixture was treated with H_2O, extracted with EA. Organic layers were washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4 and evaporated till dryness. Purification by HPFC (SiO_2, 80 g, cHex/ EtOAc 5 → 35%) to yield a product **38** (750 mg, 32%) as a white solid.

R_f = 0.65 (SiO_2; cHex/EtOAc 2:1)

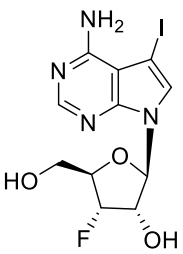
^{1}H NMR (400 MHz, Chloroform-*d*) δ 2.44 (s, 3H, CH_3-tol), 4.56 – 4.64 (m, 1H, H-4′), 4.71 – 4.86 (m, 2H, H-5′), 5.65 (ddd, *J* = 53.5, 4.7, 1.8 Hz, 1H, H-3′), 5.95 (ddd, *J* = 19.0, 7.3, 4.7 Hz, 1H, H-2′), 6.68 (dd, *J* = 7.3, 0.9 Hz, 1H, H-1′), 7.29 – 7.34 (m, 2H, H-tol), 7.43 (t, *J* = 7.8, 7.8 Hz, 2H, H-Bz), 7.48 (s, 1H, H-6), 7.54 – 7.62 (m, 1H, H-Bz), 7.95 – 8.04 (m, 4H, H-Bz + tol), 8.52 (s, 1H, H-2).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 21.79 (CH_3-tol), 63.08 (d, *J* = 9.4 Hz, C4 + C5), 74.18 (d, *J* = 15.5 Hz, C2), 85.58 (C1), 89.58 (d, *J* = 190.1 Hz, C3), 128.59 (C-Bz),129.63 (C-tol), 129.71(C-tol), 130.02 (C-Bz), 131.95 (CH-6), 133.95 (C-Bz), 151.24 (CH-2), 165.66 (d, *J* = 73.4 Hz, C=O).

^{19}F NMR (377 MHz, Chloroform-*d*) δ –203.91 to –191.63.

HR MS (ESI) for $C_{26}H_{20}O_5N_3ClFI$ $[M + H]^+$: calcd 636.01949; found 636.01930.
HR MS (ESI) for $C_{26}H_{20}O_5N_3ClFI$ Na $[M + Na]^+$: calcd 658.00148; found 658.00124.

4-Amino-5-iodo-7-(3′-deoxy-3′-fluoro-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (39)



The compound **38** (750 mg, 1.18 mmol) was dissolved in solution of 1:1 NH_3 (aq, 25% w/w) and 1,4-dioxane (30 ml). The mixture was transferred into a glass pressure tube and sealed. The reaction was left stirring at 80 °C for 12 h. After 12 h the mixture was evaporated and directly purified. Purification by reverse-phase HPFC (C18 column, H_2O/MeOH 5 → 100%) gave a product **39** (210 mg, 45%) as a white solid.

R_f = 0.30 (SiO_2; DCM/MeOH 9:1)

m.p. 160–162 °C

$[\alpha]^{20}_{589}$ -75.0 (c 0.180, DMSO)

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.56 – 3.64 (m, 2H, H-5′); 4.20 (dt, 1H, $J_{H,F}$ = 27.8, $J_{4',5'}$ = 3.9, H-4′); 4.66 (dddd, 1H, $J_{H,F}$ = 26.3, $J_{2',1'}$ = 8.2, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 4.2, H-2′); 5.01 (dd, 1H, $J_{H,F}$ = 54.7, $J_{3',2'}$ = 4.2, H-3′); 5.41 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.81 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2′); 6.07 (d, 1H, $J_{1',2'}$ = 8.2, H-1′); 6.73 (bs, 2H, NH_2); 7.69 (s, 1H, H-6); 8.11 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 52.73 (C-5); 61.16 (d, $J_{C,F}$ = 11.6, CH_2-5′); 72.69 (d, $J_{C,F}$ = 16.2, CH-2′); 83.41 (d, $J_{C,F}$ = 21.3, CH-4′); 85.74 (CH-1′); 93.31 (d, $J_{C,F}$ = 181.0, CH-3′); 103.55 (C-4a); 127.23 (CH-6); 150.70 (C-7a); 152.27 (CH-2); 157.50 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): –192.28 (dd, $J_{F,H}$ = 54.7, 27.8, 26.3).

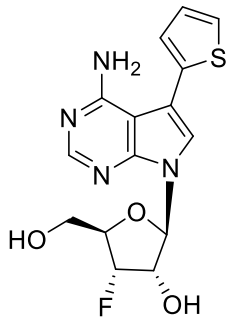
HR MS (ESI) for $C_{11}H_{13}O_3N_4FI$ [M + H]$^+$: calcd 395.00140; found 395.00109.
HR MS (ESI) for $C_{11}H_{13}O_3N_4FI$ Na [M + Na]$^+$: calcd 416.98325; found 416.98303.

5.3 Coupling reaction

5.3.1 General procedure

To a flask with nucleoside (1 equiv.) was added $Pd(OAc)_2$ (0.05 equiv.), TPPTS (0.15 equiv.), Na_2CO_3 (3 equiv.) and corresponding boronic acid (1.35 equiv.). The flask was purged with argon three times and a solution of MeCN/H_2O (1:2) was added. The mixture was stirred at 90 °C for 3 h under Ar. After that the mixture was neutralized with 1M HCl and transferred into silica gel. Reverse phase column chromatography (H_2O/MeOH 5 →100 %) gave a desired product.

4-Amino-5-(2-thiophenyl)-7-(3´-deoxy-3´-fluoro-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (40)



Following the general procedure nucleoside **39** (140 mg, 0.355 mmol), $Pd(OAc)_2$ (4.2 mg, 0.018 mmol), TPPTS (30 mg, 0.053 mmol), Na_2CO_3 (113 mg, 1.07 mmol) and 2-thiophenylboronic acid (61 mg, 0.48 mmol) were reacted in 9 ml of H2O/MeCN (2:1). Reverse phase column chromatography (C18 column, H_2O/MeOH 5 → 100%) provided the product **40** (96 mg, 77%) as a brown solid.

R_f = 0.30 (SiO_2; DCM/MeOH 19:1)

m.p. 125–128 °C

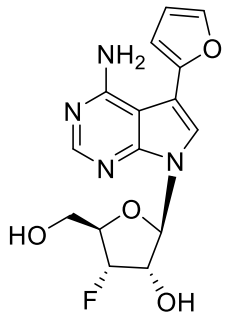
$[\alpha]^{20}_{589}$ -68.7 (c 0.114, DMSO)

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.61 (dd, 2H, $J_{5',OH}$ = 5.7, $J_{5',4'}$ = 3.8, H-5′); 4.23 (dt, 1H, $J_{H,F}$ = 27.8, $J_{4',5'}$ = 3.8, H-4′); 4.75 (dddd, 1H, $J_{H,F}$ = 26.3, $J_{2',1'}$ = 8.2, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 4.3, H-2′); 5.03 (dd, 1H, $J_{H,F}$ = 54.8, $J_{3',2'}$ = 4.3, H-3′); 5.45 (t, 1H, $J_{OH,5'}$ = 5.7, OH-5′); 5.84 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2′); 6.15 (d, 1H, $J_{1',2'}$ = 8.2, H-1′); 6.37 (bs, 2H, NH_2); 7.16 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 1.3, H-3-thienyl); 7.19 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,3}$ = 3.5, H-4-thienyl); 7.59 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,3}$ = 1.3, H-5-thienyl); 7.63 (s, 1H, H-6); 8.16 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.19 (d, $J_{C,F}$ = 11.6, CH_2-5′); 72.61 (d, $J_{C,F}$ = 16.3, CH-2′); 83.41 (d, $J_{C,F}$ = 21.2, CH-4′); 85.93 (CH-1′); 93.39 (d, $J_{C,F}$ = 181.0, CH-3′); 100.94 (C-4a); 109.05 (C-5); 122.10 (CH-6); 126.15 (CH-5-thienyl); 126.69 (CH-3-thienyl); 128.50 (CH-4-thienyl); 135.45 (C-2-thienyl); 151.13 (C-7a); 152.32 (CH-2); 157.55 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): –192.24 (dd, $J_{F,H}$ = 54.8, 27.8, 26.3).

HR MS (ESI) for $C_{15}H_{15}O_3N_4FS$ [M + H]$^+$: calcd 351.09245; found 351.09217.
HR MS (ESI) for $C_{15}H_{15}O_3N_4FS$ Na [M + Na]$^+$: calcd 373.07431; found 373.07411.



4-Amino-5-(2-furyl)-7-(3´-deoxy-3´-fluoro-*β*-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (41)

Following the general procedure nucleoside **39** (121 mg, 0.32 mmol), $Pd(OAc)_2$ (3.5 mg, 0.015 mmol), TPPTS (26 mg, 0.046 mmol), Na_2CO_3 (100 mg, 0.92 mmol) and 2-furylboronic acid (61 mg, 0.414 mmol) were reacted in 9 ml of H_2O/MeCN (2:1). Reverse phase column chromatography (C18 column, H_2O/MeOH 5 → 100%) provided the product **41** (66 mg, 64%) as a brown solid.

Rf: 0.30 (SiO2; DCM/MeOH 19:1)

m.p. 213–215 °C

$[\alpha]^{20}_{589}$ -82.6 (c 0.115, DMSO)

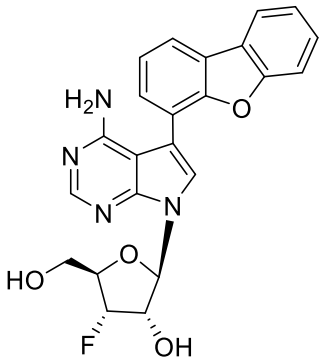
^{1}H NMR (500.0 MHz, DMSO-d_6): 3.62 (dd, 2H, $J_{5',OH}$ = 5.6, $J_{5',4'}$ = 4.1, H-5′); 4.22 (dt, 1H, $J_{H,F}$ = 27.9, $J_{4',5'}$ = 4.1, H-4′); 4.74 (dddd, 1H, $J_{H,F}$ = 26.0, $J_{2',1'}$ = 8.1, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 4.3, H-2′); 5.04 (dd, 1H, $J_{H,F}$ = 54.7, $J_{3',2'}$ = 4.3, H-3′); 5.46 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.84 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2′); 6.14 (d, 1H, $J_{1',2'}$ = 8.1, H-1′); 6.62 (dd, 1H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.9, H-4-furyl); 6.67 (dd, 1H, $J_{3,4}$ = 3.3, $J_{3,5}$ = 0.9, H-3-furyl); 6.95 (bs, 2H, NH_2); 7.80 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,3}$ = 0.9, H-5-furyl); 7.85 (s, 1H, H-6); 8.14 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.18 (d, $J_{C,F}$ = 11.5, CH_2-5′); 72.52 (d, $J_{C,F}$ = 16.3, CH-2′); 83.37 (d, $J_{C,F}$ = 21.2, CH-4′); 85.94 (CH-1′); 93.27 (d, $J_{C,F}$ = 181.1, CH-3′); 99.63 (C-4a); 105.67 (CH-3-furyl); 106.72 (C-5); 112.11 (CH-4-furyl); 120.56 (CH-6); 142.34 (CH-5-furyl); 148.57 (C-2-furyl); 151.38 (C-7a); 152.39 (CH-2); 157.53 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): –192.33 (dd, $J_{F,H}$ = 54.7, 27.9, 26.0).

HR MS (ESI) for $C_{15}H_{16}O_4N_4F$ [M + H]$^+$: calcd 335.11515; found 335.11501.
HR MS (ESI) for $C_{15}H_{16}O_4N_4F$ Na [M + Na]$^+$: calcd 357.09705; found 357.09695.

4-Amino-5-(4-(dibenzofuryl))-7-(3´-deoxy-3´-fluoro-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (42)



Following the general procedure nucleoside **39** (126 mg, 0.32 mmol), $Pd(OAc)_2$ (3.8 mg, 0.016 mmol), TPPTS (28 mg, 0.048 mmol), Na_2CO_3 (102 mg, 0.92 mmol) and 4-(dibenzofuryl)boronic acid (92 mg, 0.432 mmol) were reacted in 7.5 ml of H_2O/MeCN (2:1). Reverse phase column chromatography (C18 column, H_2O/MeOH 5 → 100%) provided the product **42** (117 mg, 84%) as a white solid.

R_f: 0.40 (SiO_2; DCM/MeOH 19:1)

m.p. 151–154 °C

$[\alpha]^{20}_{589}$ -51.7 (c 0.089, DMSO)

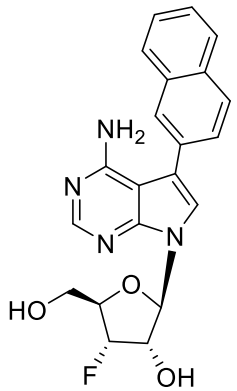
^{1}H NMR (500.0 MHz, DMSO-d_6): 3.64 (dd, 2H, $J_{5',OH}$ = 5.5, $J_{5',4'}$ = 3.9, H-5′); 4.27 (dt, 1H, $J_{H,F}$ = 27.8, $J_{4',5'}$ = 3.9, H-4′); 4.87 (dddd, 1H, $J_{H,F}$ = 26.2, $J_{2',1'}$ = 8.2, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 4.3, H-2′); 5.07 (dd, 1H, $J_{H,F}$ = 54.7, $J_{3',2'}$ = 4.3, H-3′); 5.50 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5′); 5.91 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2′); 6.10 (bs, 2H, NH_2); 6.22 (d, 1H, $J_{1',2'}$ = 8.2, H-1′); 7.43 (td, 1H, $J_{8,7}$ = $J_{8,9}$ = 7.5, $J_{8,6}$ = 1.0, H-8-dibenzofuryl); 7.49 – 7.55 (m, 3H, H-2,3,7-dibenzofuryl); 7.69 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.0, $J_{6,9}$ = 0.6, H-6-dibenzofuryl); 7.79 (s, 1H, H-6); 8.18 (m, 1H, H-1-dibenzofuryl); 8.19 (s, 1H, H-2); 8.21 (ddd, 1H, $J_{9,8}$ = 7.5, $J_{9,7}$ = 1.3, $J_{9,6}$ = 0.6, H-9-dibenzofuryl).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.30 (d, $J_{C,F}$ = 11.6, CH_2-5′); 72.59 (d, $J_{C,F}$ = 16.3, CH-2′); 83.47 (d, $J_{C,F}$ = 21.2, CH-4′); 86.48 (CH-1′); 93.50 (d, $J_{C,F}$ = 180.9, CH-3′); 101.98 (C-4a); 110.18 (C-5); 112.06 (CH-6-dibenzofuryl); 118.87 (C-4-dibenzofuryl); 120.47 (CH-1-dibenzofuryl); 121.55 (CH-9-dibenzofuryl); 122.81 (CH-6); 123.43 (CH-8-dibenzofuryl); 123.80 (CH-2-dibenzofuryl); 124.02 (C-9a-dibenzofuryl); 124.39 (C-9b-dibenzofuryl); 127.89 (CH-7-dibenzofuryl); 128.67 (CH-3-dibenzofuryl); 151.16 (C-7a); 152.10 (CH-2); 153.43 (C-4a-dibenzofuryl); 155.71 (C-5a-dibenzofuryl); 157.78 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): –192.27 (dd, $J_{F,H}$ = 54.7, 27.8, 26.2).

HR MS (ESI) for $C_{23}H_{19}O_4N_4F$ [M + H]$^+$: calcd 435.14575; found 435.14631.
HR MS (ESI) for $C_{23}H_{19}O_4N_4F$ Na [M + Na]$^+$: calcd 457.12766; found 457.12825.

4-Amino-5-(2-naphtyl)-7-(3′-deoxy-3′-fluoro-β-D-ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin (43)



Following the general procedure nucleoside **39** (123 mg, 0.312 mmol), $Pd(OAc)_2$ (3.8 mg, 0.016 mmol), TPPTS (27 mg, 0.047 mmol), Na_2CO_3 (15 mg, 0.92 mmol) and 2-naphtylboronic acid (72 mg, 0.432 mmol) were reacted in 7.5 ml of H_2O/MeCN (2:1). Reverse phase column chromatography (C18 column, H_2O/MeOH 5 → 100%) provided the product **43** (97 mg, 79%) as white solid.

R_f: 0.40 (SiO_2; DCM/MeOH 19:1)

m.p. 138–140 °C

$[\alpha]^{20}_{589}$ -43.0 (c 0.114, DMSO)

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.63 (dd, 2H, $J_{5',OH}$ = 5.6, $J_{5',4'}$ = 4.0, H-5′); 4.24 (dt, 1H, $J_{H,F}$ = 27.9, $J_{4',5'}$ = 4.0, H-4′); 4.81 (dddd, 1H, $J_{H,F}$ = 26.2, $J_{2',1'}$ = 8.2, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 4.3, H-2′); 5.05 (dd, 1H, $J_{H,F}$ = 54.8, $J_{3',2'}$ = 4.3, H-3′); 5.47 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.85 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2′); 6.20 (d, 1H, $J_{1',2'}$ = 8.2, H-1′); 6.25 (bs, 2H, NH_2); 7.54 (ddd, 1H, $J_{6,5}$ = 8.4, $J_{6,7}$ = 6.8, $J_{6,8}$ = 1.4, H-6-naphth); 7.56 (ddd, 1H, $J_{7,8}$ = 8.4, $J_{7,6}$ = 6.8, $J_{7,5}$ = 1.4, H-7-naphth); 7.65 (dd, 1H, $J_{3,4}$ = 8.4, $J_{3,1}$ = 1.8, H-3-naphth); 7.69 (s, 1H, H-6); 7.96 – 8.00 (m, 3H, H-1,5,8-naphth); 8.04 (d, 1H, $J_{4,3}$ = 8.4, H-4-naphth); 8.18 (s, 1H, H-2).

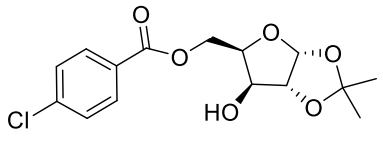
^{13}C NMR (125.7 MHz, DMSO-d_6): 61.25 (d, $J_{C,F}$ = 11.5, CH_2-5′); 72.57 (d, $J_{C,F}$ = 16.3, CH-2′); 83.33 (d, $J_{C,F}$ = 21.2, CH-4′); 86.00 (CH-1′); 93.40 (d, $J_{C,F}$ = 181.0, CH-3′); 100.92 (C-4a); 116.91 (C-5); 121.56 (CH-6); 126.15 (CH-6-naphth); 126.77 (CH-7-naphth); 126.87 (CH-1-naphth); 127.14 (CH-3-naphth); 127.86 (CH-5-naphth); 128.03 (CH-8-naphth); 128.72 (CH-4-naphth); 131.96 (C-2-naphth); 132.06 (C-4a-naphth); 133.42 (CH-8a-naphth); 151.55 (C-7a); 152.07 (CH-2); 157.71 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): –192.21 (dd, $J_{F,H}$ = 54.8, 27.9, 26.2).

HR MS (ESI) for $C_{21}H_{20}O_3N_4F$ $[M + H]^+$: calcd 395.15147; found 395.15140.
HR MS (ESI) for $C_{21}H_{20}O_3N_4F$ Na $[M + Na]^+$: calcd 417.13341; found 417.13334.

5.4 Synthesis of compounds used during optimizations.

5-*O*-(4-Chlorobenzoyl)-1,2-*O*-isopropylidene-*α*-D-xylofuranose (10)

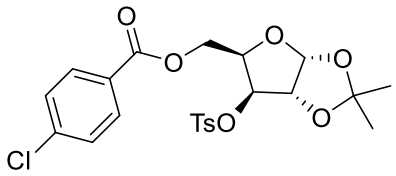


To a solution of compound **4** (2 g, 10.52 mmol) in dry pyridine (11 ml) a solution of 4-chlorobenzoyl chloride (1.5 ml, 10.52 mmol) in dry pyridine (2 ml) was added dropwise with rapid stirring at –20 °C under Ar. The reaction was left stirring at –20 °C for 2 h. The mixture was diluted with DCM and washed with NH_4Cl and H_2O. The organic layer was dried over Na_2SO_4 and evaporated. The residue was co-evaporated with toluene to remove traces of pyridine. The residue was purified by column chromatography on silica gel (cHex/EtOAc 3:1) to give product **10** (3.75g, 80%) as a yellow solid.

R_f = 0.20 (SiO_2; cHex/EtOAc 4:1)

^{1}H NMR is in agreement with literature.[74]

5-*O*-(4-Chlorobenzoyl)-3-*O*-tosyl-1,2-*O*-isopropylidene-*α*-D-xylofuranose (12)



To the solution of **10** (1 g, 3.05 mmol) in dry pyridine (6.8 ml) was added tosyl chloride (872 mg, 4.57 mmol). The mixture was stirred at r. t. for 12 h. The mixture was then poured into ice-cold water and extracted with DCM. The residue was co-evaporated with toluene. Purification by HPFC (SiO_2, 40 g, cHex/ EtOAc 20%) gave product **12** (0.9 g, 61%) as a white foam.

R_f = 0.40 (SiO_2; cHex/EtOAc 4:1)

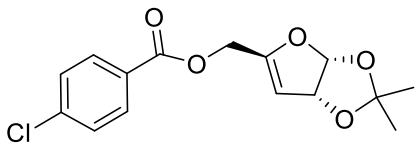
^{1}H NMR (400 MHz, Chloroform-*d*) δ 1.33 (s, 3H, CH_3), 1.52 (s, 3H), 2.34 (s, 3H), 4.31 (dd, *J* = 11.5, 6.4 Hz, 1H), 4.42 (dd, *J* = 11.4, 6.2 Hz, 1H), 4.55 (td, *J* = 6.3, 6.3, 2.9 Hz, 1H), 4.79 (d, *J* = 3.7 Hz, 1H), 4.96 (d, *J* = 2.9 Hz, 1H), 5.99 (d, *J* = 3.7 Hz, 1H), 7.26 (s, 2H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 1H).

5-*O*-benzyl-1,2-*O*-isopropylidene-D-xylofuranose (17)

To a solution of NaH (0.54 g, 22.42 mmol) in THF (45 ml) was added a solution of **4** (3.87 g, 20.38 mmol) dropwise at –78 °C. The resulting mixture was stirred for 30 min. TBAI (3.9 g, 10.6 mmol) was added to the mixture followed by BnBr (2.66 ml, 22.42 mmol). The mixture was stirred at –78 °C to r. t. for 22 h. The mixture was cooled to 0 °C and treated with sat. NH_4Cl. The mixture then was diluted with H_2O and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and evaporated till dryness. Purification by HPFC (SiO_2, 4 g cHex/EtOAc 1:1) gave product **17** (351 mg, 49%).

^{1}H NMR is in agreement with literature.[67]

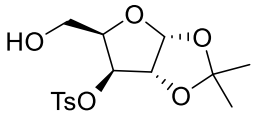
Fluorination of compounds 10 and 12 using TBAF and DAST



DAST (90 µl, 0.67 mmol) was added slowly to a mixture of compound **10** (200 mg, 0.61 mmol) in dry DCM (3 ml) and dry pyridine (178 µl, 2.20 mmol) at –10 °C. The reaction was left stirring for 4 days at r. t. The mixture was washed with H_2O, dried over $MgSO_4$ and evaporated. Purification by HPFC (SiO_2, 4 g cHex/EtOAc 15:1 → 11:1 + 0.5% MeOH) to give product **15** (39 mg, 21%).

R_f = 0.50 (SiO_2; cHex/EtOAc 4:1)

^{1}H NMR is in agreement with literature.[75]



Compound **12** (587 mg, 1.22 mmol) was dissolved in MeCN (2.5 ml) and TBAF (3.7 ml, 3.66 mmol) was added. The reaction was left stirring at 60 °C for 16 h. The mixture was poured into EtOAc and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated till dryness. Purification by HPFC (SiO_2, 4 g cHex/EtOAc 15:1 → 11:1 + 0.5% MeOH) gave product **13** (351 mg, 87%).

R_f = 0.90 (SiO_2; DCM/MeOH 19:1)

^{1}H NMR is in agreement with literature.[76]

6 References

(1) Moffatt, B. A.; Ashihara, H. Purine and Pyrimidine Nucleotide Synthesis and Metabolism. *Arab. Book* **2002**, *1*.

(2) Seley-Radtke, K. L.; Yates, M. K. The Evolution of Nucleoside Analogue Antivirals: A Review for Chemists and Non-Chemists. Part 1: Early Structural Modifications to the Nucleoside Scaffold. *Antiviral Res.* **2018**, *154*, 66–86.

(3) Rosemeyer, H. The Chemodiversity of Purine as a Constituent of Natural Products. *Chem. Biodivers.* **2004**, *1* (3), 361–401.

(4) Evans, Ben.; Wolfenden, Richard. Potential Transition State Analog for Adenosine Deaminase. *J. Am. Chem. Soc.* **1970**, *92* (15), 4751–4752.

(5) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. Impact of Natural Products on Developing New Anti-Cancer Agents †. *Chem. Rev.* **2009**, *109* (7), 3012–3043.

(6) Eyer, L.; Nencka, R.; de Clercq, E.; Seley-Radtke, K.; Růžek, D. Nucleoside Analogs as a Rich Source of Antiviral Agents Active against Arthropod-Borne Flaviviruses. *Antivir. Chem. Chemother.* **2018**, *26*, 204020661876129.

(7) Perlíková, P.; Hocek, M. Pyrrolo[2,3- *d*]Pyrimidine (7-Deazapurine) as a Privileged Scaffold in Design of Antitumor and Antiviral Nucleosides. *Med. Res. Rev.* **2017**, *37* (6), 1429–1460.

(8) De Coen, L. M.; Heugebaert, T. S. A.; García, D.; Stevens, C. V. Synthetic Entries to and Biological Activity of Pyrrolopyrimidines. *Chem. Rev.* **2016**, *116* (1), 80–139.

(9) Acs, G.; Reich, E.; Mori, M. BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE ANALOGUE ANTIBIOTIC TUBERCIDIN. *Proc. Natl. Acad. Sci.* **1964**, *52* (2), 493–501.

(10) McCarty, R. M.; Bandarian, V. Biosynthesis of Pyrrolopyrimidines. *Bioorganic Chem.* **2012**, *43*, 15–25.

(11) Suhadolnik, R. J.; Uematsu, T.; Uematsu, H. Toyocamycin: Phosphorylation and Incorporation into RNA and DNA and the Biochemical Properties of the Triphosphate. *Biochim. Biophys. Acta BBA - Nucleic Acids Protein Synth.* **1967**, *149* (1), 41–49.

(12) Brdar, B.; Reich, E. Biochemical and Biological Properties of 5-Bromotubercidin: Differential Effects on Cellular DNA-Directed and Viral RNA-Directed RNA Synthesis. *Bioorg. Med. Chem.* **2008**, *16* (3), 1481–1492.

(13) Nishioka, H.; Sawa, T.; Hamada, M.; Shimura, N.; Imoto, M.; Umezawa, K. Inhibition of Phosphatidylinositol Kinase by Toyocamycin. *J. Antibiot. (Tokyo)* **1990**, *43* (12), 1586–1589.

(14) Loomis, C. R.; Bell, R. M. Sangivamycin, a Nucleoside Analogue, Is a Potent Inhibitor of Protein Kinase C. *J. Biol. Chem.* **1988**, *263* (4), 1682–1692.

(15) Nauš, P.; Pohl, R.; Votruba, I.; Džubák, P.; Hajdúch, M.; Ameral, R.; Birkuš, G.; Wang, T.; Ray, A. S.; Mackman, R.; Cihlar, T.; Hocek, M. 6-(Het)Aryl-7-Deazapurine Ribonucleosides as Novel Potent Cytostatic Agents. *J. Med. Chem.* **2010**, *53* (1), 460–470.

(16) Spáčilová, P.; Nauš, P.; Pohl, R.; Votruba, I.; Snášel, J.; Zábranská, H.; Pichová, I.; Ameral, R.; Birkuš, G.; Cihlář, T.; Hocek, M. CycloSal-Phosphate Pronucleotides of Cytostatic 6-(Het)Aryl-7-Deazapurine Ribonucleosides: Synthesis, Cytostatic Activity, and Inhibition of Adenosine Kinases. *ChemMedChem* **2010**, *5* (8), 1386–1396.

(17) Bourderioux, A.; Nauš, P.; Perlíková, P.; Pohl, R.; Pichová, I.; Votruba, I.; Džubák, P.; Konečný, P.; Hajdúch, M.; Stray, K. M.; Wang, T.; Ray, A. S.; Feng, J. Y.; Birkus, G.; Cihlar, T.; Hocek, M. Synthesis and Significant Cytostatic Activity of 7-Hetaryl-7-Deazaadenosines. *J. Med. Chem.* **2011**, *54* (15), 5498–5507.

(18) Perlíková, P.; Rylová, G.; Nauš, P.; Elbert, T.; Tloušťová, E.; Bourderioux, A.; Slavětínská, L. P.; Motyka, K.; Doležal, D.; Znojek, P.; Nová, A.; Harvanová, M.; Džubák, P.; Šiller, M.; Hlaváč, J.; Hajdúch, M.; Hocek, M. 7-(2-Thienyl)-7-Deazaadenosine (AB61), a New Potent Nucleoside Cytostatic with a Complex Mode of Action. *Mol. Cancer Ther.* **2016**, *15* (5), 922–937.

(19) Snášel, J.; Nauš, P.; Dostál, J.; Hnízda, A.; Fanfrlík, J.; Brynda, J.; Bourderioux, A.; Dušek, M.; Dvořáková, H.; Stolaříková, J.; Zábranská, H.; Pohl, R.; Konečný, P.; Džubák, P.; Votruba, I.; Hajdúch, M.; Řezáčová, P.; Veverka, V.; Hocek, M.; Pichová, I. Structural Basis for Inhibition of Mycobacterial and Human Adenosine Kinase by 7-Substituted 7-(Het)Aryl-7-Deazaadenine Ribonucleosides. *J. Med. Chem.* **2014**, *57* (20), 8268–8279.

(20) Perlíková, P.; Eberlin, L.; Ménová, P.; Raindlová, V.; Slavětínská, L.; Tloušťová, E.; Bahador, G.; Lee, Y.-J.; Hocek, M. Synthesis and Cytostatic and Antiviral Activities of 2'-Deoxy-2',2'-Difluororibo- and 2'-Deoxy-2'-Fluororibonucleosides Derived from 7-(Het)Aryl-7-Deazaadenines. *ChemMedChem* **2013**, *8* (5), 832–846.

(21) Dykstra, K. D.; Ichiishi, N.; Krska, S. W.; Richardson, P. F. Emerging Fluorination Methods in Organic Chemistry Relevant for Life Science Application. In *Fluorine in Life Sciences: Pharmaceuticals, Medicinal Diagnostics, and Agrochemicals*; Elsevier, 2019; pp 1–90.

(22) Tressaud, A. Fluorine, a Key Element for the 21st Century. In *Fluorine*; Elsevier, 2019; pp 77–150.

(23) Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. Metabolism of Fluorine-Containing Drugs. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41* (1), 443–470.

(24) Liu, P.; Sharon, A.; Chu, C. K. Fluorinated Nucleosides: Synthesis and Biological Implication. *J. Fluor. Chem.* **2008**, *129* (9), 743–766.

(25) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the Development of Nucleoside and Nucleotide Analogues for Cancer and Viral Diseases. *Nat. Rev. Drug Discov.* **2013**, *12* (6), 447–464.

(26) Galmarini, C. M.; Mackey, J. R.; Dumontet, C. Nucleoside Analogues: Mechanisms of Drug Resistance and Reversal Strategies. *Leukemia* **2001**, *15* (6), 875–890.

(27) de Sousa Cavalcante, L.; Monteiro, G. Gemcitabine: Metabolism and Molecular Mechanisms of Action, Sensitivity and Chemoresistance in Pancreatic Cancer. *Eur. J. Pharmacol.* **2014**, *741*, 8–16.

(28) Shelton, J.; Lu, X.; Hollenbaugh, J. A.; Cho, J. H.; Amblard, F.; Schinazi, R. F. Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev.* **2016**, *116* (23), 14379–14455.

(29) Parker, W. B.; Gandhi, V. Clofarabine: Structure, Mechanism of Action, and Clinical Pharmacology. In *Chemotherapy for Leukemia*; Ueda, T., Ed.; Springer Singapore: Singapore, 2017; pp 261–286.

(30) Zhenchuk, A.; Lotfi, K.; Juliusson, G.; Albertioni, F. Mechanisms of Anti-Cancer Action and Pharmacology of Clofarabine. *Biochem. Pharmacol.* **2009**, *78* (11), 1351–1359.

(31) Kline, J. P.; Larson, R. A. Clofarabine in the Treatment of Acute Myeloid Leukaemia and Acute Lymphoblastic Leukaemia: A Review. *Expert Opin. Pharmacother.* **2005**, *6* (15), 2711–2718.

(32) Ghanem, H.; Jabbour, E.; Faderl, S.; Ghandhi, V.; Plunkett, W.; Kantarjian, H. Clofarabine in Leukemia. *Expert Rev. Hematol.* **2010**, *3* (1), 15–22.

(33) Cavaliere, A.; Probst, K. C.; Westwell, A. D.; Slusarczyk, M. Fluorinated Nucleosides as an Important Class of Anticancer and Antiviral Agents. *Future Med. Chem.* **2017**, *9* (15), 1809–1833.

(34) Kimberlin, D. W. Antiviral Agents. In *Principles and Practice of Pediatric Infectious Diseases*; Elsevier, 2018; pp 1551-1567.

(35) Burke, L. A.; Marks, K. M. Drugs to Treat Viral Hepatitis. In *Infectious Diseases*; Elsevier, 2017; pp 1327-1332.

(36) Jang, J.-H.; Kim, J.-W.; Jeong, S.-H.; Myung, H.-J.; Kim, H. S.; Park, Y. S.; Lee, S. H.; Hwang, J.-H.; Kim, N.; Lee, D. H. Clevudine for Chronic Hepatitis B: Antiviral Response, Predictors of Response, and Development of Myopathy: Clevudine for CHB. *J. Viral Hepat.* **2011**, *18* (2), 84–90.

(37) Kim, B. K.; Oh, J.; Kwon, S. Y.; Choe, W. H.; Ko, S. Y.; Rhee, K. H.; Seo, T. H.; Lim, S. D.; Lee, C. H. Clevudine Myopathy in Patients with Chronic Hepatitis B. *J. Hepatol.* **2009**, *51* (4), 829–834.

(38) Murakami, E.; Tolstykh, T.; Bao, H.; Niu, C.; Steuer, H. M. M.; Bao, D.; Chang, W.; Espiritu, C.; Bansal, S.; Lam, A. M.; Otto, M. J.; Sofia, M. J.; Furman, P. A. Mechanism of Activation of PSI-7851 and Its Diastereoisomer PSI-7977. *J. Biol. Chem.* **2010**, *285* (45), 34337–34347.

(39) Lam, A. M.; Espiritu, C.; Murakami, E.; Zennou, V.; Bansal, S.; Micolochick Steuer, H. M.; Niu, C.; Keilman, M.; Bao, H.; Bourne, N.; Veselenak, R. L.; Reddy, P. G.; Chang, W.; Du, J.; Nagarathnam, D.; Sofia, M. J.; Otto, M. J.; Furman, P. A. Inhibition of Hepatitis C Virus Replicon RNA Synthesis by PSI-352938, a Cyclic Phosphate Prodrug of β-d-2′-Deoxy-2′-α-Fluoro-2′-β- *C* -Methylguanosine. *Antimicrob. Agents Chemother.* **2011**, *55* (6), 2566–2575.

(40) Ray, A. S.; Vela, J. E.; Boojamra, C. G.; Zhang, L.; Hui, H.; Callebaut, C.; Stray, K.; Lin, K.-Y.; Gao, Y.; Mackman, R. L.; Cihlar, T. Intracellular Metabolism of the Nucleotide Prodrug GS-9131, a Potent Anti-Human Immunodeficiency Virus Agent. *Antimicrob. Agents Chemother.* **2008**, *52* (2), 648–654.

(41) Smee, D. F.; Morris, J. L. B.; Barnard, D. L.; Van Aerschot, A. Selective Inhibition of Arthropod-Borne and Arenaviruses in Vitro by 3′-Fluoro-3′-Deoxyadenosine. *Antiviral Res.* **1992**, *18* (2), 151–162.

(42) Van Aerschot, A.; Herdewijn, P.; Janssen, G.; Cools, M.; De Clercq, E. Synthesis and Antiviral Activity Evaluation of 3'-Fluoro-3'-Deoxyribonucleosides: Broad-Spectrum Antiviral Activity of 3'-Fluoro-3'-Deoxyadenosine. *Antiviral Res.* **1989**, *12* (3), 133–150.

(43) Mikhailopulo, I. A.; Poopeiko, N. E.; Prikota, 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), 2195–2202.

(44) Plagemann, P. G. W.; Wohlhueter, R. M.; Woffendin, C. Nucleoside and Nucleobase Transport in Animal Cells. *Biochim. Biophys. Acta BBA - Rev. Biomembr.* **1988**, *947* (3), 405–443.

(45) 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), 139–141.

(46) Vorbrüggen Glycosylation: (Vorbrüggen Reaction, Vorbrüggen Glycosidation, Vorbrüggen Coupling). In *Comprehensive Organic Name Reactions and Reagents*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2010.

(47) Vorbrüggen, H.; Ruh-Pohlenz, C. Synthesis Of Nucleosides. In *Organic Reactions*; John Wiley & Sons, Inc., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 1999; pp 1–630.

(48) Sun, J.; Dou, Y.; Ding, H.; Yang, R.; Sun, Q.; Xiao, Q. First Total Synthesis of a Naturally Occurring Iodinated 5'-Deoxyxylofuranosyl Marine Nucleoside. *Mar. Drugs* **2012**, *10* (4), 881–889.

(49) Ugarkar, B. G.; DaRe, J. M.; Kopcho, J. J.; Browne, C. E.; Schanzer, J. M.; Wiesner, J. B.; Erion, M. D. Adenosine Kinase Inhibitors. 1. Synthesis, Enzyme Inhibition, and Antiseizure Activity of 5-Iodotubercidin Analogues. *J. Med. Chem.* **2000**, *43* (15), 2883–2893.

(50) Downey, A. M.; Pohl, R.; Roithová, J.; Hocek, M. Synthesis of Nucleosides through Direct Glycosylation of Nucleobases with 5-O-Monoprotected or 5-Modified Ribose: Improved Protocol, Scope, and Mechanism. *Chem. Weinh. Bergstr. Ger.* **2017**, *23* (16), 3910–3917.

(51) Downey, A. M.; Pohl, R.; Roithová, J.; Hocek, M. Synthesis of Nucleosides through Direct Glycosylation of Nucleobases with 5- *O* -Monoprotected or 5-Modified Ribose: Improved Protocol, Scope, and Mechanism. *Chem. - Eur. J.* **2017**, *23* (16), 3910–3917.

(52) Pankiewicz, K. W. Fluorinated Nucleosides. *Carbohydr. Res.* **2000**, *327* (1), 87–105.

(53) Reichman, U.; Watanabe, K. A.; Fox, J. J. A Practical Synthesis of 2-Deoxy-2-Fluoro-d-Arabinofuranose Derivatives. *Carbohydr. Res.* **1975**, *42* (2), 233–240.

(54) Elzagheid, M. I.; Viazovkina, E.; Damha, M. J. Synthesis of Protected 2′-Deoxy-2′-Fluoro-β-D-Arabinonucleosides. *Curr. Protoc. Nucleic Acid Chem.* **2002**, *10* (1), 1.7.1-1.7.19.

(55) *Organo-Fluorine Compounds*, 4th ed.; Baasner, Hagemann, Tatlow, Eds.; Georg Thieme Verlag: Stuttgart, 1998.

(56) Baudoux, J.; Cahard, D. Electrophilic Fluorination with N-F Reagents. In *Organic Reactions*; John Wiley & Sons, Inc., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2008; pp 1–326.

(57) Welch, J. T.; Eswarakrishnan, S. *Fluorine in Bioorganic Chemistry*; Wiley: New York, 1991.

(58) Levy, S.; Livni, E.; Elmaleh, D.; Curatolo, W. Direct Displacement with Anhydrous Fluoride of the C-2 Trifluoromethanesulphonate of Methyl 4,6-O-Benzylidene-3-O-Methyl-2-O-Trifluoromethylsulphonyl-β- D -Mannopyranoside. *J Chem Soc Chem Commun* **1982**, *0* (17), 972–973.

(59) Haradahira, T.; Maeda, M.; Kai, Y.; Omae, H.; Kojima, M. Improved Synthesis of 2-Deoxy-2-Fluoro-D-Glucose Using Fluoride Ion. *Chem. Pharm. Bull. (Tokyo)* **1985**, *33* (1), 165–172.

(60) Card, P. J. Fluorinated Carbohydrates. Use of DAST in the Synthesis of Fluorinated Sugars. *J. Org. Chem.* **1983**, *48* (3), 393–395.

(61) Denavit, V.; Lainé, D.; Bouzriba, C.; Shanina, E.; Gillon, É.; Fortin, S.; Rademacher, C.; Imberty, A.; Giguère, D. Stereoselective Synthesis of Fluorinated Galactopyranosides as Potential Molecular Probes for Galactophilic Proteins: Assessment of Monofluorogalactoside–LecA Interactions. *Chem. – Eur. J.* **2019**, *25* (17), 4478–4490.

(62) Yan, N.; Lei, Z.-W.; Su, J.-K.; Liao, W.-L.; Hu, X.-G. Ring Opening of Sugar-Derived Epoxides by TBAF/KHF 2 : An Attractive Alternative for the Introduction of Fluorine into the Carbohydrate Scaffold. *Chin. Chem. Lett.* **2017**, *28* (2), 467–470.

(63) Kasuya, M. C.; Ito, A.; Hatanaka, K. Simple and Convenient Synthesis of a Fluorinated GM4 Analogue. *J. Fluor. Chem.* **2007**, *128* (5), 562–565.

(64) Mikhailopulo, I. A.; Sivets, G. G. A Novel Route for the Synthesis of Deoxy Fluoro Sugars and Nucleosides. *Helv. Chim. Acta* **1999**, *82* (11), 2052–2065.

(65) Hansske, F.; Madej, D.; Robins, M. J. 2’ And 3’-Ketonucleosides and Their Arabino and Xylo Reduction Products: Convenient Access via Selective Protection and Oxidation of Ribonucleosides. *Tetrahedron* **1984**, *40* (1), 125–135.

(66) Song, Y.; Ding, H.; Dou, Y.; Yang, R.; Sun, Q.; Xiao, Q.; Ju, Y. Efficient and Practical Synthesis of 5′-Deoxytubercidin and Its Analogues via Vorbrüggen Glycosylation. *Synthesis* **2011**, *2011* (09), 1442–1446.

(67) De, S.; De Jonghe, S.; Herdewijn, P. Synthesis of a 3′-Fluoro-3′-Deoxytetrose Adenine Phosphonate. *J. Org. Chem.* **2017**, *82* (18), 9464–9478.

(68) Krzeminski, J.; Nawrot, B.; Pankiewicz, K. W.; Watanabe, K. A. Synthesis of 9-(2-Deoxy-2-Fukuoro-β-D- Abinoruranosyl)Hypoxhine. The First Direct Introduction of A 2′-β-Fldoro Substituent in Ppepormed Purine Nucleosides. Studies Directed Toward the Synthesis of 2′-DEOXY-2′-Substituted Arabppmocebosides. 8. [1]. *Nucleosides Nucleotides* **1991**, *10* (4), 781–798.

(69) Pankiewicz, K. W.; Krzeminski, J.; Ciszewski, L. A.; Ren, W. Y.; Watanabe, K. A. A Synthesis of 9-(2-Deoxy-2-Fluoro-.Beta.-D-Arabinofuranosyl)Adenine and -Hypoxanthine. An Effect of C3'-Endo to C2'-Endo Conformational Shift on the Reaction Course of 2'-Hydroxyl Group with DAST. *J. Org. Chem.* **1992**, *57* (2), 553–559.

(70) Tewson, T. J.; Welch, M. J. New Approaches to the Synthesis of 3-Deoxy-3-Fluoro-D-Glucose. *J. Org. Chem.* **1978**, *43* (6), 1090–1092.

(71) Fleckenstein, C. A.; Plenio, H. Efficient Suzuki−Miyaura Coupling of (Hetero)Aryl Chlorides with Thiophene- and Furanboronic Acids in Aqueous n-Butanol. *J. Org. Chem.* **2008**, *73* (8), 3236–3244.

(72) Arzel, L.; Dubreuil, D.; Dénès, F.; Silvestre, V.; Mathé-Allainmat, M.; Lebreton, J. Synthesis of Ribonucleosidic Dimers with an Amide Linkage from D-Xylose. *J. Org. Chem.* **2016**, *81* (22), 10742–10758.

(73) Arzel, L.; Dubreuil, D.; Dénès, F.; Silvestre, V.; Mathé-Allainmat, M.; Lebreton, J. Synthesis of Ribonucleosidic Dimers with an Amide Linkage from D -Xylose. *J. Org. Chem.* **2016**, *81* (22), 10742–10758.

(74) Nomura, M.; Sato, T.; Washinosu, M.; Tanaka, M.; Asao, T.; Shuto, S.; Matsuda, A. Nucleosides and Nucleotides. Part 212: Practical Large-Scale Synthesis of 1-(3-C-Ethynyl-β-d-Ribo-Pentofuranosyl)Cytosine (ECyd), a Potent Antitumor Nucleoside. Isobutyryloxy Group as an Efficient Anomeric Leaving Group in the Vorbrüggen Glycosylation Reaction. *Tetrahedron* **2002**, *58* (7), 1279–1288.

(75) Nowak, I.; Robins, M. J. Synthesis of 3'-Deoxynucleosides with 2-Oxabicyclo[3.1.0]Hexane Sugar Moieties: Addition of Difluorocarbene to a 3',4'-Unsaturated Uridine Derivative and 1,2-Dihydrofurans Derived from d- and l-Xylose1. *J. Org. Chem.* **2007**, *72* (9), 3319–3325.

(76) Yadav, J. S.; Subba Reddy, B. V. A Mild and Selective Cleavage of Trityl Ethers by CBr4–MeOH. *Carbohydr. Res.* **2000**, *329* (4), 885–888.