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Modifikované nukleosidové deriváty pyrimido[4,5-*b*]indolu

Modified nucleosides derived from pyrimido[4,5-*b*]indole

Diplomová práce

Vedoucí závěrečné práce/Školitel: Prof. Ing. Michal Hocek, CSc., DSc.

Praha 2016

Prohlášení:

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

V Praze, 8.5.2016

Podpis

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

Abstract

Syntheses of two series of 2’-sugar-modified pyrimido[4,5-*b*]indole nucleosides were developed. The synthetic strategy was based on functional group transformations of the 2’-hydroxy group of the 3’,5’-protected ribonucleoside. The key intermediate was prepared via stereoselective nucleobase anion glycosylation of the known 4,6-dichloropyrimido[4,5-*b*]indole nucleobase with 2,3-*O*-isopropylidene-5-*O*-TBS-protected halogenose, subsequent deprotection under acidic conditions and protection of 3’- and 5’-hydroxy groups with Markiewicz reagent. Pyrimidoindole arabinonucleoside was then synthesized using a sequence of oxidation-reduction reactions of the 2’-hydroxy stereocenter. The synthesis of pyrimidoindole 2’-deoxy-2’-fluororibonucleoside was achieved by stereoselective S_N2 fluorination of the THP-protected arabinoside followed by acidic deprotection. For the biological activity testing, two series of 4-substituted arabinonucleosides and 2’-deoxy-2’-fluororibonucleosides were synthesized employing nucleophilic substitution or Pd-catalyzed cross-coupling reactions.

Keywords

nucleosides, heterocycles

Abstrakt

Byly vyvinuty syntézy dvou sérií pyrimido[4,5-*b*]indolových nukleosidů modifikovaných v poloze 2'. Syntetický postup byl založen na transformaci 2'-hydroxylové skupiny 3',5'-chráněného ribonukleosidu. Klíčový intermediát byl připraven stereoselektivní glykosylací pomocí aniontu známé 4,6-dichloropyrimido[4,5-*b*]indolové nukleobáze a 2,3-*O*-isopropyliden-5-*O*-TBS-chráněné halogenosy, následované deprotekcí v kyselém prostředí a chráněním 3'- a 5'-hydroxylových skupin Markiewiczovým reagentem. Pyrimidoindolový arabinonukleosid byl připraven sekvencí oxidace-redukce 2'-hydroxy stereogenního centra. Syntéza pyrimidoindolového 2'-deoxy-2'-fluororibonukleosidu byla završena stereoselektivní S_N2 fluorací THP-chráněného arabinosidu a následnou deprotekcí kyselou hydrolýzou. Pro testy biologické aktivity pak byly použitím nukleofilní substituce nebo Pd-katalyzovaných cross-couplingových reakcí připraveny dvě série 4-substituovaných arabinonukleosidů a 2'-deoxy-2'-fluororibonukleosidů.

Klíčová slova

nukleosidy, heterocykly

List of abbreviations

Ac	acetyl
aq.	aqueous
ATP	adenosine 5'-*O*-triphosphate
ATR	attenuated total reflection
Bn	benzyl
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bz	benzoyl
cAMP	cyclic adenosine 3',5'-*O*-monophosphate
CCRF-CEM	human T-lymphoblastoid cell line
cGMP	cyclic guanosine 3',5'-*O*-monophosphate
CoA	coenzyme A
DAST	diethylaminosulfur trifluoride
DCE	1,2-dichloroethane
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMP	Dess–Martin periodinane
DNA	deoxyribonucleic acid
EGFR	epidermal growth factor receptor
eq.	equivalent
FAD	flavin adenine dinucleotide
FDA	U.S. Food and Drug Administration
GTP	guanosine 5'-*O*-triphosphate
HBV	hepatitis B virus
HCV	hepatitis C virus
HeLa S3	human cervix carcinoma cell line
HIV	human immunodeficiency virus
HMPT	tris(dimethylamino)phosphine
HPFC	high performance flash chromatography
IC_{50}	the half maximal inhibitory concentration

IMC	invasive mammary carcinoma
iPr	isopropyl
KOD	*Pyrococcus kodakaraensis* DNA polymerase
L1210	mouse lymphocytic leukemia cell line
LG	leaving group
^{MD}A	methoxybenzodeazaadenine
^{MD}I	methoxybenzodeazainosine
MS	mass spectrometry
MTB	*Mycobacterium tuberculosis*
NAD^+	nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NS5B	nonstructural protein 5B
PE	petroleum ether
PI-3 kinase	phosphatidylinositol-4,5-bisphosphate 3 kinase
PG	protecting group
Ph	phenyl
Pr	propyl
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
r.t.	room temperature
SAM	*S*-adenosyl methionine
SAR	structure-activity relationship
SM	starting material
SNP	single-nucleotide polymorphism
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TDA-1	tris[2-(2-methoxyethoxy)ethyl]amine
TFA	trifluoroacetic acid
THP	tetrahydropyran-2-yl
$TIPDSCl_2$	1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane
TPPTS	3,3’,3’’-phosphanetriyltris(benzenesulfonic acid) trisodium salt
TsOH	*p*-toluenesulfonic acid

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1 Introduction

1.1 Purine nucleosides and their biological function in nature

Purine derivatives play an important role in various biochemical processes and can be found everywhere in nature. Despite the fact that purine (**1**) is the most widely occurring N-heterocycle in nature, its unsubstituted form is not present there.[1] The simplest existing form of purine in nature is the *β*-D-ribonucleoside nebularine (**2**) isolated from the mushroom *Agaricus nebularis* exhibiting antibiotic activity.[2] Purine nucleosides adenosine (**3**) and guanosine (**4**) are building blocks of nucleic acids (DNA and RNA) which are responsible for carrying and expression of genetic information of all living organisms and viruses. Furthermore, many purine derivatives are involved in numerous metabolic pathways. Adenosine 5'-*O*-triphosphate (ATP) is an energy rich compound used for transport and storage of chemical energy in many cellular processes and it also functions as a substrate for kinases, enzymes which have a crucial role in signal transmission and regulation of different processes in cells. Guanosine 5'-*O*-triphosphate (GTP) activates the G alpha subunit of the G-protein-coupled receptors in eukaryotic cell membranes. In signal transduction cascades, second messengers cyclic adenosine 3',5'-*O*-monophosphate (cAMP) and cyclic guanosine 3',5'-*O*-monophosphate (cGMP) trigger different physiological processes via binding induced activation of corresponding protein kinases. And some adenosine derivatives such as flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$), coenzyme A (CoA) and *S*-adenosyl methionine (SAM) participate in many enzymatic reactions as essential cofactors. Moreover, adenosine receptors can be found in all organs of the human body and are targets of importance in the research of purine therapeutic agents.[3]

This shows that purine analogs have potential to interfere with cellular mechanisms, and therefore, design and development of novel pharmaceutically active purine derivatives has been of great interest to the field of medicinal and pharmaceutical chemistry. And for new biologically active compounds, naturally occurring substances usually function as lead structures.

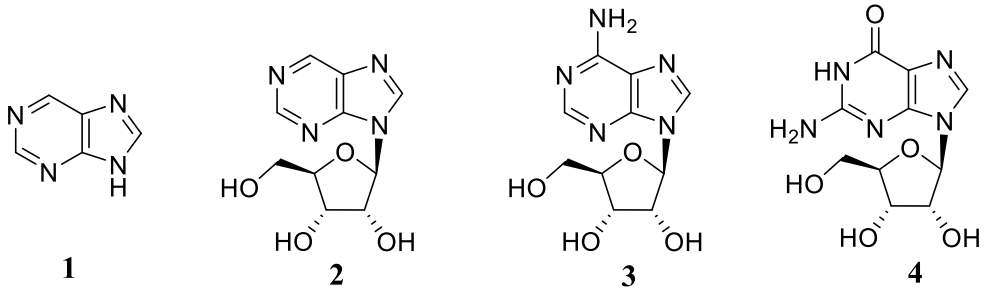


Figure 1 Purine (**1**) and some examples of its nucleoside derivatives **2**–**4**

1.1.1 Biologically active natural purine and 7-deazapurine nucleosides

There are few mentionable examples of purine nucleoside analogs to be found in nature possessing interesting biological activities. In plants, cytokines are a group of hormones, which regulate many cellular processes.[4] It has been shown that their ribosides exhibit anticancer activity both *in vitro* and *in vivo* against a broad panel of human cancer cell lines, with *ortho*-topolin riboside (**5**) being the most active with IC_{50} value of 0.5–11.6 μM.[5] Spongosine (**6**) was first isolated from demosponge *Cryptotethya crypta*[6] and its triphosphate form is known as an inhibitor of DNA-polymerases.[1] Arabino analog of adenosine, vidarabine (**7**), is active against herpes simplex virus encephalitis and herpes zoster virus infections.[7] This nucleoside was first synthesized in 1960[8] prior to its isolation from *Streptomyces herbaceous*.[9] Poor solubility of vidarabine in water decreases its potential usefulness, however, this problem has been solved by using its soluble 5'-*O*-monophospate form.[7] In combination with 2'-deoxycoformycin (**8**), it was reported as an effective agent in the clinical treatment of acute monocytic leukemia.[10]

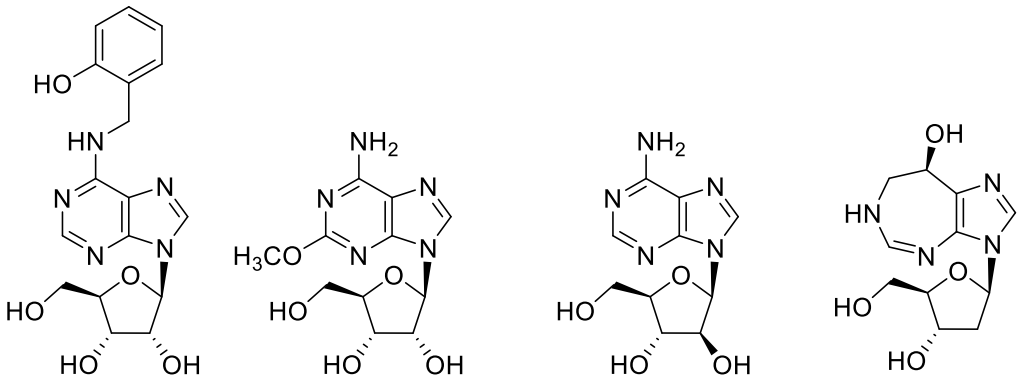


Figure 2 Naturally occurring purine analogs **5**–**8**

Replacement of one of the nitrogen atoms by carbon atom in purine nucleosides leads to another important class of compounds titled deazapurine nucleosides. This simple modification offers an extra valence for the introduction of various substituents and functional groups and therefore gives a rise to many different classes of biologically active compounds. In the next section, some examples of natural 7-dezapurine[11] products with biological significance are given.

7-Deaza analog of adenosine, tubercidin (**9**), is a natural antibiotic obtained from culture filtrates of *Streptomyces tubercidius*.[12] Among antibacterial, tubercidin has also exhibited antiviral activities against vaccina (DNA-virus), Reovirus III and Mengovirus (RNA-viruses).[13] It inhibits the growth of *Mycobacterium tuberculosis* and has shown antifungal activity against *Candida albicans*.[14] Tubercidin has also displayed antiproliferative effects, which are explained by its incorporation into nucleic acids (both DNA and RNA) that leads to the inhibition of multiple metabolic processes.[13] More specific in their biological activity are toyocamycin (**10**) and sangivamycin (**11**), the C-5 analogs of tubercidin (**9**), both produced by different species of *Streptomyces* bacteria.[15] Toyocamycin (**10**) possesses cytostatic activity against variety of human cancers,[16] but its ability to inhibit viral RNA replication in some viruses including murine retrovirus, adenovirus and vesicular stomatitis virus was proved to be more significant.[17] Moreover, it is also known inhibitor of PI-3 kinase,[18] a regulatory protein that controls key cell-functions such as cell growth, proliferation and differentiation. On the other hand, the antitumor effect and mechanism of action of sangivamycin (**11**) is via inhibition of protein kinase C, which is a transducer of

extracellular signals.[19] In addition, cadeguomycin (**12**), 7-deaza derivative of guanosine, was isolated together with tubercidin from *Streptomyces hygroscopicus*.[20] This nucleoside antibiotic inhibited growth of subcutaneous solid IMC carcinoma and pulmonary metastasis of Lewis lung carcinoma in mice.[21]

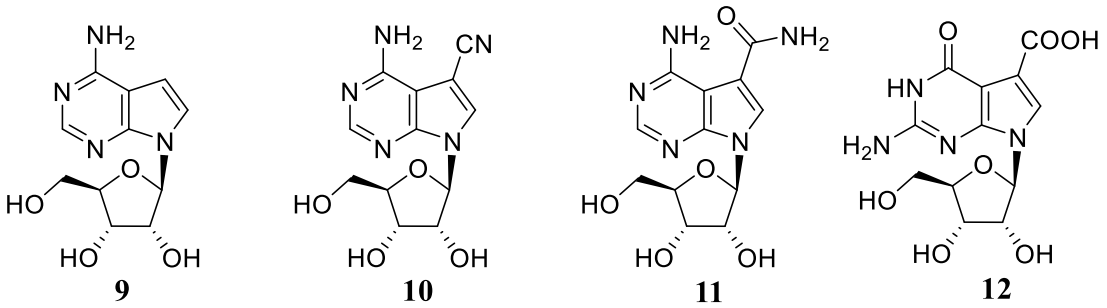


Figure 3 Biologically active natural 7-deazapurine nucleosides **9**–**12**

Although natural 7-deazapurines possess broad range of interesting features, they often suffer from high toxicity, which makes them not suitable for use in clinical practice. Nevertheless, they remain an inspiration in the design and synthesis of novel types of nucleoside analogs leading to substances with improved biological effects.

1.2 Synthetic analogs of nucleosides

During the extensive study of purine nucleosides and their analogs over the past decades, a number of active compounds with significant antiviral and antitumor properties has been discovered.[22,23] Few examples are given in **Figure 4**. Didanosine (**13**) and entecavir (**14**) belong to a group of antiretroviral drugs known as nucleoside reverse-transcriptase inhibitors and are being used in the treatment of viral infections such as HIV and HBV, respectively. Some purine derivatives are used as clinical therapeutics in the treatment of various types of leukemia (clofarabine (**15**) and cladribine (**16**)). Despite systematic research, there still remains a space for the design and development of novel types of nucleoside-based

therapeutics, mainly to overcome issues like resistance, poor oral bioavailability or long-term toxicity of the administered ones.[24]

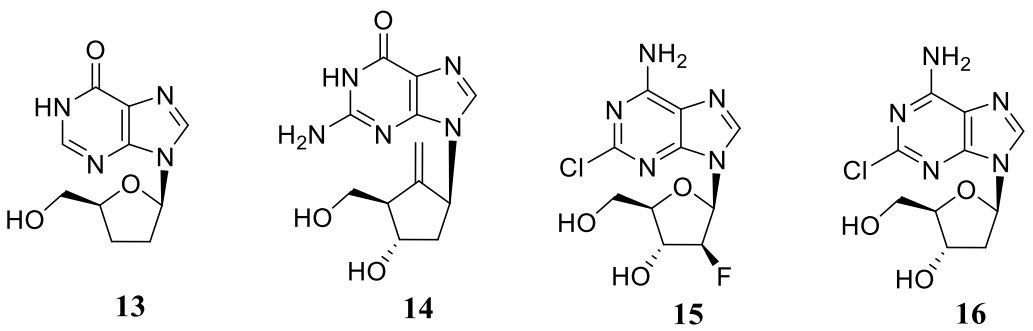
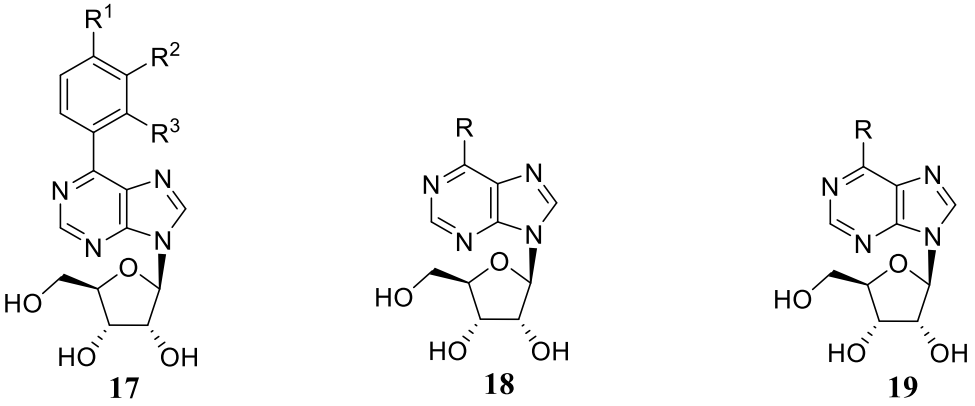


Figure 4 Clinically used synthetic purine analogs **13**–**16**

1.2.1 Purine and 7-deazapurine nucleosides developed in our laboratory

Biological activities of modified purine and 7-deazapurine nucleobases and nucleosides have been systematically studied in our research group for almost two decades. During these years, several classes of purine nucleosides analogs with interesting antiviral or cytostatic features have been discovered.

In the year 2000, a series of 6-phenylpurine nucleosides **17** was prepared and some of the title compounds exerted micromolar cytostatic activity in CCRF-CEM, HeLa S3, and L1210 cell lines.[25] Additionally, their sugar-modified (5'-deoxyribo-, 2'-deoxyribo- and 3'-deoxyribonucleoside) analogs were prepared but none of them has shown any considerable cytostatic activity.[26] Introduction of any substituent to position 2 or 8 of 6-phenylpurine ribonucleosides **17** led to the loss of their cytostatic activity.[27] The loss of the activity was also observed by their L-ribonucleoside analogs.[28] In another work, structure-activity relationship study led to a discovery of 6-hetarylpurine nucleosides **18**, which showed higher activity in CCRF-CEM and L1210 cell lines than parent nucleosides **17**.[29] This 6-hetaryl series was later extended and all the compounds were tested for cytostatic and anti-HCV activity.[30] The most active in this series were nucleosides **19** bearing simple five-membered heterocyclic substituents exerting sub-micromolar anti-HCV activity, accompanied by significant inhibition of cellular rRNA.



R^1, R^2, R^3 = H, F, Me, OMe, OEt, Cl

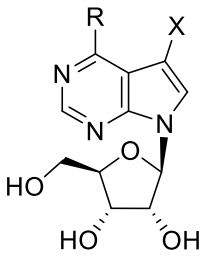
R = pyridin-2-yl, furan-2-yl, thiophen-2-yl

R = furan-2-yl, pyrrol-3-yl

Figure 5 Biologically active 6-substituted purine nucleosides **17**–**19**

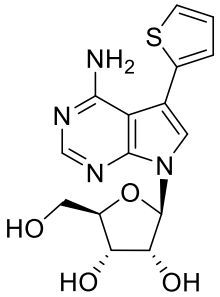
Extensive study of 7-deazapurine nucleosides in our laboratory has revealed few classes of compounds with interesting biological properties. 6-Hetaryl-7-dezapurine ribonucleosides have been introduced as the first class of novel potent cytostatic agents.[31] The highest activities were observed with 7-H or 7-F derivatives of 6-furyl- or 6-thienyl-7-deazapurines **20**, which have displayed low nanomolar cytostatic effects with the potency comparable to known cytostatic clofarabine (**15**).[32] In following works,[33] their *cyclo*Sal-phosphate and phosphoramidate prodrugs were prepared. Unfotunately, they were found to be less active than parent ribonucleosides due to increased efflux from cells. Similarly high antiproliferative effects were displayed by the class of analogs of natural cytostatic tubercidin (**9**).[34] 2-Thienyl derivative (AB61) **21** appeared to be the most promising compound of this series with high cytotoxicity against leukemic and solid tumor cancer cell lines and without any cytoxicity against normal human fibroblasts. In more specific preclinical studies, the detailed mechanism of action of AB61 (**21**) was investigated together with its *in vivo* antitumor activity against xenotransplanted human solid tumors in mice.[35] It has been found that selectivity of AB61 (**21**) is caused by its more efficient phosphorylation in the leukemic CCRF-CEM cell line than in normal human fibroblast. AB61 triphosphate is then incorporated into both DNA and RNA where it induces damage of DNA and inhibits protein translation/folding machinery. Series of 7-hetaryl-7-deazapurine has been later extended with various substituents added at position 6.[36] In this series,

7-deazahypoxanthine derivatives and 2-substituted derivatives were completely inactive and only several 6-substituted 7-(het)aryl- or 7-ethynyl-7-deazapurine ribonucleosides **22** have displayed nanomolar cytostatic effects similar to those obtained in 7-hetaryl-7-deazadenosine series. All three classes of compounds **20**–**22** have also displayed high but nonspecific antiviral activity against HCV.[31,34,36]



20

X = H or F
R = furan-2-yl, furan-3-yl, thiophen-2-yl



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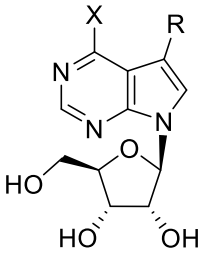
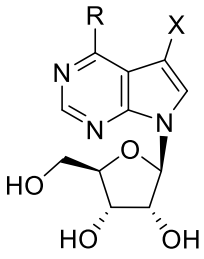


22

X = OMe, SMe, Me, NHMe or NMe_2
R = furan-2-yl or ethynyl

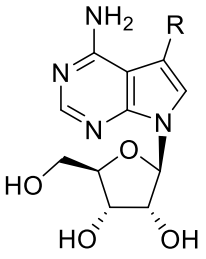
Figure 6 Cytostatic 7-deazapurine nucleosides **20**–**22**

Moreover, some of 6-substituted 7-dezapurine nucleosides **23** and 7-substituted 7-deazaadenosine nucleosides **24** with bulky substituents were evaluated as strong and selective *in vitro* inhibitors of either human or MTB adenosine kinase and therefore were found to be potent antimycobacterial agents.[37] Additionally, some 2,6-disubstituted 7-deazapurine ribonucleosides **25** were reported also as potent and selective inhibitors of MTB adenosine kinase.[38]



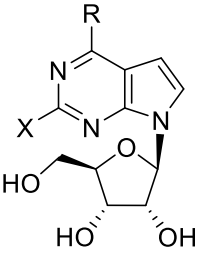
23

X = H or F
R = Me, (het)aryl



24

R = ethynyl, *trans*-2-phenylvinyl, dibenzofuran-4-yl, 2-naphtyl, 4-phenoxathiine



25

X = Cl or F
R = thiophen-3-yl, phenyl, benzofuran-2-yl

Figure 7 Antimycobacterial 7-deazapurine nucleosides **23**–**25**

These studies have shown that modifications at position 7 can significantly affect the biological activity of 7-dezapurine nucleosides. Inspired by this fact and knowing that there is still some more space for further derivatization, pyrimido[4,5-*b*]indole ribonucleosides were designed and synthesized.

1.2.2 Pyrimido[4,5-b]indole nucleobases and nucleosides

Pyrimidoindole nucleobases can be described as benzo-fused analogs of 7-deazapurines and their first synthesis dates back to 1972.[39] Later, their interesting biological properties have been discovered and studied by various research groups.[40–47] Some pyrimidoindoles have been reported as A1-adenosine receptor antagonists,[40] whereas other ones inhibited EGFR tyrosine kinase.[41,42] Zaware *et al.* reported pyrimidoindole derivative **26** as a strong inhibitor of *Toxoplasma gondii* thymidylate synthase, which is an enzyme crucial for nucleotide synthesis.[43] Compound **26** has shown high 122-fold selectivity for *Toxoplasma gondii* thymidylate synthase over human thymidylate synthase. The same laboratory has introduced **27** as an antimitotic agent[44] or **28** as a vascular endothelial growth factor receptor-2 inhibitor in solid tumors.[45] Moreover, 5-[(4-methylphenyl)thio]-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (**29**) was recognized as a substance that combines vascular endothelial growth factor receptor-2 inhibitory activity and platelet-derived growth factor receptor-β inhibitory activity along with inhibition of human thymidylate synthase and thus affords unique chemotherapeutic potential in a single agent.[46] In preclinical studies, this agent *in vitro* induced rapid cellular necrosis through the mitochondria and *in vivo* inhibited tumor growth and reduced lung metastases in two breast cancer mouse models better than clinically used docetaxel.[47]

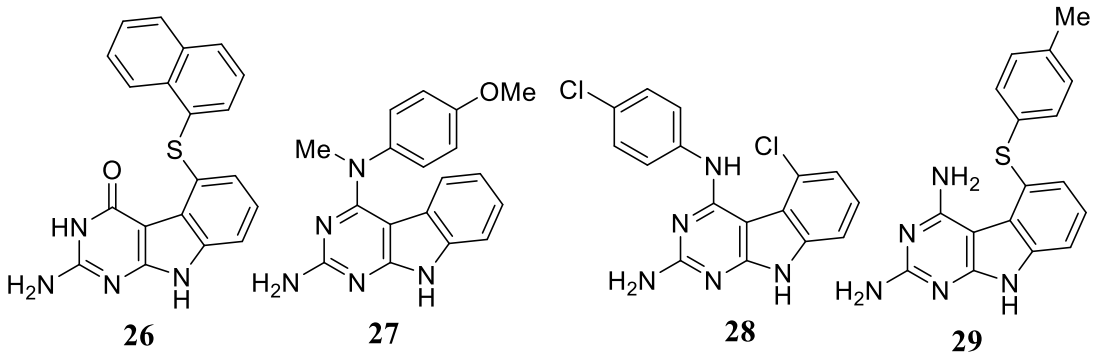


Figure 8 Some examples of pyrimidoindoles **26**–**29**

During the years 2003–2006, Saito's group reported synthesis and few biochemical applications of 2'-deoxypyrimidoindole ribonucleosides.[48–51] After incorporation into DNA using DNA synthesizer, methoxybenzodeazaadenine (^{MD}A) (**30**) showed a remarkably high hole transport ability and potential usage as DNA nanowire in the development of bionanomaterials.[48] Later, enzymatic incorporation of ^{MD}A triphosphate into DNA using KOD Dash polymerase was achieved.[49] In other works, ^{MD}A (**30**) and ^{MD}I (**31**) were discovered as new base-discriminating fluorescent nucleosides for the detection of single nucleotide alteration[50] and used for the development of a new photoelectrochemical SNP typing method which uses hole-transporting DNA immobilized on gold electrodes.[51]

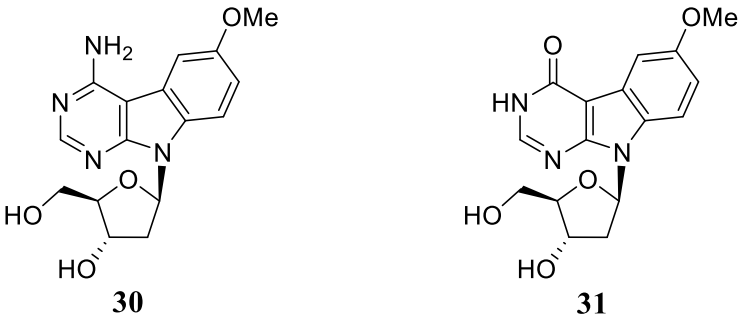
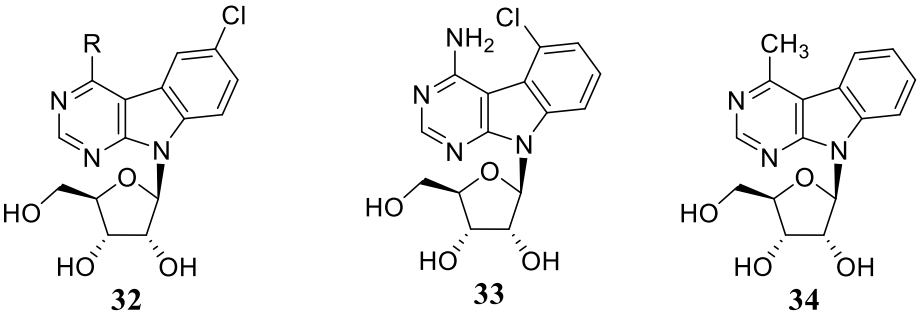


Figure 9 Fluorescent 2'-deoxyribonucleosides **30** and **31**

In order to complement the SAR of modified purine and deazapurine nucleosides in our group, pyrimido[4,5-*b*]indole ribonucleosides were synthesized and their biological activities were evaluated.[52,53] From the first class of

pyrimido[4,5-*b*]indole ribonucleosides, derivatives **32** exerted submicromolar anti-Dengue virus activities.[52] In the related work,[53] compounds **33** and **34** displaying antiviral activity against HCV and Dengue virus were discovered. The antiviral activity of 4-amino derivative **33** was unfortunately accompanied by cytotoxicity, but 4-methyl nucleoside **34** has shown only low cytotoxicity. Compared to 7-deazapurine ribonucleosides **20**–**22** (**Figure 6**), more specific anti-HCV activity but no significant activity against leukemia or cancer cell lines was observed in this class of base-modified ribonucleosides. These results show that further derivatization can raise the selectivity towards RNA-viruses and reduce the cytotoxicity of the parent 7-deazapurine ribonucleosides.



R = furan-2-yl, thiophen-2-yl, benzofuran-2-yl

Figure 10 Pyrimidoindole ribonucleosides **32**–**34** with antiviral activity

1.2.3 Sugar-modified nucleosides

The ribose moiety of the nucleoside offers numerous possibilities for further derivatization of these biologically active molecules and its modification is frequently used in the discovery of novel nucleoside analogs. Replacement of hydroxy groups or other atoms of the furanose ring, addition of various functional groups and atoms or simple inversion of configuration of stereogenic centers often leads to compounds with improved clinical features like higher antitumor activity, better selectivity towards target proteins or lower cytotoxicity. In nature, modification of the 2'-position of sugar part of the nucleosides causes big difference in the structure and functionality of DNA and RNA, which are main

targets in the development of novel antiviral or cytostatic agents. Therefore, some examples of bioactive 2'-modified nucleosides will be discussed in the next section.

Arabinonucleosides are considered as 2'-deoxyribonucleoside analogs and two of their purine derivatives have been approved for the treatment of cancer.[23] 9-[*β*-D-Arabinofuranosyl]-2-fluoroadenine (fludarabine) (**35**) was first synthesized in 1969[54] and later its cytostatic properties were discovered.[55] However, due to its poor solubility in water, fludarabine is clinically used as a 5'-*O*-monophosphate prodrug **36** and is effective in the treatment of chronic lymphocytic leukemia.[56] The mechanism of action of fludarabine starts with its dephosphorylation to the free nucleoside which is then transported into cells where it is phosphorylated to the triphosphate and incorporated into both DNA and RNA. This leads to the inhibition of nucleic acid synthesis.[57] In enzyme assays, it has been shown that triphoshate of **35** also inhibits DNA polymerase and ribonucleotide reductase.[58] Furthermore, fludarabine has also demonstrated effectivity in the therapy in hairy cell leukemia.[59] Nelarabine (**38**), an arabino analog of guanosine (**4**), was approved by FDA for the treatment of T-cell acute lymphoblastic leukemia.[60] This more water-soluble prodrug is first demethylated by adenosine deaminase[61] to form cytotoxic arabinosyl guanine (**37**).[62] After intracellular phosphorylation, which occurs preferentially in T-cells rather than in B-cells,[63] arabinosyl guanine triphoshate is formed and its incorporation into DNA by DNA polymerase leads to the apoptosis of T-lymphoblastic cells.[64]

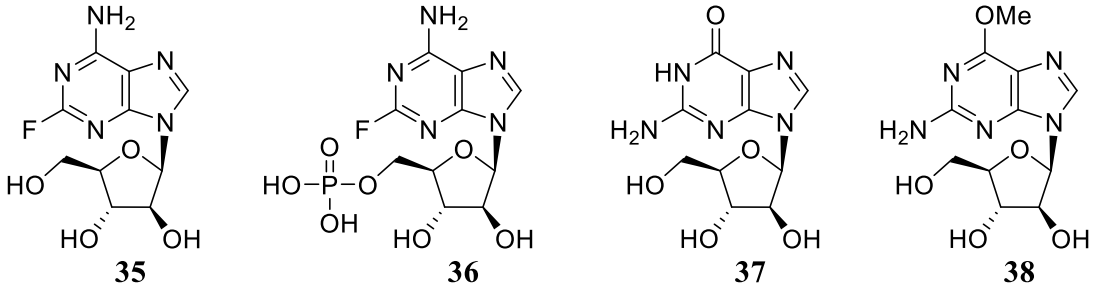


Figure 11 Cytotoxic arabinonucleosides **35**, **37** and their clinically used prodrugs **36**, **38**

In medicinal chemistry, introduction of a fluorine atom into a molecule of a nucleoside is often an important strategy used to improve biological and pharmacokinetic properties of these bioactive compounds. The special nature of fluorine is based on its unique characteristics.[65] Fluorine is a small and electronegative atom, which can mimic hydrogen atom or hydroxy group, as it is also a hydrogen bond acceptor. Also, increased C-F bond strength and polarization often leads to dramatic changes in biological activity and more stable substances. There are several fluorinated analogs of ribonucleosides, which have shown significant antiviral activities. From the series of 2'-deoxy-2'-fluororibosides, 2'-deoxy-2'-fluoroguanosine (**39**) displayed the highest anti-influenza activity against influenza strains A and B and *in vivo*, it was more effective for the influenza therapy than ribavirin or amantadine.[66] In *in vitro* assays, Maruyama *et al.* reported two purine 2'-deoxy-2'-fluororibonucleosides **40** as active against HIV-1 virus type.[67] Additionally, pyrimidine analog, 2'-deoxy-2'-fluorocytidine (**41**) possessed micromolar antiviral activity against influenza A virus subtypes (H5N1, H1N1, H3N1)[68] and it was also found to be active against both I and II type of the herpes virus.[69] In another work, **41** has displayed anti-HCV activity, mostly through the inhibition of viral NS5B polymerase.[70]

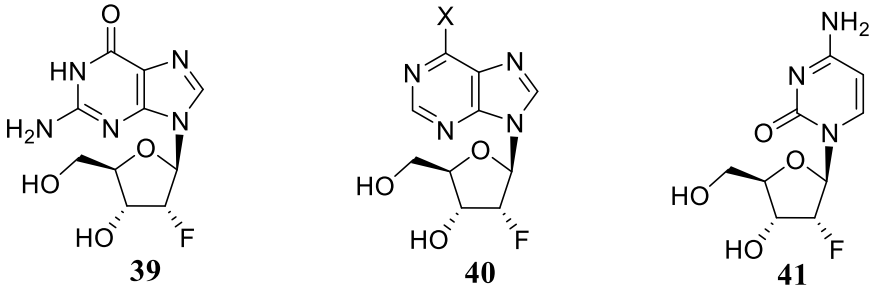
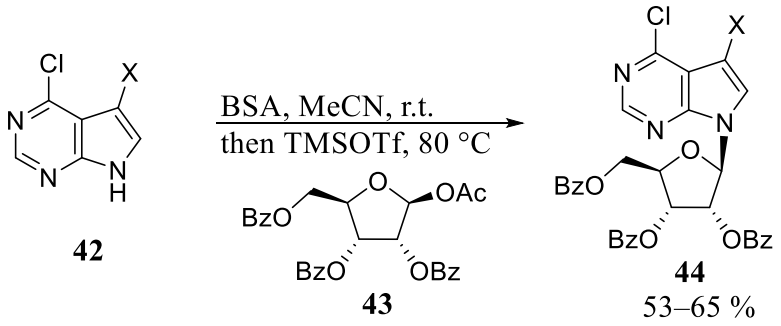


Figure 12 Antiviral 2'-deoxy-2'-fluororibonucleosides **39**–**41**

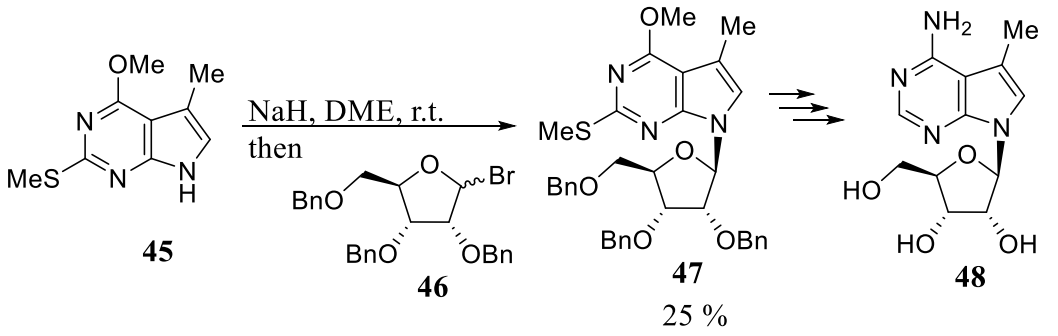
1.3 Synthetic approaches towards modified purine nucleosides

The most important synthetic step in the preparation of novel nucleoside analogs is usually the coupling of the nucleobase and the sugar derivative. There is a need for the stereoselective formation of N-glycosidic bond as *β*-anomers are usually the ones possessing biological activities. Various protocols have been introduced and optimized throughout the years in order to achieve this selectivity. Among them, the most widely used nucleoside forming reaction is the Silyl-Hilbert-Johnson reaction using Vorbrüggen conditions, which were used in the synthesis 7-halogenated 7-deazapurine ribonucleosides **44** (**Scheme 1**).[71] In this one-pot protocol, the nucleobase **42** is first silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in MeCN at r.t. Silylated base then reacts with acyloxonium ion of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**43**)**,** which is formed in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the Lewis acid catalyst. Nucleophilic attack of the silylated nucleobase is then forced from the opposite, *β*-face of the molecule according to Baker's trans rule which leads exclusively to *β*-nucleoside.[72]



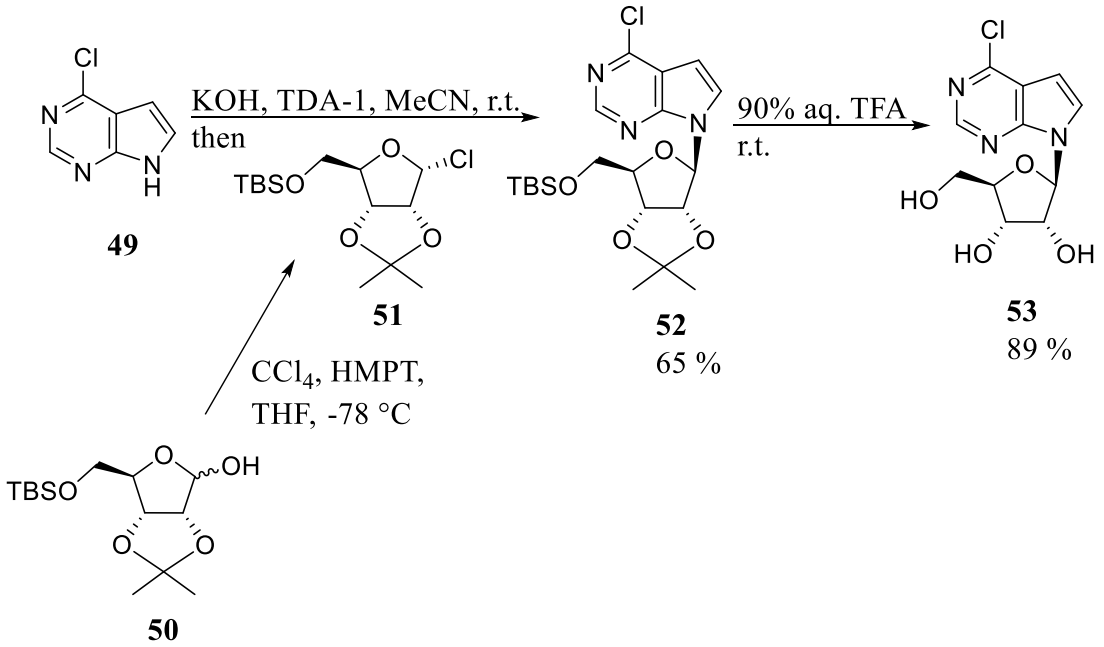
Scheme 1 Synthesis of 7-deazapurine nucleosides **44** using Vorbrüggen reaction

Another frequently used reaction in the synthesis of nucleosides is the nucleobase anion glycosylation. In the sodium salt procedure, the nucleobase anion is generated in the presence of NaH and coupled with protected sugar derivative. Glycosylation of the anion of nucleobase **45** with bromo sugar **46** was used in the synthesis of 5-methyltubercidin (**48**) and gave the desired *β*-nucleoside **47** in a 25% yield together with its *α*-anomer.[73]



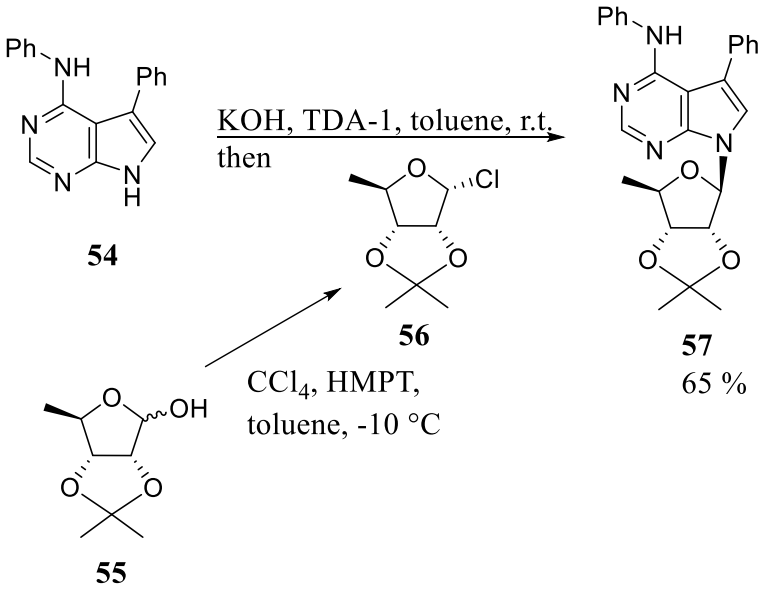
Scheme 2 Nucleobase anion glycosylation using sodium salt procedure

The nucleobase anion glycosylation under solid-liquid conditions has been extensively studied in Seela's laboratory and this method was used in the synthesis of tubercidin and its derivatives.[74] In this protocol, the anion of the nucleobase **49** is prepared using powdered KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as a phase-transfer catalyst, which increases the nucleophilicity of the nucleobase anion by complexation of the potassium cation. To this solution in MeCN, the freshly prepared solution of *α*-D-halogenose **51** in THF is added. The synthesis of anomerically pure halogenose **51** from lactol **50** by Appel's chlorination was previously reported by Wilcox *et al.*[75] Treatment of protected nucleoside **52** with aqueous TFA (90 % *v*/*v*) affords the free nucleoside **53**.



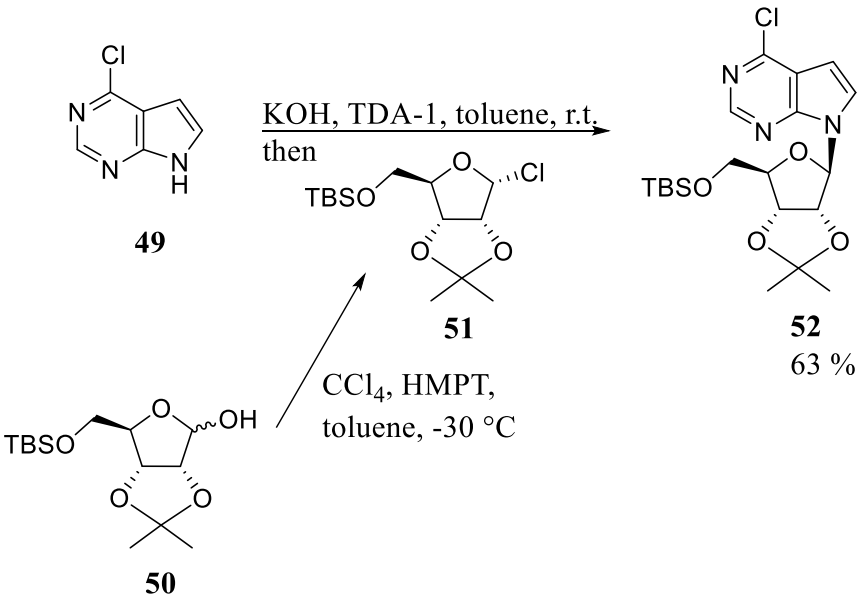
Scheme 3 Nucleobase anion glycosylation under solid-liquid conditions

In order to improve poor yields for the glycosylation of various nucleobases with different α-chloro sugars, modified conditions for this procedure were developed during the studies of adenosine kinase inhibitors by Ugarkar *et al.*[76] Investigation of different conditions has shown that the chlorination of protected sugar **55** proceeds well in toluene at higher temperature (-10 °C) compared to chlorination in THF (-78 °C). Also, washing the reaction mixture with an ice-cold brine and drying over $MgSO_4$ prolonged the half-life of the unstable α-halogenose **56** to >48 hours at ≤4 °C. Toluene was also used as a solvent for the glycosylation of **54** with 1 eq. of TDA-1, 2 eq. of KOH and 2 eq. of sugar **56**, the yield of the desired nucleoside **57** was raised from 30–35 % to 65 % and the yield of the unwanted α-nucleoside was reduced to trace amounts only.



Scheme 4 Optimized nucleobase anion glycosylation procedure

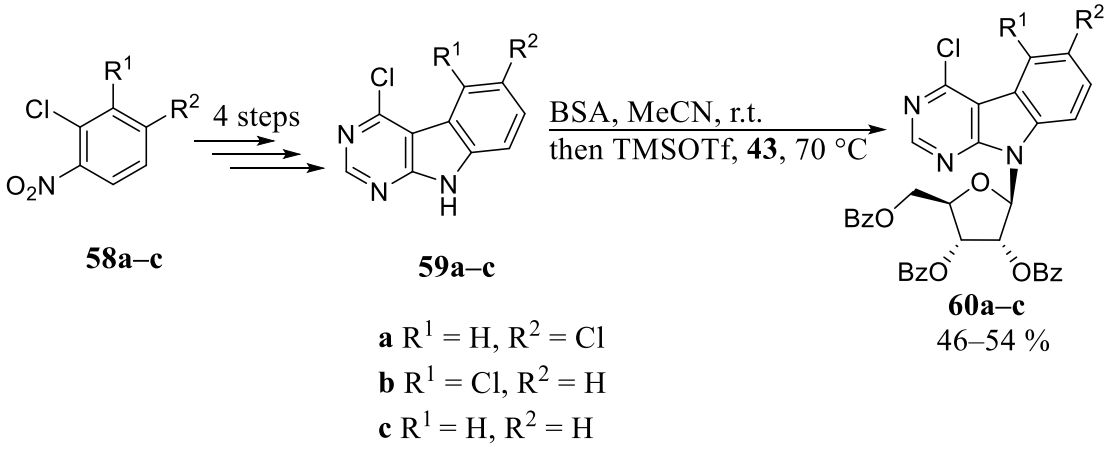
When this method was applied for the synthesis of a series of 6-substituted 7-deazapurine nucleosides, the protected nucleoside **52** was prepared by the glycosylation of 7-deazapurine **49** with α-halogenose **51** in 63% yield as a single β-anomer.[31]



Scheme 5 Ugarkar's conditions in the synthesis of 7-deazapurine ribonucleosides

1.3.1 Synthesis of pyrimido[4,5-b]indole ribonucleosides

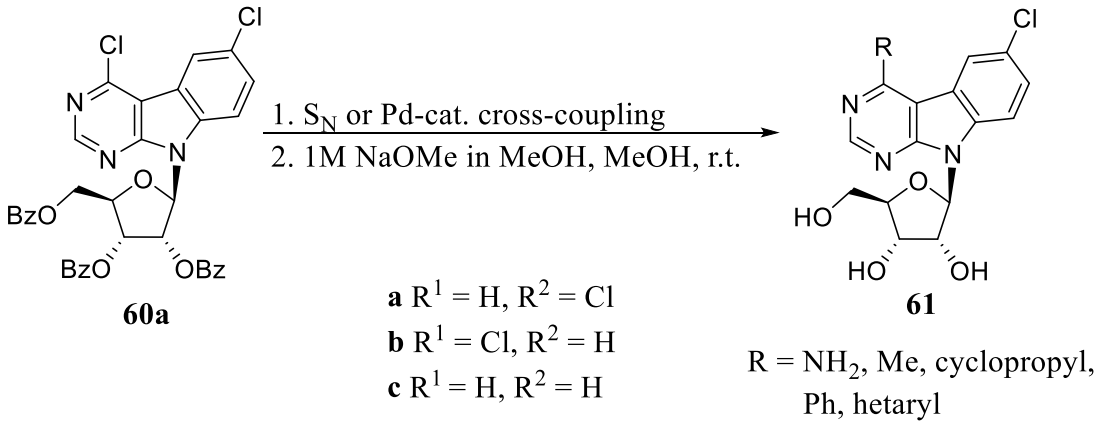
The synthesis of pyrimido[4,5-*b*]indole ribonucleosides **60a–c**, reported by Tichý *et al.*, was achieved using one-pot Vorbrüggen conditions.[52,53] First, the corresponding heterocyclic nucleobases **59a–c** were constructed from commercially available chloronitrobenzenes **58a–c**. Nucleobases **59a–c** were then silylated with BSA for 15 min at r.t. and reacted with protected *β*-ribofuranose **43** and TMSOTf in MeCN at 70 °C for 8 hours. This resulted in the formation of protected *β*-ribonucleosides **60a–c** in yields around 50 %.



a R^1 = H, R^2 = Cl
b R^1 = Cl, R^2 = H
c R^1 = H, R^2 = H

Scheme 6 Synthesis of protected pyrimido[4,5-*b*]indole ribonucleosides **60a–c**

Various substituents were introduced to the position 4 of benzoylated chloropyrimidoindole nucleosides **60a** employing nucleophilic substitution or Pd-catalyzed cross-coupling reactions. 4-Phenyl and 4-hetaryl derivatives were prepared by Suzuki-Miyaura or Stille cross-coupling, 4-methyl derivative was prepared by Pd-catalyzed alkylation with trimethylaluminium and cyclopropyl group was introduced by Negishi reaction. The deprotection of nucleosides was then performed under Zemplén deacetylation conditions affording the target free 4-substituted ribonucleosides **61** in very good yields (70–90 %).[52,53]



60a

a R^1 = H, R^2 = Cl
b R^1 = Cl, R^2 = H
c R^1 = H, R^2 = H

61

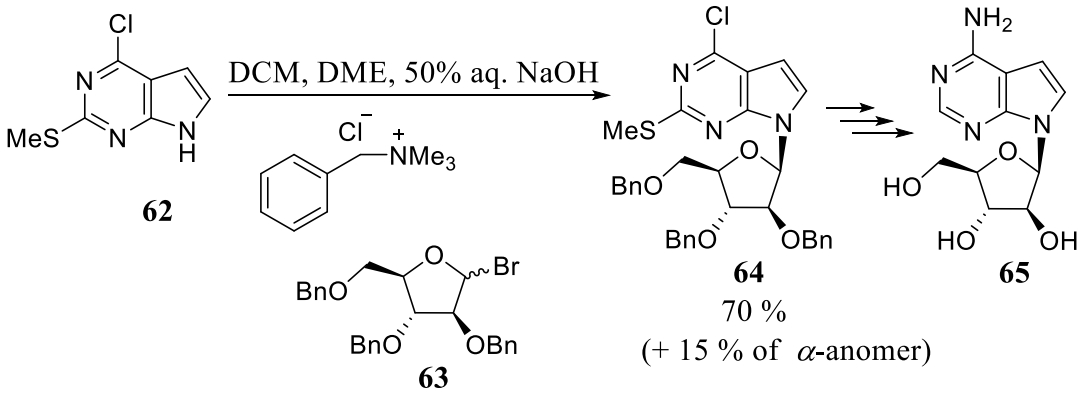
R = NH_2, Me, cyclopropyl, Ph, hetaryl

Scheme 7 Synthesis of 4-substituted pyrimido[4,5-*b*]indole ribonucleosides **61**

1.3.2 Synthesis of 2'-sugar-modified nucleosides

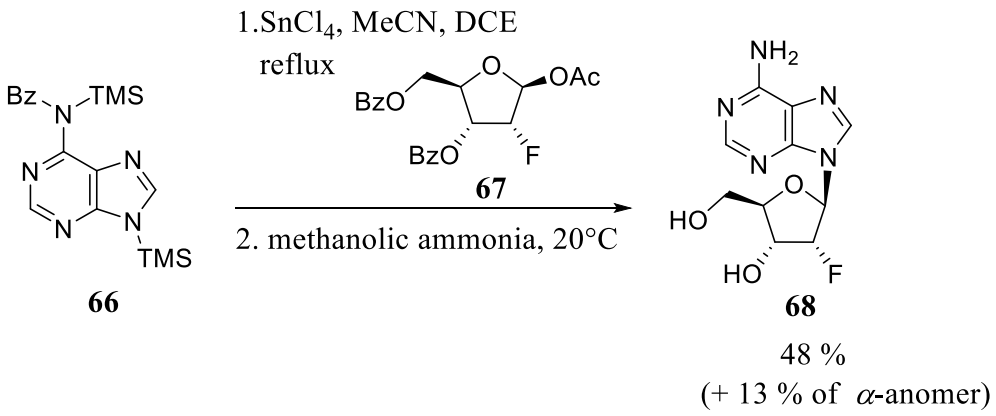
In the synthetic approach towards 2'-modified nucleosides, the glycosylation reaction is not very frequently used. This is mainly caused by the low selectivity of the coupling of nucleobase and 2-modified furanose. Absence of the acyl-protected hydroxyl group for the neighboring group participation, which is required for the Vorbrüggen reaction, or steric factors often result in the mixture of *α*- and *β*-anomers. Nonetheless, some examples for the glycosylations with 2-modified sugar derivatives can be found in the literature. One of them is the synthesis of 4-amino-7-(*β*-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**65**), arabino analog of tubercidin (**9**), via nucleobase anion glycosylation.[77] The nucleobase **62** reacted with benzyl-protected *α*-bromose **63** in the mixture of DCM, DME, 50% aqueous NaOH and benzyltrimethylammonium chloride as the phase-transfer catalyst.

The target protected *β*-arabinonucleoside **64** was obtained in 70% yield with 15 % of its *α*-anomer.



Scheme 8 Synthesis of *arabino*-tubercidin (**65**) via nucleobase anion glycosylation

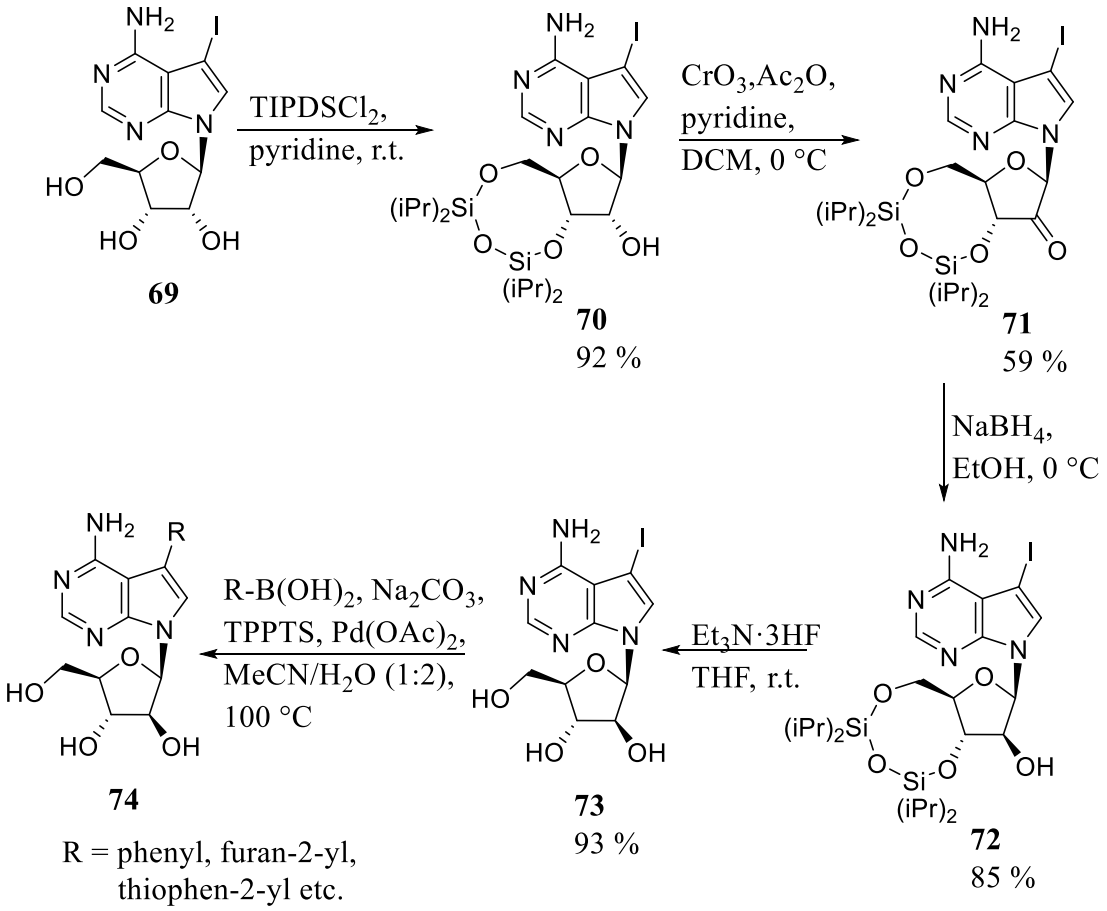
Another example of glycosylation reaction used in the preparation of 2'-modified nucleosides is the synthesis of 2'-deoxy-2'-fluoroadenosine (**68**).[78] This method used persilylated N^6-benzoyladenine **66**, which is coupled with 1-*O*-acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-*β*-D-ribofuranose (**67**) in the presence of $SnCl_4$ in the mixture of MeCN and DCE. Resulting anomeric mixture of protected nucleosides is subsequently deprotected to give the free 2'-deoxy-2'-fluoro-*β*-ribonucleoside **68** in 48% yield accompanied by 13 % of its *α*-anomer.



Scheme 9 Glycosylation used in the synthesis of 2'-deoxy-2'-fluoroadenosine (**68**)

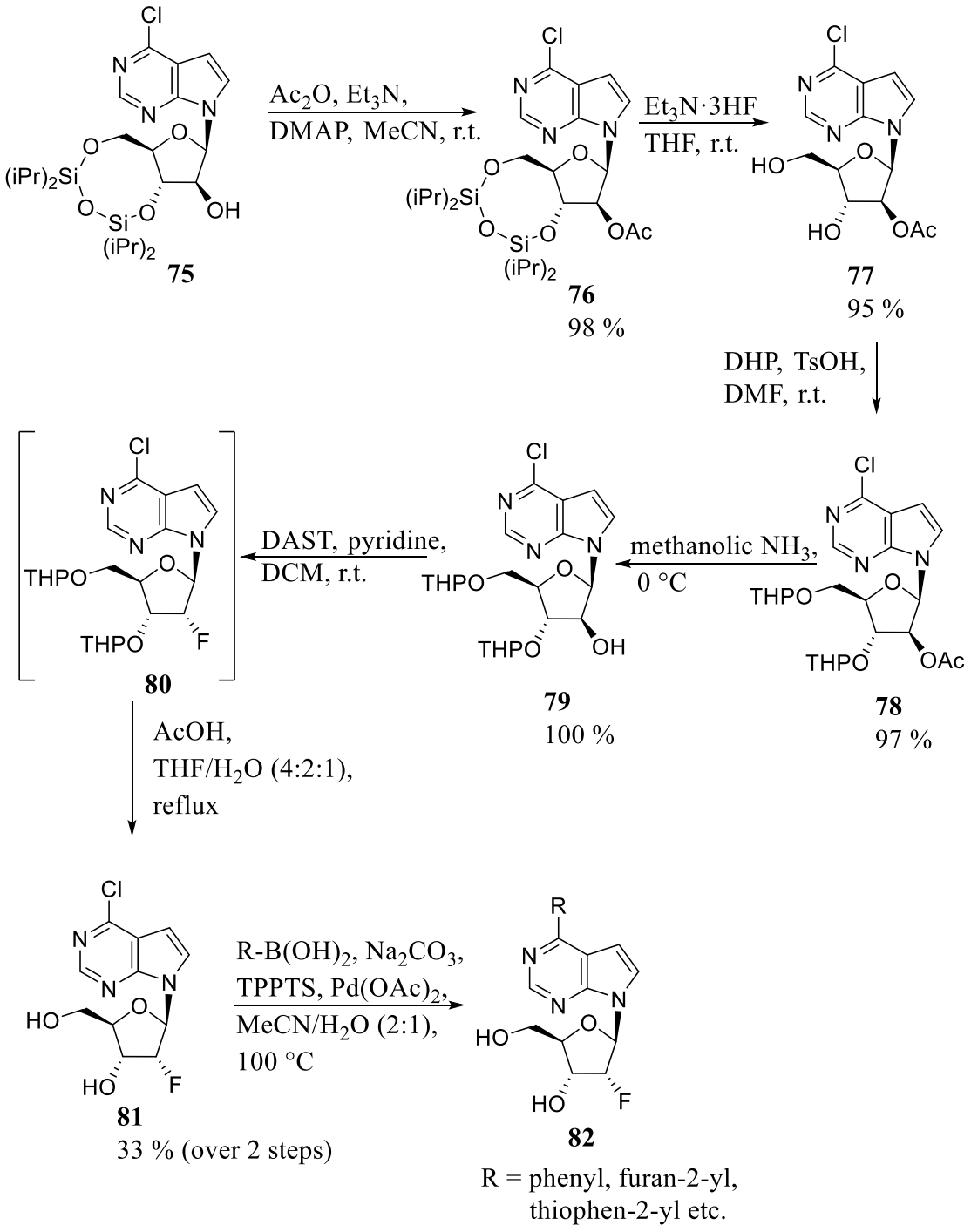
Difficult separation of the anomeric mixtures can be avoided by the direct modification of the sugar moiety of the ribonucleoside. In this approach, selective

protection of 3’- and 5’-hydroxy group is required in order to regioselectively manipulate with the functional groups at the position 2’ of the nucleoside. This procedure was used in the synthesis of 2’-modified (arabino and 2’-deoxy-2’-fluororibo) analogs of cytostatic 6-(het)aryl-7-deazapurine[79,80] and 7-(het)aryl-7-deazaadenosine nucleosides.[81,82] The synthesis of 7-(het)aryl-7-deazaadenine arabinonucleosides **74** was achieved using inversion of configuration at the 2’-position. The first step was the protection of 3’- and 5’-hydroxy groups of 7-iodotubercidin **69** using the Markiewicz reagent, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. Oxidation by CrO_3 in the presence of acetic anhydride and pyridine gave the ketone **71**, which was stereoselectively reduced to the arabinonucleoside **72** using $NaBH_4$. The last step was the deprotection of 3’- and 5’-hydroxy groups by $Et_3N{\cdot}3HF$, which afforded the free nucleoside **73** in high, 93% yield. The final 7-substituted arabinonucleosides **74** were prepared by aqueous Suzuki cross-coupling reactions with various hetaryl- or arylboronic acids, Na_2CO_3, $Pd(OAc)_2$ and TPPTS as ligand.[81]



Scheme 10 Synthesis of 7-(het)aryl-7-deazaadenine arabinonucleosides **74**

The synthetic route towards 6-(het)aryl-7-deazapurine 2'-deoxy-2'-fluororibonucleosides **81** used 3',5'-protected arabinonucleoside **75** as the starting material. In the first step, the free 2'-hydroxy group was acetylated and then the silyl-protecting groups were replaced by THP-protecting groups to give THP-protected acetate **78**. Treatment of **78** with methanolic ammonia at 0 ºC afforded arabinonucleoside **79**. S_N2 fluorination of arabinonucleoside **79** with DAST gave the crude 2'-deoxy-2'-fluororibo nucleoside **80**, which was then deprotected under acidic conditions to provide the free nucleoside **81** in 33% yield over two steps. Various aryl and hetaryl substituents were then introduced via aqueous Suzuki cross-coupling reaction.[80]



Scheme 11 Sequence for the synthesis of 6-(het)aryl-7-deazapurine 2'-deoxy-2'-fluororibonucleosides **82**

2 Specific aims of the thesis

1. Synthesis of 4,6-dichloropyrimido[4,5-*b*]indole arabinonucleoside.
2. Synthesis of 4,6-dichloropyrimido[4,5-*b*]indole 2'-deoxy -2'-fluoro-ribonucleoside.
3. Synthesis of two series of 2'-modified 4-substituted pyrimido[4,5-*b*]indole nucleoside derivatives for biological activity testing.

2.1 Rationale of the specific aims

During the long-term medicinal chemistry project of base-modified nucleobase and nucleoside analogs in Hocek research group, two classes of 7-deazapurine ribonucleosides with nanomolar cytostatic activities have been discovered.[31,34] In order to improve their biological properties, cytostatic and/or antiviral activities, their 2'-sugar-modified nucleoside derivatives were prepared, but most of the compounds were inactive or less active than the parent 7-deazapurine ribonucleosides.[79–82] Further modification at position 7 of 7-dezapurine moiety led to the design and synthesis of series of pyrimido[4,5-*b*]indole ribonucleosides, from which some of the compounds have shown promising anti-viral activities against HCV or Dengue virus.[52,53]

In order to investigate the effect of modification of the ribose moiety of the pyrimido[4,5-*b*]indole ribonucleosides on their biological activity and to complement the SAR for this class of benzo-fused analogs of 7-deazapurine ribonucleosides, my task was the synthesis of 4-substituted pyrimido[4,5-*b*]indole arabinonucleosides and 4-substituted pyrimido[4,5-*b*]indole 2'-deoxy-2'-fluororibonucleoside derivatives (**Figure 13**) for the biological activity screening.

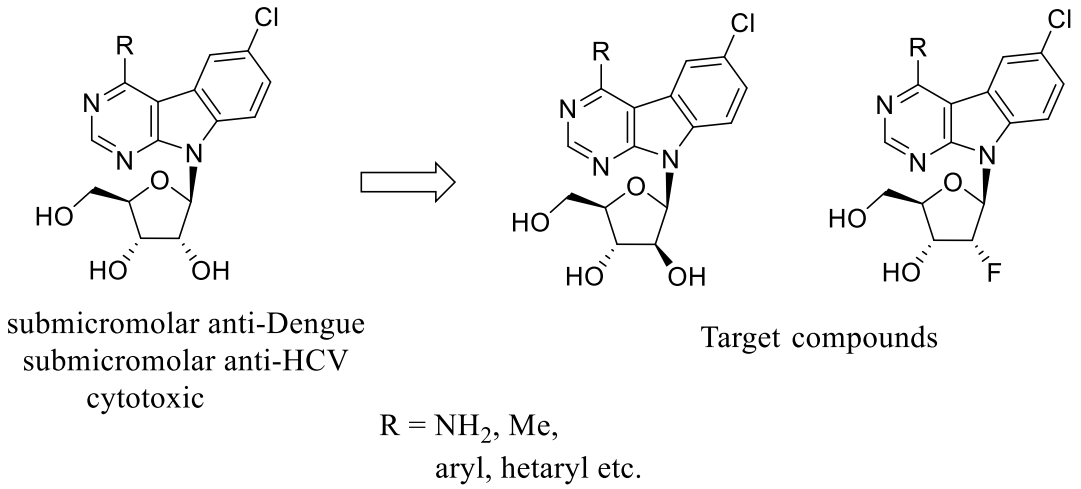
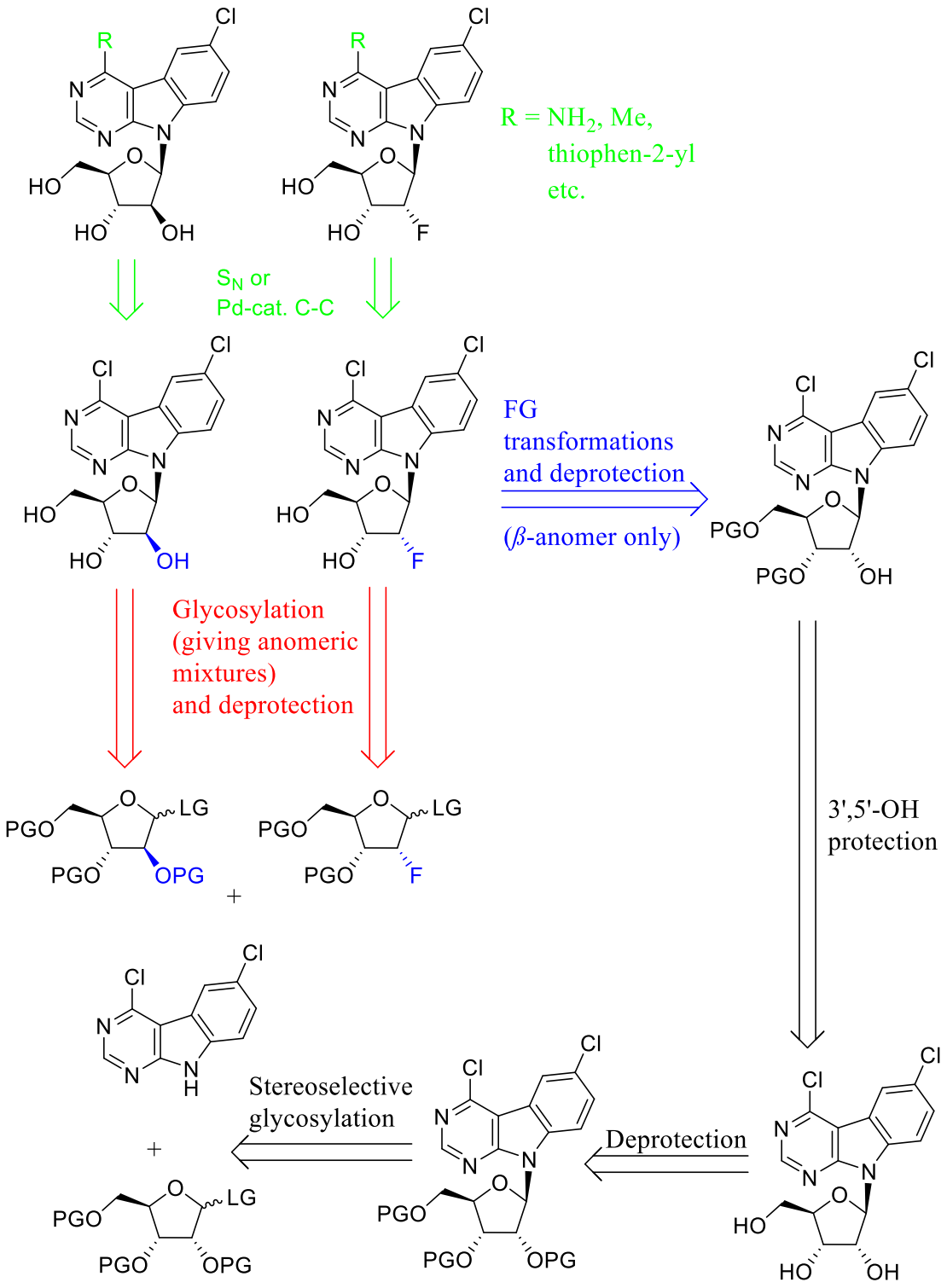


Figure 13 Parent ribonucleosides and target 4-substituted 2'-modified nucleosides

The arabino motif is inspired by clinically used cytostatics fludarabine[55] and nelarabine.[60] The inspiration for the 2'-deoxy-2'fluororibo motif is based on reported bioactive compounds, which have possessed interesting antiviral activities.[66–69]

3 Results and discussion

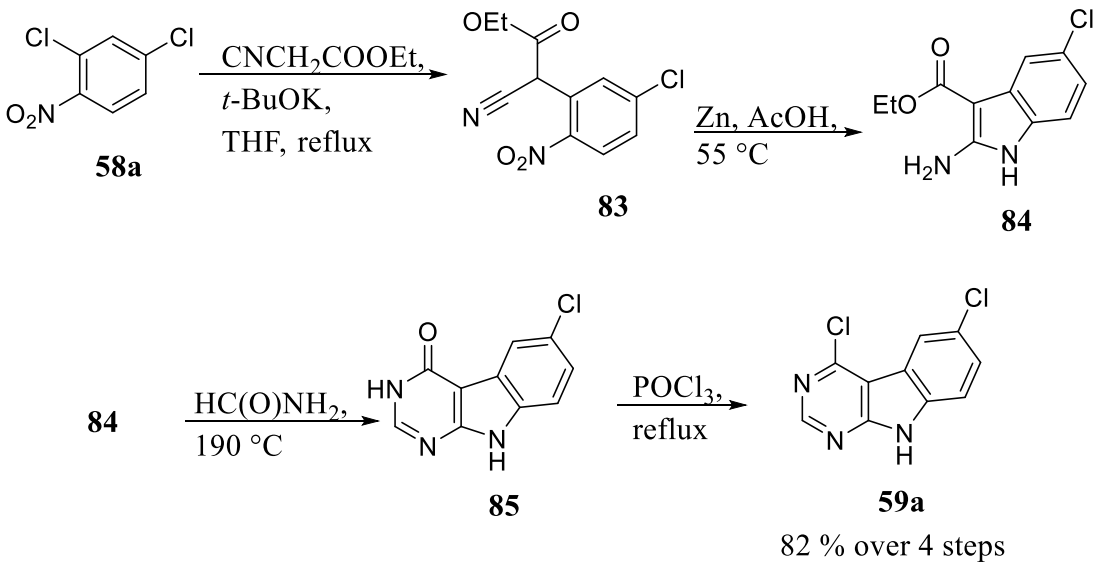
The synthetic pathway towards target 4-substituted pyrimido[4,5-*b*]indole arabinonucleosides and 2'-deoxy-2'-fluororibonucleosides is summarized in **Scheme 12**. The strategy for the preparation of final derivatives was based on nucleophilic substitutions or palladium catalyzed cross-coupling reactions of the corresponding nucleoside derivatives. Because the glycosylations with protected arabinose or 2'-deoxy-2'-fluororibose derivatives often lead to anomeric mixtures, we decided to directly modify the ribose moiety of the nucleoside. This approach was used mainly to avoid laborious separations of anomers and in order to achieve the desired regioselectivity of reactions, 3'- and 5'-hydroxy groups had to be selectively protected. Hence, 2'-modified intermediates were prepared using functional group transformations of the 2'-hydroxy group of the 3',5'-protected ribonucleoside derivative. The free nucleoside intermediate was accessed via deprotection of the protected nucleoside, which had to be prepared by the glycosylation of the pyrimidoindole nucleobase with commercially or synthetically available sugar derivative. Therefore, the synthetic route started with the construction of the heterocyclic nucleobase.



Scheme 12 Retrosynthetic approach towards 4-substituted 2'-modified nucleosides

3.1 Synthesis of 4,6-dichloropyrimido[4,5-*b*]indole

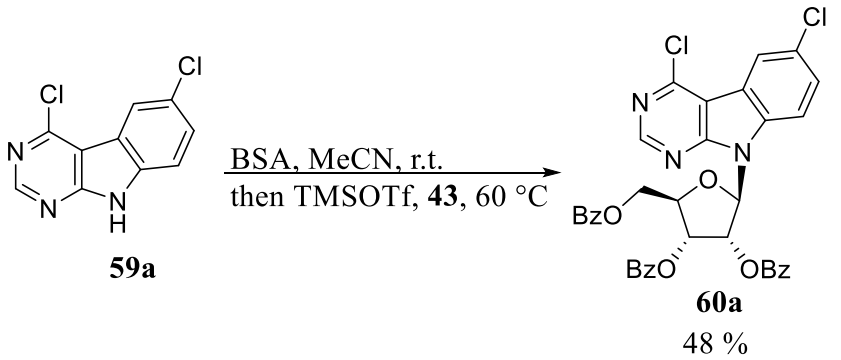
The first intermediate, 4,6-dichloropyrimido[4,5-*b*]indole (**59a**), was prepared according to the procedure which was published for the synthesis of pyrimido[4,5-*b*]indole ribonucleosides **60a–c** (**Scheme 13**).[52] The starting material for the construction of the heterocyclic nucleobase **59a**, was the commercially available 2,4-dichloronitrobenzene (**58a**). In the first step, chlorine at position 2 was substituted by potassium salt of ethyl cyanoacetate using the conditions published by Gangjee *et al*.[46] This aromatic substitution proceeded regioselectively to give ethyl-2-(2-nitrophenyl)-cyanoacetate (**83**). Reduction of **83** by zinc dust in acetic acid followed by spontaneous cyclization gave the indole derivative **84**. Like in the published procedure,[52] no external heating was needed because the reaction is exothermic enough to reach temperature of 55 ºC, which was necessary for the reaction to proceed.[46] The cyclocondenstation of **84** with formamide at 190 ºC afforded the pyrimidoindole derivative **85**. In this step, the formamide was used as well as the solvent and the precipitated product was isolated by filtration. Finally, the chlorination of indole **85** with $POCl_3$ for two days under reflux, resulted in the dichloropyrimido[4,5-*b*]indole **59a**. The nucleobase **59a** was prepared using this 4-step synthesis in very good 82 % overall yield and after drying under reduced pressure, it was used for the following glycosylation reactions without further purification.



Scheme 13 Synthesis of 4,6-dichloropyrimidoindole **59a**

3.2 Synthesis of 4,6-dichloropyrimido[4,5-*b*]indole ribonucleoside

To be able to introduce various substituents to the position 4 of 2'-modified nucleosides, we needed an access to free ribonucleoside with chlorine at position 4. The debenzoylation with the simultaneous amination of the protected pyrimidoindole ribonucleosides has been reported before.[53] Yet, the deprotection of the 4,6-dichloropyrimidoindole ribonucleoside **60a** without the nucleophilic substitution at position 4 has not been performed. Therefore, the first attempts at the synthesis of the free pyrimidoindole ribonucleoside were starting from the known benzoylated nucleoside **60a**,[52] which was prepared using one-pot Vorbrüggen reaction in analogy to the published procedure. The nucleobase **59a** was silylated by *N*,*O*-bis(trimethylsilyl)acetamide in MeCN and then reacted with commercially available protected *β*-ribofuranose **43** and TMSOTf at 60 ºC. The benzoyl-protected *β*-nucleoside **60a** was obtained in good, 48% yield (**Scheme 14**).



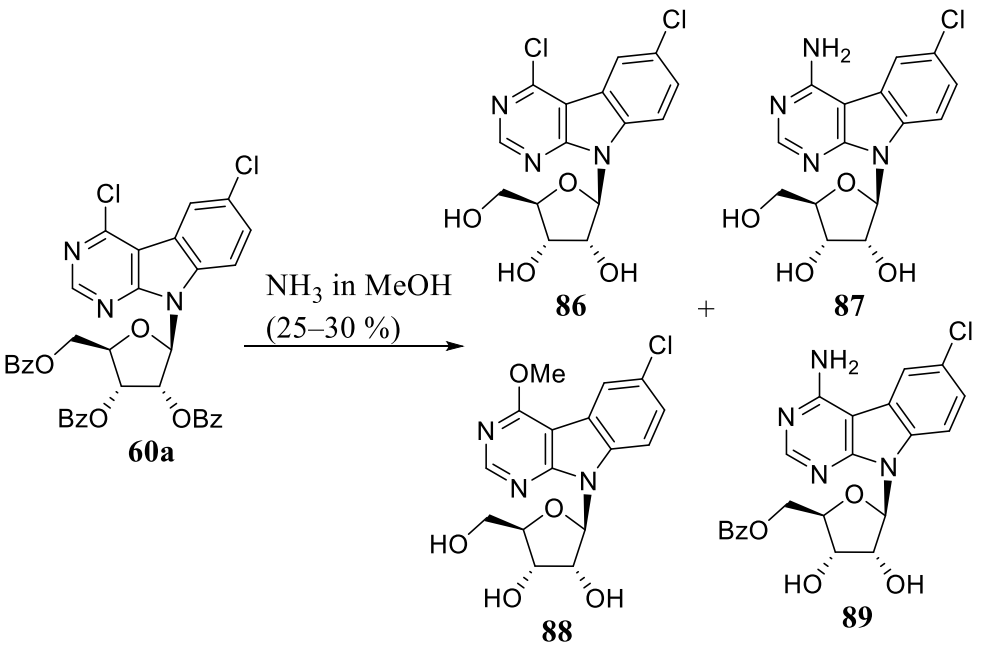
59a **60a**

48 %

Scheme 14 Synthesis of protected 4,6-dichloropyrimidoindole nucleoside **60a**

For the deprotection of the nucleoside **60a**, the conditions, which were applied in the synthesis of 2'-*C*-methyl purine ribonucleosides reported by Eldrup *et al.*, were tried first.[83] In that work, the deprotection of benzoylated 2'-*C*-methyl purine ribonucleoside was achieved in saturated methanolic ammonia at r.t. without the substitution of chlorine at position 6.

Applying these conditions for 2.5 days, free 4-amino **87** and 4-methoxy **88** derivatives were the only products of this deprotection. Shorter reaction times gave again products **87** and **88**, together with small amounts of the target free nucleoside **86** (**Table 1**, Entries 2 and 3) and 3 hours were not enough for the complete deprotection of the starting nucleoside derivative **60a**. We tried to lower the reaction temperature (**Table 1**, Entries 5–7), but this was not sufficient for the cleavage of all benzoyl-protecting groups. Also previously, during the experiments for the amination and subsequent debenzoylation of the protected dichloro ribonucleosides **60a–c** was found, that at temperatures around 100 ºC, only partial deprotection appears and the amount of partially protected nucleoside **89** was three times higher than that of **87**.[84] This only supported the fact, that this derivative is highly activated towards nucleophilic aromatic substitution at the position 4, which occurs more readily than the cleavage of the benzoyl-protecting groups. The results of performed experiments are summarized in **Table 1**.



Scheme 15 Deprotection of the ribonucleoside **60a**

Table 1 Attempts at the deprotection of the nucleoside **60a**

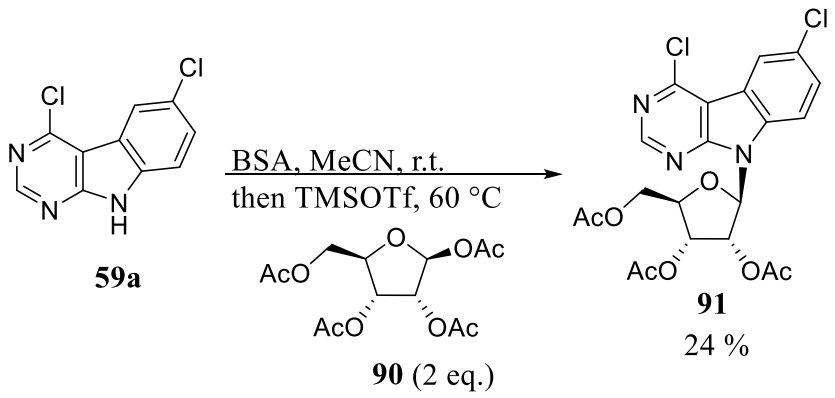
Entry	Temp.	Time	Products[a]
1	r.t.	2.5 days	**87**, **88**
2	r.t.	24 hours	**86** (6 %)[b], **87**, **88**
3	r.t.	5 hours	**86** (traces), **87**, **88**
4	r.t.	3 hours	**60a**, **86**, **87**, partially protected **60a**
5	-20 ºC	5 days	**60a**[c]
6	4 ºC	17 hours	**89**[d]
7	0 ºC	3 hours	**60a**, **86**, partially protected **60a**

[a]Determined by MS from the crude reaction mixture; [b]Isolated yield; [c]No reaction, only starting material recovered; [d]Structure based on previously performed experiments.[84]

Because of these findings and also the fact, that separation of the protected nucleoside **60a** from the unreacted pyrimidoindole **59a** after the glycosylation reaction (**Scheme 14**) on a larger scale (8 mmol of **59a**) was not entirely possible, we decided to use the acetyl-protected nucleoside **91** as the intermediate for the

synthesis of the target free nucleoside **86**. Acetyl-protecting groups are less stable than benzoyl-protecting groups and therefore their cleavage should be easier under the basic conditions.

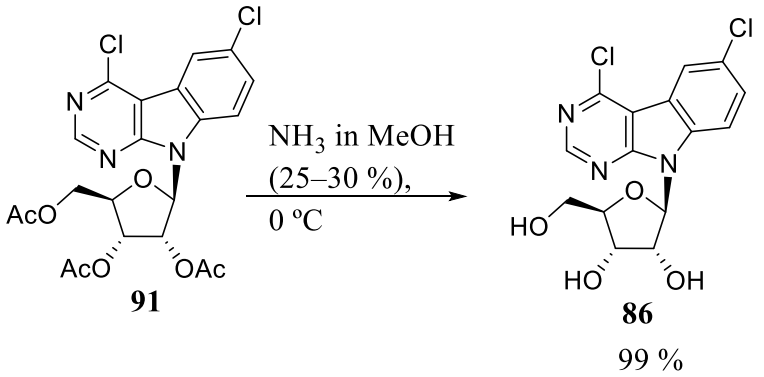
The synthesis of the acetyl-protected dichloro-pyrimidoindole ribonucleoside has not been reported yet. Nevertheless, the Vorbrüggen conditions for the glycosylation of 6-chloro-3-deazapurine with 1,2,3,5-tetra-*O*-acetyl-*β*-D-ribofuranose (**90**) have been applied previously.[85] In analogy with this procedure, the nucleobase was silylated by BSA at r.t. and glycosylated with commercially available ribofuranose **90** to give the acetyl-protected *β*-nucleoside **91** in low, 17% yield. When 2 eq. of the sugar derivative **90** were used, the yield of the nucleoside **91** was slightly raised to 24 % (**Scheme 16**). In this case, the separation of the product from the unreacted base **59a** was even more difficult than by the benzoylated pyrimidoindole ribonucleoside **60a** and on the larger scale (>4 mmol of the nucleobase **59a**) it was not possible to separate **91** from **59a**.



Scheme 16 Synthesis of the acetyl-protected ribonucleoside **91**

Despite all that, the deprotection of the nucleoside **91** was tried out using the solution of methanolic ammonia. At r.t., the complete conversion of the starting material was observed after 1 hour, but both 4-chloro and 4-amino derivatives **86** and **87** were present in the reaction mixture. At lower temperature (0 ºC), the protected nucleoside **91** was again consumed within 1 hour and after 3 hours it was fully deprotected to give the desired 4-chloro nucleoside **86** quantitatively (**Scheme 17**). Moreover, 4-amino derivative **87** was not formed at this temperature,

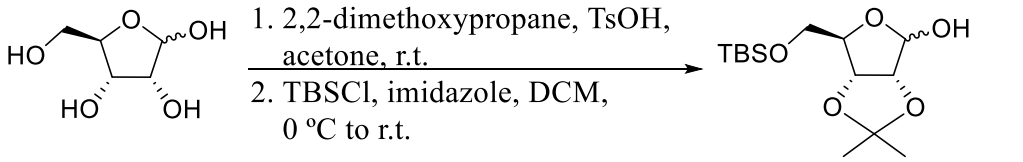
proving that deprotection of this system proceeds in a smoother manner than that of the benzoyl-protected nucleoside **60a**.



Scheme 17 Deprotection of the acetyl-protected nucleoside **91**

Although the deprotection of the acetyl-protected nucleoside **91** was successful, the complications with larger scale synthesis and the troublesome separation of the product from the unreacted base **59a** made this approach not suitable for the synthesis of free 4,6-dichloro nucleoside **86**. In the next attempts, we decided to use different hydroxyl protecting groups for the nucleoside. This was mainly because we wanted to avoid basic deprotection conditions under which the nucleophilic substitution of the activated chlorine at the position 4 takes place. This was possible employing the approach in which the nucleobase **59a** was glycosylated with known 2,3-*O*-isopropylidene-5-*O*-TBS-protected halogenose[75] **51** via nucleobase anion glycosylation and the deprotection of nucleoside was then performed under acidic conditions.

The protected sugar intermediate **50** was prepared from D-ribose (**92**) following the literature procedure (**Scheme 18**).[86] The 2,3-hydroxy groups of D-ribose (**92**) were protected with 2,2-dimethoxypropane in the presence of catalytic amount of *p*-toluenesulfonic acid, followed by the protection of the 5-hydroxy group with *tert*-butyldimethylsilyl chloride. The protected D-ribose **50** was obtained in good yield (52 %) over two steps.

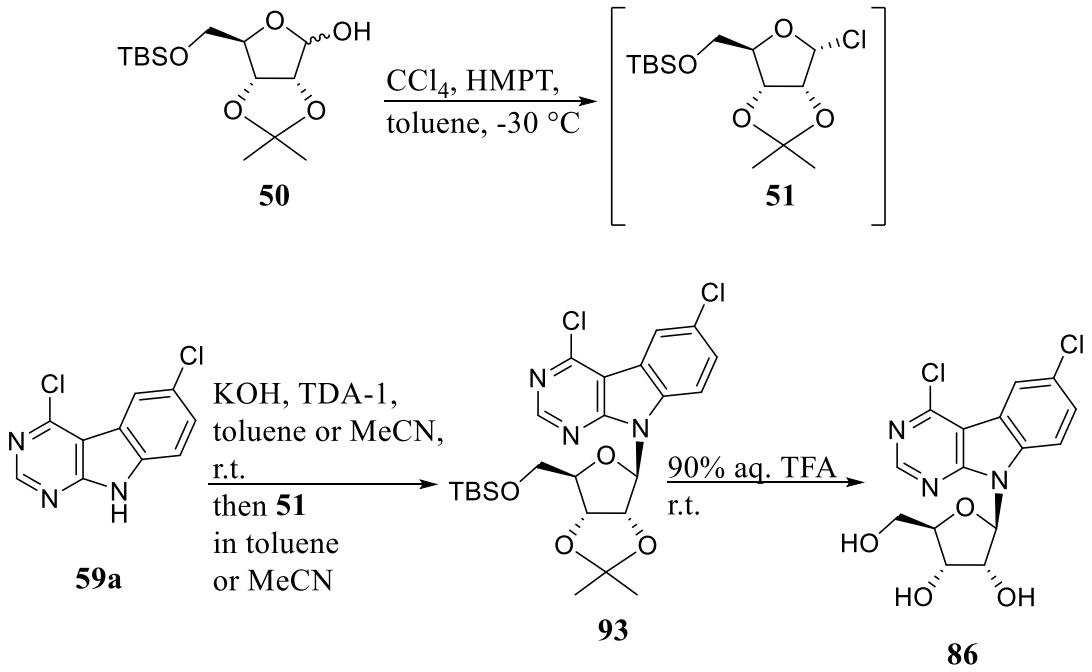


92 **50**

52 % over two steps

Scheme 18 Synthesis of the sugar intermediate **50**

The unstable, "nonparticipating" *α*-chloro sugar **51** was then prepared by Appel's chlorination from the lactol **50** by conditions, which were first described by Ugarkar *et al.* utilizing tris(dimethylamino)phosphine (HMPT) and carbon tetrachloride.[76] It has been found that using toluene as the solvent for this reaction allows to use higher temperature for the chlorination compared to reported procedure with THF at -78 ºC[74] and it also prolongs the half-life of the unstable halogenose **51**. This crude halogenose **51** was then used in the displacement reaction with the potassium salt of the pyrimidoindole nucleobase **59a**, which was generated using KOH and tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) as the phase-transfer catalyst in MeCN (**Scheme 19**). After stirring for 24 hours, work-up and purification by column chromatography, the product **93** was obtained, together with some unreacted sugar material. It has never been possible to get rid of all the impurities and entirely purify the nucleoside **93**, not on a small, nor on a larger scale synthesis. Therefore, the crude mixture, which was received after the column chromatography, was used in the deprotection with 90% aqueous trifluoroacetic acid. After the evaporation of solvents and recrystallization from a mixture of water and MeOH, the free nucleoside **86** was prepared as a single *β*–anomer. Low yields of this synthesis led to attempts at the optimization of reaction conditions, of which results are highlighted in **Table 2**.



Scheme 19 Nucleobase anion glycosylation of the nucleobase **59a** with **51**

Table 2 Optimization of glycosylation of the nucleobase **59a** with the sugar **51**

Entry	**50** (eq.)	CCl_4 (eq.)	HMPT (eq.)	**59a** (eq.)	KOH (eq.)	TDA-1 (eq.)	Glycosylation solvent	Yield of **86**[a]
1	1.5	1.5	1.3	1	2.2	0.5	toluene	18 %
2	1.5	1.5	1.3	1	2.2	0.5	MeCN	21 %[b]
3	1.8	1.5	1.3	1	2.2	0.5	MeCN	29 %[b]
4	1.8	1.5	1.3	1	2.2	1	MeCN	29 %[b]
5	2	2	1.7	1	2.2	0.5	toluene	25 %
6	1.5	1.5	0.9	1	3	1	toluene	29 %
7	2	2	1.7	1	2	1	toluene	21 %

[a]After the deprotection (2 steps); [b]With some amounts of α-ribonucleoside.

When literature conditions[31] were applied, with 1.5 eq. of sugar **50**, 2.2 eq. of KOH and 0.5 eq. of TDA-1 used in toluene, the free nucleoside **86** was obtained in 18% yield as a single *β*-anomer. In the next experiment, MeCN was tried as the solvent for the glycosylation. In this case, the yield of the target nucleoside **86** was higher than in toluene, which might be caused by better solubility of the nucleobase **59a** in MeCN compared to toluene. However, under these conditions, some amounts of the unwanted *α*-anomer of **93** were found in the reaction mixture. This could be caused by the need of evaporation of toluene during the work-up of the chlorination, after which the crude halogenose **51** was redissolved in MeCN and added to the anion of the nucleobase **59a**. This usually required manipulation with the reaction mixture at r.t. over some period of time (<1 hour). During this time, anomerization of the unstable *α*-chlorosugar **51** to its thermodynamically more stable *β*-anomer could take place.[74] Another factor, which could play an important role is the higher polarity of MeCN compared to toluene. It has been found that the half-life of the chlorosugar **51** is much shorter in more polar THF than in toluene[76] and that this can affect the yields of the desired *β*-nucleoside **86**. In the next experiment (**Table 2**, Entry 3), addition of more sugar derivative **50** led to slightly higher yield of **86** (29 %). The next step was the investigation of the effect of the amount of TDA-1. TDA-1 is a phase-transfer catalyst, which's presence strongly increases the nucleophilicity of the anion of nucleobase **59a** by complexation of the potassium cation and thus improves its reactivity. Therefore, in the next attempt, one full eq. of TDA-1 was used, but it did not have any effect on the reactivity, probably because of the sufficient solubility of the nucleobase **59a** in MeCN. In both cases, the *α*-nucleoside was formed too. Trying to prevent the formation of the undesirable co-product, attention was turned back to use of toluene for the glycosylation as the *α*-anomer of the nucleoside **93** was not formed in this solvent before. Increasing the amount of the sugar derivative **50** led to higher, 25% yield and when 1 eq. of TDA-1 was used with 1.5 eq. of ribose **50**, the yield of the *β*-nucleoside **86** was 29 %. This slight increase of the yield could be caused by better solubility of the nucleobase in toluene in the presence of more TDA-1. In the last experiment (**Table 2**, Entry 7), the amounts of HMPT and CCl_4 were raised and with 2 eq. of the sugar derivative **50**, 1 eq. of TDA-1 in toluene, the desired

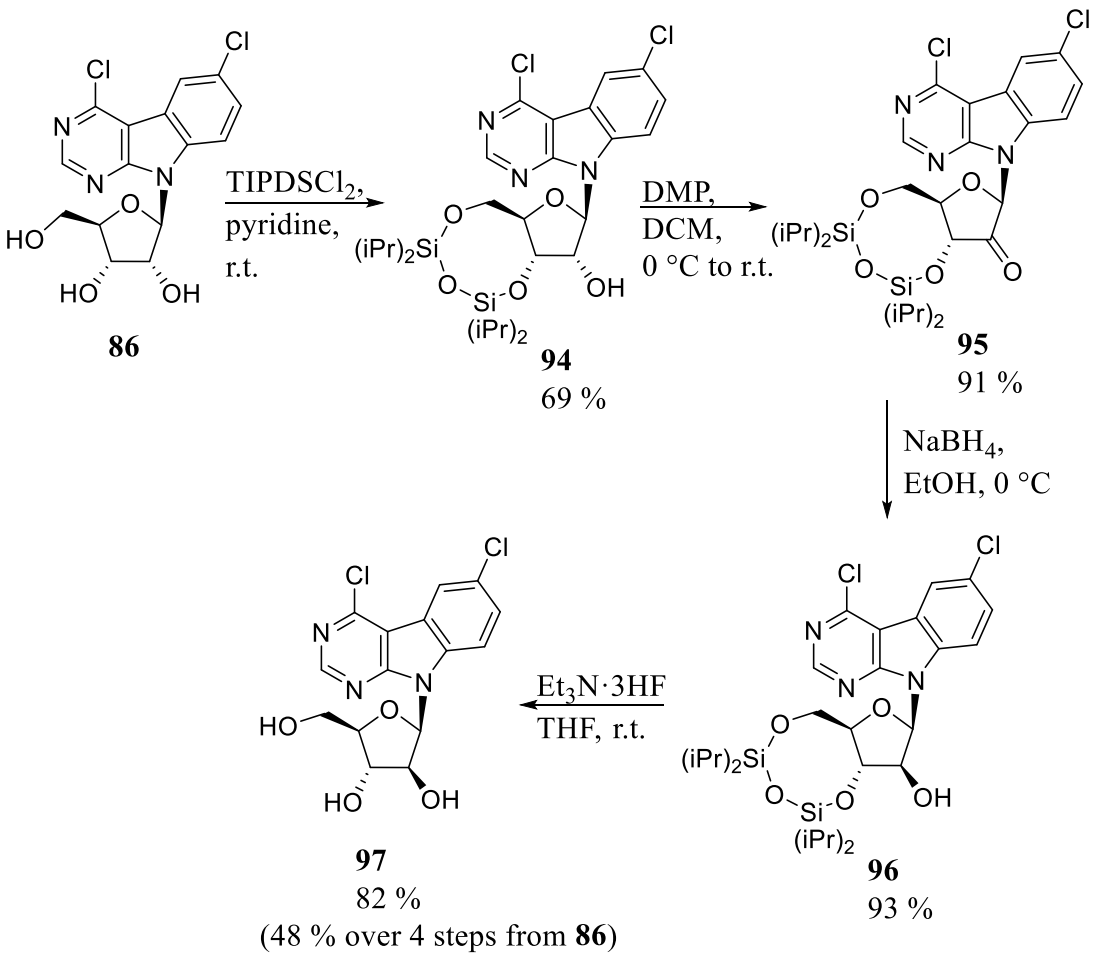
4,6-dichloropyrimidoindole *β*-ribonucleoside **86** was prepared in 21% yield after the deprotection and recrystallization. This only showed that yields of this glycosylation are variable and higher than 29% yield of the free nucleoside **86** turned out to be difficult to reach. The most probable reason for this is the instability of the halogenose **51**. Although the yields were significantly lower compared to glycosylation of 6-chloro-7-deazapurine,[31] the scale-up synthesis provided enough supply of the free nucleoside **86** necessary for the following synthetic steps.

Based on previous findings, acyl-protected ribonucleosides are not suitable compounds for the synthesis of the nucleoside intermediate **86**. Basic Zemplén deacytelytion conditions or deprotection using methanolic ammonia, which are necessary for the cleavage of benzoyl- or acetyl-protecting groups, result in unwanted nucleophilic substitution of the chlorine at position 4. Additionally, the problematic separation of the product of glycosylation from the unreacted material on the larger scale synthesis makes this method not convenient for the preparation of the target nucleoside **86**. Therefore, the best synthetic route towards the 4,6-dichloropyrimido[4,5-*b*]indole ribonucleoside **86** proved to be the nucleobase anion glycosylation of pyrimidoindole **59a** with 2,3-*O*-isopropylidene-5-*O*-TBS-protected halogenose **51** followed by the deprotection under acidic conditions. Even though the product of the glycosylation could not be entirely purified, the subsequent deprotection and recrystallization from the crude reaction mixture gave the pure *β*-anomer of **86**.

3.3 Synthesis of pyrimido[4,5-*b*]indole arabinonucleosides

As Vorbrüggen conditions cannot be used for the glycosylation with derivatives of arabinose, because the neighbouring group participation at 2'-carbon would lead to the steroselective formation of *α*-anomer and the nucleobase anion glycosylation usually gives an unseparable mixture of two anomers, the synthetic pathway towards pyrimidoindole arabinonuclesoide was based on the inversion of configuration of the corresponding ribonucleoside.[79,81] For this, protection of 3'- and 5'-hydroxy groups of the free nucleoside **86** was required. This was

achieved using the Markiewicz reagent ($TIPDSCl_2$). The 3',5'-protected ribonucleoside **94** was prepared in 69% yield. In the next step, 2'-hydroxy group was oxidized using Dess–Martin periodinane (DMP) in DCM to give ketone **95** in 91% yield. When this oxidation was performed with CrO_3 in the presence of acetic anhydride and pyridine, **95** was obtained in a slightly lower yield (88 %). The subsequent reduction of the oxo group of **95** was performed by sodium borohydride in ethanol and provided arabinoside **96** stereoselectively, in 93% yield. The reason for this stereoselectivity is that the attack of borohydride anion on the oxo group of the ketone **95** occurs from the *re* face of the 2'-carbon, which is caused by the steric hindrance of the pyrimidoindole part of the molecule. The silyl-protecting groups were then cleaved with $Et_3N{\cdot}3HF$, affording the free arabinonucleoside **97** in very good, 82% yield. This reagent afforded the product **97** in much better yield compared to the deprotection using TBAF, which gave only 56 % of **97**. Applying this 4-step procedure (**Scheme 20**), the free arabino intermediate **97** was prepared in good, 48% overall yield on the 2 mmol scale.



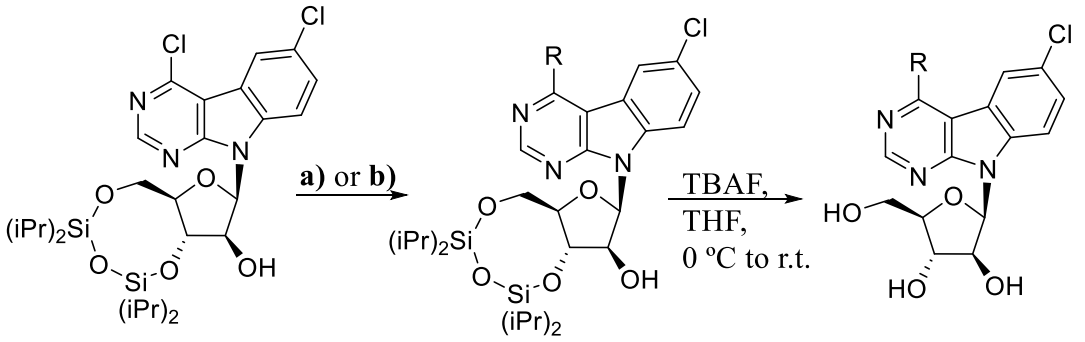
Scheme 20 Sequence for the synthesis of arabinonucleoside **97**

3.3.1 Synthesis of final 4-substituted arabinonucleosides

Previously investigated regioselectivity of the Suzuki-Miyaura and Stille cross-coupling reactions of pyrimido[4,5-*b*]indole ribonucleosides has shown a selective formation of 4-substituted nucleosides.[52] This could be explained by the higher reactivity of the chlorine at position 4, which is caused by electron-poor nature of the pyrimidine part of the molecule. Moreover, the distribution of the electrons makes this benzofused system accessible also for the nucleophilic substitution at position 4. On the other hand, the benzene part of the molecule makes the chlorine at position 6 much less reactive and in order to attach another substituent at this position, cross-coupling reactions under harsher conditions using X-Phos ligands in DMF had to be performed.[52] However, none of the 4,6-disubstituted nucleosides showed any significant antiviral activity and therefore we focused only on the synthesis of 4-substituted derivatives. Hence, the final

pyrimidoindole arabinonucleoside derivatives **99a–f** were prepared employing nucleophilic substitution or Pd-catalyzed cross-coupling reactions (**Table 3**, **Table 4** and **Table 5**).

At first, experiments with protected arabinoside **96** were performed in order to test its reactivity. Protected 4-methyl nucleoside **98a** was synthesized by palladium-catalyzed cross coupling reaction of **96** with trimethylaluminium in 93% yield. In this case, deprotection of **98a** with TBAF in THF was used and it afforded the free 4-methyl arabinonucleoside **102a** in 61% yield. Nucleophilic substitution was used for the synthesis of 4-methylsulfanyl derivative **99b**. Nucleoside **96** reacted with sodium thiomethoxide in EtOH and was subsequently deprotected to give the final free nucleoside **99b** in good, 63% yield over two steps (**Scheme 21**).



96 **98a,b** **99a,b**

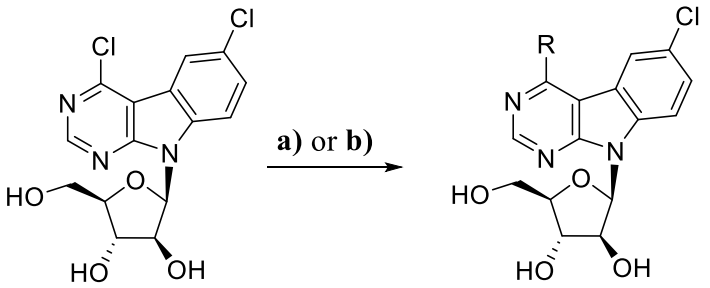
Scheme 21 Synthesis of the final compounds **99a,b**. Reagents: **a)** $(Me)_3Al$ (2M in toluene, 2 eq.), $Pd(PPh_3)_4$ (0.05 eq.), THF; 70 ºC; **b)** NaSMe (2 eq.), EtOH, r.t.

Table 3 Yields of final nucleosides **99a,b**

			Product (yield)	
98,99	R	Conditions[a]	Protected	Deprotected
a	Me	**a)**	**98a** (93 %)	**99a** (61 %)
b	SMe	**b)**	n.i.	**99b** (63 %)[b]

n.i. = not isolated; [a]Given in **Scheme 21**; [b]Overall yield after deprotection.

The other final derivatives were prepared from the free arabinoside **97**. Nucleophilic substitution with aqueous ammonia in dioxane or sodium methoxide in MeOH afforded the 4-amino and 4-methoxy derivatives **99c** and **99d** in good yields (85 % and 64 % respectively) (**Scheme 22**).



97 **99c,d**

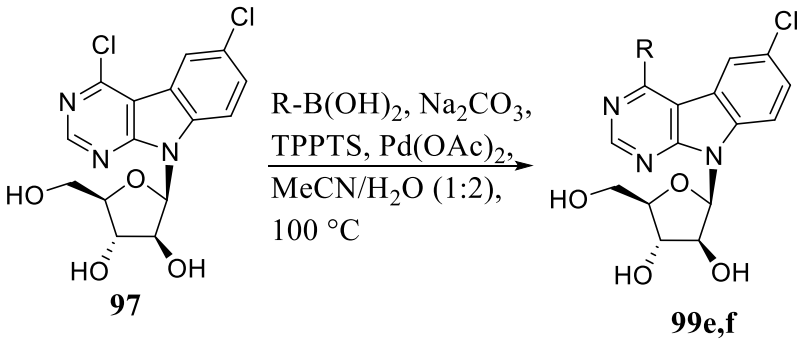
Scheme 22 Nucleophilic substitution of the free **97**. Reagents and conditions: **a)** aq. NH_3, dioxane, 100 ºC; **b)** 1M NaOMe in MeOH, MeOH, r.t.

Table 4 Yields of final nucleosides **99c,d**

99	R	Conditions[a]	Yield of product
c	NH_2	**a)**	**99c** (85 %)
d	OMe	**b)**	**99d** (64 %)

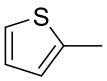
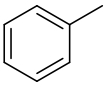
[a]Given in **Scheme 22**.

Phenyl and 2-thiophenyl substituents were introduced by Pd-catalyzed aqueous-phase Suzuki cross-coupling reactions[87] using corresponding aryl- or hetaryl-boronic acids in the presence of Na_2CO_3 as a base, $Pd(OAc)_2$ as a catalyst and TPPTS as a ligand in water-MeCN mixture (2:1) at 100 °C. The arabinonucleosides **99e** (70 %) and **99f** (58 %) were obtained in good yields after the reversed-phase HPFC purification and recrystallization (**Scheme 23**).



Scheme 23 Aqueous Suzuki cross-couplings of **97**

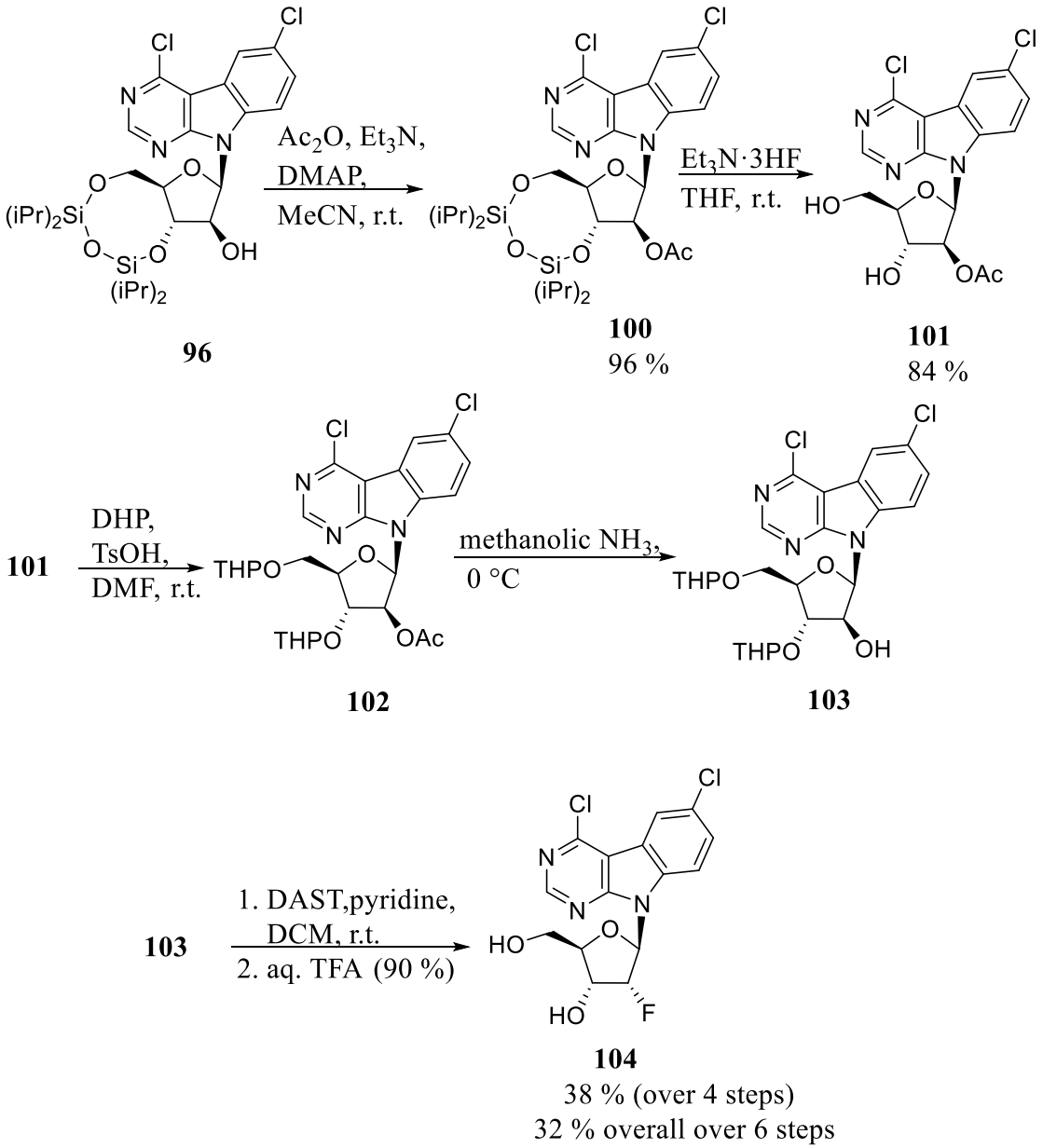
Table 5 Aqueous Suzuki cross-coupling reactions

99	R	Yield of product
e		**99e** (70 %)
f		**99f** (58 %)

3.4 Synthesis of pyrimido[4,5-*b*]indole 2'-deoxy-2'-fluororibonucleosides

To avoid the difficult separation of the anomeric mixture, which frequently arises from the glycosylation of the nucleobase with 2-modified ribose derivatives, the direct modification of the ribose moiety of nucleoside was chosen for the synthesis of the 2'-deoxy-2'-fluororibonucleoside **104**. Intermediate **104** was prepared utilizing stereoselective S_N2 fluorination of the 3',5'-THP-protected arabinonicleoside **103**. This sequence of reactions started from 3',5'-disilyl-protected arabinoside **96** (**Scheme 24**). Because the silyl protecting groups are not stable under fluorination conditions, they had to be replaced by THP-protecting groups. To achieve selective protection, in the first step, the free 2'-hydroxy group of **96** was acetylated to give acetate **100**, using acetic anhydride in the presence of Et_3N and catalytic amount of DMAP. Removal of the silyl protecting groups by treatment with $Et_3N{\cdot}3HF$ in THF afforded compound **101** in 84% yield. 3'- and 5'-hydroxy groups of the acetate **101** were then protected by reaction with 3,4-dihydro-2*H*-pyran in the presence of TsOH in DMF, leading to the

THP-protected acetate **102**. In the next step, aminolysis of the acetyl group by methanolic ammonia at 0 ºC gave the 3',5'-THP-protected arabinonucleoside **103**. THP-intermediates **102** and **103** were not characterized by NMR, but the formation of products was in both cases confirmed by mass spectrometry and crude mixtures with some impurities were used in following steps. Stereoselective S_N2 fluorination of the protected arabinoside **103** with DAST in the presence of pyridine in DCM and subsequent cleavage of THP-protecting groups under acidic conditions provided the free pyrimidoindole 2'-deoxy-2'-fluororibonucleoside **104** in moderate yield (38 % over four steps from **101**). This 6-step sequence of reactions led to the target free fluoro intermediate **104** in 32% overall yield on the 2 mmol scale.

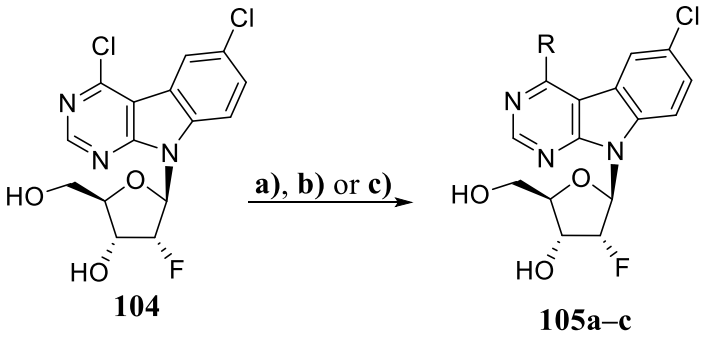


Scheme 24 Sequence for the synthesis of 2'-deoxy-2'-fluororibonucleoside **104**

3.4.1 Synthesis of final 4-substituted 2'-deoxy-2'-fluororibonucleosides

A series of 4-substituted derivatives was then prepared from the fluororibo intermediate **104** via nucleophilic substitution reactions or Pd-catalyzed cross-coupling reactions (**Table 6** and **Table 7**). Reaction of **104** with aqueous ammonia in dioxane at 100 ºC afforded the 4-amino nucleoside **105a** in 82% yield. 4-Methoxy and 4-methylsulfanyl derivatives **105b** and **105c** were prepared by

reaction of **104** with sodium methoxide and sodium thiomethoxide respectively, in very good yields (80 % for **105b** and 87 % for **105c**) (**Scheme 25**).



104 **105a–c**

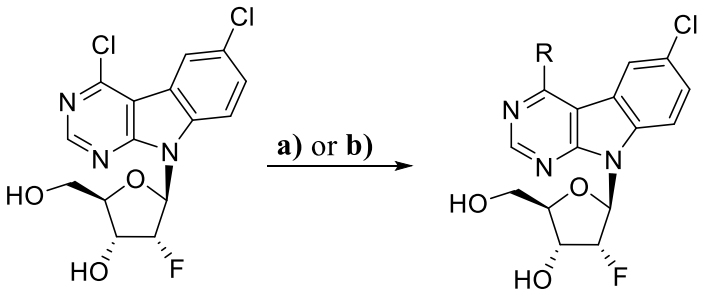
Scheme 25 Nucleophilic substitution of the free **104**. Reagents and conditions: **a)** aq. NH_3, dioxane, 100 ºC; **b)** 1M NaOMe in MeOH, MeOH, r.t.; **c)** NaSMe (2 eq.), EtOH, r.t.

Table 6 Yields of final nucleosides **105a–c**

105	R	Conditions[a]	Yield of product
a	NH_2	**a)**	**105a** (82 %)
b	OMe	**b)**	**105b** (80 %)
c	SMe	**c)**	**105c** (87 %)

[a]Given in **Scheme 25**.

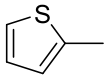
Pd-catalyzed methylation of intermediate **104** with trimethylaluminium led to the 4-methyl fluororibonucleoside **105d** in good, 65% yield. Aqueous-phase Suzuki cross-coupling reaction was used for the synthesis of the final thiophene-2-yl nucleoside **105e**. This reaction was performed using corresponding hetaryl boronic acid in the presence of Na_2CO_3, TPPTS as the ligand and palladium (II) acetate as the catalyst in a mixture of water and MeCN (2:1) at 100 °C. The thiophene-2-yl derivative **105e** was obtained in very good 89% yield (**Scheme 26**).



104 **105d,e**

Scheme 26 Synthesis of the final 2'-deoxy-2'-fluororibonucleosides **105d,e**. Reagents and conditions: **a)** $(Me)_3Al$ (2M in toluene, 2 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), THF; 70 ºC; **b)** R-$B(OH)_2$ (1.5 eq.), Na_2CO_3 (3 eq.), $Pd(OAc)_2$ (0.05 eq.), TPPTS (0.12 eq.), H_2O/MeCN (2:1), 100 °C.

Table 7 Yields of final nucleosides **105d,e**

105	R	Conditions[a]	Yield of product
d	Me	**a)**	**105d** (65 %)
e		**b)**	**105e** (89 %)

[a]Given in **Scheme 26**.

4 Conclusion

Syntheses of two series of 2'-sugar modified pyrimido[4,5-*b*]indole nucleosides were developed using multistep sequence of reactions, which started from the unprotected 4,6-dichloropyrimidoindole ribonucleoside. Initial attempts at the synthesis of this intermediate via deprotection of the known 4,6-dichloro-9-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole failed as the chlorine at position 4 was substituted under basic deprotection conditions. Although, the basic deprotection of the acetyl-protected pyrimidoindole nucleoside without the unwanted substitution of chlorine at position 4 was achieved, the larger scale synthesis of this nucleoside could not be done due to problematic purification of the product. Therefore the key ribonucleoside intermediate was prepared via stereoselective nucleobase anion glycosylation of the 4,6-dichloropyrimido[4,5-*b*]indole nucleobase with 2,3-*O*-isopropylidene-5-*O*-TBS-protected halogenose and subsequent deprotection under acidic conditions. Small optimization of the reaction conditions for this glycosylation provided the desired ribonucleoside in 29% yield as a single *β*-anomer. Pyrimido[4,5-*b*]indole arabinonucleoside was then prepared by inversion of the configuration at the 2'-carbon of the 3',5'-protected ribonucleside using the sequence of oxidation-reduction reactions in good 48% yield over 4 steps. Pyrimido[4,5-*b*]indole 2'-deoxy-2'-fluororibonucleoside intermediate was obtained in 32% overall yield by the 6-step synthesis concluded by stereoselective S_N2 fluorination of the THP-protected arabinoside followed by acidic deprotection. Different substituents were then introduced into position 4 of the pyrimido[4,5-*b*]indole arabinonucleoside and 2'-deoxy-2'-fluororibonucleoside by nucleophilic substitution or Pd-catalyzed cross-coupling reactions in good to very good yields (58–89 %). Screening of biological activities of final 4-substituted 2'-modified pyrimido[4,5-*b*]indole nucleosides against viruses (HCV, RSV, HSV-1, HIV, Dengue, coxsackie B3 virus, influenza, ebola), cancer cell lines (HepG2, HL 60, HeLaS3, CCRF-CEM) and microbes (*Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* CCM 3953, *Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* MRSA 4591, *Staphylococcus haemolyticus* A/16568, *Escherichia coli* C/16702, *Pseudomonas aeruginosa* A/16575) is now in progress.

5 Experimental section

5.1 General remarks

All the reagents and solvents were purchased from commercial suppliers and used as recieved.

HPFC purifications were performed on ISCO Combiflash Rf system-1 apparatus with RediSep Rf Gold Silica Gel Disposable columns for normal-phase or Reverse Phase (C18) RediSep Rf columns for reversed-phase HPFC. Merck Silica gel 60 was used for column chromatography. Monitoring of reactions was performed using TLC Silica gel 60 F254 plates. Compounds were detected using shortwave (254 nm) UV lamp or by a solution of 4-anisaldehyde in ethanol and 10 % of sulfuric acid. NMR spectra were recorded on Bruker Avance 400 MHz spectrometer (400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C) or on Bruker Avance 500 MHz spectrometer (500 MHz for ^{1}H, 125.7 MHz for ^{13}C and 470.3 MHz for ^{19}F), in $CDCl_3$ (TMS was used as internal standard) or DMSO-*d6* (referenced to the residual solvent signal). 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). Melting points were measured on Stuart automatic melting point SMP40 and are uncorrected. IR spectra (wavenumbers in cm^{-1}) were recorded on Brucker ALPHA FT-IR spectrometer using attenuated total reflection (ATR). Optical rotations were measured at 25 °C in DMSO on Autopol IV (Rudolps Research Analytical) polarimeter, $[\alpha]_D^{20}$ values are given in 10^{-1} $deg \cdot cm^2 \cdot g^{-1}$. Purity of all final compounds (unprotected nucleosides) was determined by clean NMR spectra and analytical HPLC.

5.1.1 General procedure for the aqueous Suzuki cross-coupling reaction:[38]

An argon-purged mixture of free nucleoside (1 eq.), the appropriate boronic acid (1.5 eq.), Na_2CO_3 (3 eq.), $Pd(OAc)_2$ (0.05 eq.), and 3,3',3''-phosphanetriyltris-(benzenesulfonic acid) trisodium salt (TPPTS; 0.12 eq.) in H_2O/MeCN (2:1) was stirred at 100 °C for 4 hours. After cooling down, the mixture was neutralized by the addition of aqueous HCl (1M) and diluted with MeOH (20 ml). Solvents were removed under reduced pressure and the residue was purified by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O).

5.2 Synthesis of 4,6-dichloropyrimido[4,5-*b*]indole and protected ribose

Ethyl 2-(5-chloro-2-nitrophenyl)-2-cyanoacetate (83)

Compound **83** was prepared according to modified literature conditions.[46] Ice-cooled solution of ethyl cyanoacetate (30.7 ml, 0.26 mol) in anhydrous THF (250 ml) under the argon atmosphere was treated with potassium tert-butoxide (29.2 g, 0.26 mol). The suspension was stirred for 20 min and then 2,4-dichloronitrobenzene (**58a**) (25 g, 0.13 mol) was added. Reaction mixture was heated to 75 °C for 20 hours, then diluted with water and acidified to pH~2 with 2M HCl. This mixture was extracted with ether (3×150 ml). Combined organic layers were dried over Na_2SO_4 and solvents were evaporated. After drying under reduced pressure, compound **83** (30 g) was obtained as a brown oil. Crude material was used directly for the next step. ^{1}H NMR is in agreement with literature.[88]

Ethyl 2-amino-5-chloro-1*H*-indole-3-carboxylate (84)

Compound **84** was prepared according to literature conditions.[52] Crude **83** (30 g) was dissolved in glacial acetic acid (350 ml) and zinc dust (30 g) was added by 5 parts during 45 min. Resulting mixture was stirred for 2 hours without external heating, filtered through a pad of celite and the pad was washed well with 200 ml of acetic acid. Solvent was evaporated and residue was washed with 600 ml of water. After drying under reduced pressure compound **84** (33 g) was obtained as a brown

powder. Crude material was used directly for the next step. ^{1}H NMR is in agreement with literature.[89]

6-Chloro-3*H*-pyrimido[4,5-*b*]indol-4(9*H*)-one (85)

Compound **85** was prepared according to literature conditions.[52] Crude **84** (33 g) was dissolved in formamide (110 ml) and heated to 185 °C for 20 hours. Cooled reaction mixture was filtered and washed well with 1.5 l of water. After drying under reduced pressure compound **85** (38 g) was obtained as a dark powder. Crude material was used directly for the next step. ^{1}H NMR is in agreement with literature.[52]

4,6-Dichloro-9*H*-pyrimido[4,5-*b*]indole (59a)

Pyrimidoindole **59a** was prepared according to literature conditions.[52] Crude **85** (38 g) was dissolved in $POCl_3$ (330 ml) and heated to 120 °C for 3 days. $POCl_3$ was evaporated under reduced pressure, residue was diluted with ice-cold water and cooled with ice. Solution was slowly neutralized with aqueous ammonia to pH~7, filtered and washed with cold water, 2M hydrochloric acid (200 ml) and again with cold water until neutral pH. After drying under reduced pressure compound **59a** (25.5 g) was obtained as a dark powder. Crude material was used for the glycosylation reaction without purification. ^{1}H NMR is in agreement with literature.[52] Overall yield of the 4-step synthesis of the compound **59a** was 82 %.

2,3-*O*-Isopropylidene-5-*O*-tert-butyldimethylsilyl-D-ribofuranose (50)

Protected sugar **50** was prepared according to modified literature conditions.[86] D-Ribose (**92**) (20 g, 0.13 mol) was dissolved in acetone (70 ml) and treated with 2,2-dimethoxypropane (25 ml) and $TsOH{\cdot}H_2O$ (950 mg, 5 mmol). The reaction mixture was stirred for 1 hour at r.t., then neutralized with solid $NaHCO_3$ (600 mg, 7.1 mmol) and filtered. The filtrate was concentrated in vacuo, dissolved in anhydrous DCM (250 ml) and cooled to 0 °C. Then, *tert*-butyldimethylsilyl chloride (20.77 g, 0.14 mol) and imidazole (21.65 g, 0.32 mol) were added, the reaction mixture was allowed to warm to r.t. and stirred for 2 hours. The reaction mixture was washed with water (2×100 ml), organic layer was dried over Na_2SO_4,

solvent was evaporated under reduced pressure and residue was purified by column chromatography on silica gel (PE-EtOAc 7:1) to give **50** (21.2 g, 52 %) as an anomeric mixture, which spontaneously crystallized to a white crystalline solid. ^{1}H NMR is in agreement with literature.[86]

5.3 Synthesis of pyrimido[4,5-*b*]indole arabinonucleosides

4,6-Dichloro-9-(2,3-*O*-isopropylidene-5-*O*-tert-butyldimethylsilyl-*β*-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (93)

To a solution of protected ribose **50** (960 mg, 3.2 mmol) and carbon tetrachloride (0.45 ml, 6.7 mmol) in anhydrous toluene (10 ml) at -30 °C, tris(dimethylamino)phosphine (0.5 ml, 2.8 mmol) was added dropwise. After 10 min of vigorous stirring at -30 °C, the reaction mixture was quickly washed with ice-cold brine (10 ml), dried over $MgSO_4$ and added to a vigorously stirred suspension of pyrimidoindole **59a**, powdered KOH (355 mg, 6.3 mmol), TDA-1 (0.7 ml, 2.1 mmol) in anhydrous toluene (15 ml). The reaction mixture was stirred for 24 hours at r.t. After filtration and evaporation under reduced pressure, the reaction mixture was purified by HPFC (silica column, 0→5 % EtOAc in PE) to give the crude nucleoside **93** (400 mg) as a yellow oil, which was used directly in the next step.

^{1}H NMR (500.0 MHz, DMSO-d_6): -0.054, -0.048 (2 × s, 2 × 3H, CH_3Si); 0.81 (s, 9H, $(CH_3)_3CSi$); 1.32, 1.58 (2 × s, 2 × 3H, $(CH_3)_3C$); 3.73 (dd, 1H, J_{gem} = 11.3, $J_{5'b,4'}$ = 5.3, H-5'b); 3.81 (dd, 1H, J_{gem} = 11.3, $J_{5'a,4'}$ = 4.4, H-5'a); 4.18 (ddd, 1H, $J_{4',5'}$ = 5.3, 4.4, $J_{4',3'}$ = 4.2, H-4'); 5.10 (dd, 1H, $J_{3',2'}$ = 6.7, $J_{3',4'}$ = 4.2, H-3'); 5.54 (dd, 1H, $J_{2',3'}$ = 6.7, $J_{2',1'}$ = 3.2, H-2'); 6.64 (d, 1H, $J_{1',2'}$ = 3.2, H-1'); 7.67 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.1, H-7); 8.02 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.33 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.93 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): -5.32 (CH_3Si); 18.23 ($(CH_3)_3$**C**Si); 25.50 (($\mathbf{C}H_3)_2C$); 25.91 (($\mathbf{C}H_3)_3CSi$); 27.29 (($\mathbf{C}H_3)_2C$); 62.80 (CH_2-5'); 80.27 (CH-3'); 82.25 (CH-2'); 85.57 (CH-4'); 88.48 (CH-1'); 111.32 (C-4a); 114.32 (($CH_3)_2$**C**); 114.46 (CH-8); 119.59 (C-4b); 121.92 (CH-5); 127.50 (C-6); 128.69 (CH-7);

136.58 (C-8a); 152.55 (C-4); 154.70 (CH-2); 155.36 (C-9a).ESI MS m/z (rel. %): 524 (6) [M+H], 546 (25) [M+Na].

HR MS (ESI) for $C_{24}H_{31}O_4N_3Cl_2NaSi$ [M+Na]: calcd 546.13531; found 546.13532.

4,6-Dichloro-9-(*β*-D-ribonofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (86)

Crude protected nucleoside **93** (400 mg) was treated with aqueous TFA (90 % *v*/*v*, 5 ml) and stirred at r.t. for 30 min. Then, the volatiles were removed under reduced pressure and the residue was co-evaporated few times with MeOH. Free nucleoside **86** (225 mg, 29 % over 2 steps) was obtained as a white solid after recrystallization (MeOH-H_2O 4:1).

m.p. 253–256 °C.

$[\alpha]_D^{20}$ -49.3 (c 0.209, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.69 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 3.7, H-5′b); 3.72 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.3, H-5′a); 4.01 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.3, $J_{4',3'}$ = 2.8, H-4′); 4.23 (dd, 1H, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 2.8, H-3′); 4.72 (dd, 1H, $J_{2',1'}$ = 7.4, $J_{2',3'}$ = 5.7, H-2′); 5.12 - 5.42 (bm, 3H, OH-2′,3′,5′); 6.49 (d, 1H, $J_{1',2'}$ = 7.4, H-1′); 7.69 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 8.25 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.33 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.91 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.62 (CH_2-5′); 70.15 (CH-3′); 70.90 (CH-2′); 85.94 (CH-4′); 87.37 (CH-1′); 111.06 (C-4a); 115.51 (CH-8); 119.65 (C-4b); 121.73 (CH-5); 127.22 (C-6); 128.60 (CH-7); 136.52 (C-8a); 152.37 (C-4); 154.61 (CH-2); 156.23 (C-9a).

ESI MS m/z (rel. %): 370 (100) [M+H], 392 (67) [M+Na].

HR MS (ESI) for $C_{15}H_{14}O_4N_3Cl_2$ [M+H]: calcd 370.03559; found 370.03566.

IR (ATR): ν = 3257, 1590, 1551, 1445, 1228, 1106, 1075, 1055, 1031, 1004, 835, 621, 430 cm^{-1}.

4,6-Dichloro-9-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*β*-D-ribofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (94)

Free nucleoside **86** (1.39 g, 3.7 mmol) was dissolved in anhydrous pyridine (40 ml) and $TIPDSCl_2$ (1.2 ml, 3.7 mmol) was added. The reaction mixture was stirred at r.t. for 4 hours and then solvent was removed under reduced pressure. Residue

was dissolved in EtOAc (50 ml) and extracted with water (50 ml). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by HPFC (silica column, 0→10% EtOAc in PE) to give nucleoside **94** (1.6 g, 69 %) as a yellowish solid.

m.p. 145–147 °C.

^{1}H NMR (500.0 MHz, $CDCl_3$): 0.88 – 1.22 (m, 28H, $(CH_3)_2CHSi$); 3.22 (bs, 1H, OH-2′); 4.04 – 4.088 (m, 3H, H-4′,5′); 4.94 (td, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 2.0, H-2′); 5.28 (m, 1H, H-3′); 6.30 (d, 1H, $J_{1',2'}$ = 2.0, H-1′); 7.57 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.64 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.36 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.72 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, $CDCl_3$): 12.58, 12.76, 13.01, 13.27 ($(CH_3)_2$**C**HSi); 16.93, 17.00, 17.02, 17.15, 17.27, 17.35, 17.38, 17.42 (($\mathbf{C}H_3)_2$CHSi); 61.60 (CH_2-5′); 70.72 (CH-3′); 73.44 (CH-2′); 81.56 (CH-4′); 89.40 (CH-1′); 111.95 (CH-8); 112.28 (C-4a); 119.80 (C-4b); 122.90 (CH-5); 128.40 (C-6); 128.79 (CH-7); 137.12 (C-8a); 153.20 (C-4); 153.92 (CH-2); 155.43 (C-9a).

ESI MS m/z (rel. %): 612 (100) [M+H], 634 (85) [M+Na].

HR MS (ESI) for $C_{27}H_{40}O_5N_3Cl_2Si_2$ [M+H]: calcd 612.18781; found 612.18798.

IR (ATR): ν = 2875, 1440, 108/7, 1032, 868, 693, 615, 449 cm^{-1}.

4,6-Dichloro-9-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*β*-D-*erythro*-pentofuran-2-ulosyl]-9*H*-pyrimido[4,5-*b*]indole (95)

Dess-Martin periodinane (3.13 g, 7.4 mmol) was dissolved in anhydrous DCM (20 ml) and cooled to 0 °C and then the solution of **94** (1.51 g, 2.46 mmol) in anhydrous DCM (20 ml) was added. The reaction mixture was stirred 10 min at 0 °C and then it was allowed to warm to r.t. and stirred overnight. Reaction mixture was then diluted with DCM (60 ml) and the solution of $Na_2S_2O_3 \cdot 5H_2O$ (19.2 g) in aqueous $NaHCO_3$ (saturated, 150 ml) was added. The organic phase was washed with water, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by HPFC (silica column, 0→10% EtOAc in PE) afforded the product **95** (1.37 g, 91 %) as a yellowish foam.

^{1}H NMR (500.0 MHz, $CDCl_3$): 0.99 – 1.26 (m, 28H, $(CH_3)_2CHSi$); 4.08 (ddd, 1H, $J_{4',3'}$ = 9.9, $J_{4',5'}$ = 2.9, 2.5, H-4′); 4.15 (dd, 1H, J_{gem} = 13.2, $J_{5'b,4'}$ = 2.9, H-5′b); 4.20 (dd, 1H, J_{gem} = 13.2, $J_{5'a,4'}$ = 2.5, H-5′a); 5.62 (d, 1H, $J_{3',4'}$ = 9.9, H-3′); 6.01 (s, 1H,

H-1′); 7.52 (d, 1H, $J_{8,7}$ = 8.8, H-8); 7.58 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 8.34 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.60 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, $CDCl_3$): 12.40, 12.49, 12.90, 13.46 ((CH_3)$_2$**C**HSi); 16.74, 16.77, 16.81, 16.91, 17.19, 17.27, 17.29, 17.31 (($\mathbf{C}H_3$)$_2$CHSi); 60.56 (CH_2-5′); 72.27 (CH-3′); 78.59 (CH-4′); 79.56 (CH-1′); 110.80 (CH-8); 112.32 (C-4a); 119.95 (C-4b); 123.14 (CH-5); 128.92 (C-6); 128.99 (CH-7); 137.15 (C-8a); 153.31 (C-4); 153.77 (CH-2); 155.26 (C-9a); 206.22 (C-3′).

ESI MS m/z (rel. %): 610 (23) [M+H], 632 (12) [M+Na].

HR MS (ESI) for $C_{27}H_{38}O_5N_3Cl_2Si_2$ [M+H]: calcd 610.17216; found 610.17223.

4,6-Dichloro-9-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*β*-D-arabinofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (96)

Ketone **95** (1.7 g, 2.8 mmol) was dissolved in ethanol (99 %, 50 ml) and cooled to 0 °C. Then the solution of sodium borohydride (212 mg, 5.6 mmol) in ethanol (99 %, 50 ml) was slowly added and the reaction mixture was stirred at r.t. for 1 hour. Then, aqueous NH_4Cl (saturated, 30 ml) was added and the reaction mixture was extracted with EtOAc (150 ml). The organic layer was washed with water (100 ml), dried over $MgSO_4$ and evaporated under reduced pressure. After the purification by HPFC (silica column, 0→15% EtOAc in PE), the arabinonucleoside **96** (1.62 g, 93 %) was obtained as a white foam.

^{1}H NMR (500.0 MHz, $CDCl_3$): 0.97 – 1.18 (m, 28H, (CH_3)$_2$CHSi); 3.81 (ddd, 1H, $J_{4',3'}$ = 7.7, $J_{4',5'}$ = 4.1, 3.4, H-4′); 3.98 (dd, 1H, J_{gem} = 12.7, $J_{5'b,4'}$ = 4.1, H-5′b); 4.03 (dd, 1H, J_{gem} = 12.7, $J_{5'a,4'}$ = 3.4, H-5′a); 4.76 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.6, H-2′); 4.85 (dd, 1H, $J_{3',4'}$ = 7.7, $J_{3',2'}$ = 6.6, H-3′); 6.55 (d, 1H, $J_{1',2'}$ = 6.6, H-1′); 7.56 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.1, H-7); 7.82 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.36 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.72 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, $CDCl_3$): 12.45, 13.01, 13.08, 13.55 ((CH_3)$_2$**C**HSi); 17.01, 17.04, 17.09, 17.10, 17.39, 17.45, 17.54 (($\mathbf{C}H_3$)$_2$CHSi); 61.18 (CH_2-5′); 76.40 (CH-3′); 78.35 (CH-2′); 80.49 (CH-4′); 83.83 (CH-1′); 112.55 (C-4a); 113.80 (CH-8); 119.97 (C-4b); 122.72 (CH-5); 128.65 (C-6); 129.06 (CH-7); 138.20 (C-8a); 153.46 (CH-2); 153.53 (C-4); 155.39 (C-9a).

ESI MS m/z (rel. %): 612 (46) [M+H], 634 (100) [M+Na].

HR MS (ESI) for $C_{27}H_{39}O_5N_3Cl_2NaSi_2$ [M+Na]: calcd 634.16975; found 634.16989.

4,6-Dichloro-9-(*β*-D-arabinofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (97)

$Et_3N \cdot 3HF$ (655 µl, 4 mmol) was added to a solution of silyl-protected nucleoside **96** (1.2 g, 2 mmol) in anhydrous THF (30 ml). The reaction mixture was stirred overnight at r.t. and evaporated under reduced pressure. Purification by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O) afforded the free arabinonucleoside **97** (610 mg, 82 %) as a white solid after recrystallization from H_2O/MeOH (4:1).

m.p. 217–219 °C.

$[\alpha]_D^{20}$ -19.1 (c 0.204, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.76 – 3.88 (m, 3H, H-4′,5′); 4.15 (td, 1H, $J_{3',4'}$ = $J_{3',OH}$ = 4.8, $J_{3',2'}$ = 3.3, H-3′); 4.23 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 4.8, $J_{2',3'}$ = 3.3, H-2′); 5.16 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.33 (d, 1H, $J_{OH,2'}$ = 4.8, OH-2′); 5.62 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 6.81 (d, 1H, $J_{1',2'}$ = 4.9, H-1′); 7.62 (dd, 1H, $J_{7,8}$ = 9.0, $J_{7,5}$ = 2.2, H-7); 8.16 (d, 1H, $J_{8,7}$ = 9.0, H-8); 8.26 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.89 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 60.98 (CH_2-5′); 76.44 (CH-3′); 77.69 (CH-2′); 84.18 (CH-4′); 86.02 (CH-1′); 110.88 (C-4a); 117.86 (CH-8); 119.29 (C-4b); 121.02 (CH-5); 126.63 (C-6); 128.12 (CH-7); 138.42 (C-8a); 152.02 (C-4); 154.53 (CH-2); 155.58 (C-9a).

ESI MS m/z (rel. %): 392 (100) [M+Na].

HR MS (ESI) for $C_{15}H_{13}O_4N_3Cl_2Na$ [M+Na]: calcd 392.01753; found 392.01764.

IR (ATR): ν = 3302, 1590, 1442, 1296, 1219, 1157, 1064, 1033, 830, 566, 426 cm^{-1}.

5.3.1 Synthesis of 4-substituted arabinonucleosides

6-Chloro-4-methyl-9-[3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*β*-D-arabinofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (98a)

Nucleoside **96** (250 mg, 0.41 mmol) and $Pd(PPh_3)_4$ (24 mg, 0.021 mmol) were dissolved in anhydrous THF (4 ml) and Me_3Al (2M in toluene, 410 µl) was added.

The reaction mixture was stirred for 18 hours at 70 °C. After evaporation of solvents under reduced pressure and HPFC purification (silica column, 10→50% EtOAc in PE), the product **98a** (225 mg, 93 %) was obtained as a white foam.

^{1}H NMR (500.0 MHz, DMSO-d_6): 0.94 – 1.23 (m, 28H, $(CH_3)_2CHSi$); 2.95 (s, 3H, CH_3); 3.84 (ddd, 1H, $J_{4',3'}$ = 8.6, $J_{4',5'}$ = 3.4, 3.1, H-4′); 4.03 (dd, 1H, J_{gem} = 12.9, $J_{5'b,4'}$ = 3.1, H-5′b); 4.20 (dd, 1H, J_{gem} = 12.9, $J_{5'a,4'}$ = 3.4, H-5′a); 4.50 (ddd, 1H, $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 6.6, $J_{2',OH}$ = 5.8, H-2′); 4.63 (dd, 1H, $J_{3',4'}$ = 8.6, $J_{3',2'}$ = 6.6, H-3′); 5.46 (d, 1H, $J_{OH,2'}$ = 5.8, OH-2′); 6.86 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 7.40 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.97 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.19 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.84 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 12.10, 12.62, 12.85, 13.05 ($(CH_3)_2$**C**HSi); 16.95, 17.02, 17.06, 17.14, 17.42, 17.48, 17.57, 17.67 (($\mathbf{C}H_3)_2$CHSi); 22.92 (CH_3); 60.70 (CH_2-5′); 76.24 (CH-2′); 76.80 (CH-3′); 79.09 (CH-4′); 83.20 (CH-1′); 111.37 (C-4a); 115.49 (CH-8); 121.19 (C-4b); 122.12 (CH-5); 126.08 (C-6); 126.37 (CH-7); 137.76 (C-8a); 154.32 (CH-2); 154.89 (C-9a); 160.70 (C-4).

ESI MS m/z (rel. %): 592 (100) [M+H], 614 (73) [M+Na].

HR MS (ESI) for $C_{28}H_{43}O_5N_3ClSi_2$ [M+H]: calcd 592.24243; found 592.24254.

9-(*β*-D-Arabinofuranosyl)-6-chloro-4-methyl-9*H*-pyrimido[4,5-*b*]indole (99a)

Protected nucleoside **98a** (185 mg, 0.31 mmol) was dissolved in anhydrous THF (5 ml) and solution of tetrabutylammonium fluoride (195 mg, 0.62 mmol) in ahydrous THF (0.5 ml) was added dropwise at 0 °C. The reaction mixture was then stirred at r.t. for 30 min., solvent was evaporated under reduced pressure and the crude product was purified by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O). Recrystallization (H_2O/MeOH 4:1) afforded product **99a** (67 mg, 61 %) as a white crystalline solid.

m.p. 233–235 °C.

$[\alpha]_D^{20}$ -10.7 (c 0.206, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.97 (s, 3H, CH_3); 3.76 – 3.87 (m, 3H, H-4′,5′); 4.16 (dd, 1H, $J_{3',4'}$ = *4.5*, $J_{3',2'}$ = 3.3, H-3′); 4.22 (td, 1H, $J_{2',1'}$ = 4.9, $J_{2',3'}$ = 3.3, H-2′); 6.81 (d, 1H, $J_{1',2'}$ = 4.9, H-1′); 7.53 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 8.11 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.17 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.93 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 22.48 (CH_3O); 61.06 (CH_2-5′); 76.59 (CH-3′); 77.64 (CH-2′); 83.99 (CH-4′); 85.50 (CH-1′); 111.50 (C-4a); 117.31 (CH-8); 120.94 (C-4b); 121.67 (CH-5); 126.12 (C-6); 126.86 (CH-7); 138.01 (C-8a); 153.70 (CH-2); 154.55 (C-9a); 159.89 (C-4).

ESI MS m/z (rel. %): 350 (12) [M+H], 372 (100) [M+Na].

HR MS (ESI) for $C_{16}H_{16}O_4N_3ClNa$ [M+Na]: calcd 372.07215; found 372.07232.

IR (ATR): ν = 3271, 1564, 1475, 1068, 1030, 836, 567, 425 cm^{-1}.

9-(*β*-D-Arabinofuranosyl)-6-chloro-4-methylsulfanyl-9*H*-pyrimido[4,5-*b*]indole (99b)

Nucleoside **96** (250 mg, 0.41 mmol) and sodium thiomethoxide (60 mg, 0.86 mmol) were dissolved in anhydrous EtOH (10 ml) and stirred for 1.5 hours at r.t. The solvent was evaporated under reduced pressure, the crude product **98b** was dissolved in anhydrous THF (7 ml) and cooled to 0 °C. Then, the solution of tetrabutylammonium fluoride (260 mg, 0.82 mmol) in ahydrous THF (1 ml) was added dropwise. The reaction mixture was stirred at r.t. for 1 hour, solvent was removed under reduced pressure and the crude product was purified by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O). After recrystallization (H_2O/MeOH 4:1), the product **99b** (100 mg, 63 % over two steps) was furnished as a white solid.

m.p. 181–183 °C.

$[\alpha]_D^{20}$ -14.9 (c 0.276, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.80 (s, 3H, CH_3S); 3.76 – 3.85 (m, 3H, H-4′,5′); 4.15 (td, 1H, $J_{3',4'} = J_{3',OH} = 4.8$, $J_{3',2'} = 3.2$, H-3′); 4.20 (td, 1H, $J_{2',1'} = J_{2',OH} = 4.9$, $J_{2',3'} = 3.2$, H-2′); 5.11 (t, 1H, $J_{OH,5'} = 5.2$, OH-5′); 5.30 (d, 1H, $J_{OH,2'} = 4.9$, OH-2′); 5.56 (d, 1H, $J_{OH,3'} = 4.8$, OH-3′); 6.79 (d, 1H, $J_{1',2'} = 4.9$, H-1′); 7.52 (dd, 1H, $J_{7,8} = 8.9$, $J_{7,5} = 2.2$, H-7); 8.01 (d, 1H, $J_{5,7} = 2.2$, H-5); 8.10 (d, 1H, $J_{8,7} = 8.9$, H-8); 8.87 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 11.83 (CH_3S); 61.05 (CH_2-5′); 76.54 (CH-3′); 77.68 (CH-2′); 83.99 (CH-4′); 85.57 (CH-1′); 109.33 (C-4a); 117.38 (CH-8); 120.28 (C-4b); 120.87 (CH-5); 125.89 (C-6); 126.40 (CH-7); 137.38 (C-8a); 153.10 (C-9a); 154.03 (CH-2); 162.32 (C-4).

ESI MS m/z (rel. %): 404 (100) [M+Na].

HR MS (ESI) for $C_{16}H_{16}O_4N_3ClNaS$ [M+Na]: calcd 404.04423; found 404.04433.

IR (ATR): ν = 3278, 1557, 1473, 1433, 1292, 1236, 1163, 1119, 1072, 943, 844, 802, 593, 565 cm^{-1}.

4-Amino-9-(*β*-D-arabinofuranosyl)-6-chloro-9*H*-pyrimido[4,5-*b*]indole (99c)

Arabinoside **97** (120 mg, 0.32 mmol) was dissolved in dioxane (3 ml) and aqueous ammonia (30%, 3 ml) was added. The reaction mixture was stirred in screw-cap pressure glass tube at 100 °C for 20 h and then solvents were evaporated under reduced pressure. Purification by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O) and recrystallization from H_2O/MeOH mixture (4:1) afforded the 4-amino arabinonucleoside **99c** (95 mg, 85 %) as a white solid.

m.p. 298–301 °C.

$[\alpha]_D^{20}$ 0 (c 0.228, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.69 – 3.85 (m, 3H, H-4′,5′); 4.10 – 4.17 (m, 2H, H-2′,3′); 5.14 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5′); 5.33 (d, 1H, $J_{OH,2'}$ = 5.2, OH-2′); 5.51 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 6.69 (d, 1H, $J_{1',2'}$ = 4.7, H-1′); 7.30 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.37 (bs, 2H, NH_2); 7.92 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.30 (s, 1H, H-2); 8.42 (d, 1H, $J_{5,7}$ = 2.2, H-5).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.24 (CH_2-5′); 76.73 (CH-3′); 77.67 (CH-2′); 83.75 (CH-4′); 85.32 (CH-1′); 94.95 (C-4a); 116.22 (CH-8); 120.06 (CH-5); 121.56 (C-4b); 123.99 (CH-7); 125.41 (C-6); 136.09 (C-8a); 155.21 (CH-2); 155.53 (C-9a); 157.78 (C-4).

ESI MS m/z (rel. %): 351 (100) [M+H], 373 (56) [M+Na].

HR MS (ESI) for $C_{15}H_{16}O_4N_4Cl$ [M+H]: calcd 351.08546; found 351.08557.

IR (ATR): ν = 3120, 1654, 1597, 1460, 1319, 1218, 1033, 907, 855, 795, 525 cm^{-1}.

9-(*β*-D-Arabinofuranosyl)-6-chloro-4-methoxy-9*H*-pyrimido[4,5-*b*]indole (99d)

A mixture of nucleoside **97** (145 mg, 0.39 mmol) and sodium methoxide (1M in MeOH, 2 ml) in anhydrous MeOH (5 ml) was stirred for 4 hours at r.t. After evaporation of the solvent, purification by reversed-phase HPFC (C18 column,

10→100% MeOH in H_2O) and recrystallization (H_2O/MeOH 4:1), the product **99d** (90 mg, 64 %) was obtained as a white solid.

m.p. 238–241 °C.

$[\alpha]_D^{20}$ -8.4 (c 0.298, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.75 – 3.85 (m, 3H, H-4′,5′); 4.15 (td, 1H, $J_{3',4'}$ = $J_{3',OH}$ = 4.8, $J_{3',2'}$ = 3.3, H-3′); 4.20 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 4.9, $J_{2',3'}$ = 3.3, H-2′); 4.20 (s, 3H, CH_3O); 5.32 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.32 (d, 1H, $J_{OH,2'}$ = 4.9, OH-2′); 5.55 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 6.77 (d, 1H, $J_{1',2'}$ = 4.9, H-1′); 7.45 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.95 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.04 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.68 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 54.38 (CH_3O); 61.12 (CH_2-5′); 76.57 (CH-3′); 77.67 (CH-2′); 83.93 (CH-4′); 85.65 (CH-1′); 98.31 (C-4a); 117.22 (CH-8); 120.15 (C-4b); 120.61 (CH-5); 125.68 (CH-7); 125.82 (C-6); 136.92 (C-8a); 154.85 (CH-2); 156.33 (C-9a); 163.81 (C-4).

ESI MS m/z (rel. %): 388 (100) [M+Na].

HR MS (ESI) for $C_{16}H_{16}O_5N_3ClNa$ [M+Na]: calcd 388.06707; found 388.06722.

IR (ATR): ν = 3299, 1598, 1566, 1460, 1323, 1294, 1192, 1124, 1052, 943, 769, 722, 588, 560 cm^{-1}.

9-(*β*-D-Arabinofuranosyl)-6-chloro-4-(thiophene-2-yl)-9*H*-pyrimido[4,5-*b*]indole (99e)

Arabinoside **97** (120 mg, 0.32 mmol) was reacted with 2-thienylboronic acid (62 mg, 0.48 mmol) in 3 ml of H_2O/MeCN (2:1) according to the general procedure for aqueous Suzuki-cross coupling. The product **99e** (94 mg, 70 %) was obtained as a tan solid after recrystallization (H_2O/MeOH 4:1).

m.p. 192–194 °C.

$[\alpha]_D^{20}$ +18.1 (c 0.260, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.77 – 3.87 (m, 3H, H-4′,5′); 4.16 (td, 1H, $J_{3',4'}$ = $J_{3',OH}$ = 4.8, $J_{3',2'}$ = 3.2, H-3′); 4.25 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 4.8, $J_{2',3'}$ = 3.2, H-2′); 5.13 (t, 1H, $J_{OH,5'}$ = 5.0, OH-5′); 5.35 (d, 1H, $J_{OH,2'}$ = 4.8, OH-2′); 5.59 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 6.88 (d, 1H, $J_{1',2'}$ = 4.8, H-1′); 7.43 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.7, H-4-thienyl); 7.54 (dd, 1H, $J_{7,8}$ = 9.0, $J_{7,5}$ = 2.2, H-7); 7.99 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.1,

H-5-thienyl); 8.06 (dd, 1H, $J_{3,4}$ = 3.7, $J_{3,5}$ = 1.1, H-3-thienyl); 8.15 (d, 1H, $J_{8,7}$ = 9.0, H-8); 8.20 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.98 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.06 (CH_2-5′); 76.66 (CH-3′); 77.69 (CH-2′); 84.06 (CH-4′); 85.71 (CH-1′); 108.77 (C-4a); 117.61 (CH-8); 120.16 (C-4b); 120.58 (CH-5); 125.65 (C-6); 127.36 (CH-7); 128.69 (CH-4-thienyl); 129.83 (CH-3-thienyl); 131.48 (CH-5-thienyl); 138.42 (C-8a); 141.16 (C-2-thienyl); 153.07 (C-4); 154.30 (CH-2); 156.06 (C-9a).

ESI MS m/z (rel. %): 418 (100) [M+H].

HR MS (ESI) for $C_{19}H_{17}O_4N_3ClS$ [M+H]: calcd 418.06228; found 418.06235.

IR (ATR): ν = 3358, 1562, 1447, 1217, 1172, 1138, 1057, 918, 707, 632, 483 cm^{-1}.

9-(*β*-D-Arabinofuranosyl)-6-chloro-4-phenyl-9*H*-pyrimido[4,5-*b*]indole (99f)

Arabinoside **97** (120 mg, 0.32 mmol) was reacted with phenylboronic acid (59 mg, 0.48 mmol) in 3 ml of H_2O/MeCN (2:1) according to the general procedure for aqueous Suzuki-cross coupling. The product **99f** (76 mg, 58 %) was obtained as a tan solid after recrystallization (H_2O/MeOH 4:1).

m.p. 183–185 °C.

$[\alpha]_D^{20}$ -6.5 (c 0.168, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.78 – 3.88 (m, 3H, H-4′,5′); 4.18 (td, 1H, $J_{3',4'}$ = $J_{3',OH}$ = 4.8, $J_{3',2'}$ = 3.2, H-3′); 4.26 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 4.8, $J_{2',3'}$ = 3.2, H-2′); 5.13 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.36 (d, 1H, $J_{OH,2'}$ = 4.8, OH-2′); 5.59 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 6.90 (d, 1H, $J_{1',2'}$ = 4.8, H-1′); 7.50 (dd, 1H, $J_{7,8}$ = 9.0, $J_{7,5}$ = 2.2, H-7); 7.64 (d, 1H, $J_{5,7}$ = 2.2, H-5); 7.67 – 7.72 (m, 3H, H-*m*,*p*-Ph); 7.88 (m, 2H, H-*o*-Ph); 8.13 (d, 1H, $J_{8,7}$ = 9.0, H-8); 9.08 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.10 (CH_2-5′); 76.67 (CH-3′); 77.68 (CH-2′); 84.10 (CH-4′); 85.66 (CH-1′); 110.16 (C-4a); 117.61 (CH-8); 120.30 (C-4b); 120.50 (CH-5); 125.44 (C-6); 127.21 (CH-7); 128.91 (CH-*o*-Ph); 129.09 (CH-*m*-Ph); 130.62 (CH-*p*-Ph); 137.83 (C-*i*-Ph); 138.39 (C-8a); 154.64 (CH-2); 155.66 (C-9a); 159.88 (C-4).

ESI MS m/z (rel. %): 412 (100) [M+H], 434 (59) [M+Na].

HR MS (ESI) for $C_{21}H_{19}O_4N_3Cl$ [M+H]: calcd 412.10586; found 412.10594.

IR (ATR): ν = 3365, 1561, 1448, 1219, 1174, 1058, 918, 883, 767, 702, 640, 601, 483 cm^{-1}.

5.4 Synthesis of pyrimido[4,5-*b*]indole 2'-deoxy-2'-fluororibonucleosides

4,6-Dichloro-9-[2-*O*-acetyl-3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-*β*-D-arabinofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (100)

Protected arabinonucleoside **96** (3.49 g, 5.7 mmol) was dissolved in anhydrous MeCN (100 ml) and Et_3N (0.95 ml, 6.8 mmol), DMAP (70 mg, 0.57 mmol) and acetic anhydride (0.65 ml, 6.8 mmol) were added. The reaction mixture was stirred at r.t. for 1 hour and then evaporated under reduced pressure. Residue was dissolved in EtOAc (60 ml), extracted with water (50 ml) and aqueous $NaHCO_3$ (saturated, 30 ml), dried under $MgSO_4$ and after evaporation under reduced pressure, acetate **100** (3.62 g, 96 %) was obtained as a yellowish foam.

^{1}H NMR (500.0 MHz, $CDCl_3$): 0.98 – 1.28 (m, 28H, $(CH_3)_2CHSi$); 1.32 (s, 3H, CH_3CO); 3.90 (ddd, 1H, $J_{4',3'}$ = 8.5, $J_{4',5'}$ = 3.8, 3.2, H-4′); 4.14 (dd, 1H, J_{gem} = 12.7, $J_{5'b,4'}$ = 3.2, H-5′b); 4.27 (dd, 1H, J_{gem} = 12.7, $J_{5'a,4'}$ = 3.8, H-5′a); 5.11 (bdd, 1H, $J_{3',4'}$ = 8.5, $J_{3',2'}$ = 6.5, H-3′); 5.56 (dd, 1H, $J_{2',1'}$ = 6.9, $J_{2',3'}$ = 6.5, H-2′); 7.00 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 7.51 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.79 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.33 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.80 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, $CDCl_3$): 12.44, 13.03, 13.13, 13.33 ($(CH_3)_2$**C**HSi); 16.80, 16.84, 16.91, 16.96, 17.36, 17.43, 17.52, 17.54 ((**C**$H_3)_2$CHSi); 19.56 (**C**H_3CO); 60.85 (CH_2-5′); 74.09 (CH-3′); 79.19 (CH-2′); 80.02 (CH-4′); 81.74 (CH-1′); 111.57 (C-4a); 114.54 (CH-8); 119.80 (C-4b); 122.42 (CH-5); 128.29 (C-6); 128.51 (CH-7); 137.09 (C-8a); 153.02 (C-4); 154.11 (CH-2); 155.89 (C-9a); 169.49 (**C**OCH_3).

ESI MS m/z (rel. %): 676 (100) [M+Na].

HR MS (ESI) for $C_{29}H_{41}O_6N_3Cl_2NaSi_2$ [M+Na]: calcd 676.18032; found 676.1804215.

4,6-Dichloro-9-(2-*O*-acetyl-*β*-D-arabinofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (101)

To a solution of acetate **100** (3.55 g, 5.4 mmol) in anhydrous THF (80 ml), $Et_3N{\cdot}3HF$ (1.77 ml, 10.8 mmol) was added. The reaction mixture was stirred overnight at r.t. After evaporation under reduced pressure and HPFC purification (silica column, 0→2% MeOH in DCM), the product **101** (1.86 g, 84 %) was obtained as a yellowish foam.

^{1}H NMR (500.0 MHz, DMSO-d_6): 1.25 (s, 3H, CH_3CO); 3.79 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 5.1, H-5′b); 3.84 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.5, H-5′a); 3.91 (ddd, 1H, $J_{4',3'}$ = 6.4, $J_{4',5'}$ = 5.1, 3.5, H-4′); 4.48 (bdd, 1H, $J_{3',4'}$ = 6.4, $J_{3',2'}$ = 3.8, H-3′); 5.51 (bs, 1H, OH-5′); 5.27 (dd, 1H, $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 3.8, H-2′); 5.92 (bs, 1H , OH-3′); 7.02 (d, 1H, $J_{1',2'}$ = 5.4, H-1′); 7.70 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 8.10 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.27 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.92 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 19.71 (**C**H_3CO); 60.25 (CH_2-5′); 73.58 (CH-3′); 79.91 (CH-2′); 82.94 (CH-1′); 83.29 (CH-4′); 110.77 (C-4a); 116.88 (CH-8); 119.31 (C-4b); 121.37 (CH-5); 127.16 (C-6); 128.48 (CH-7); 137.16 (C-8a); 152.27 (C-4); 154.71 (CH-2); 155.54 (C-9a); 169.22 (**C**OCH_3).

ESI MS m/z (rel. %): 434 (100) [M+Na].

HR MS (ESI) for $C_{17}H_{15}O_5N_3Cl_2Na$ [M+Na]: calcd 434.02810; found 434.02816.

4,6-Dichloro-9-[2-*O*-acetyl-3,5-di-*O*-(tetrahydropyran-2-yl)-*β*-D-arabinofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (102)

Compound **101** (1.83 g, 4.4 mmol) and $TsOH{\cdot}H_2O$ (840 mg, 8.8 mmol) were dissolved in anhydrous DMF (50 ml) and the reaction mixture was cooled to 0 °C. 3,4-Dihydro-2*H*-pyran (12.2 ml, 134 mmol) was added and the reaction mixture was allowed to warm to r.t. and stirred overnight. The reaction mixture was diluted with EtOAc (100 ml) and extracted with aqueous $NaHCO_3$ (saturated, 50 ml). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. HPFC purification (silica column, 10→50% EtOAc in PE) furnished the crude product **102** (2.43 g) as a yellow oil. This intermediate was not characterized by NMR and was used directly in the next step.

ESI MS m/z (rel. %): 580 (14) [M+H], 602 (100) [M+Na].

HR MS (ESI) for $C_{27}H_{31}O_7N_3Cl_2Na$ [M+Na]: calcd 602.14313; found 602.14319.

4,6-Dichloro-9-[3,5-di-*O*-(tetrahydropyran-2-yl)-*β*-D-arabinofuranosyl]-9*H*-pyrimido[4,5-*b*]indole (103)

Crude THP-protected acetate **102** (2.4 g) was dissolved in methanolic ammonia (27 %, 200 ml) at 0 °C and stirred for 4 hours at 0 °C. The evaporation of the solution under reduced pressure resulted in crude THP-protected arabinoside **103** (2.1 g) as a yellow oil. This intermediate was not characterized by NMR and was used directly in the next step.

ESI MS m/z (rel. %): 538 (14) [M+H], 560 (100) [M+Na].

HR MS (ESI) for $C_{25}H_{29}O_6N_3Cl_2Na$ [M+Na]: calcd 560.13256; found 560.13257.

4,6-Dichloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (104)

THP-protected arabinonucleoside **103** (2.1 g, 3.9 mmol) was dissolved in anhydrous DCM (55 ml) and anhydrous pyridine was added (2.4 ml, 9.75 ml). The reaction mixture was cooled to 0 °C and DAST (2.6 ml; 19.7 mmol) was added. The reaction mixture was allowed to warm to r.t. and stirred overnight. Then, it was diluted with DCM (60 ml) and neutralized with aqueous $NaHCO_3$ (saturated, 90 ml). The organic phase was washed with water (90 ml), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was then dissolved in aqueous TFA (90 % *v*/*v*, 20 ml) and stirred at r.t. for 2 hours. The solution was evaporated under reduced pressure and several times co evaporated with MeOH. After reversed-phase HPFC purification (C18 column, 10→100% MeOH in H_2O) and recrystallization (H_2O/MeOH 3:1), the free fluororibonucleoside **104** (640 mg, 38 % over four steps) was obtained as a beige solid.

m.p. 249–251 °C.

$[\alpha]_D^{20}$ -26.2 (c 0.240, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.61 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.5, H-5'b); 3.74 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 2.6, H-5'a); 4.02 (dddd, 1H, $J_{4',3'}$ = 6.3, $J_{4',5'}$ = 4.5, 2.6, $J_{H,F}$ = 1.0, H-4'); 4.63 (ddd, 1H, $J_{H,F}$ = 14.8, $J_{3',4'}$ = 6.3, $J_{3',2'}$ = 5.4, H-3'); 5.71

(ddd, 1H, $J_{H,F}$ = 53.6, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 3.9, H-2'); 6.72 (dd, 1H, $J_{H,F}$ = 19.3, $J_{1',2'}$ = 3.9, H-1'); 7.71 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.1, H-7); 8.18 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.31 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.91 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 60.78 (CH_2-5'); 68.45 (d, $J_{C,F}$ = 15.7, CH-3'); 84.20 (CH-4'); 86.02 (d, $J_{C,F}$ = 34.0, CH-1'); 91.75 (d, $J_{C,F}$ = 187.0, CH-2'); 111.41 (C-4a); 114.60 (CH-8); 119.53 (C-4b); 121.85 (CH-5); 127.55 (C-6); 128.92 (CH-7); 136.78 (C-8a); 152.54 (C-4); 154.70 (CH-2); 155.67 (C-9a).

^{19}F NMR (470.4 MHz, DMSO-d_6): -198.47 (ddd, $J_{F,H}$ = 53.6, 19.3, 14.8).

ESI MS m/z (rel. %): 372 (100) [M+H].

HR MS (ESI) for $C_{15}H_{13}O_3N_3ClF$ [M+H]: calcd 372.03125; found 372.03137.

IR (ATR): ν = 3279, 1589, 1443, 1295, 1227, 1104, 1057, 1028, 1003, 833, 808, 537 cm^{-1}.

5.4.1 Synthesis of 4-substituted 2'-deoxy-2'-fluororibononucleosides

4-Amino-6-chloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (105a)

Fluororiboside **104** (90 mg, 0.24 mmol) was dissolved in dioxane (3 ml) and aqueous ammonia (30%, 3 ml) was added. The reaction mixture was stirred in screw-cap pressure glass tube at 100 °C for 20 h and then solvents were evaporated under reduced pressure. Purification by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O) and recrystallization from H_2O/MeOH mixture (3:1) afforded the 4-amino derivative **105a** (69 mg, 82 %) as a white solid.

m.p. 275–278 °C.

$[\alpha]_D^{20}$ -44.9 (c 0.207, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.59 (ddd, 1H, J_{gem} = 12.2, $J_{5'b,OH}$ = 5.9, $J_{5'b,4'}$ = 4.5, H-5'b); 3.72 (ddd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 2.6, H-5'a); 3.97 (dddd, 1H, $J_{4',3'}$ = 5.8, $J_{4',5'}$ = 4.5, 2.6, $J_{H,F}$ = 1.2, H-4'); 4.56 (dddd, 1H, $J_{H,F}$ = 13.0, $J_{3',OH}$ = 6.2, $J_{3',4'}$ = 5.8, $J_{3',2'}$ = 5.5, H-3'); 5.16 (dd, 1H, $J_{OH,5'}$ = 5.9, 5.2, OH-5′); 5.65 (ddd, 1H, $J_{H,F}$ = 54.1, $J_{2',3'}$ = 5.5, $J_{2',1'}$ = 4.4, H-2'); 5.69 (d, 1H, $J_{OH,3'}$ = 6.2, OH-3′); 6.60 (dd, 1H, $J_{H,F}$ = 19.3, $J_{1',2'}$ = 4.4, H-1'); 7.41 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.51

(bs, 2H, NH_2); 7.90 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.31 (s, 1H, H-2); 8.51 (d, 1H, $J_{5,7}$ = 2.1, H-5).

^{13}C NMR (125.7 MHz, DMSO-d_6): 61.18 (CH_2-5'); 68.61 (d, $J_{C,F}$ = 15.5, CH-3'); 84.09 (CH-4'); 85.45 (d, $J_{C,F}$ = 33.3, CH-1'); 91.45 (d, $J_{C,F}$ = 186.9, CH-2'); 95.37 (C-4a); 112.85 (CH-8); 120.93 (CH-5); 121.28 (C-4b); 124.88 (CH-7); 126.48 (C-6); 134.57 (C-8a); 155.50 (CH-2); 155.67 (C-9a); 157.99 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): -199.28 (ddd, $J_{F,H}$ = 54.1, 19.3, 13.0).

ESI MS m/z (rel. %): 353 (100) [M+H], 375 (31) [M+Na].

HR MS (ESI) for $C_{15}H_{15}O_3N_4ClF$ [M+H]: calcd 353.08112; found 353.08124.

IR (ATR): ν = 3163, 1575, 1463, 1303, 1198, 1059, 901, 857, 799, 775, 425 cm^{-1}.

6-Chloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-4-methoxy-9*H*-pyrimido[4,5-*b*]indole (105b)

Nucleoside **104** (90 mg, 0.24 mmol) was dissolved in dry MeOH (5 ml) and sodium methoxide (1M in MeOH, 2 ml) was added. The reaction mixture was stirred for 3 hours at r.t. After evaporation of the solvent, purification by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O) and recrystallization (H_2O/MeOH 3:1), the product **105b** (71 mg, 80 %) was obtained as a white solid.

m.p. 201–205 °C.

$[\alpha]_D^{20}$ -33.6 (c 0.217, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.60 (ddd, 1H, J_{gem} = 12.2, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 4.6, H-5'b); 3.73 (dd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 2.6, H-5'a); 4.00 (dddd, 1H, $J_{4',3'}$ = 6.3, $J_{4',5'}$ = 4.6, 2.6, $J_{H,F}$ = 1.0, H-4'); 4.20 (s, 3H, CH_3O); 4.60 (dtd, 1H, $J_{H,F}$ = 13.8, $J_{3',4'}$ = $J_{3',OH}$ = 6.3, $J_{3',2'}$ = 5.4, H-3'); 5.07 (dd, 1H, $J_{OH,5'}$ = 5.6, 5.3, OH-5′); 5.69 (ddd, 1H, $J_{H,F}$ = 53.6, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 4.2, H-2'); 5.73 (d, 1H, $J_{OH,3'}$ = 6.3, OH-3′); 6.67 (dd, 1H, $J_{H,F}$ = 19.2, $J_{1',2'}$ = 4.2, H-1'); 7.54 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.2, H-7); 8.00 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.05 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.71 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 54.52 (CH_3O); 60.97 (CH_2-5'); 68.52 (d, $J_{C,F}$ = 15.6, CH-3'); 84.11 (CH-4'); 85.74 (d, $J_{C,F}$ = 33.5, CH-1'); 91.64 (d, $J_{C,F}$ = 187.1, CH-2'); 98.91 (C-4a); 113.96 (CH-8); 120.39 (C-4b); 121.45 (CH-5); 126.59 (CH-7); 126.86 (C-6); 135.39 (C-8a); 155.20 (CH-2); 156.39 (C-9a); 164.06 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): -199.04 (ddd, $J_{F,H}$ = 53.6, 19.2, 13.8).

ESI MS m/z (rel. %): 368 (79) [M+H], 390 (100) [M+Na].

HR MS (ESI) for $C_{16}H_{15}O_4N_3ClFNa$ [M+Na]: calcd 390.06273; found 390.06282.

IR (ATR): ν = 3313, 1600, 1460, 1327, 1189, 1109, 1069, 1045, 894, 851, 536, 432 cm^{-1}.

6-Chloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-4-methylsulfanyl-9*H*-pyrimido[4,5-*b*]indole (105c)

Nucleoside **104** (90 mg, 0.24 mmol) and sodium thiomethoxide (35 mg, 0.50 mmol) were dissolved in anhydrous EtOH (10 ml) and stirred for 2 hours at r.t. The solvent was then evaporated under reduced pressure. The purification by reversed-phase HPFC (C18 column, 10→100% MeOH in H_2O) and recrystallization (H_2O/MeOH 3:1) afforded the product **105c** (80 mg, 87 %) as a white solid.

m.p. 205–209 °C.

$[\alpha]_D^{20}$ -34.6 (c 0.188, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.80 (s, 3H, CH_3S); 3.59 (ddd, 1H, J_{gem} = 12.2, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 4.6, H-5'b); 3.73 (dd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 2.6, H-5'a); 4.00 (dddd, 1H, $J_{4',3'}$ = 6.2, $J_{4',5'}$ = 4.6, 2.6, $J_{H,F}$ = 1.3, H-4'); 4.62 (dtd, 1H, $J_{H,F}$ = 13.8, $J_{3',4'}$ = $J_{3',OH}$ = 6.2, $J_{3',2'}$ = 5.4, H-3'); 5.07 (dd, 1H, $J_{OH,5'}$ = 5.5, 5.3, OH-5′); 5.69 (ddd, 1H, $J_{H,F}$ = 53.8, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 4.0, H-2'); 5.75 (d, 1H, $J_{OH,3'}$ = 6.2, OH-3′); 6.69 (dd, 1H, $J_{H,F}$ = 19.5, $J_{1',2'}$ = 4.0, H-1'); 7.62 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 8.04 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.11 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.88 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 11.92 (CH_3S); 60.91 (CH_2-5'); 68.50 (d, $J_{C,F}$ = 15.6, CH-3'); 84.08 (CH-4'); 85.74 (d, $J_{C,F}$ = 33.8, CH-1'); 91.75 (d, $J_{C,F}$ = 186.8, CH-2'); 109.74 (C-4a); 114.15 (CH-8); 120.51 (C-4b); 121.71 (CH-5); 126.94 (C-6); 127.32 (CH-7); 135.84 (C-8a); 153.24 (C-9a); 154.27 (CH-2); 163.16 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): -198.57 (ddd, $J_{F,H}$ = 53.8, 19.5, 13.8).

ESI MS m/z (rel. %): 384 (81) [M+H], 406 (100) [M+Na].

HR MS (ESI) for $C_{16}H_{15}O_3N_3ClFNaS$ [M+Na]: calcd 406.03989; found 406.03999.

IR (ATR): ν = 3247, 1558, 1472, 1433, 1295, 1235, 1049, 966, 906, 839, 794, 594, 535 cm^{-1}.

6-Chloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-4-methyl-9*H*-pyrimido[4,5-*b*]indole (105d)

Nucleoside **104** (90 mg, 0.24 mmol) and $Pd(PPh_3)_4$ (14 mg, 0.012 mmol) were dissolved in anhydrous THF (4 ml) and Me_3Al (2M in toluene, 250 µl) was added. The reaction mixture was stirred at 70 °C for 24 hours. After evaporation of solvents under reduced pressure, reversed-phase HPFC purification (C18 column, 10→100% MeOH in H_2O) and recrystallization (H_2O/MeOH 3:1) the product **105d** (55 mg, 65 %) was obtained as a white solid.

m.p. 247–251 °C.

$[\alpha]_D^{20}$ -45.2 (c 0.179, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 2.95 (s, 3H, CH_3); 3.59 (ddd, 1H, J_{gem} = 12.2, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 4.7, H-5'b); 3.73 (dd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 2.6, H-5'a); 4.00 (dddd, 1H, $J_{4',3'}$ = 6.2, $J_{4',5'}$ = 4.7, 2.6, $J_{H,F}$ = 1.0, H-4'); 4.62 (dtd, 1H, $J_{H,F}$ = 14.0, $J_{3',4'}$ = $J_{3',OH}$ = 6.2, $J_{3',2'}$ = 5.4, H-3'); 5.08 (dd, 1H, $J_{OH,5'}$ = 5.5, 5.3, OH-5′); 5.71 (ddd, 1H, $J_{H,F}$ = 53.9, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 4.1, H-2'); 5.75 (d, 1H, $J_{OH,3'}$ = 6.2, OH-3′); 6.70 (dd, 1H, $J_{H,F}$ = 19.4, $J_{1',2'}$ = 4.1, H-1'); 7.62 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 8.09 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.23 (d, 1H, $J_{5,7}$ = 2.1, H-5); 8.89 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 23.03 (CH_3); 60.97 (CH_2-5'); 68.53 (d, $J_{C,F}$ = 15.6, CH-3'); 84.07 (CH-4'); 85.54 (d, $J_{C,F}$ = 33.6, CH-1'); 91.57 (d, $J_{C,F}$ = 186.9, CH-2'); 111.84 (C-4a); 113.92 (CH-8); 121.27 (C-4b); 122.59 (CH-5); 126.94 (C-6); 127.56 (CH-7); 136.30 (C-8a); 154.60 (CH-2); 154.73 (C-9a); 161.33 (C-4).

^{19}F NMR (470.4 MHz, DMSO-d_6): -198.88 (ddd, $J_{F,H}$ = 53.9, 19.4, 14.0).

ESI MS m/z (rel. %): 352 (100) [M+H], 374 (60) [M+Na].

HR MS (ESI) for $C_{16}H_{16}O_3N_3ClF$ [M+H]: calcd 352.08587; found 352.08597.

IR (ATR): ν = 3309, 1580, 1449, 1371, 1299, 1183, 1133, 1103, 1068, 1024, 859, 828, 808, 621, 525, 480, 425, 389 cm^{-1}.

6-Chloro-9-(2-deoxy-2-fluoro-*β*-D-ribofuranosyl)-4-(thiophene-2-yl)-9*H*-pyrimido-[4,5-*b*]indole (105e)

Nucleoside **104** (90 mg, 0.24 mmol) was reacted with 2-thienylboronic acid (46 mg, 0.36 mmol) in 3 ml of H_2O/MeCN (2:1) according to the general procedure for aqueous Suzuki-cross coupling. The product **105e** (94 mg, 70 %) was obtained as a tan solid after recrystallization (H_2O/MeOH 3:1).

m.p. 206–211 °C.

$[\alpha]_D^{20}$ -37.1 (c 0.186, DMSO).

^{1}H NMR (500.0 MHz, DMSO-d_6): 3.62 (ddd, 1H, J_{gem} = 12.2, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 4.6, H-5'b); 3.75 (ddd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 2.6, H-5'a); 4.02 (dddd, 1H, $J_{4',3'}$ = 6.3, $J_{4',5'}$ = 4.6, 2.6, $J_{H,F}$ = 1.0, H-4'); 4.65 (dtd, 1H, $J_{H,F}$ = 14.4, $J_{3',4'}$ = $J_{3',OH}$ = 6.3, $J_{3',2'}$ = 5.4, H-3'); 5.09 (dd, 1H, $J_{OH,5'}$ = 5.5, 5.3, OH-5′); 5.74 (ddd, 1H, $J_{H,F}$ = 53.8, $J_{2',3'}$ = 5.4, $J_{2',1'}$ = 4.0, H-2'); 5.78 (d, 1H, $J_{OH,3'}$ = 6.3, OH-3′); 6.77 (dd, 1H, $J_{H,F}$ = 19.7, $J_{1',2'}$ = 4.0, H-1'); 7.43 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.7, H-4-thienyl); 7.65 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 8.01 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.1, H-5-thienyl); 8.09 (dd, 1H, $J_{3,4}$ = 3.7, $J_{3,5}$ = 1.1, H-3-thienyl); 8.15 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.25 (d, 1H, $J_{5,7}$ = 2.1, H-5); 9.00 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): 60.95 (CH_2-5'); 68.52 (d, $J_{C,F}$ = 15.6, CH-3'); 84.11 (CH-4'); 85.75 (d, $J_{C,F}$ = 34.0, CH-1'); 91.67 (d, $J_{C,F}$ = 186.8, CH-2'); 109.17 (C-4a); 114.26 (CH-8); 120.52 (C-4b); 121.55 (CH-5); 126.67 (C-6); 128.30 (CH-7); 128.80 (CH-4-thienyl); 130.17 (CH-3-thienyl); 131.84 (CH-5-thienyl); 136.80 (C-8a); 140.94 (C-2-thienyl); 153.70 (C-4); 154.52 (CH-2); 156.24 (C-9a).

^{19}F NMR (470.4 MHz, DMSO-d_6): -198.28 (ddd, $J_{F,H}$ = 53.8, 19.7, 14.4).

ESI MS m/z (rel. %): 420 (100) [M+H], 442 (79) [M+Na].

HR MS (ESI) for $C_{19}H_{16}O_3N_3ClFS$ [M+H]: calcd 420.05794; found 420.05797.

IR (ATR): ν = 3090, 1569, 1443, 1291, 1051, 965, 804, 717, 539, 436 cm^{-1}.

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