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Nové modifikované nukleosidy s protivirovou nebo cytostatickou aktivitou
Novel modified nucleosides with antiviral or cytostatic activity

Disertační práce

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Prohlášení

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

V Praze, 30.09.2020

Podpis

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Abstract

A general and modular synthetic approach to 4-substituted phenyl, 2-substituted pyridin-5-yl and 5-substituted pyridin-2-yl 2′-*C*-methyl-*C*-ribonucleosides as potential anti-HCV agents was developed. Addition of halo(het)aryllithium reagents to benzylated 2-*C*-methyl-D-ribonolactone gave the corresponding hemiketals, which were subsequently converted to the β-anomeric benzyl-protected bromo(het)aryl-*C*-nucleosides via either direct reduction (in the case of phenyl derivative) or acetylation followed by reduction of the resulting hemiketal acetates (in the case of pyridyl derivatives). The key halogenated (het)aryl-*C*-nucleoside intermediates were further transformed by Pd-catalyzed cross-coupling, hydroxylation and amination reactions affording series of protected *C*-nucleosides with small hydrophilic and hydrophobic substituents. The final protecting group removal was rather problematic, and different debenzylation methods, such as hydrogenation on Pd/C or treatment with BCl_3, had to be optimized for each derivative to minimize the formation of side-products. The final *C*-nucleosides were also converted into their 5′-*O*-triphosphates, and biological activity screenings revealed that none of the free *C*-nucleosides possesses any antiviral activity in the HCV replicon assay, and none of their NTPs significantly inhibits the HCV RNA polymerase.

Two series of isomeric pyrrolo-fused 7-deazapurine ribonucleosides were prepared in order to extend the promising class of biologically active modified nucleosides featuring 7-deazapurine nucleobase with annulated five-membered heteroaromatic ring. The synthetic strategy was based on thermal heterocyclizations of 4-azido-6-chloro-5-pyrrolylpyrymidines to construct the tricyclic nucleobases, followed by glycosylation and further derivatization via various cross-coupling and nucleophilic substitution reactions. The final nucleosides bearing hetaryl, amino, dimethylamino, methyl, methoxy and methylsulfanyl groups were then screened for antiviral and cytotoxic activities. While pyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine nucleosides were devoid of any cytotoxic activity, their isomeric pyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine analogues exerted submicromolar cytotoxicity, and within this series, methyl, methoxy and methylsulfanyl derivatives showed the highest activity with good selectivity toward cancer cells. It was also shown that the nucleosides are intracellularly phosphorylated and then get incorporated into RNA and DNA where they cause DNA damage. Some pyrrolo-fused 7-deazapurine ribonucleosides also showed submicromolar anti-HCV activities, but there is still a need for further deeper studies to prove the mechanism and biological targets.

Abstrakt

V této disertační práci je popsán vývoj obecného a modulárního syntetického přístupu pro 4-substituované fenyl, 2-substituované pyridin-5-yl a 5-substituované pyridin-2-yl 2′-*C*-methyl-*C*-ribonukleosidy, potenciální inhibitory HCV. Nejprve byly přidáním halo(het)aryllithných činidel k benzylovaným 2-*C*-methyl-D-ribonolaktonům získány odpovídající hemiketaly. Ty byly následně převedeny buď přímou redukcí (v případě fenylových derivátů), nebo acetylací a následnou redukcí vzniklých hemiketalových acetátů (v případě pyridylových derivátů) na benzylované β-anomerické bromo(het)aryl-*C*-nukleosidy. Transformace klíčových halogenovaných (het)aryl-*C*-nukleosidových meziproduktů palladiem katalyzovanými cross-couplingovými reakcemi, hydroxylacemi a aminacemi poskytly sérii chráněných *C*-nukleosidů s malými hydrofilními a hydrofobními substituenty. Kvůli tvorbě vedlejších produktů při závěrečném odstranění chránicích skupin musely být pro každý derivát optimalizovány různé debenzylační metody, jako je hydrogenace na Pd/C nebo reakce s BCl_3. Nakonec byly finální C-nukleosidy převedeny na jejich 5′-*O*-trifosfáty. Testování biologické aktivity odhalilo, že žádný z volných *C*-nukleosidů nemá protivirovou aktivitu vůči HCV (test na HCV replikonu) a žádný z nukleosidtrifosfátů významně neinhibuje HCV RNA polymerasu.

Dále bylo cílem rozšířit slibnou skupinu biologicky aktivních modifikovaných nukleosidů obsahujících 7-deazapurinovou nukleobázi s anelovanými pětičlennými cykly a byly proto připraveny dvě série izomerních pyrrolo-kondenzovaných 7-deazapurinových ribonukleosidů. Prvním krokem syntézy byla konstrukce tricyklických nukleobází heterocyklizacemi 4-azido-6-chlor-5-pyrrolylpyrymidinů. Následně byly takto syntetizované heterocykly glykosylovány a vzniklé nukleosidy derivatizovány různými cross-couplingovými reakcemi a nukleofilními substitucemi. Finální nukleosidy nesoucí hetarylovou, aminovou, dimethylaminovou, methylovou, methoxy a methylsulfanylovou skupinu byly testovány na antivirovou a cytotoxickou aktivitu. Zatímco pyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidinové nukleosidy neměly žádnou cytotoxickou aktivitu, izomerní pyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidinové analogy prokázaly submikromolární cytotoxicitu. Nejlepší aktivity měly deriváty obsahující methylovou, methoxy a methylsulfanylovou skupinu. Tyto látky jsou zároveň selektivní nádorovým buňkám. Bylo také prokázáno, že nukleosidy jsou v buňkách fosforylovány a poté jsou začleněny do RNA a DNA, kde způsobují poškození DNA. Některé pyrrolo-kondenzované 7-deazapurinové ribonukleosidy také vykazovaly submikromolární aktivity proti HCV, stále je však potřeba dalších studií, které by prokázaly mechanismus a přesné biologické cíle.

List of publications relevant to this thesis

1. Tokarenko, A.; Poštová Slavětínská, L.; Klepetářová, B.; Hocek, M.: "Synthesis of Benzene and Pyridine 2'-*C*-Methyl-*C*-ribonucleosides and -nucleotides" *Eur. J. Org. Chem.* **2015**, *36*, 7962–7983.

2. Tokarenko, A.; Lišková, B.; Smoleń, S.; Táborská, N.; Tichý, M.; Gurská, S.; Perlíková, P.; Frydrych, I.; Tloušťová, E.; Znojek, P.; Mertlíková-Kaiserová, H.; Poštová Slavětínská, L.; Pohl, R.; Klepetářová, B.; Khalid, N.; Wenren, Y.; Laposa, R. R.; Džubák, P.; Hajdúch, M.; Hocek, M.: "Synthesis and Cytotoxic and Antiviral Profiling of Pyrrolo- and Furo-Fused 7-Deazapurine Ribonucleosides" *J. Med. Chem.* **2018**, *61*, 9347–9359.

List of abbreviations

Ac	acetyl
ADK	adenosine kinase
AIDS	acquired immune deficiency syndrome
aq.	aqueous
ATP	adenosine triphosphate
ATR	attenuated total reflection
BAR	benzamide ribonucleoside
Bn	benzyl
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bz	benzoyl
calcd	calculated
cAMP	cyclic adenosine monophosphate
CCR5	C-C motif chemokine receptor type 5
cGMP	cyclic guanosine monophosphate
compd.	compound
COSY	correlation spectroscopy (NMR)
CTP	cytidine triphosphate
Cy-JohnPhos	(2-biphenyl)dicyclohexylphosphine
DAAs	direct antiviral agents (also direct-acting antivirals)
dATP	2'-deoxyadenosine triphosphate
dba	dibenzylideneacetone
decomp.	decomposition
DENV	dengue virus
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dsDNA	double-stranded deoxyribonucleic acid
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
ED-XRF	energy dispersive X-ray fluorescence
ESI	electrospray ionization
Et	ethyl
FAD	flavin adenine dinucleotide

FDA	Food and Drug Administration
GTP	guanosine triphosphate
HMBC	heteronuclear multiple bond correlation (NMR)
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPFC	high performance flash chromatography
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence (NMR)
HSV	herpes simplex virus
IMPDH	inosine 5′-monophosphate dehydrogenase
iPr	isopropyl
IR	infrared
ISTI	integrase strand transfer inhibitors
IUPAC	International Union of Pure and Applied Chemistry
J	coupling constant (NMR)
JohnPhos	(2-biphenyl)-di-*tert*-butylphosphine
LiHMDS	lithium bis(trimethylsilyl)amide
Me	methyl
$Me_4(t\text{-}Bu)_2XPhos$	2-di-*tert*-butylphosphino-3,4,5,6-tetramethyl-2′,4′,6′-triisopropylbiphenyl
m.p.	melting point
NAD	nicotinamide adenine dinucleotide
n-Bu	but-1-yl
NI	nucleoside and nucleotide NS5B polymerase inhibitors
NMR	nuclear magnetic resonance
NNI	non-nucleoside NS5B polymerase inhibitors
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NS	nonstructural protein
NTP	nucleoside triphosphate
PAMPA	parallel artificial membrane permeability assay

PAPS	3'-phosphoadenosine-5'-phosphosulfate
PG	protecting group
Ph	phenyl
PI	protease inhibitors
PNP	purine nucleoside phosphorylase
ppm	parts per million (NMR)
RdRp	RNA dependent RNA polymerase
RNA	ribonucleic acid
ROESY	rotating frame Overhauser effect spectroscopy (NMR)
rRNA	ribosomal ribonucleic acid
RSV	respiratory syncytial virus
r.t.	room temperature
SAM	S-adenosyl methionine
sat.	saturated
TAD	thiazole-4-carboxamide adenine dinucleotide
TBAB	tetrabutylammonium bromide
TBDMS	*tert*-butyldimethylsilyl
TBE	tris/borate/EDTA
t-Bu	*tert*-butyl
TCRB	2,5,6-trichloro-1-(β-D-ribofuranosyl)-1*H*-benzo[*d*]imidazole
TDA-1	tris[2-(2-methoxyethoxy)ethyl]amine
TEAB	triethylammonium bicarbonate
Tf	triflate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidine
TMS	trimethylsilyl
tRNA	transfer ribonucleic acid
UTP	uridine triphosphate
VZV	varicella zoster virus

Contents

1 Introduction

1.1 Nucleoside analogues as antiviral and anticancer agents

Nucleosides and nucleotides are endogenous compounds that are involved in numerous cellular processes. These essential biomolecules serve as building blocks of both RNA and DNA. Natural nucleosides consist of a ribose-based sugar part and a nucleobase (mainly substituted pyrimidine or purine heterocycle), whereas nucleotides are nucleosides that also contain at least one phosphate (or phosphate-like) group. There are eight canonical nucleosides of which RNA and DNA are constructed (**Figure 1**). Four of them – uridine, cytidine, thymidine, and 2′-deoxycytidine – contain a pyrimidine nucleobase, and the other four – adenosine, guanosine, 2′-deoxyadenosine, and 2′-deoxyguanosine – are derivatives of purine. As for the sugar part, D-ribose is a component of RNA, while 2-deoxy-D-ribose is a component of DNA.

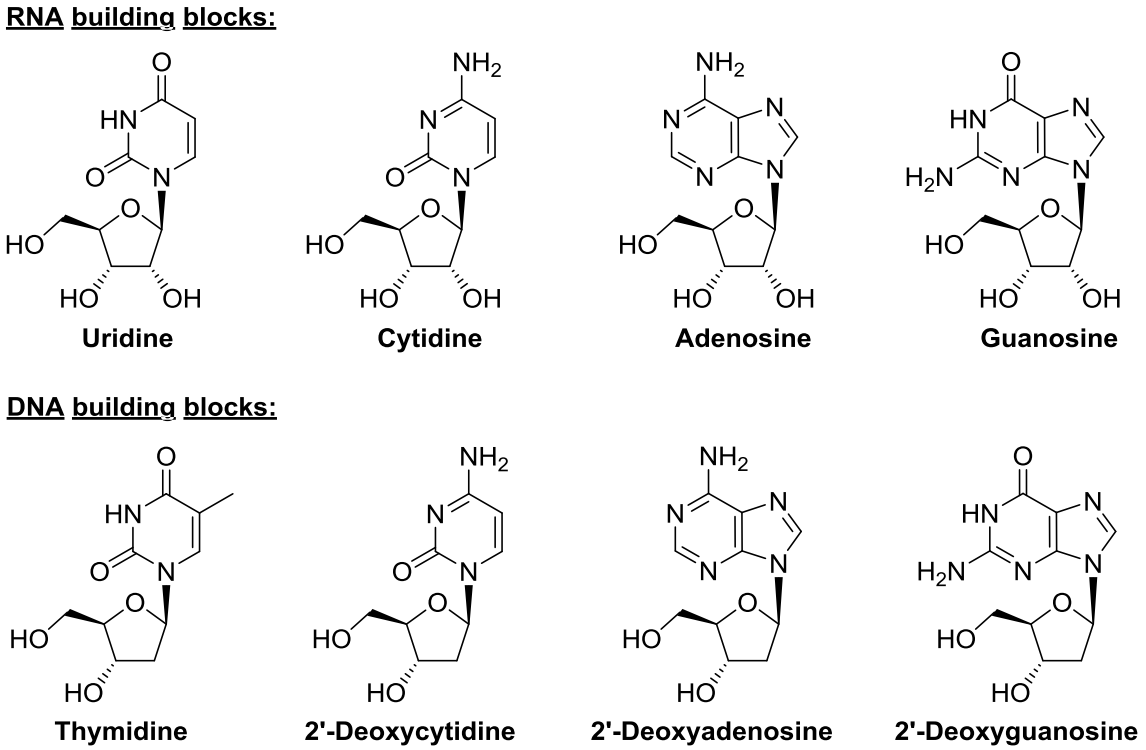


Figure 1. Naturally occurring canonical RNA and DNA nucleoside building blocks.

Nucleosides and their analogs play an important role in various cellular processes such as synthesis of nucleic acids and their components, cell signaling, enzyme regulation, and metabolism.[1,2] Many nucleoside derivatives (ATP, GTP, CTP, coenzyme A, NAD, FAD, SAM, PAPS) serve as enzyme cofactors. Nucleoside 5′-triphosphates ATP, GTP and UTP

have the role of activators of substrates in metabolic reactions and are also crucial in energetic metabolism since they are used for temporary storage of energy. GTP and a few other nucleoside derivatives, such as cyclic monophosphates cAMP and cGMP, are involved in cellular signal transduction: GTP is essential to activation of the G-protein-coupled receptor signaling pathway, whereas cAMP and cGMP activate protein kinases and serve as second messengers. All these facts show that nucleosides and their analogues have great potential to interfere with various cellular mechanisms, and nucleoside involvement in numerous critical biological processes makes these compounds a unique starting point for drug design.

1.1.1 Biologically active modified nucleosides found in nature

There are many natural modified nucleosides and some of them display interesting biological activities. For example, pyrimidine nucleoside analogues spongouridine (araU) and spongothymidine (araT), found in the marine sponge *Cryptotethia crypta*,[3,4] are derivatives of D-arabinose and show activity against herpes simplex virus (HSV) (**Figure 2**).[5] While neither of these compounds ever became a useful drug, their discovery led to the development of other arabinose-based nucleoside analogues with potent antiviral and anticancer activities.

Nebularine and pentostatin are examples of biologically active purine nucleoside analogues found in nature (**Figure 2**). Nebularine, the simplest purine nucleoside, is an antibiotic isolated from *Leptista nebularis* and *Streptomyces yokosukanensis*.[6] Pentostatin was identified in a culture broth of *Streptomyces antibioticus* and was found to be a potent anticancer agent.[7] This nucleoside irreversibly inhibits adenosine deaminase leading to a high level of dATP and to activation of the apoptotic pathway. Pentostatin is now approved for the treatment of several types of leukemia.[8,9]

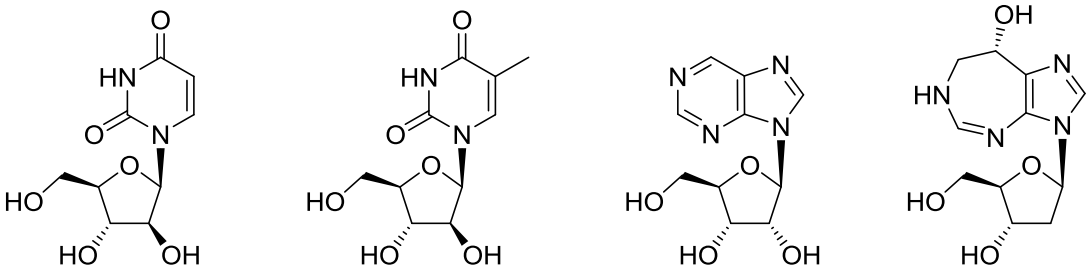


Figure 2. Examples of biologically active modified nucleosides found in nature.

In addition to a large number of natural modified nucleosides, many more synthetic analogues have been prepared and studied for their biological activities. This thesis will focus

mainly on antiviral and anticancer effects of unnatural modified nucleosides and their analogues.

1.1.2 Synthetic modified nucleosides with antiviral activity

Viral infections cause millions of deaths worldwide every year. Viruses often lead to serious illnesses that require aggressive pharmacological intervention. Human immunodeficiency virus (HIV) is one of the most dangerous since it causes acquired immune deficiency syndrome (AIDS). There are six major classes of anti-HIV drugs: nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), CCR5 inhibitors, integrase strand transfer inhibitors (ISTI), and fusion inhibitors.[10] Reverse transcriptase is the key viral enzyme that is responsible for the synthesis of double stranded DNA from viral RNA, and nucleoside analogues are obvious candidates for the development of the reverse transcriptase inhibitors. **Figure 3** shows modified nucleosides and their analogues that are currently used to treat HIV-infection. All the compounds are inhibitors of the viral reverse transcriptase but feature various structural modifications. Didanosine and zidovudine were among first 2′,3′-dideoxy analogues with good chain terminating properties, but these compounds suffered from various unwanted side effects, problems with toxicity, and the development of viral resistance.[11,12] The efforts to find new and better chain terminators led to the discovery of numerous analogues including stavudine, lamivudine, and emtricitabine.[13,14] Like didanosine and zidovudine, stavudine is a 2′,3′-dideoxy derivative, but it also features unsaturation at the sugar moiety. On the other hand, lamivudine and emtricitabine are examples of L-nucleosides that also contain a sulfur atom instead of 3′ carbon in the sugar ring. Another successful anti-HIV agent fabacavir has an additional modification to the sugar scaffold – it is a carbocyclic analogue. The main advantage of carbocyclic nucleosides is increased stability due to the change in the nature of the glycosidic bond.[15] Further attempts to modify the sugar part led to the development of a different class of antiviral drugs – acyclic nucleosides and their analogues. One of the most effective anti-HIV drugs tenofovir is an acyclic adenosine analogue that also features another important structural modification – a phosphonate group. Nucleoside phosphonates were designed to overcome the problem of modified nucleoside recognition by kinases during the first phosphorylation step.[16,17] Unfortunately, tenofovir is associated with low bioavailability, but this issue was improved by the addition of a prodrug moiety. Tenofovir disoproxil demonstrated a higher potency and greater efficacy than the parent tenofovir.[18] However, it

was recently largely replaced by another prodrug – tenofovir alafenamide – that required lower dosage and provided higher levels of the nucleotide inside infected cells.[19]

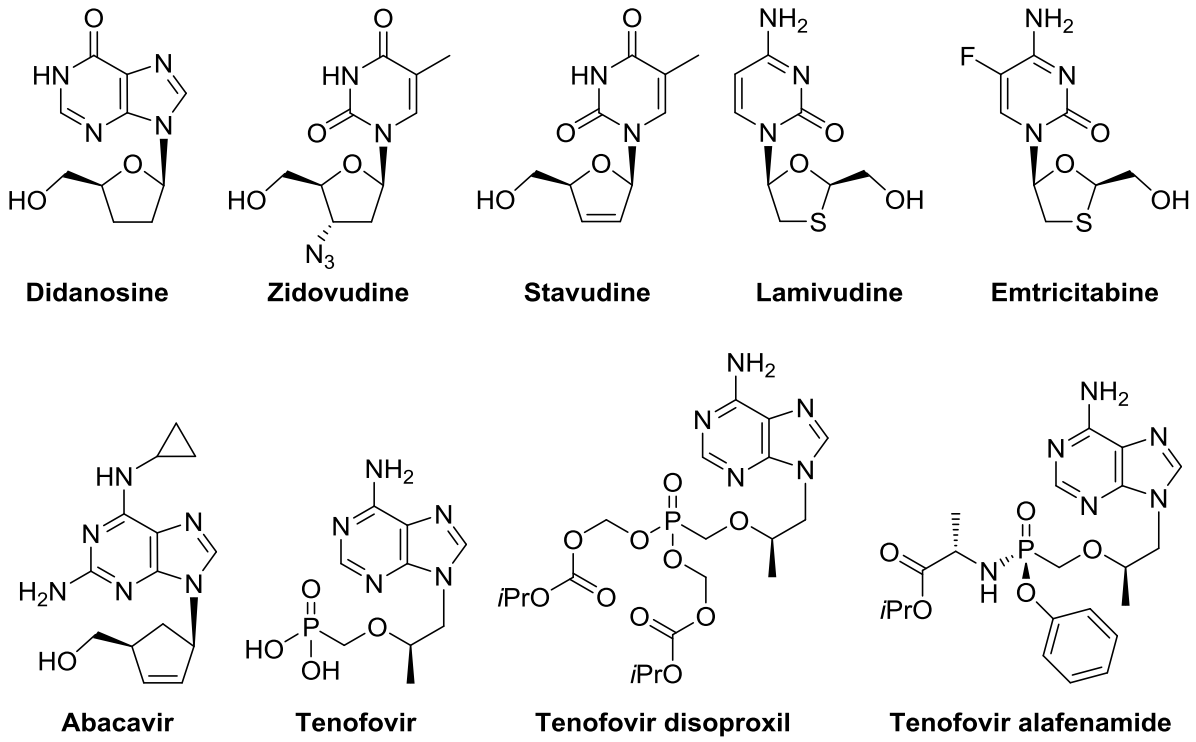


Figure 3. Nucleoside analogues currently used to treat HIV.

Hepatitis B virus (HBV) therapy is also mainly based on the treatment with nucleoside analogues. HBV is a DNA virus, and the main therapeutic target is viral DNA polymerase. Above-mentioned compounds lamivudine and tenofovir disoproxil are approved for the treatment of both HIV and HBV infections. Two other modified nucleosides entecavir and telbivudine are used mostly as anti-HBV agents (**Figure 4**). Entecavir is one of the most important examples of the carbocyclic nucleosides that retains 3′-OH group but in addition possesses a unique exocyclic double bond. This double bond was found to increase the binding affinity since it fits into a hydrophobic pocket of the HBV polymerase.[20] Similar to lamivudine and emtricitabine, telbivudine is an L-nucleoside, and is potent in the treatment of chronic hepatitis B infections.[21] This modified nucleoside is the L-enantiomer of naturally occurring thymidine, and after incorporation by viral DNA polymerase, induces chain termination. Adefovir dipivoxil, a prodrug of an acyclic nucleoside phosphonate, was first utilized in the treatment of HIV, however, it suffered from toxicity and adverse side effects.[22] Later, this compound proved to be very effective and relatively cheap treatment for chronic hepatitis B infections.[23]

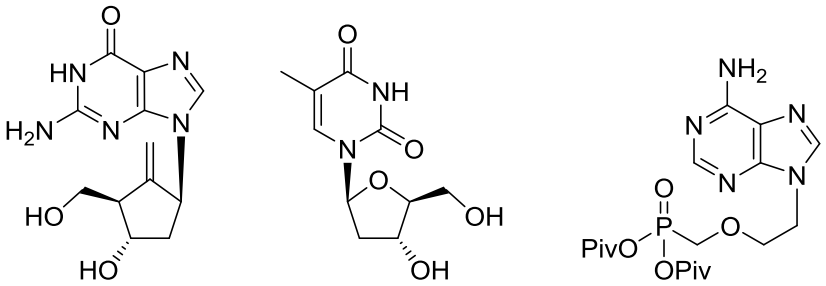


Figure 4. Modified nucleosides and their analogues as anti-HBV agents.

Nucleoside analogues are successfully used for the treatment of other DNA viruses, such as HSV, varicella zoster virus (VZV), and human cytomegalovirus (HCMV). There are three pyrimidine nucleoside analogues approved for the cure of HSV or VZV: idoxuridine, trifluridine, and brivudine (**Figure 5**). All these compounds are 2′-deoxynucleoside analogues modified at the C5 position of the pyrimidine ring. Such modification results in a blockage of base pairing and inhibition of DNA synthesis after the nucleoside is incorporated during the replication. Idoxuridine was initially developed as an antitumor drug, but it later became the first antiviral agent to be used clinically for the treatment of HSV.[24] Similarly to idoxuridine, trifluridine displayed both antiviral and anticancer activities, and it is currently used to cure HSV[25] as well as colorectal cancer.[26] As for brivudine, it was found to be active against both HSV and VZV, and is now clinically used for the treatment of these viruses.[27]

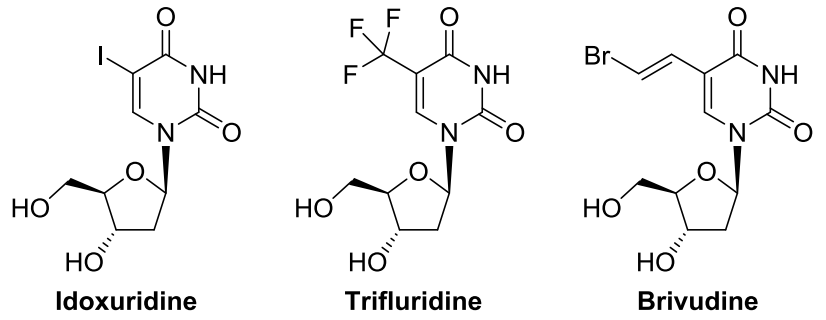


Figure 5. Pyrimidine nucleoside analogues as anti-HSV or anti-VZV agents.

Figure 6 contains examples of purine nucleoside analogues approved for the treatment of both HSV and VZV infections (with the exception of penciclovir that is now used to exclusively cure HSV). Aciclovir is an acyclic guanosine mimic that is very efficiently phosphorylated and incorporated by the viral DNA polymerase inhibiting DNA synthesis.[28] Unfortunately, like many nucleoside analogues, it suffers from low bioavailability that could be improved by the addition of a prodrug moiety. Indeed, the problem was solved with the

synthesis of valaciclovir, a valine ester prodrug of aciclovir.[29] Similarly to aciclovir, a carbocyclic guanosine analogue penciclovir demonstrated high antiviral activity, but low bioavailability.[30] Attempts to find a suitable prodrug led to the development of famciclovir that features an acetate prodrug moiety as well as a nucleobase lacking a carbonyl group.[31]

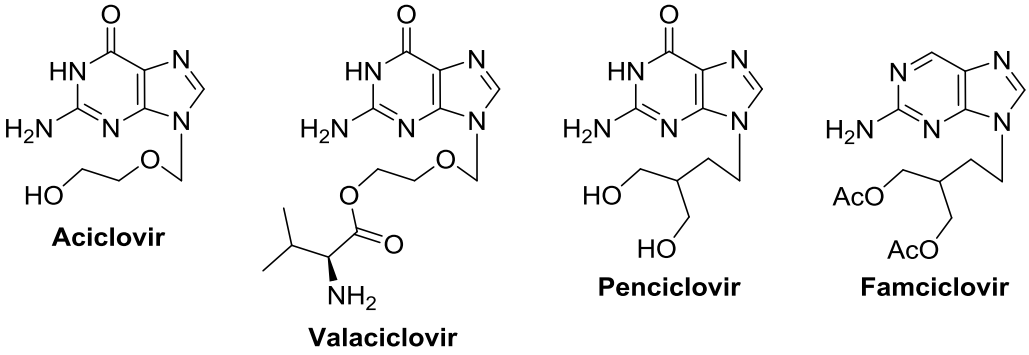


Figure 6. Purine nucleoside analogues with anti-HSV and anti-VZV activity.

Three other nucleoside analogues are currently used to treat HCMV infection (**Figure 7**). Like valaciclovir, valganciclovir is a valine prodrug with a higher bioavailability than the parent ganciclovir.[32] Cidofovir, a cytosine acyclic phosphonate analogue, retains the 2′-OH group and has shown activity against numerous DNA viruses[33] but its primary use is to cure HCMV retinitis in AIDS patients.[34] The mechanism of action of ganciclovir, valganciclovir, and cidofovir is similar to the other acyclic analogues: these compounds are phosphorylated giving the corresponding triphosphates, which are subsequently incorporated into the viral DNA and thus inhibit viral DNA polymerase.

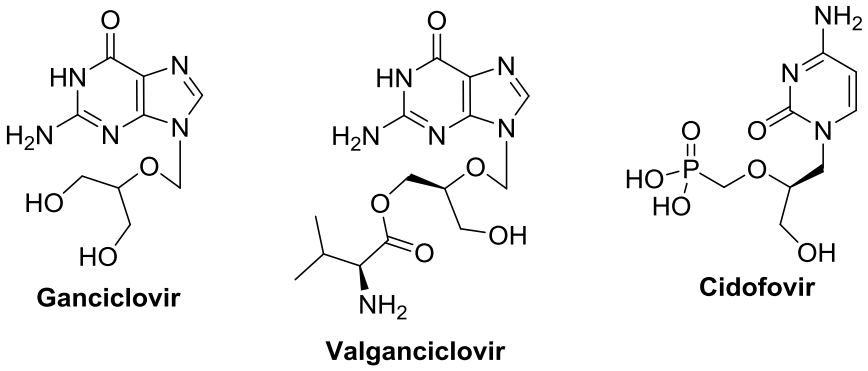


Figure 7. Modified nucleosides as anti-HCMV agents.

Modified nucleosides and their analogues also help to fight against a dangerous RNA virus – hepatitis C virus (HCV). Hepatitis C infection is a global health problem. Acute

infection often leads to chronic hepatitis C that can cause hepatic fibrosis and liver cirrhosis, which can in turn progress to hepatocellular carcinoma. Until recently, a combination of PEGylated interferon-α and ribavirin was used as the main cure (**Figure 8**). Ribavirin is a synthetic guanosine analog with a broad-spectrum antiviral activity against both DNA and RNA viruses, but it is primarily used to treat chronic hepatitis C.[35] The mechanism of action of ribavirin still remains unclear, even though there is evidence for a number of proposed direct (targeting the virus) or indirect (targeting host cells) mechanisms, such as inhibition of the viral polymerase,[36] RNA mutagenesis (error catastrophe),[37] inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH),[38] immunomodulation,[39] and potentiation of induction of interferon-stimulated genes.[40] Unfortunately, the PEGylated interferon/ribavirin therapy required treatment for a long period, was associated with substantial toxicity and numerous side effects, and gave only moderate cure rates.[41] This drove a search for less toxic and more effective anti-HCV agents, and the research in this area has led to a revolution in the treatment of HCV infection – discovery and clinical development of new molecules called direct antiviral agents (DAAs, also termed direct-acting antivirals). In comparison with interferon-α and ribavirin, DAAs were designed to directly target one of the viral essential proteins or protein complexes. Combining DAAs for the treatment of HCV resulted in a shorter duration of the therapy, fewer side effects and much higher cure rates. Currently, there are four major classes of DAAs: NS3/4A protease inhibitors (PIs), NS5A inhibitors, nucleoside and nucleotide NS5B polymerase inhibitors (NIs), and non-nucleoside NS5B polymerase inhibitors (NNIs).[42] Among these four classes of DAAs, NIs proved to be the most successful due to high antiviral potency, broad genotypic coverage and high barrier to the development of resistance.[43] These compounds are ribonucleoside analogues that inhibit the key enzyme in the viral replication – viral RNA dependent RNA polymerase (RdRp) NS5B[44] – by mimicking the natural polymerase substrates and acting as chain terminators.

Sofosbuvir,[45] mericitabine,[46] valopicitabine,[47] and BMS-986094[48] are examples of potent nucleoside NS5B inhibitors that entered or underwent clinical trials (**Figure 8**). All of these compounds have two important structural features. On one hand, they contain a methyl group at the 2′-position of the sugar, and presence of this group is known to prevent an incoming nucleoside triphosphate from binding to the active site of the viral polymerase. It was shown that such structural modification can yield selective HCV polymerase inhibitors with excellent chain terminating properties.[49] On the other hand, since most sugar-modified nucleosides are known to be poor substrates for nucleoside kinases, they are often delivered as various prodrugs, which are then metabolized to the active nucleoside triphosphates.[50] For example,

Sofosbuvir, an effective and relatively safe compound that is now used clinically to treat HCV, is a phosphate prodrug of 2′-methyl-2′-fluororibonucleoside.

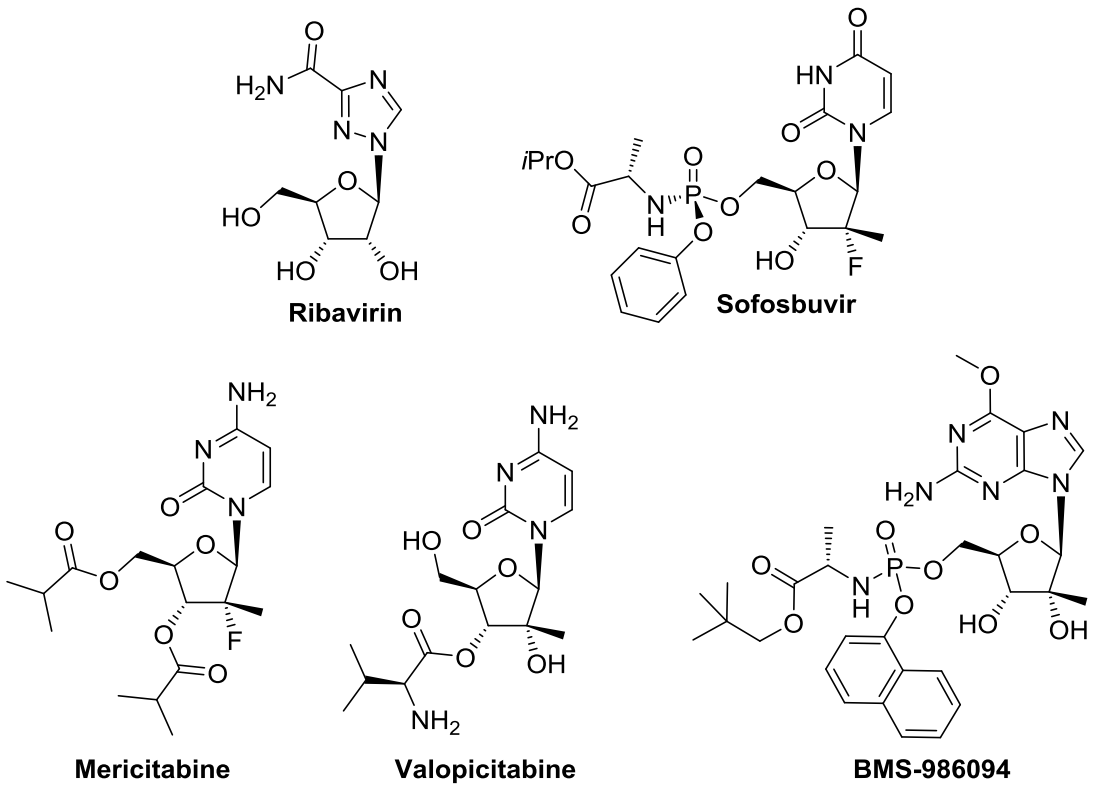


Figure 8. Anti-HCV nucleoside analogues that underwent or entered clinical trials.

Although many nucleoside analogues are successfully used for the treatment of various DNA and RNA viruses, such treatment is often associated with unwanted side effects, and there is always a threat of the development of viral resistance. Furthermore, there are other viruses for which there is no treatment available, so the search for new nucleoside antiviral drugs is still a worthwhile goal.

1.1.3 Unnatural nucleoside analogues in antitumor therapy

Cancer is one of the leading causes of death in the world with almost 10 million cancer deaths in 2018. Cytotoxic nucleobases and nucleoside analogues were among the first chemotherapeutic agents to be introduced for the medical treatment of cancer. These agents act as antimetabolites, compete with natural nucleosides, and interact with numerous intracellular targets to induce cytotoxic effect. Currently, there are 12 nucleoside analogues approved by FDA for the treatment of cancer.[51] Two of them – pentostatin and trifluridine – were already discussed above. **Figure 9** shows the structures of six synthetic pyrimidine nucleoside antitumor agents. Cytarabine (araC) and gemcitabine are cytidine derivatives that

feature a sugar moiety modified at 2′-position. These compounds are incorporated by DNA polymerases and cause delayed chain termination thus inhibiting DNA synthesis.[52,53] Other cytidine analogues azacytidine and decitabine contain a modified nucleobase with a nitrogen atom instead of a carbon atom at position 5. These aza analogues were developed as epigenetic drugs: they are incorporated into the DNA but cannot be further methylated by DNA methyltransferase, which can influence epigenetic gene regulation.[54] The last two pyrimidine nucleoside analogues floxuridine and capecitabine contain a fluorine atom at the position 5 of the nucleobase. These derivatives are metabolized to 5-fluorouracil, which in turn inhibits thymidylate synthase – the enzyme required for the synthesis of thymidine monophosphate that is an essential DNA building block.[55]

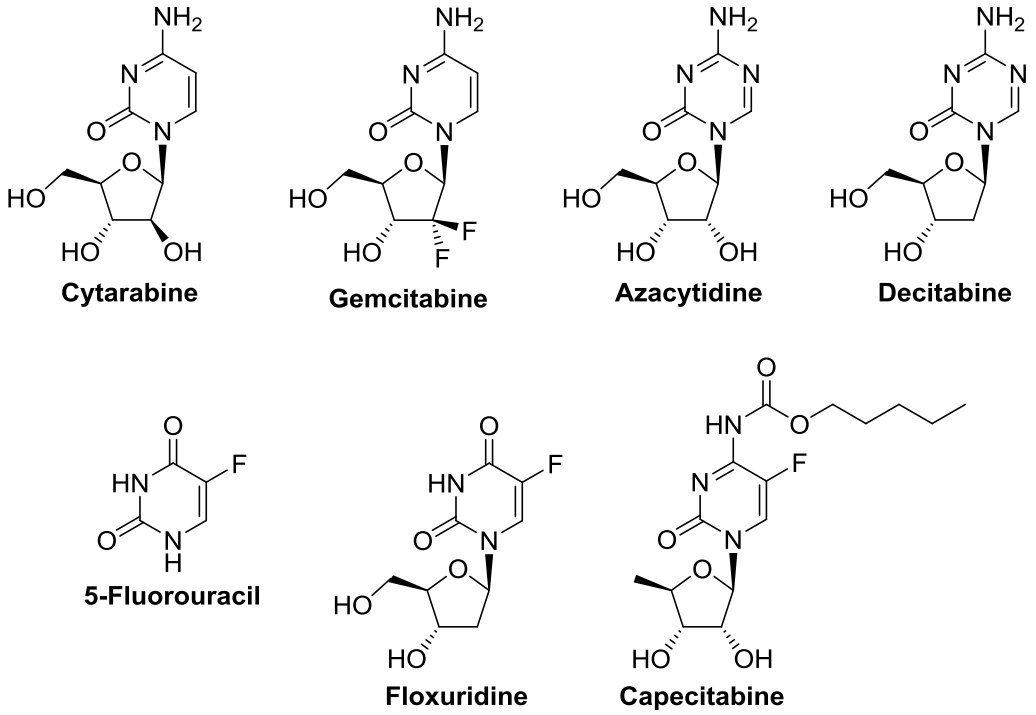


Figure 9. Structures of pyrimidine nucleoside anticancer drugs, and 5-fluorouracil.

As for the synthetic purine nucleoside analogues, there are four compounds approved for the treatment of cancer: fludarabine-5′-monophosphate, cladribine, clofarabine, and nelarabine (**Figure 10**). The first three derivatives have a similar mechanism of action: on one hand, they inhibit ribonucleotide reductase that is the key enzyme in biosynthesis of 2′-deoxynucleotides, and on the other hand, they act as chain terminators upon incorporation into the DNA.[56,57] The presence of a halogen atom at position 2 of the nucleobase prevents hydrolysis of the amino group by adenosine deaminase.[58] The last derivative, guanosine analogue nelarabine, is a prodrug of araG with an improved solubility compared to the parent

compound.[59] Like other purine nucleoside derivatives, araG is incorporated into the DNA causing inhibition of DNA replication.

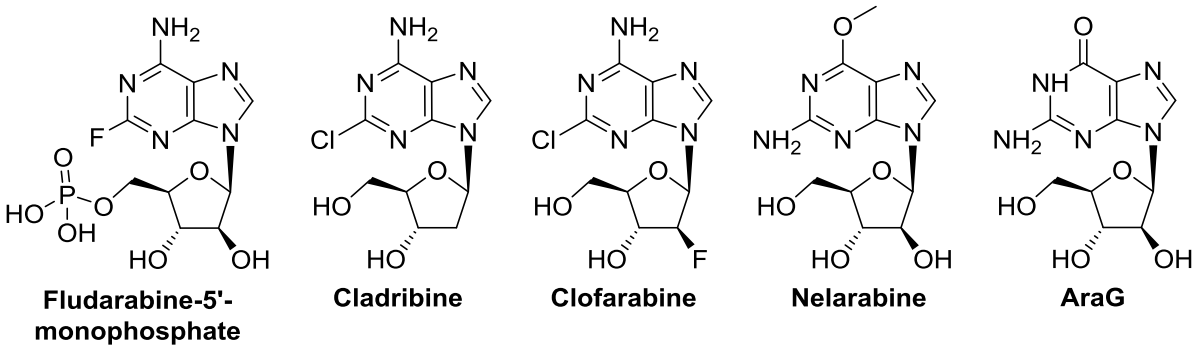


Figure 10. Purine nucleoside analogues with antitumor activity.

Although a number of nucleoside analogues are used as anticancer agents, they usually suffer from severe adverse effects such as immunosuppression, neurotoxicity, and hepatotoxicity, so further development of novel more selective cytotoxic modified nucleosides is needed.

1.2 7-Deazapurine scaffold in design of biologically active nucleosides

Synthesis and biological evaluation of diverse purine nucleoside derivatives and analogues have been one of the major research areas in our group for many years. The purine motive could be in principle modified by substitution at positions 2, 6 and 8, as well as by replacement of the nitrogen atoms with carbons leading to deazapurine analogues, which may be further substituted at the newly introduced carbon atoms (**Figure 11**). A particularly interesting class of base-modified nucleosides is derived from 7-deazapurine – a purine-like heterocycle that on one hand contains a more electron rich five-membered ring, and on the other hand provides a suitable position (C7) for introduction of various substituents, that would point out to the major groove when the nucleoside is incorporated into DNA or RNA. Such alterations could result in increased base-pairing[60]or in better binding to enzymes,[61-63] leading to compounds with promising biological activities. According to the IUPAC nomenclature rules, 7-deazapurine should be called 7*H*-pyrrolo[2,3-*d*]pyrimidine, but in this thesis the more illustrative semitrivial name – 7-deazapurine – and the custom purine numbering will be used.

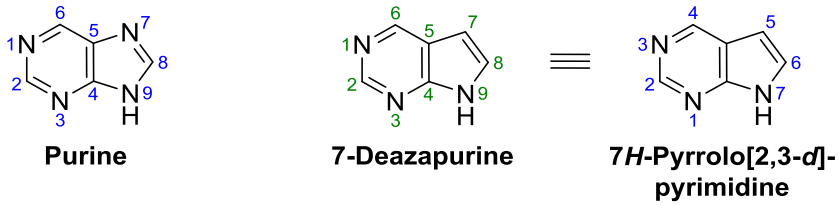


Figure 11. The structures and numbering of purine and 7-deazapurine (systematic IUPAC numbering shown in blue, and custom numbering shown in green).

1.2.1 Naturally occurring 7-deazapurine nucleosides

Some 7-deazapurine nucleosides can be found in nature both as free nucleosides and as nucleic acid components. Queuosine[64] and archaeosine,[65] for example, are 7-deazaguanosine analogues present in tRNA of prokaryotic and eukaryotic organisms and archaea, respectively (**Figure 12**). Three other compounds – tubercidin (7-deazaadenosine) and its 7-substituted derivatives toyocamycin and sangivamycin – are potent cytotoxic nucleosides isolated from *Streptomyces* cultures.[66,67] All three nucleosides are phosphorylated by cellular kinases, and the resulting nucleotides are incorporated into RNA and DNA affecting their functions.[68-71] Tubercidin was also found to impair numerous cellular processes, including purine synthesis,

mitochondrial respiration, methylation of tRNA, and rRNA processing.[72,73] Moreover, it showed activity against *Mycobacterium tuberculosis* and against a number of viruses, such as mengovirus, reovirus, and vaccinia virus.[68] A cyano derivative toyocamycin in turn inhibits synthesis and maturation of rRNA;[74] and it also interferes with inositol triphosphate signaling pathway via inhibition of phosphatidylinositol 4-kinase.[75] As for the last example, carbamoyl analogue sangivamycin, its antitumor effect is mainly based on selective inhibition of protein kinase C.[76] Unfortunately, none of the naturally occurring 7-deazapurine nucleosides proceeded to clinical use due to the toxicity issues.

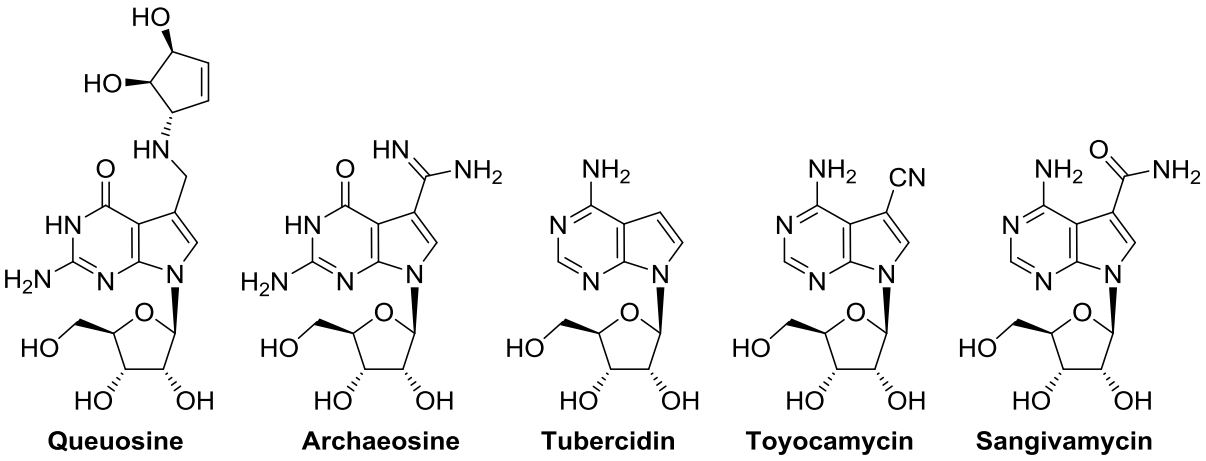


Figure 12. Natural 7-deazapurine nucleosides.

1.2.2 Antiviral effects of synthetic 7-deazapurine nucleosides

Since 7-deazapurine ribonucleosides closely resemble natural substrates of the viral RNA polymerases, they were extensively studied as potential antiviral candidates for various RNA viruses.[77,78] Shown in **Figure 13** are examples of the most important antiviral nucleosides that contain a 7-deazapurine core. 6-*N*,*N*-Dimethylamino-7-deazapurine ribonucleoside **I**, developed as an analogue of the previously mentioned natural 7-deazapurine antibiotic tubercidin, showed submicromolar anti-HCV activity in HCV replicon assay, and only weak cytotoxicity towards fibroblasts, which makes this compound a suitable lead structure.[79] The next two compounds – 7-deaza-2′-*C*-methyladenosine,[80] also known as MK-0608, and its 7-fluoro analogue **II**[81] – were found to be even more potent and selective inhibitors of HCV NS5B RNA polymerase. Modification of the sugar part is introduced in order to decrease the cytotoxicity that often accompanies anti-HCV activities of the corresponding 7-deazapurine ribonucleosides, and 2′-*C*-methyl group proved to be one of the most suitable for the NS5B polymerase inhibition.[49] MK-0608 has been extensively studied as an anti-HCV drug candidate, and this compound was found to be effectively phosphorylated and then

incorporated into the growing RNA chain causing its termination. Although this nucleoside showed promising results in animal studies and was advanced into phase 1 of clinical trials,[82,83] its development has been terminated for unknown reasons. Among a number of 7-substituted 7-deaza-2′-*C*-methyladenosine derivatives, a fluoro analogue **II** is one of the most potent while still non-cytotoxic: comparing to the unsubstituted nucleoside MK-0608, it possesses a fourfold higher anti-HCV activity (probably due to the improved bioavailability).[81] In general, ineffective cellular permeation and intracellular phosphorylation seem to be one of the major issues in the development of anti-HCV 7-deazapurine nucleosides. For example, 7-deazaguanosine analogues (in their triphosphate forms) were often found to be powerful NS5B RNA polymerase inhibitors but showed much lower activity in the HCV replicon assay.[80] Unfortunately, the efforts to improve bioavailability of these compounds by introducing a prodrug moiety (either to the base or to the sugar part) were unsuccessful.[84,85] Although many 7-deazapurine nucleosides and their analogues displayed promising anti-HCV activity, the drawbacks such as high toxicity and low bioavailability did not allow them to proceed to clinical use.

Replacement of the methyl group in MK-0608 with ethynyl group resulted in derivative NITD008, which was found to be a good candidate for inhibition of another RNA virus – dengue virus (DENV) (**Figure 13**). DENV is responsible for causing dengue fever, which currently has no specific treatment approved. Targeting viral RNA-dependent RNA polymerase, NITD008 is first phosphorylated in vivo and then acts as a chain inhibitor.[86] Although this 7-deazapurine nucleoside showed good results in a mouse model, it caused substantial toxicity when treatment was longer than one week.[87] Introducing a fluorine atom into position 7 led to derivative **III** with slightly higher antiviral activity, but also increased cytotoxicity.

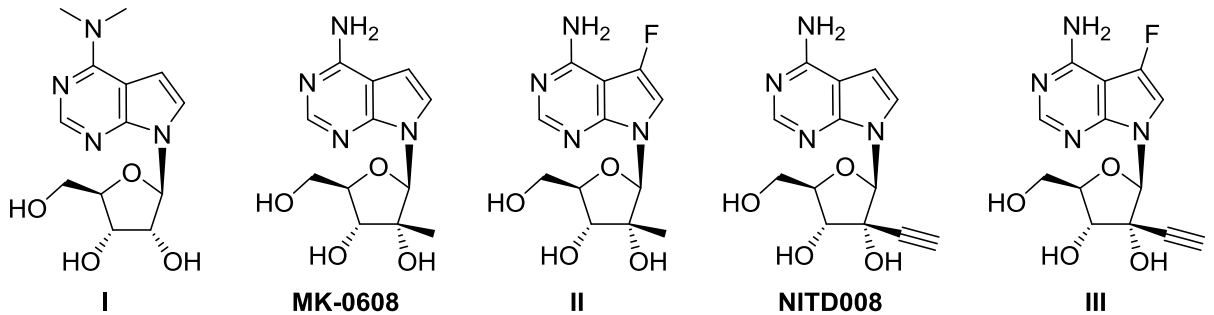


Figure 13. Examples of synthetic antiviral 7-deazapurine nucleosides.

In addition to possessing anti-HCV and anti-DENV activities, many 7-deazapurine nucleosides were found to be effective against other RNA viruses (e.g., HIV, Zika virus, tick-borne encephalitis virus) as well as DNA viruses (e.g., HSV, HBV, HCMV),[88-92] but unfortunately most of the derivatives were also cytotoxic. Nevertheless, these compounds could serve as suitable lead structures for the development of more potent and safe antivirals.

1.2.3 Unnatural 7-deazapurine nucleosides with cytostatic activity

With inspiration from the natural potent cytotoxic 7-deazapurine nucleosides, a large number of both base- and sugar-modified nucleosides featuring 7-deazapurine heterocycle have been prepared as potential anticancer agents.[67,93] Regarding the nucleobase-modified derivatives, the most promising results were achieved when substituents were introduced at positions 6 and 7, while the nucleosides modified at position 2 or 8 were mostly less active or inactive. In our laboratory, we have recently discovered a new group of nanomolar cytostatic 7-deazapurine ribonucleosides **IV** and **V** bearing aryl or hetaryl substituents at position 7, and amino[79] or other small groups (such as methyl, methoxy, or methylsulfanyl)[94] at position 6 (**Figure 14**). These compounds were found to be effective against a broad panel of leukemia and cancer cell lines in vitro, and they were also found to inhibit human and mycobacterial adenosine kinase.[95] It was shown that derivatives containing five-membered heterocycles (i.e., thiophene or furan) were the most active, while the nucleosides with phenyl substituent were inactive. 7-(Thiophen-2-yl) analogue of tubercidin, known as AB61, showed the highest anticancer activity and is now undergoing clinical trials. Its mechanism of action was studied in detail,[96] and it was revealed that the nucleoside is efficiently phosphorylated in tumor cells but not in fibroblasts, which explains its good selectivity. The resulting triphosphate then gets incorporated partly into RNA and partly into DNA leading to inhibition of protein synthesis, and to DNA damage, respectively.

Series of 6-(het)aryl and 6-alkyl-7-deazapurine ribonucleosides **VI** bearing H, F, or Cl atom in position 7 were also prepared and screened for anticancer activity[96] (**Figure 14**). Similarly to the previous case, compounds with 6-membered rings either showed very low activities or were found to be inactive, while nucleosides with 5-membered rings, as well as with alkyl groups, displayed micromolar to nanomolar cytostatic activities against a broad range of cancer cell lines. 6-(Furan-2-yl) and 6-(thiophen-2-yl) derivatives with no substituent at position 7 were the most active, and introduction of F or Cl atom led to nucleosides with similar or lower activities, respectively. The mechanism of action of 6-hetaryl-7-deazapurine

ribonucleosides has not been fully elucidated yet, but these compounds are probably phosphorylated by cellular kinases, and then the corresponding triphosphates act as inhibitors of RNA synthesis.

Another example of nanomolar anticancer base-modified 7-deazapurine nucleosides is 7-ethynyl derivative **VII**,[67] which structurally resembles natural antibiotic toyocamycin (**Figure 14**). Replacement of amino group with small substituents, such as methyl, methylsulfanyl, and dimethylamino groups, resulted in slightly lower activity, but also in improved selectivity (in case of the methylsulfanyl derivative).[79]

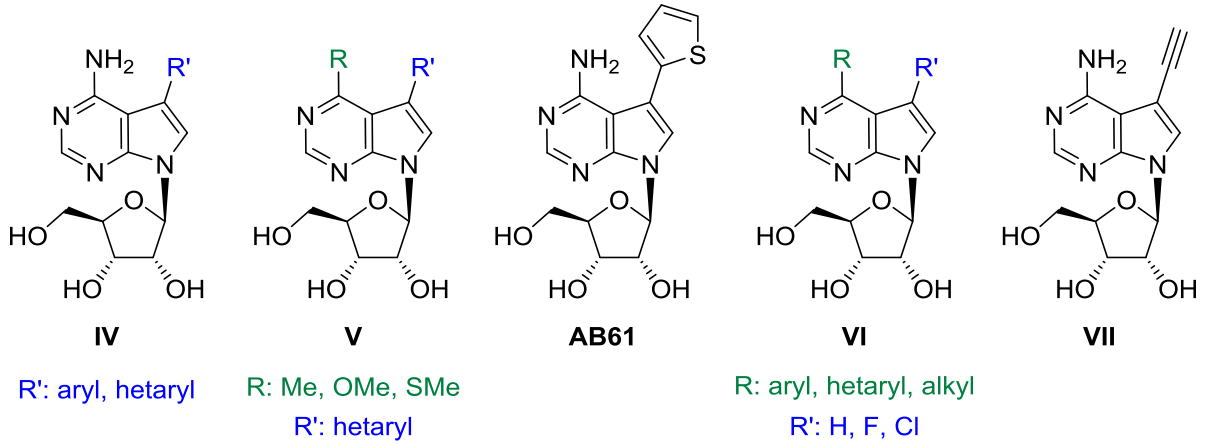


Figure 14. Potent anticancer synthetic 7-deazapurine nucleosides.

Discovery of these new classes of potent anticancer 7-deazapurine nucleosides led to their further studies, and many various sugar-modified derivatives and prodrugs were prepared in attempts to improve activity or bioavailability.[97-101] Unfortunately, such modifications mostly resulted in a decrease or even loss of cytotoxicity of the parent ribonucleosides.

1.2.4 Fused 7-deazapurine nucleosides

1.2.4.1 Biological effects of fused 7-deazapurine nucleosides

Within the class of modified 7-deazapurine nucleosides, multistep activation by kinases and complex multitarget mechanism of action[95] make rational design and further lead structure optimization very difficult. Therefore, the development of new potential anticancer or antiviral agents of this type relies mainly on systematic synthesis and biological evaluation of diverse derivatives and analogues, and on their subsequent SAR studies. Since the previous results showed that in order to keep biological activity of 7-deazapurine nucleosides it is

necessary to leave position 2 (the "minor-groove" side) of deazapurine base without any modification,[102] whereas even bulky substituents are tolerated at position 7 (the "major groove" side), we decided to further explore the space around positions 7 and 8 by introducing annulated six- and five-membered aromatic rings (**Figure 15**). First, several series of benzo-fused (pyrimidoindole) 7-deazapurine nucleosides **VIII** were prepared, and these compounds displayed moderate anti-HCV and anti-DENV activity, but only weak cytostatic activity, which means that the fused benzene ring could be already too bulky for the anticancer effect.[103,104] Indeed, replacement of the annulated benzene with a smaller thiophene ring resulted in thieno-fused 7-deazapurine nucleosides **IX** and **X** with much higher (submicromolar) cytostatic activities.[105] Among the series, derivatives bearing methyl, methoxy, and methylsulfanyl groups were the most active, with the nucleosides **X** showing higher selectivity than the corresponding isomers **IX** indicating the impact of the sulfur atom position. Initial studies of the mechanism of action revealed effective phosphorylation and subsequent inhibition of RNA synthesis.

Having this promising class of fused 7-deazapurine nucleosides in hand, we decided to prepare new types of similar compounds featuring other annulated six- and five-membered aryl and hetaryl rings, and the aim of my work was to prepare novel pyrrolo-fused analogues and to study their biological effects.

In parallel with my project, we have also designed and successfully synthesized three series of ribonucleosides **XI-XIII** bearing 7-deazapurine nucleobase fused to furan[106] and naphthalene[107] cycles, and after publishing these results, we decided to extend the class of annulated 7-deazapurine nucleosides even further by the development of benzothieno-,[108] pyrazolo-,[109] and pyrido-fused[110] analogues **XIV-XX** (**Figure 15**). Within these series, some of the furo-, pyrazolo- and pyrido-fused nucleosides showed selective submicromolar cytostatic effect, as well as submicromolar anti-HCV activity, while their benzothieno- and naphtho-fused analogues did not exert any significant cytotoxicity and mostly any antiviral activity. Interestingly, for derivatives with annulated furan and pyridine heterocycles, we have proven that the mechanism of action also involves incorporation into the DNA and its subsequent damage. The above results show that these novel types of fused 7-deazapurine nucleosides could be useful for SAR studies and for further development of novel antitumor as well as antiviral agents.

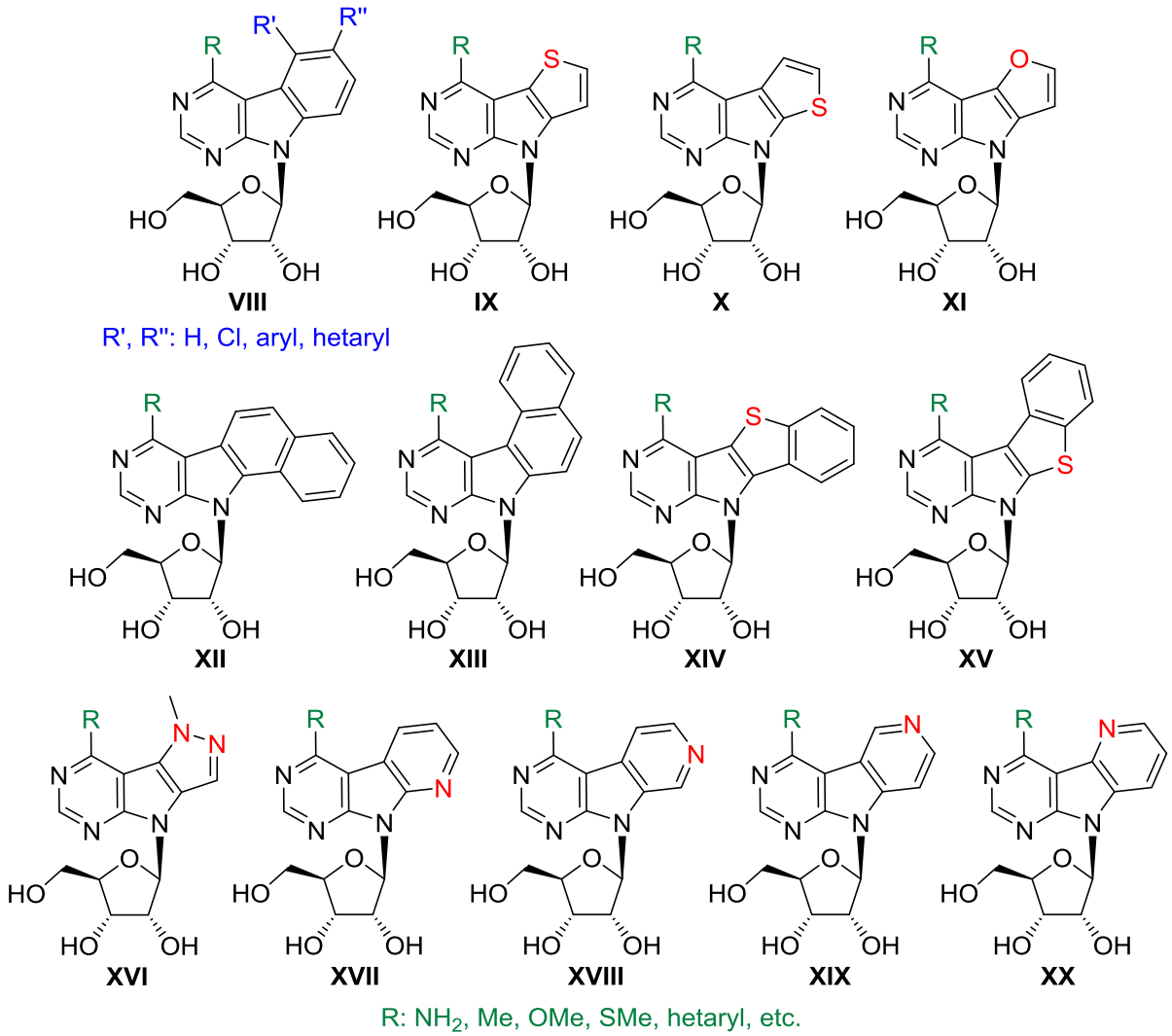


Figure 15. Novel fused 7-deazapurine ribonucleosides developed in our laboratory.

Antineoplastic nucleoside triciribine is an example of a different type of fused 7-deazapurine nucleoside derivatives in which the annulated ring is occupying space between positions 6 and 7 of the 7-deazapurine heterocycle (**Figure 16**). This compound was synthesized already in 1971,[111] and its anticancer and antiviral properties were intensively studied.[112] The mechanism of action of triciribine involves inhibition of Akt kinase[113] making this nucleoside effective in Akt-overexpressing tumors such as ovarian and pancreatic. Since triciribine suffers from poor solubility, its more soluble 5′-monophosphate prodrug, known as TCN-P, is often used, and it was also recently shown that application of phosphoramidate prodrug approach in this case can substantially improve bioavailability of the parent compound.[114] Although TCN-P showed promising results as an antineoplastic agent during phase I clinical trials, its further testing was halted due to insufficient efficacy and toxicity in high dosing. However, it is still being clinically studied in combination therapies with other antineoplastic compounds.[115,116]

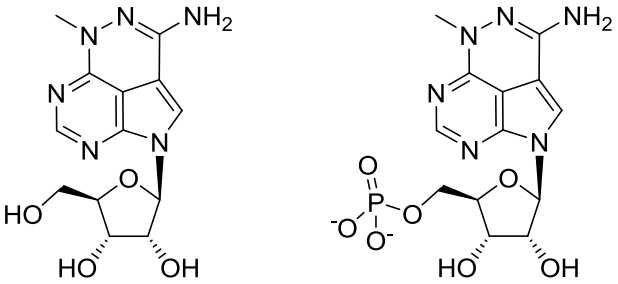
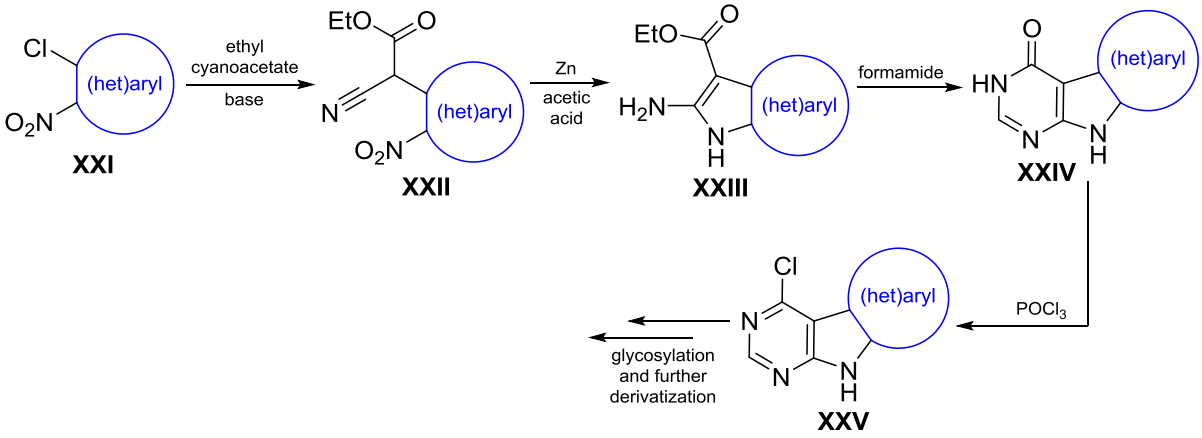


Figure 16. Structures of triciribine and TCN-P.

1.2.4.2 General synthetic approaches to fused 7-deazapurine nucleobases

Synthesis of the nucleobase moiety is the most challenging task in the preparation of fused 7-deazapurine nucleosides, and in this thesis, I will focus mainly on the general approaches, which allow preparation of a variety of 7-deazapurine heterocycles fused with six- and five-membered aromatic rings.

The first general method is a classical heterocyclization approach that is based on a multistep nucleobase build-up, and usually starts with suitable aromatics **XXI** bearing chloro and nitro groups in *ortho* position (**Scheme 1**). First, the chlorine atom is subjected to a nucleophilic aromatic substitution with ethyl cyanoacetate, and then the resulting derivative **XXII** is reduced using zinc dust in acetic acid followed by spontaneous cyclization[117] and formation of a bicyclic product **XXIII**. The subsequent cyclocondensation[118-121] with formamide or other suitable reagent affords desired tricyclic nucleobase **XXIV**, which could be further chlorinated to yield a key derivative **XXV** suitable for glycosylation and introduction of various groups and substituents via cross-coupling and substitution reactions allowing to prepare series of diversely substituted 7-deazapurine nucleosides.



Scheme 1. Classical heterocyclization approach.

Since there are many variously substituted *o*-chloronitrobenzenes available, this approach was used as a general method for the synthesis of modified benzene-fused 7-deazapurines (pyrimidoindoles) **XXVI** (**Figure 17**).[122] The strategy was also successfully employed for the preparation of two isomeric pyrido-fused analogues **XXVII** and **XXVIII**, which after subsequent substitution of the chlorine atom with various amines gave selective micromolar checkpoint kinase inhibitors with potential use for cancer treatment.[123] In our laboratory, we have successfully applied this approach for the preparation of the other two isomeric pyrido-fused 7-deazapurines **XXIX** and **XXX**,[110] as well as a tricyclic nucleobase **XXXI**[109] containing a five-membered heterocycle – pyrazole.

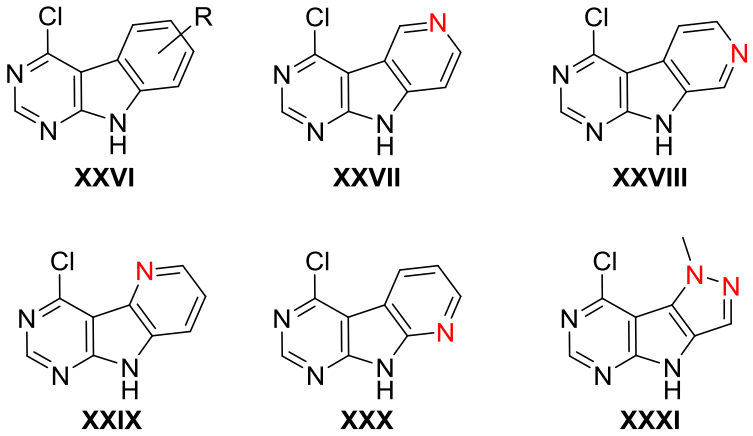
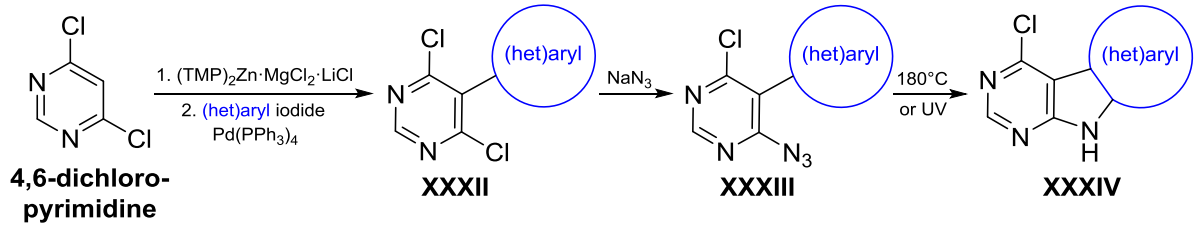


Figure 17. Fused 7-deazapurines prepared by classical heterocyclization approach.

The second general approach to the synthesis of fused 7-deazapurine nucleobases relies on cross-coupling reactions between suitable halogenated aromatics on one hand, and an organozinc species generated from 4,6-dichloropyrimidine on the other hand, followed by azidation and cyclization (**Scheme 2**). Treatment of 4,6-dichloropyrimidine with $(TMP)_2Zn{\cdot}MgCl_2{\cdot}LiCl$ (the Turbo-Hauser base) results in its zincation at position 5,[124] and the resulting organozinc compound is then subjected to Negishi cross-coupling reactions[125] with aryl iodides to afford (het)arylpyrimidines **XXXII**. Next, one chlorine atom is substituted with azido group to give derivative **XXXIII**, which exists in azide-tetrazole equilibrium. The last step of the synthesis is either thermal or photocyclization,[126] which affords target fused 7-deazapurine nucleobases **XXXIV** suitable for glycosylation and further derivatization. This method is shorter than the classical heterocyclization approach but is limited by availability and reactivity of starting (het)aryl iodides. The other problematic step could be the final cyclization, which is usually carried out under harsh conditions – either very high

temperatures in the case of thermal cyclization or presence of TFA in the case of UV-driven reaction.



Scheme 2. Synthesis of fused 7-deazapurines starting from 4,6-dichloropyrimidine.

Nevertheless, this method proved to be suitable for the preparation of chlorinated 7-deazapurine nucleobases **XXXV-XLI** featuring various fused aromatic and heteroaromatic cycles such as thiophene,[105] furan,[106], naphthalene [107] and benzothiophene[108] (**Figure 18**). We also applied this approach for the synthesis of pyrazolo-fused analogues **XXXI**[109] in order to compare this strategy with the classical heterocyclization. While the overall yields were similar in both cases, the second method has advantage of being shorter.

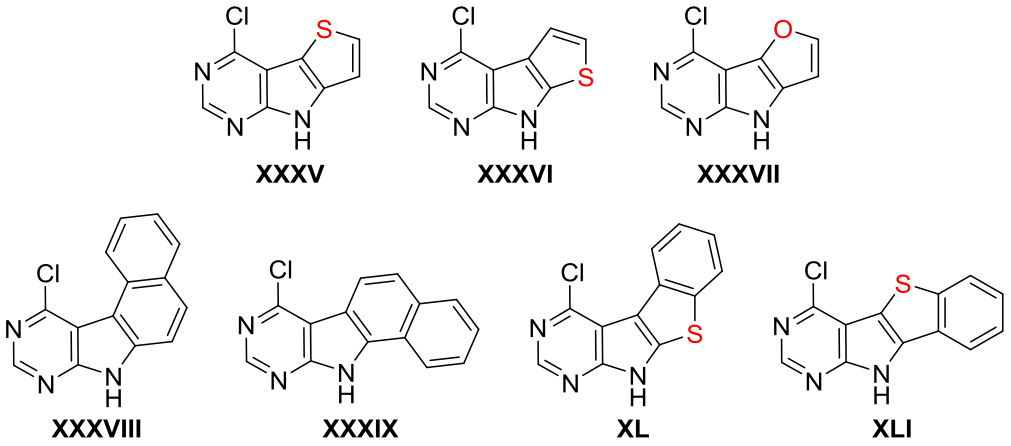


Figure 18. Fused 7-deazapurines prepared using 4,6-dichloropyrimidine and (het)aryl iodides.

Although a number of other less versatile approaches to the construction of 7-deazapurines fused to six- and five-membered aromatic cycles have been developed,[127-133] to the best of my knowledge, no analogous pyrrolo-fused 7-deazapurine nucleobases or nucleosides were known when I started working on my second Ph.D. project.

1.3 *C*-Nucleosides: medicinal chemistry and synthetic approaches

The canonical nucleosides (*N*-nucleosides) contain a heterocyclic aglycon that is linked to a carbohydrate moiety through a carbon-nitrogen glycosidic bond. Replacement of this bond with a chemically and enzymatically more stable carbon-carbon bond results in a class of compounds called *C*-nucleosides (**Figure 19**). In addition to increased stability, such change in the nature of the glycosidic bond can be accompanied by altered hydrogen bonding motifs, thus affecting molecular recognition properties. All these features, together with the broad structural variations possible in the aglycon part, offer potential for the development of new therapeutic agents and biochemical probes, allowing the applications of *C*-nucleosides in medicinal chemistry (mainly as antibacterial, antiviral or cytostatic agents) and in chemical biology (for studying and modifying biological processes, expansion of the genetic alphabet, development of the artificial bio-analogue systems, *etc.*).[134-136]

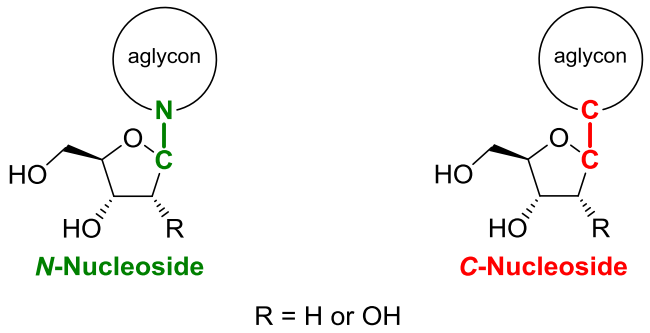


Figure 19. *C*-Nucleoside versus canonical *N*-nucleoside.

1.3.1 Natural *C*-nucleosides

The majority of naturally occurring *C*-nucleosides are antibiotics, and many also display anticancer or antiviral activity (**Figure 20**). Pseudouridine is the first discovered natural *C*-nucleoside that was isolated from the yeast tRNA.[137,138] Cellular RNAs contain over one hundred different modified nucleosides, of which pseudouridine is the most abundant.[139] This *C*-nucleoside was found to stabilize RNA stacking,[140] but it does not possess any significant biological activities.

Pyrazofurin, a *C*-nucleoside isolated from *Streptomyces candidus*, was initially explored as an antiviral agent due to its activity against a number of viruses.[141,142] Later, it was also shown to exhibit a broad spectrum of antitumor properties,[143,144] which is apparently the result of the inhibition of orotidylate decarboxylase, an enzyme involved in pyrimidine biosynthesis.[145]

Another example of naturally occurring C-nucleosides is showdomycin, an antibiotic isolated from *Streptomyces showdoensis*.[146] This compound was found to exhibit strong cytotoxic activity,[147] and to selectively inhibit various enzymes.[148,149] Such inhibition is likely due to the alkylating property of the maleimide aglycon moiety, which is known to specifically react with the thiol groups of the enzyme.

Natural antibiotics formycin A and formycin B, which resemble purine nucleosides, were first isolated from the rice mold *Nocardia interforma*.[150,151] These compounds display various biological activities, such as antitumor,[152,153] antiviral,[154] and antiparasitic.[155-157] Although their mechanism of action has not been fully elucidated, it was proved that formycins strongly inhibit purine nucleoside phosphorylase (PNP)[158,159] and nucleosidases.[160]

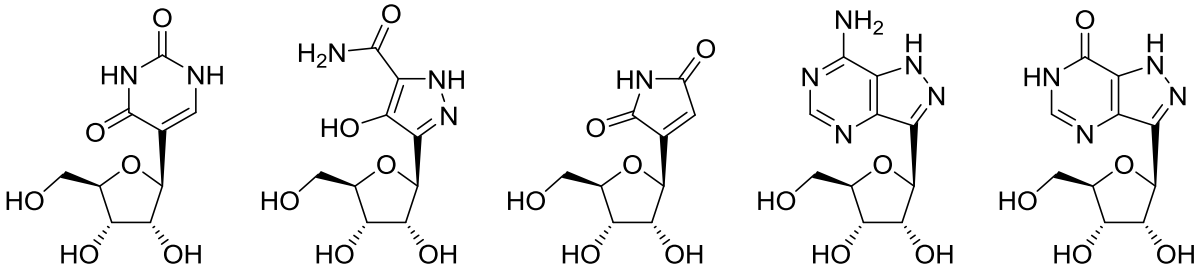
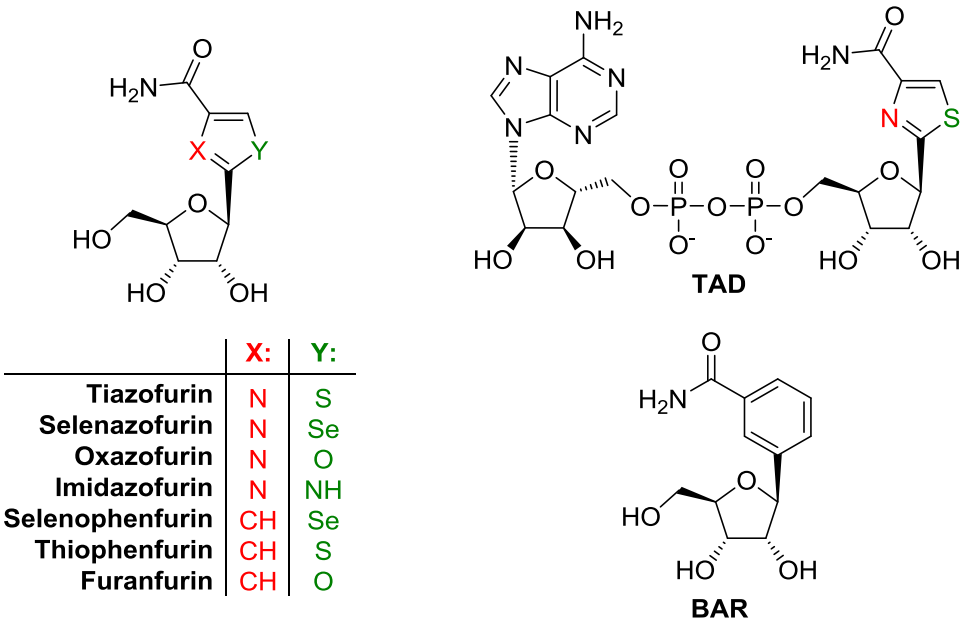


Figure 20. Examples of *C*-nucleosides found in nature.

1.3.2 Synthetic *C*-nucleosides with cytostatic activity

Shown in **Figure 21** are examples of unnatural cytostatic *C*-nucleosides that contain a carbamoyl group attached to the aglycon part. Tiazofurin, a potent anticancer agent,[161,162] is a thiazole carboxamide *C*-nucleoside. This compound was found to be metabolized to thiazole-4-carboxamide adenine dinucleotide (TAD, analogous to NAD), which is an IMPDH inhibitor.[163] The same mechanism of action and similar or even higher activity have been demonstrated for a number of other related *C*-nucleosides, among them benzamide ribonucleoside (BAR),[164,165] selenazofurin,[166] oxazofurin,[167] imidazofurin,[168] selenophenfurin,[169] thiophenfurin, and furanfurin.[170]



	X:	Y:
Tiazofurin	N	S
Selenazofurin	N	Se
Oxazofurin	N	O
Imidazofurin	N	NH
Selenophenfurin	CH	Se
Thiophenfurin	CH	S
Furanfurin	CH	O

Figure 21. *C*-Nucleoside inhibitors of IMPDH.

Immucillins, another group of synthetic *C*-nucleosides with a potent anticancer activity, act similarly to natural formycins: these compounds were found to be powerful transition-state analogue inhibitors of PNP (**Figure 22**).[171] Structurally, immucillins are aza-*C*-nucleoside analogues featuring azaribose as a sugar component, and some of them in addition contain an 8-aza-modified nucleobase.

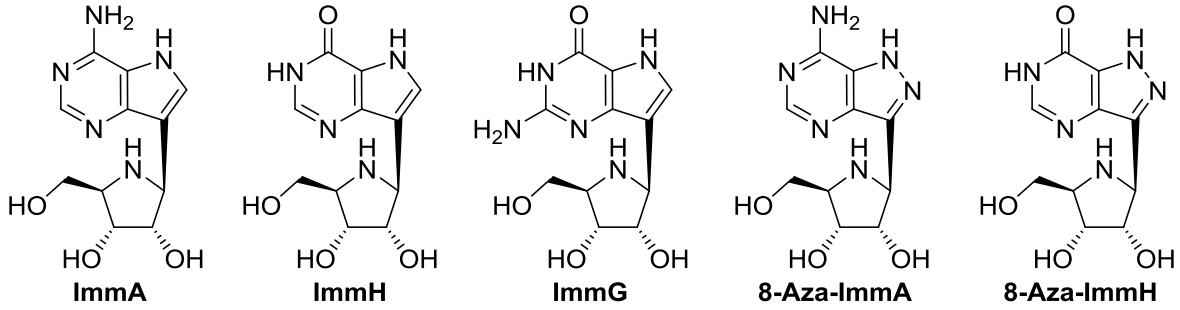


Figure 22. Structures of immucillins.

1.3.3 Unnatural *C*-nucleosides as antiviral agents

Several types of polyhalogenated indole[172] and quinoline[173] *C*-nucleosides were prepared as potential inhibitors of HCMV and HSV (**Figure 23**). These compounds were designed as more stable carba-analogues of 2,5,6-trichloro-1-(β-D-ribofuranosyl)-1*H*-benzo[*d*]imidazole (TCRB), a potent anti-HCMV inhibitor, which was found to disappear rapidly from the bloodstream due to the hydrolysis of its labile C-N glycosidic bond.[174] It was showed that

indole *C*-ribonucleoside **XLII** and quinoline derivative **XLIII** possess moderate activity against HCMV, but no activity against HSV-1. However, compound **XLII** was also found to be cytotoxic.

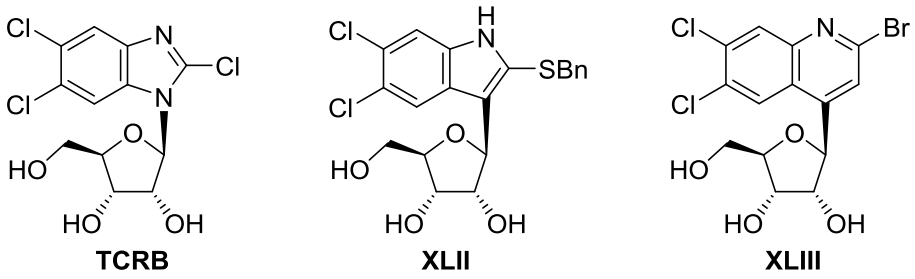


Figure 23. Nucleosides with anti-HCMV activity.

C-Nucleosides have also shown promising results in the treatment of HCV infection. Although, as discussed earlier, the majority of clinically tested nucleoside and nucleotide NS5B polymerase inhibitors (NIs) are derivatives of *N*-nucleosides, *C*-nucleosides have also shown good potential in the development of new anti-HCV agents. Synthesis and biological evaluation of carba-analogues of 2′-*C*-methyladenosine demonstrated that the *C*-nucleoside scaffold could be tolerated for the anti-HCV activity (**Figure 24**).[175] Although 2′-*C*-methyladenosine was found to inhibit HCV RNA replication without significant toxicity,[49] its potential therapeutic use was limited by the rapid metabolic degradation. Since one way to increase the metabolic stability of a nucleoside could be replacement of its hydrolyzable C-N glycosidic bond with a nonhydrolyzable C-C bond, carba-analogues of 2′-*C*-methyladenosine **XLIV** and **XLV** were prepared and studied for their anti-HCV activities. 9-Deaza analogue **XLIV** was found to possess only weak activity probably due to the change in the character of the 7-N from a hydrogen bond acceptor to a hydrogen bond donor as the result of altered tautomeric populations of the aglycon. Indeed, the swap of this 7-N in **XLIV** for oxygen (a hydrogen bond acceptor) yields *C*-nucleoside **XLV**, which is a moderately active inhibitor of HCV RNA replication.

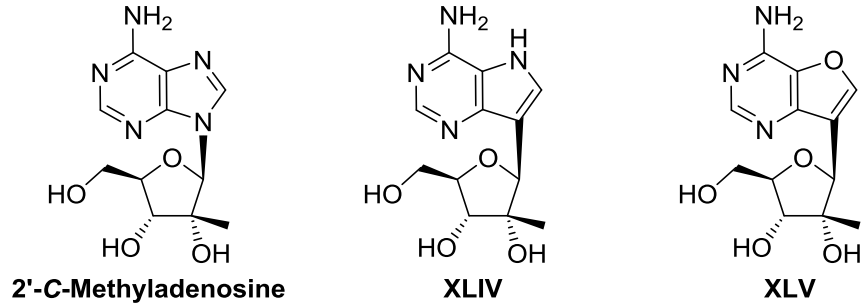


Figure 24. Structures of 2′-*C*-methyladenosine and its carba-analogues.

Later, a series of other 2′-*C*-methyl branched pyrimidine and purine *C*-nucleosides was prepared and tested for anti-HCV activity (**Figure 25**).[176,177] Pyrimidine derivatives **XLVI** and **XLVII** showed only poor enzymatic potency, while enzyme active guanosine *C*-nucleoside XL**VIII**, together with its phosphate prodrug, was inefficiently metabolized and thus inactive in the cell-based replicon assay. Adenosine *C*-nucleosides **XLIX** and **L** (and their triphosphates) showed the most promising results: these derivatives were found to exhibit selective replicon activity and to be potent inhibitors of the NS5B polymerase. Furthermore, compound **L** showed excellent cross-genotype activity, good pharmacokinetic properties, and low in vitro toxicity. However, it was also found to cause adverse effects in rat safety studies.

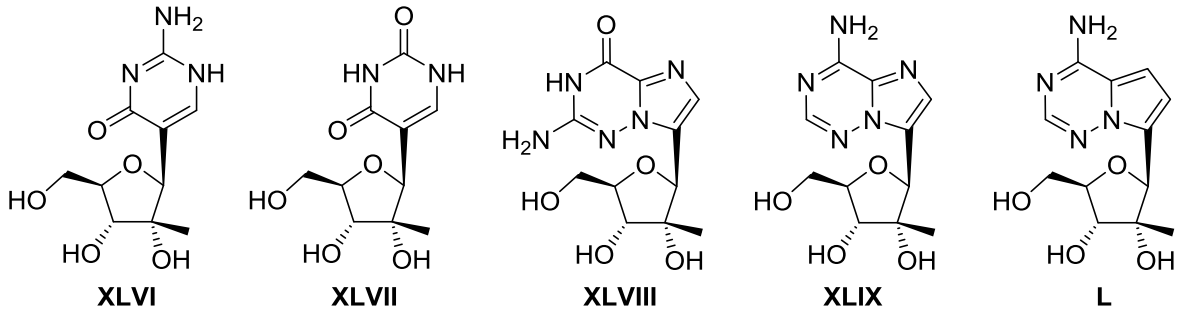
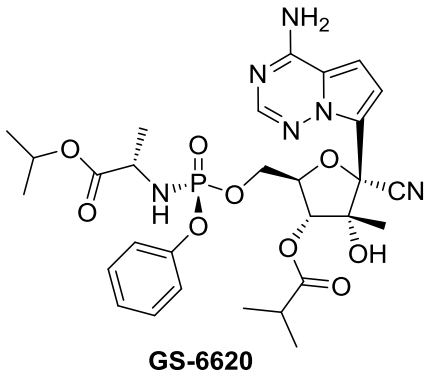


Figure 25. 2′-*C*-Methyl branched pyrimidine and purine *C*-nucleosides.

Attempts to improve cellular selectivity of **L** by introducing further structural modifications on the sugar moiety led to the discovery of GS-6620, the first *C*-nucleoside HCV polymerase inhibitor to enter clinical development (**Figure 26**).[178] This compound is a phosphoramidate prodrug of 2′-*C*-methyl-*C*-ribonucleoside that additionally contains a cyano group at the 1′-position of the sugar – yet another useful structural feature in the development of novel *C*-nucleoside antivirals.[179] In the phase I clinical trials, GS-6620 showed potential for high anti-HCV activity, but its clinical utility was limited by high intra- and interpatient pharmacokinetics and pharmacodynamics variability.



GS-6620

Figure 26. Structure of the first NI *C*-nucleoside clinical candidate GS-6620.

All these results demonstrate that development of new *C*-nucleosides as potential anti-HCV drugs is still worthwhile, and to the best of our knowledge, no report on the synthesis and biological evaluation of 2′-*C*-methyl-*C*-ribonucleosides analogous to Sofosbuvir has been published before I started to work on my first Ph.D. project.

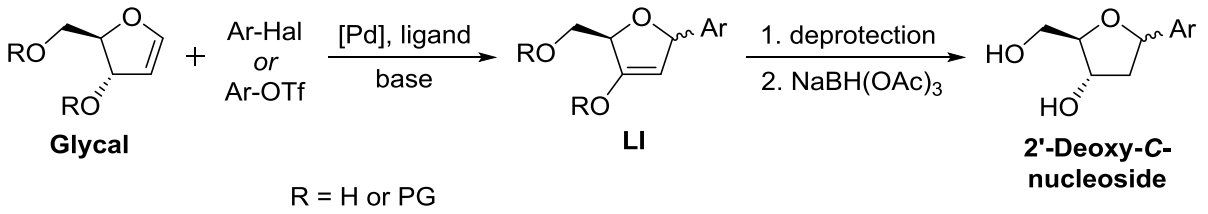
1.3.4 Synthetic approaches to *C*-nucleosides

Plenty of different synthetic approaches for the preparation of *C*-nucleosides were developed and summarized in several comprehensive reviews.[181-182] In general, there are five major synthetic strategies that could be applied for the synthesis of *C*-nucleosides: 1) stepwise construction of the aglycon unit upon a preformed carbohydrate part; 2) appropriate functionalization of a preformed aglycon followed by a construction of the sugar moiety; 3) direct connection of a preformed carbohydrate with an aglycon part; 4) modular approach; and 5) various modifications of the existing *C*-nucleosides. The first two strategies usually are laborious linear multistep sequences that are aimed at a particular derivative of interest,[175,183] and in this thesis, I will focus mainly on the other approaches, which are more general and versatile, and allow preparation of larger series of compounds.

1.3.4.1 Heck-type coupling reaction

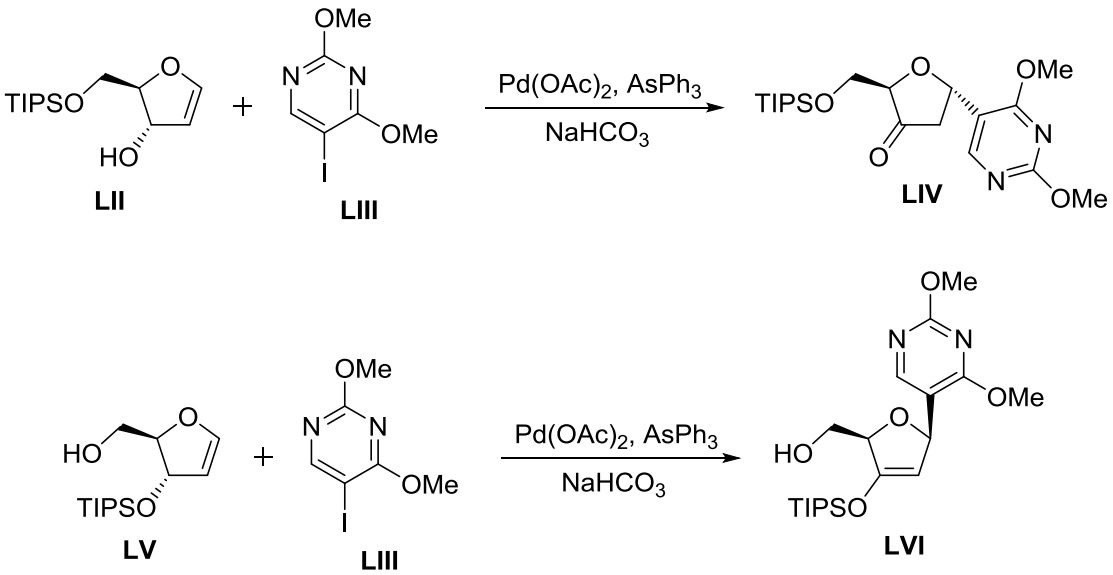
Heck-type coupling reaction is one of the most important methods for the synthesis of 2′-deoxy-*C*-nucleosides.[184,185] This reaction is used to form a new C-C bond between the anomeric carbon of glycals and functionalized (hetero)aromatics with high regio- and stereoselectivity (**Scheme 3**). General procedure typically involves the use of Pd(0) source ($Pd(OAc)_2$, Pd_2dba_3), phosphine or arsine ligand ($AsPh_3$, PPh_3, $P(C_6F_5)_3$, *etc.*), and a base (such as Et_3N, Bu_3N, iPr_2EtN, Ag_2CO_3 or $NaHCO_3$). After the coupling step, intermediate **LI** is first deprotected and then reduced using $NaBH(OAc)_3$ affording the final 2′-deoxy-*C*-

nucleoside. The selectivity of the reduction is caused by complexation of the reducing agent to 5′-hydroxy group of the carbohydrate moiety.



Scheme 3. Synthesis of *C*-nucleosides using Heck-type reaction.

The stereoselectivity of the anomeric bond formation can be controlled by the presence of an appropriate bulky protecting group on the 3- or 5-hydroxyl function of the carbohydrate.[186,187] For example, when 5-monosililated glycal **LII** is used for a coupling with aglycon **LIII**, the organopalladium reagent attacks **LII** from the α-face resulting in a formation of 1′-α-*C*-nucleoside **LIV** as a single product (**Scheme 4**). On the other hand, if 3-monoprotected glycal **LV** is used as a starting compound, the organopalladium reagent approaches it from the β-face, and 1′-β-*C*-nucleoside **LVI** is formed exclusively.

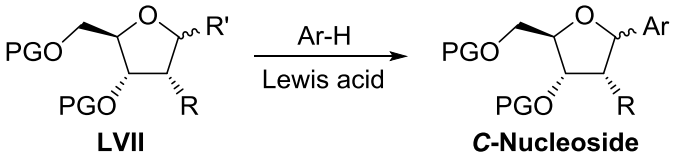


Scheme 4. Influence of protecting group position on stereoselectivity of the coupling reaction.

1.3.4.2 Lewis acid mediated electrophilic substitution

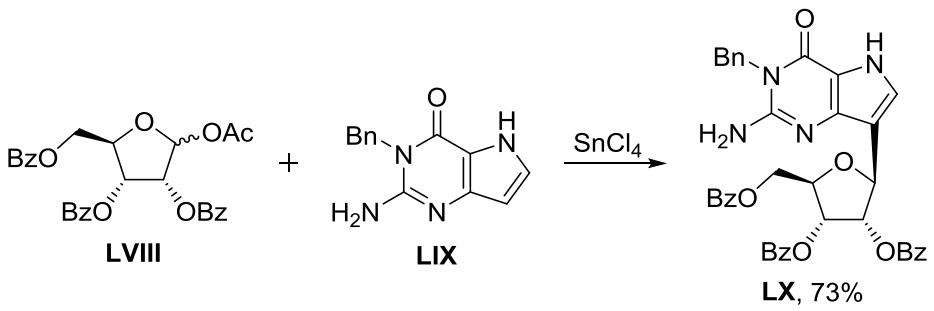
Coupling reaction of a preformed carbohydrate **LVII** and a (hetero)aryl in the presence of a Lewis acid is similar to Friedel-Crafts electrophilic substitution and provides another method for the construction of *C*-nucleosides (**Scheme 5**).[188,189] This methodology could be

applied for the synthesis of *C*-ribonucleosides as well as 2′-deoxy-*C*-ribonucleosides, but on the other hand, it is usually limited to electron rich aglycons. The method is simple but has a few disadvantages, such as low regio- and stereoselectivity (often, a mixture of α- and β-anomers is obtained), and a risk of double arylation of the sugar. $SnCl_4$ is the most widely used Lewis acid, however, other reagents, such as $BF_3 \cdot Et_2O$ and $AgBF_4$, could be also employed.



Scheme 5. Preparation of *C*-nucleosides using Lewis acid mediated electrophilic substitution.

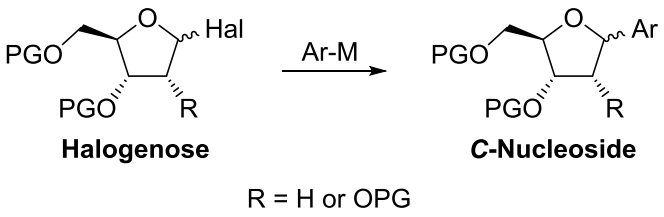
The stereoselectivity and the yield of this type of reactions are influenced by all three components (the protected sugar, the aglycon, and the Lewis acid), whereas the regioselectivity is fully controlled by the nature of the (hetero)aryl. Furthermore, stereoselectivity is usually higher in case of ribose-based sugar component comparing to 2-deoxyribose due to possible participation of the protecting group at position 2. For instance, coupling of a benzoylated ribose **LVIII** with a benzylated 9-deazaguanine **LIX** in the presence of $SnCl_4$ afforded protected *C*-ribonucleoside **LX** as a pure β-anomer (**Scheme 6**).[190] The intermediate **LX** was subsequently used for the preparation of 2- and 7-substituted 9-deazaadenosine analogues as potential cytostatic agents.



Scheme 6. Stereoselective Lewis acid mediated synthesis of a *C*-nucleoside guanosine analogue.

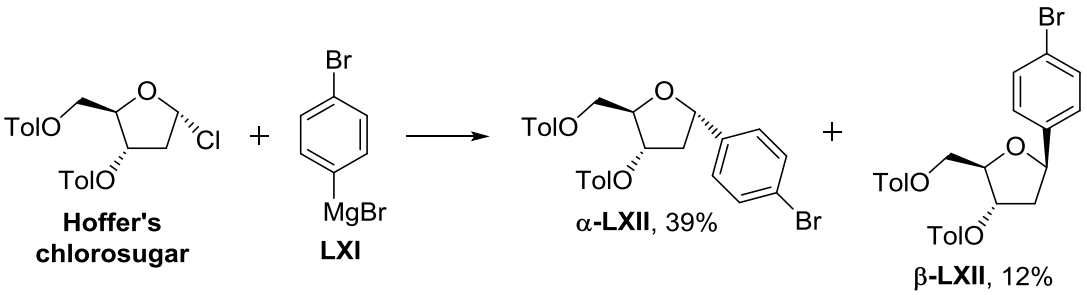
1.3.4.3 Coupling with halogenoses

Coupling reaction of organometallic reagents with halogenoses is one of the oldest ways of synthesis of *C*-nucleosides (**Scheme 7**). This method is suitable for the preparation of *C*-ribonucleosides as well as 2′-deoxy-*C*-ribonucleosides, and a variety of organometallic species, such as zinc,[191,192] cadmium,[193,194] lithium,[195] magnesium,[196,197] copper,[198] and mercury[199] reagents, could be used. Unfortunately, this approach usually allows preparation of C-nucleosides in only low yields and with an undesired α-anomer as a major product.



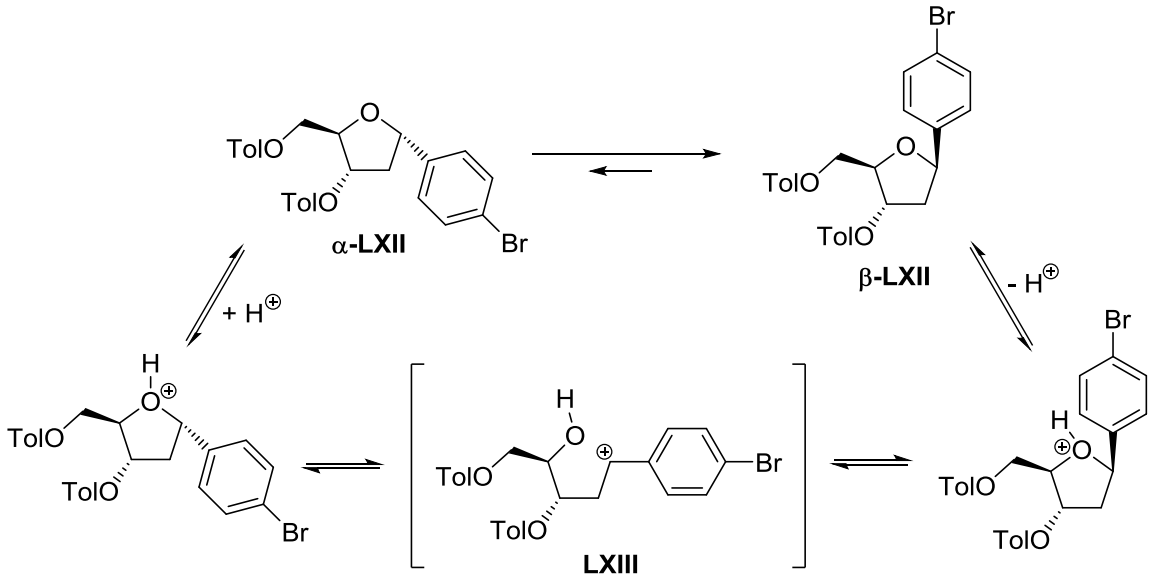
Scheme 7. Coupling of halogenoses with organometallic reagents.

Scheme 8 shows an example of the synthesis of a 2′-deoxy-*C*-nucleoside employing Hoffer's chlorosugar, one of the most widely used halogenoses. Coupling with Grignard reagent **LXI** afforded a separable mixture of anomers **α-LXII** and **β-LXII** in 39% and 12% yield, respectively.[200]



Scheme 8. Coupling of Hoffer's chlorosugar with Grignard reagent.

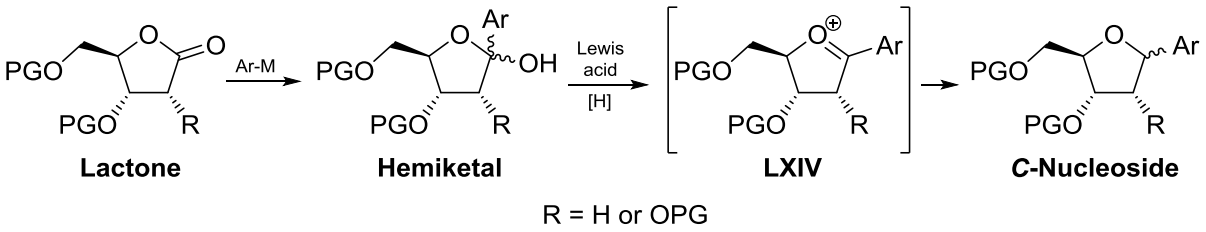
To overcome the lack in anomeric selectivity towards desired β-isomer, an acid catalyzed epimerization could be carried out (**Scheme 9**).[201,202] Protonation of the α-isomer results in the sugar ring opening and in formation of a stabilized carbocation **LXIII** that, after the ring closure, gives thermodynamically more stable β-anomer. Three cycles of **α-LXII** epimerization using a TFA/BSA mixture allowed to obtain the desired product **β-LXII** in higher 40% yield.



Scheme 9. Acid catalyzed epimerization of *C*-nucleosides.

1.3.4.4 Nucleophilic addition to lactones

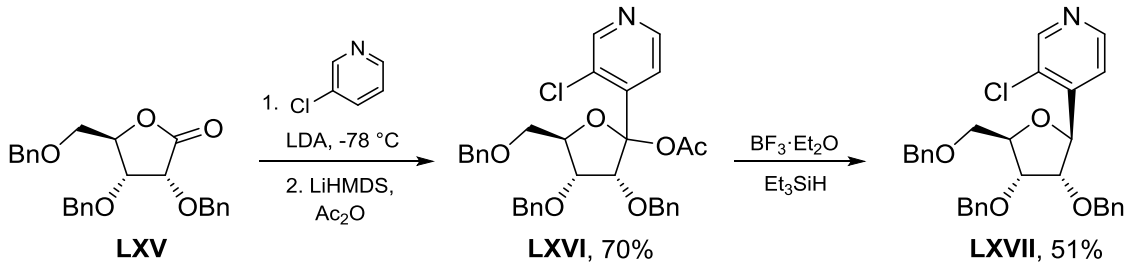
One of the most frequently used synthetic strategies for the preparation of *C*-nucleosides is addition of organometallic nucleophiles to lactones (**Scheme 10**). Reaction of suitably protected furanolactones with (het)aryl lithium reagents at low temperatures affords hemiketals that are next subjected to reduction. First, the hemiketal is deoxygenated by Lewis acid yielding an oxonium intermediate **LXIV**, which is then reduced using silane. Typically, a combination of $BF_3 \cdot Et_2O$ and Et_3SiH is used for this purpose. Stereoselectivity of the reduction step is controlled by the nature of aglycon and by the lactone protecting groups but is usually very good and allows preparation of β-anomeric *C*-nucleosides as major or exclusive products.[203,204] Conversion of hemiketals to *C*-nucleosides is also possible to accomplish by an alternative method that is based on the reduction of hemiketals to diols with their subsequent cyclization.[205-207]



Scheme 10. Nucleophilic addition to lactone followed by silane reduction.

Lactone protecting group choice is influenced by both addition and reduction step: the group should be compatible with a highly reactive organometallic agent as well as with a strong Lewis acid. On the other hand, these groups often direct nucleophilic attack during the addition step, and the attack of silane during the reduction, thus affecting stereoselectivity of the whole sequence.[208] Various silyl and benzyl groups are among the most common protecting groups used for the synthesis of *C*-nucleosides by this method.

As for the aglycon component, its nature can also have significant effect on the selectivity and efficacy of both steps. For instance, it was shown that reduction of some hemiketals bearing nitrogenous heterocycles was more efficient or more stereoselective if the hemiketal was converted into the corresponding hemiketal acetate.[209-211] Shown in **Scheme 11** is the synthesis of a chloropyridinyl *C*-nucleoside as an intermediate for the preparation of potential anti-influenza agents.[212] Coupling of a benzylated lactone **LXV** with 3-chloropyridine via nucleophilic addition in the presence of LDA afforded hemiketal, which was converted into 1′-*O*-acetate **LXVI** by treatment with LiHMDS and Ac_2O. Reduction of **LXVI** using a combination of $BF_3 \cdot Et_2O$ and Et_3SiH was more efficient than analogous reduction of the corresponding hemiketal, and allowed preparation of the desired protected *C*-nucleoside **LXVII** in 51% yield as a pure β-isomer. Hemiketal acetate synthesis does not necessarily require isolation of the hemiketal intermediate but could also be accomplished if Ac_2O is used directly for the work-up of the reaction mixture obtained after nucleophilic addition.[209]

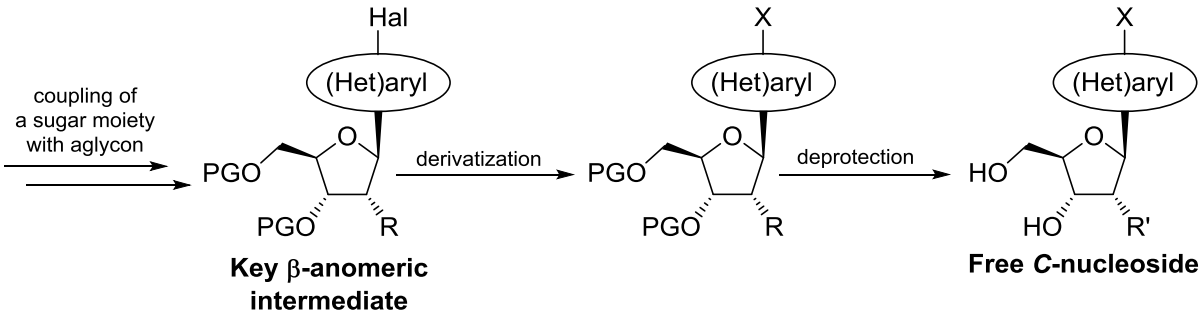


Scheme 11. Example of *C*-nucleoside synthesis employing hemiketal acetate reduction.

1.3.4.5 Modular approach

Previously mentioned synthetic strategies for the preparation of *C*-nucleosides often suffer from low anomeric selectivity, poor yields, and necessity to optimize conditions for the synthesis of each particular derivative. Modular approaches could be a nice alternative, since they allow preparation of wide series of compounds. In our group, for example, a new modular approach based on the dividing of a target nucleoside into three "modules" – sugar part, aglycon and functional groups – was developed and successfully applied for the

synthesis of a large number of *C*-nucleosides. First, a protected β-anomeric halo(het)aryl-*C*-nucleoside is prepared using one of the suitable synthetic approaches described in the previous chapters (**Scheme 12**). This key intermediate can be further derivatized by the means of various palladium-catalyzed transformations and other reactions to introduce miscellaneous substituents into the molecule. The final deprotection step affords series of diversely substituted free *C*-nucleosides.

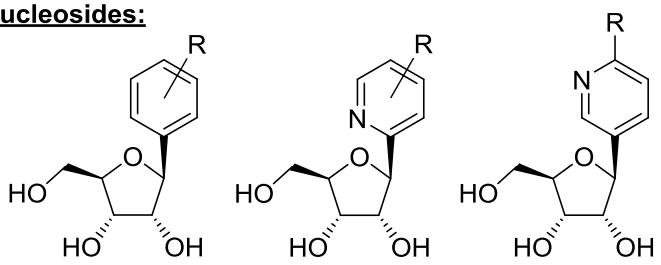


R = H or OPG; R' = H or OH; Hal = Cl or Br; X: (het)aryl, alkyl, amino, hydroxy, etc.

Scheme 12. Synthesis of *C*-nucleosides using modular approach.

This modular strategy was used in our laboratory for the preparation of large series of both *C*-ribonucleosides and 2′-deoxy-*C*-ribonucleosides bearing mono- and disubstituted six-membered (benzene,[213,214] pyridine[215-218], and pyrimidine[219,220]) or five-membered (thiophene[221] and furan[222]) rings (**Figure 27**). All these compounds contain a modified nucleobase, but the sugar part is untouched – it is the same as in canonical natural nucleosides. To the best of our knowledge, no report on modular synthesis of 2′-*C*-methyl-*C*-ribonucleosides has been published when I started my Ph.D.

***C*-Ribonucleosides:**



2'-Deoxy-*C*-ribonucleosides:

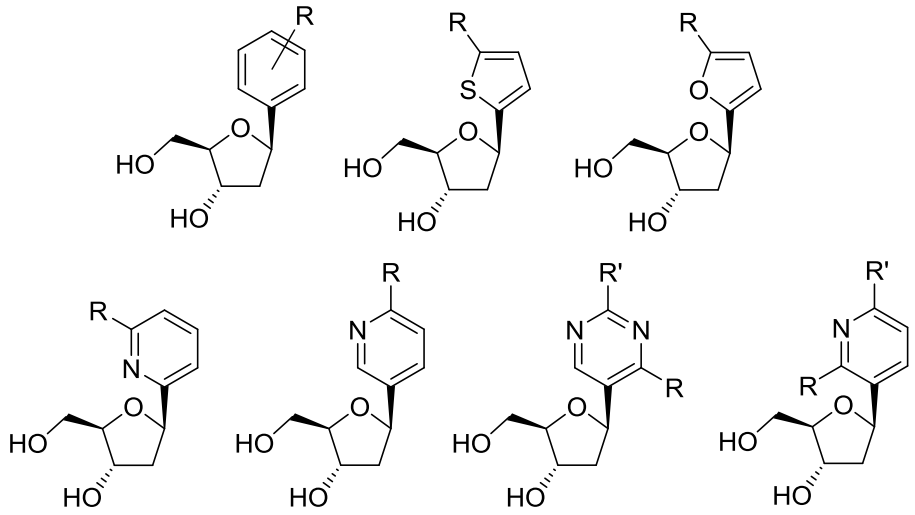


Figure 27. *C*-Nucleosides prepared by modular approach.

2 Specific aims of the thesis

1. Development of modular methodology for the synthesis of substituted phenyl and pyridyl 2′-*C*-methyl-*C*-ribonucleosides and their triphosphates.
2. Synthesis of pyrrolo-fused 7-deazapurine ribonucleosides substituted in position 4.

2.1 Rationale for the specific aims

Introduction of a methyl group into the 2′-position of ribose to generate modified nucleotides proved to be a good approach to achieving specific inhibition of the HCV RNA polymerase.[49] Sofosbuvir,[45] a nucleoside phosphate prodrug that is clinically used to treat HCV, also features this structural modification, and even though it seems to be a very efficient drug, there is always a threat of the development of viral resistance, so the search for new potent nucleoside antivirals is still a worthwhile goal. Although the majority of clinically tested nucleoside and nucleotide HCV RNA polymerase inhibitors are derivatives of *N*-nucleosides, some of their carba-analogues with a nonhydrolyzable C-C glycosidic bond – *C*-nucleosides – also showed good potential in the development of new anti-HCV agents.[175-177] The majority of those *C*-nucleosides were synthesized employing laborious linear multistep strategies unsuitable for the preparation of larger series of derivatives.[175,183] We have recently developed a general modular approach to *C*-nucleosides[213-220] based on the synthesis and functionalization of the key halo(het)aryl-*C*-ribonucleoside intermediates, and the aim of my work was to apply this method to the synthesis of three types of new substituted benzene and pyridine 2′-*C*-methyl-*C*-ribonucleosides (and their triphosphates) as carba- and dicarba-analogues of parent pyrimidine nucleosides related to Sofosbuvir.

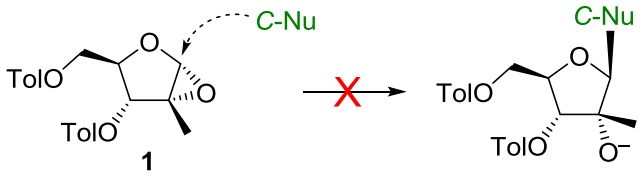
Since all three series of 2′-*C*-methyl-*C*-ribonucleosides and their triphosphates where found to be devoid of anti-HCV activity, I turned my attention to a different type of promising anticancer and antiviral modified nucleosides recently developed in our group – annulated 7-deazapurine ribonucleosides whose design was inspired by nanomolar cytostatic 7-hetaryl-7-deazapurine nucleosides previously discovered in our laboratory.[95,96] We decided to explore the space around positions 7 and 8 of 7-deazapurine by introducing a fused benzene[103,104] or thiophene[105] ring, and some of the resulting ribonucleosides showed good and selective cytotoxic effect as well as some antiviral activity, with thieno-fused derivatives being the most active. Therefore, my goal was to extend this class of 7-deazapurine nucleosides comprising annulated five-membered heterocycles and to develop synthesis of novel pyrrolo-fused analogues.

3 Results and discussion

3.1 Modular synthesis of phenyl and pyridyl 2′-*C*-methyl-*C*-ribonucleosides

3.1.1 Synthesis of the key bromo intermediates

The goal of my first project was to develop a novel general modular methodology suitable for the synthesis of larger series of diverse substituted phenyl and pyridyl 2′-*C*-methyl-*C*-ribonucleosides as potential anti-HCV agents. To form a glycosidic C-C bond, I first wanted to exploit stereoselective epoxide ring opening of toluoyl-protected 1,2-anhydro-2-*C*-methyl-α-D-ribofuranose **1**, which was successfully used for the preparation of some modified *N*-nucleosides.[99,223] Epoxide **1** was synthesized according to the literature procedure,[99] and then its ring opening by various *C*-nucleophiles was studied (**Scheme 13**). I tested a number of Grignard reagents as well as organocopper, organozinc and organolithium compounds, but unfortunately all my attempts to form C-C glycosidic bond by this method were unsuccessful: either no reaction occurred or complex mixtures of unidentified products were formed.

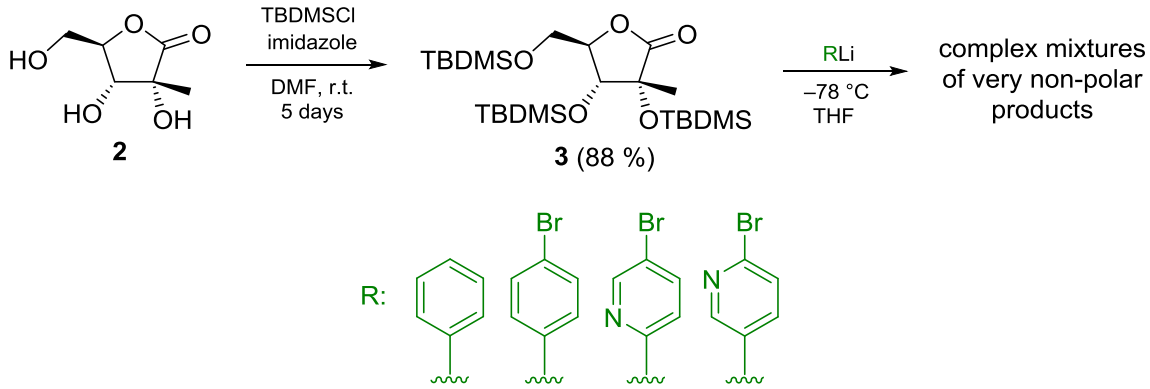


C-Nu: PhMgBr, PhMgCl, Ph_2CuLi, Ph_2CuMgI, Et_2Zn, Ph_2Zn, PhLi

Scheme 13. Attempts to open the epoxide ring with C-nucleophiles.

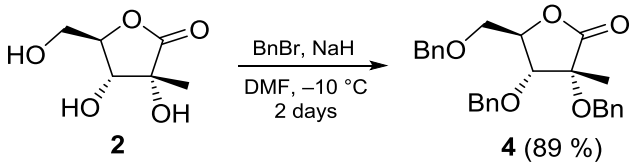
Since in our laboratory we have recently developed a general modular approach to *C*-ribonucleosides based on the synthesis of the key halogenated C-nucleosides via addition of organolithium compounds to a suitable protected lactone followed by reduction of the resulting hemiketals,[214-216] I decided to apply this method for the synthesis of 2′-*C*-methyl-*C*-ribonucleosides. In analogy to our previous works, I initially tried to use a silyl-protected lactone as a starting compound. 2-*C*-Methyl-D-ribono-1,4-lactone **2**, available in two steps from D-glucose,[224] was successfully protected with TBDMS protecting groups by treatment with TBDMSCl and imidazole in DMF affording lactone **3** in very good 88 % yield (**Scheme 14**). In order to attach the aromatic nucleobase moiety, I studied addition reactions

of various aryl and hetaryllithium compounds to this silylated lactone **3**, but complex inseparable mixtures of very non-polar products were obtained.



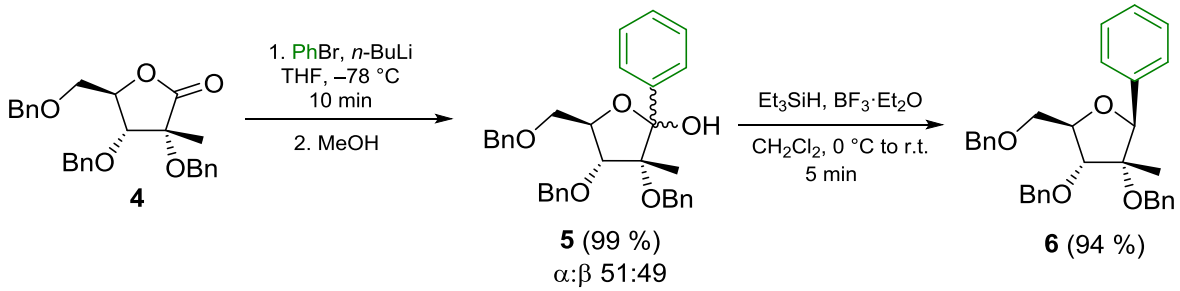
Scheme 14. Approach based on a silyl-protected lactone.

I decided to change protecting group with a less lipophilic one, and similar synthetic sequence utilizing analogous perbenzylated lactone **4** showed more promising results. Benzyl protecting groups were successfully introduced by treatment of the lactone **2** with BnBr and NaH in DMF at −10 °C to give benzylated building block **4** in 89 % yield on a multigram scale (**Scheme 15**).



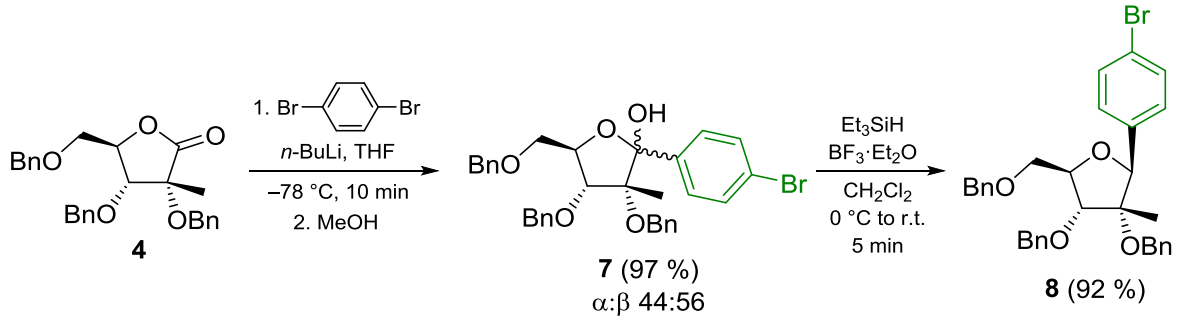
Scheme 15. Introduction of benzyl protecting groups.

I first subjected lactone **4** to an addition reaction with phenyllithium generated from bromobenzene by treatment with *n*-BuLi in THF at −78 °C, and this transformation led to formation of hemiketal **5** in excellent 99 % yield as an inseparable mixture of two anomers (α:β 51:49) (**Scheme 16**). Interestingly, epimerization of **5** was observed when its solution in DMSO-d_6 was stored at 4 °C for 2 weeks: the ratio of anomers changed to α:β 31:69. Similarly to our previously developed procedure,[214] **5** was reduced using Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 to give the benzylated *C*-nucleoside **6** in excellent 94 % yield as a single desired β-anomer. The stereoselectivity of this reduction is similar to our previous work[216], and is probably the result of formation of oxonium intermediate **LXIV** (**Scheme 10**), which is then reduced by silane.



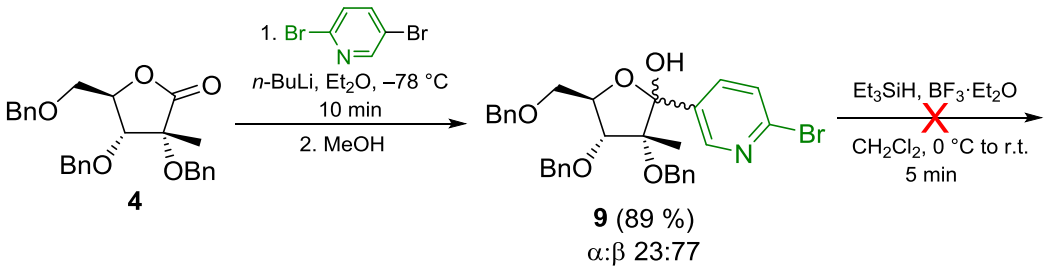
Scheme 16. Synthesis of benzylated phenyl *C*-nucleoside.

Then I applied the same synthetic strategy for the preparation of the key 4-bromophenyl analogue. Treatment of 1,4-dibromobenzene with *n*-BuLi in THF at −78 °C generated 4-bromophenyllithium that was subsequently added to the lactone **4** resulting in a formation of hemiketal **7** in excellent 97 % yield (**Scheme 17**). As in the case of phenyl derivative **5**, an inseparable mixture of anomers (α:β 44:56) was obtained, and similar epimerization was observed when solution of **7** in DMSO-d_6 was stored at 4 °C – after 2 weeks, the ratio of anomers changed to α:β 8:92. Reduction of **7** by treatment with a mixture of Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 afforded desired benzyl-protected *C*-nucleoside **8** in 92 % yield as a pure β-anomer.



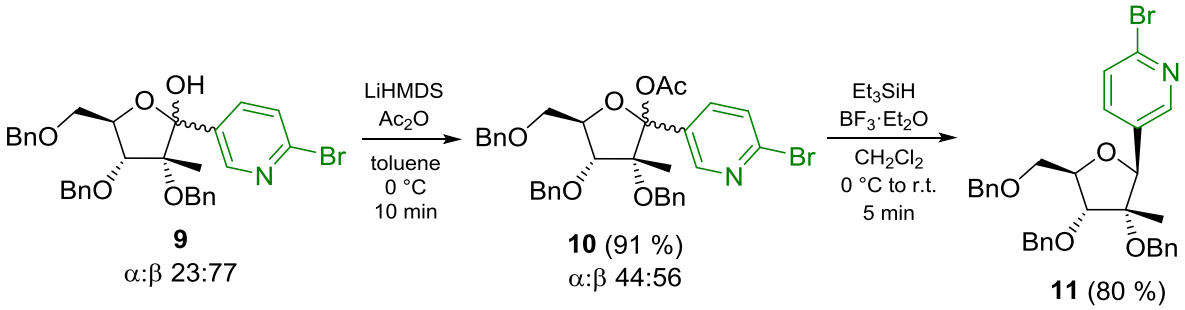
Scheme 17. Synthesis of the key 4-bromophenyl *C*-nucleoside.

Preparation of the key isomeric benzylated 2-bromopyridin-5-yl and 5-bromopyridin-2-yl analogues was based on known dichotomy[225] in regioselective lithiation of 2,5-dibromopyridine by treatment with *n*-BuLi in various solvents at −78 °C in analogy to our work.[216] Thus, in Et_2O – a coordinating solvent – 2-bromo-5-lithiopyridine is the main species that reacts with the lactone **4** to provide 2-bromopyridin-5-yl hemiketal **9** in 89 % yield (**Scheme 18**). Similarly to the previous cases, inseparable mixture of two anomers was formed (α:β 23:77). All my attempts to reduce **9** using Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 were unsuccessful – reaction did not proceed, and only starting material was isolated.



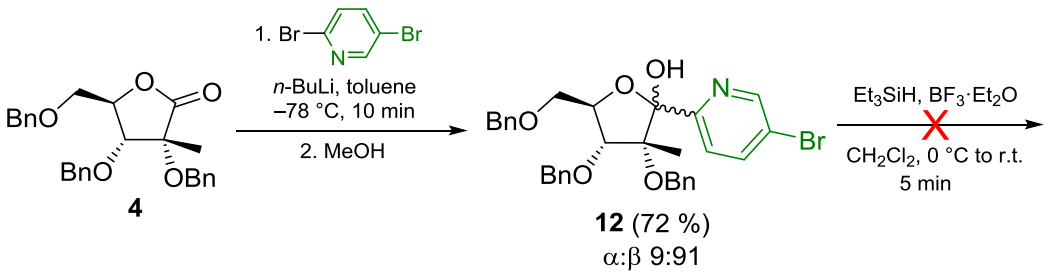
Scheme 18. Synthesis of the 2-bromopyrydin-5-yl hemiketal.

Analogously to our previous paper,[216] I tried to convert 2-bromopyrydin-5-yl hemiketal **9** to its acetate **10**, which should be more reactive towards reduction with Et_3SiH and Lewis acid. Deprotonation of **9** with LiHMDS in toluene at 0 °C, followed by addition of Ac_2O gave hemiketal-acetate **10** in very good 91 % yield as an inseparable mixture of two anomers (α:β 44:56) (**Scheme 19**). Subsequent reduction of **10** by treatment with Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_2Cl_2 proceeded smoothly resulting in a formation of the desired *C*-nucleoside **11** in 80 % yield as a single β-anomer.



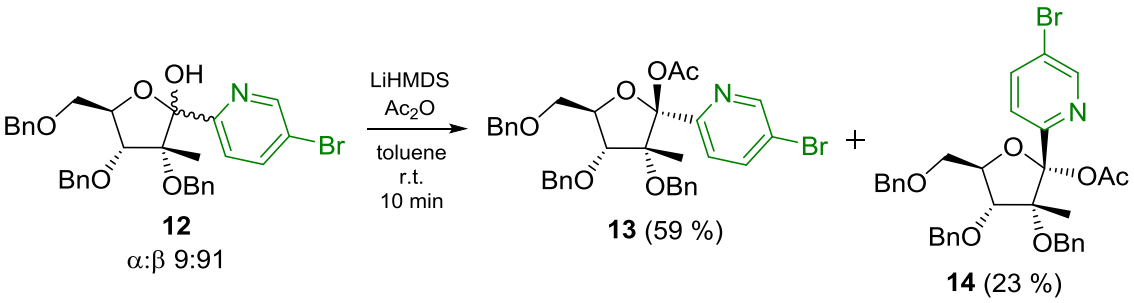
Scheme 19. Reduction of the 2-bromopyrydin-5-yl analogue.

If a non-coordinating solvent, such as toluene, is used, lithiation of 2,5-dibromopyridine yields another regioisomer – 5-bromo-2-lithiopyridine,[225] whose addition to the lactone **4** afforded 5-bromopyrydin-2-yl hemiketal **12** in 72 % yield as an inseparable mixture of two anomers (α:β 9:91) (**Scheme 20**). As in the case of isomeric 2-bromopyridin-5-yl hemiketal **9**, direct reduction of **12** using a mixture of Et_3SiH and $BF_3{\cdot}Et_2O$ was unsuccessful.



Scheme 20. Synthesis of the 5-bromopyrydin-2-yl hemiketal.

To facilitate reduction of this hemiketal, I used the same strategy as for the derivative **9** – conversion to the corresponding acetate using LiHMDS and Ac_2O in toluene, and in this case, the reaction proceeded more efficiently when the deprotonation step was performed at room temperature (**Scheme 21**). This transformation allowed me to obtain a mixture of anomers **13** (59 %) and **14** (23 %), which were successfully separated using flash column chromatography. Structure of the β-anomer **13** was also confirmed by single crystal X-ray diffraction analysis (**Figure 28**).



Scheme 21. Synthesis of the 5-bromopyrydin-2-yl hemiketal acetate.

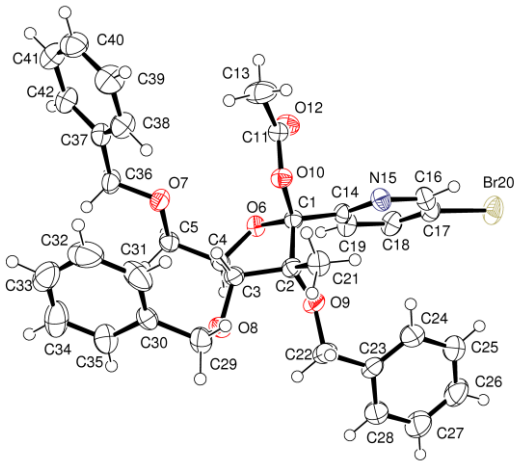
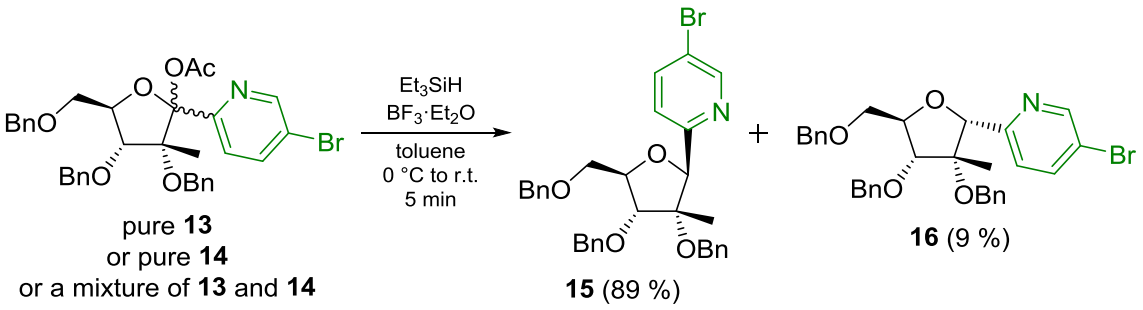


Figure 28. X-ray structure (ORTEP[226] drawing) of hemiketal acetate **13** with the atom numbering scheme. Thermal ellipsoids are shown at the 50 % probability level.

With both anomers **13** and **14** available in pure form, I studied their reduction using a standard procedure (Et_3SiH and $BF_3 \cdot Et_2O$ in CH_2Cl_2). Interestingly, the stereoselectivity of this reaction was always the same: no matter what was utilized as a starting material – pure **13**, pure **14** or their mixture – the same ratio of anomeric *C*-nucleoside products **15** and **16** was obtained (**Scheme 22**). However, I found out that the ratio slightly depends on the solvent, and the best results – 89 % of the desired β-anomer **15** and only 9 % of unwanted α-anomeric side-product **16** – were achieved when reduction was performed in toluene (comparing to 81 % of **15** and 14 % of **16** if CH_2Cl_2 was used as a solvent). The two anomeric products **15** and **16** were easily separable using flash column chromatography.



Scheme 22. Reduction of 5-bromopyridin-2-yl hemiketal acetates.

3.1.2 Derivatization by cross-coupling, amination and hydroxylation reactions

With the key bromo intermediates **8**, **11** and **15** in hand in sufficient amounts, I turned my attention to their derivatization by the means of various Pd-catalyzed cross-coupling, hydroxylation and amination reactions in analogy to our previous work on ribonucleosides (**Scheme 23**, **Table 1**).[214-216]

Cross-couplings of **8**, **11** and **15** with trimethylaluminium and $Pd(PPh_3)_4$ in THF at 66 °C were performed to prepare methyl derivatives **17a**, **18a** and **19a** in very good to excellent yields (91, 95 and 77 % respectively).

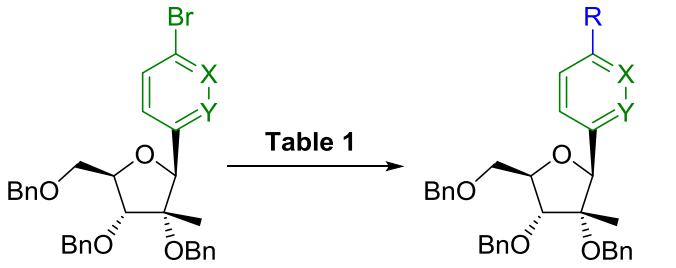
To introduce primary and tertiary amino groups, Pd-catalyzed Hartwig-Buchwald amination reactions[227] were employed. Treatment of compounds **8** and **11** with LiHMDS in the presence of Pd_2dba_3 and Cy-JohnPhos[228,229] afforded aniline or aminopyridine nucleosides **17b** and **18b** in 85 and 94 % yields correspondingly. To obtain derivative **19b** form **15**, P(*t*-Bu)$_3 \cdot HBF_4$ was used instead of Cy-JohnPhos as a ligand to get acceptable yield of 73 %.

Dimethylamino group was installed by coupling reaction with dimethylamine in toluene in the presence of Pd_2dba_3 and JohnPhos and with *t*-BuONa as a base. Using these conditions, dimethylamino derivatives **17c**, **18c** and **19c** were prepared in good yields of 83 to 87 %.

To introduce hydroxyl group, Pd-catalyzed hydroxylation reactions using KOH in the presence of Pd_2dba_3 and $Me_4(t\text{-}Bu)_2XPhos$ were applied.[230] Reactions were carried out in the mixture of 1,4-dioxane and water (3:1) at 80 °C, and after heating for 2–4 hours compounds **17d**, **18d** and **19d** were obtained in excellent 93 to 98 % yields.

Similarly to our previous work,[231] hydroxy derivatives **17d**, **18d** and **19d** were used as starting compounds for the preparation of the corresponding methoxy nucleosides. The crude reaction mixtures after the synthesis of compounds **17d** and **19d** were heated with MeI in the presence of KOH and TBAB as a phase transfer catalyst at 80 °C for 30 min to yield methoxy compounds **17e** and **19e** in 81 and 66 % yields over 2 steps respectively.

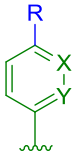
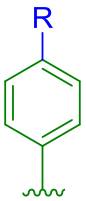
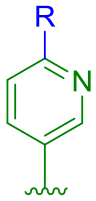
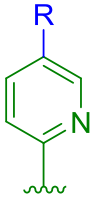
Since derivative **18d** exists mainly in a pyridone form, it could be alkylated at N- as well as O-atom. Under methylation conditions applied for the preparation of compounds **17e** and **19e** (treatment with MeI and KOH in a mixture of 1,4-dioxane with water**)**, bromo nucleoside **18d** was alkylated mainly at the *N*-site affording 92 % of unwanted derivative **20** and only 5 % of the desired *O*-methylated product **18e** (**Scheme 24**). Changing the base for Ag_2CO_3 and the solvent for CH_2Cl_2, I was able to prepare the target methoxypyridine nucleoside **18e** in 68 % yield.



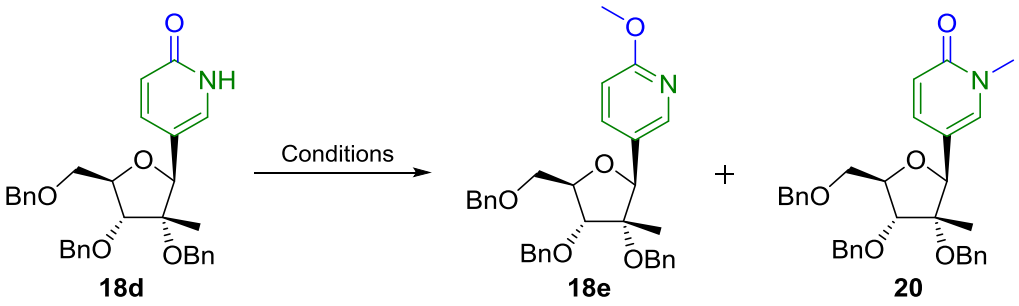
8, **11**, **15** **17a–e**, **18a–e**, **19a–e**

Scheme 23. Derivatization of the key bromo intermediates.

Table 1. Derivatization of the key bromo intermediates **8**, **11** and **15**.

	R	Reagent	Catalyst, ligand, base	Solvent; conditions	Compd. (Yield)
	Me	Me_3Al	$Pd(PPh_3)_4$	THF; 66 °C, 1 h	**17a** (91 %)
	NH_2	LiHMDS	Pd_2dba_3, Cy-JohnPhos	THF; 66 °C, 30 min[a]	**17b** (85 %)
	NMe_2	Me_2NH	Pd_2dba_3, JohnPhos, *t*-BuONa	toluene; 70 °C, 24 h	**17c** (83 %)
	OH	KOH	Pd_2dba_3, $Me_4(t\text{-Bu})_2$XPhos	1,4-dioxane/H_2O; 80 °C, 2 h	**17d** (95 %)
	OMe[b]	CH_3I	KOH, TBAB	1,4-dioxane/H_2O; 80 °C, 30 min	**17e** (81 %)
	Me	Me_3Al	$Pd(PPh_3)_4$	THF; 66 °C, 1 h	**18a** (95 %)
	NH_2	LiHMDS	Pd_2dba_3, Cy-JohnPhos	THF; 70 °C, 30 min[a]	**18b** (94 %)
	NMe_2	Me_2NH	Pd_2dba_3, JohnPhos, *t*-BuONa	toluene; 70 °C, 4 h	**18c** (87 %)
	OH	KOH	Pd_2dba_3, $Me_4(t\text{-Bu})_2$XPhos	1,4-dioxane/H_2O; 80 °C, 2 h	**18d** (93 %)
	OMe[c]	CH_3I	Ag_2CO_3	CH_2Cl_2; 60 °C, 2 h	**18e** (68 %)
	Me	Me_3Al	$Pd(PPh_3)_4$	THF; 66 °C, 1 h	**19a** (77 %)
	NH_2	LiHMDS	Pd_2dba_3, $P(t\text{-Bu})_3\cdot HBF_4$	THF; 66 °C, 3 h[a]	**19b** (73 %)
	NMe_2	Me_2NH	Pd_2dba_3, JohnPhos, *t*-BuONa	toluene; 70 °C, 2 h	**19c** (84 %)
	OH	KOH	Pd_2dba_3, $Me_4(t\text{-Bu})_2$XPhos	1,4-dioxane/H_2O; 80 °C, 4 h	**19d** (98 %)
	OMe[d]	CH_3I	KOH, TBAB	1,4-dioxane/H_2O; 80 °C, 30 min	**19e** (66 %)

[a]Then 2 M HCl; [b]starting from **17d**; [c]starting from **18d**; [d]starting from **19d**.



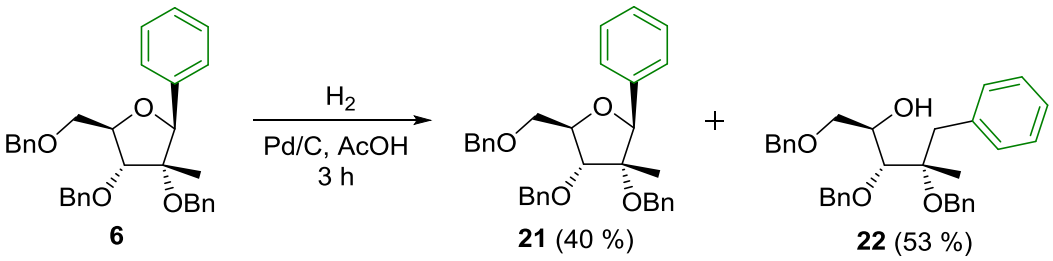
Conditions	Yield of **18e**	Yield of **20**
CH_3I, KOH, 1,4-dioxane/H_2O, 80°C, 1 h	5 %	92 %
CH_3I, Ag_2CO_3, CH_2Cl_2, 60°C, 2 h	68 %	23 %

Scheme 24. Alkylation of pyridone **18d**.

3.1.3 Removal of the benzyl protecting groups

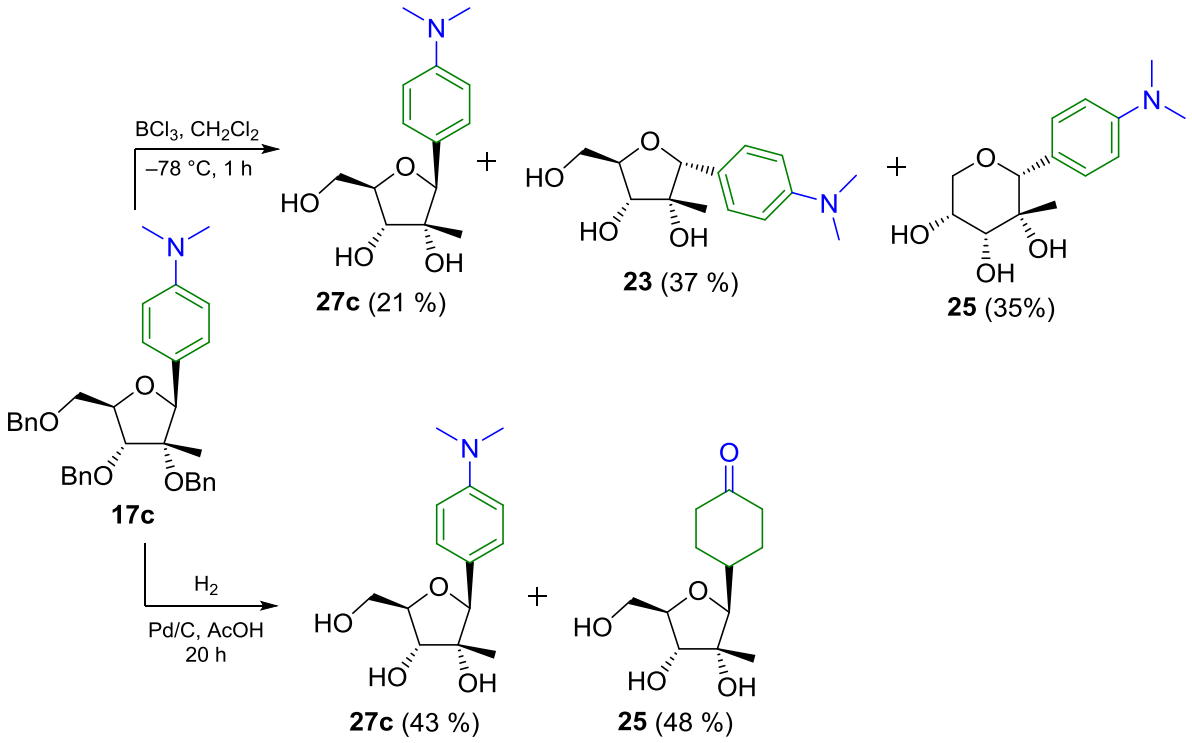
After successful preparation of all three complete series of benzylated 2′-*C*-methyl-*C*-ribonucleosides, I proceeded to the final deprotection step. The fact that aryl-*C*-nucleosides inherently comprise a cyclic benzylic ether group makes debenzylation a challenging task. I tested two common methods for benzyl protecting group removal – treatment with Lewis acid and catalytic hydrogenation. For some derivatives, either or both approaches allowed preparation of the desired β-anomeric nucleosides, but in many cases formation of unwanted side-products or decomposition of starting material was observed. Therefore, it was necessary to test and optimize debenzylation procedures for each particular derivative.

Catalytic hydrogenation of phenyl nucleoside **6** using H_2 and 10% Pd on charcoal in acetic acid afforded the desired β-anomer **21** in 40 % yield accompanied by an acyclic by-product **22** (53 %) with an overreduced sugar ring (**Scheme 25**). On the other hand, the second method – treatment with BCl_3 at −78 °C – proved to be more suitable and allowed preparation of the desired free nucleoside **21** in excellent 94 % yield (**Table 2**).



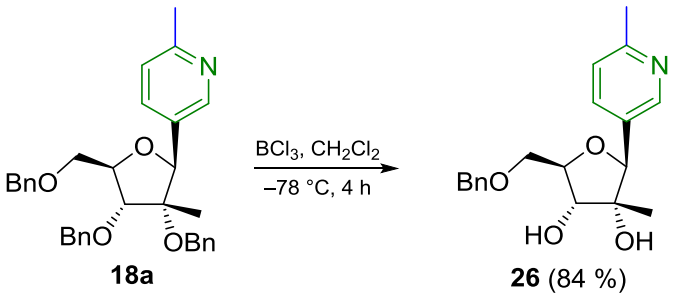
Scheme 25. Catalytic hydrogenation of phenyl *C*-nucleoside **6**.

Deprotection of the dimethylaniline derivative **17c** was the most problematic, since both debenzylation approaches led to formation of mixtures of the desired β-anomeric nucleoside with other unwanted products (**Scheme 26**). On one hand, treatment with BCl_3 at −78 °C for 1 h gave three isomeric compounds: β-anomer **27c** (21 %), α-anomer **23** (37 %) and derivative **24** (35 %) containing a pyranose ring. Catalytic hydrogenation of **17c**, on the other hand, afforded the desired nucleoside **27c** (43 %) accompanied by an unexpected side-product **25** (48 %) comprising cyclohexanone ring. This unwanted product **25** is probably formed by partial reduction of the aniline ring followed by acidic hydrolysis of the enamine intermediate. HPLC purification was required to isolate the compounds from both mixtures, and the second method – catalytic hydrogenation – was chosen for preparative purposes since it allows preparation of the desired β-anomer **27c** in higher 43 % yield.



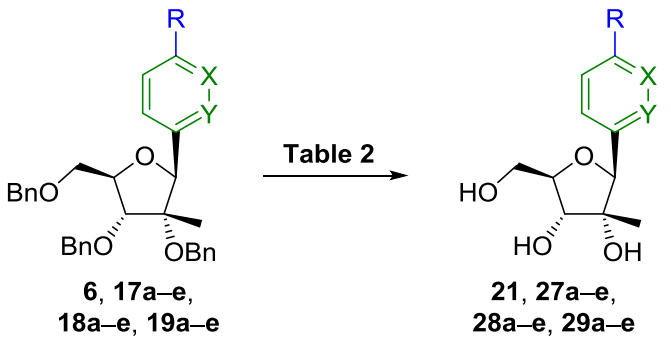
Scheme 26. Problematic deprotection of the dimethylaniline nucleoside **17c**.

Debenzylation of the derivative **18a** using BCl_3 at −78 °C was partial and led to the formation of nucleoside **26** (84 %) with a benzyl group remaining at position 5′ (**Scheme 27**). To fully deprotect this nucleoside, it was necessary to gradually warm the reaction mixture to room temperature, or to apply catalytic hydrogenation (**Table 2**).



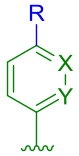
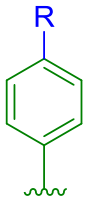
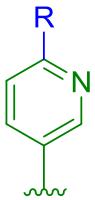
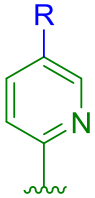
Scheme 27. Partial deprotection of compound **18a**.

Optimized procedures and conditions for debenzylation of the nucleosides **6**, **17a**–**e**, **18a**–**e** and **19a**–**e** are summarized in **Table 2** (**Scheme 28**). In general, catalytic hydrogenation was more efficient when a special type of palladium catalyst was used.[232] Compared to the usual 10% Pd on charcoal, this unusual catalyst contains unreduced form of Pd that is distributed as an eggshell on the charcoal particles. Hydrogenation using this "eggshell" unreduced form of catalyst usually proceeded faster and gave higher yield of desired free β-nucleosides.



Scheme 28. Deprotection of nucleosides **6**, **17a**–**e**, **18a**–**e** and **19a**–**e**.

Table 2. Deprotection of benzylated *C*-nucleosides.

	R	Reagent	Catalyst	Solvent	Conditions	Compd.	Yield, %
	H	BCl_3		CH_2Cl_2	−78 °C, 2 h	**21**	94
	Me	H_2	Pd/C[a]	AcOH	r.t., 6 h	**27a**	93
	NH_2	H_2	Pd/C[a]	AcOH	r.t., 3 days	**27b**	75
	NMe_2	H_2	Pd/C (10%)	AcOH	r.t., 20 h	**27c**	43
	OH	H_2	Pd/C[a]	AcOH	r.t., 2 h	**27d**	91
	OMe	H_2	Pd/C[a]	AcOH	r.t., 6 h	**27e**	91
	Me	H_2	Pd/C[a]	AcOH	r.t., 2 days	**28a**	80
	NH_2	BCl_3		CH_2Cl_2	0 °C to r.t., 2 h	**28b**	95
	NMe_2	H_2	Pd/C[a]	AcOH	r.t., 3 days	**28c**	55
	OH	BCl_3		CH_2Cl_2	−78 °C, 1 h	**28d**	92
	OMe	H_2	Pd/C[a]	AcOH	r.t., 1 day	**28e**	89
	Me	H_2	Pd/C[a]	AcOH	r.t., 1 day	**29a**	87
	NH_2	BCl_3		CH_2Cl_2	−78 °C, 1 h	**29b**	98
	NMe_2	BCl_3		CH_2Cl_2	−78 °C, 1 h	**29c**	91
	OH	H_2	Pd/C[a]	AcOH	r.t., 1 day	**29d**	97
	OMe	H_2	Pd/C[a]	AcOH	r.t., 1 day	**29e**	76

[a]5%, "eggshell", unreduced form.

Structures of all intermediates and all final free nucleosides were confirmed by NMR, and two-dimensional ROESY experiments were used for the assessment of anomeric configuration. Structure of unprotected phenol 2′-*C*-methyl-*C*-ribonucleoside **27d** was also confirmed by a single crystal X-ray structure analysis showing 3′-*endo* conformation of the sugar ring that is typical for 2′-methylribonucleosides (**Figure 29**). In contrast to the corresponding hemiketal intermediates that undergo spontaneous epimerization, the final *C*-nucleosides were found to be sufficiently stable for further transformations and testing.

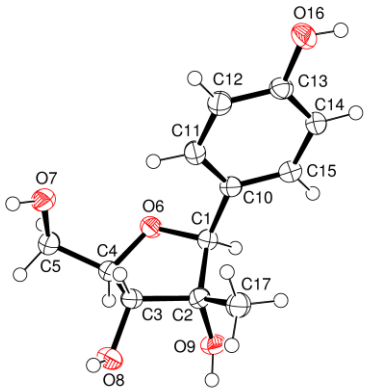
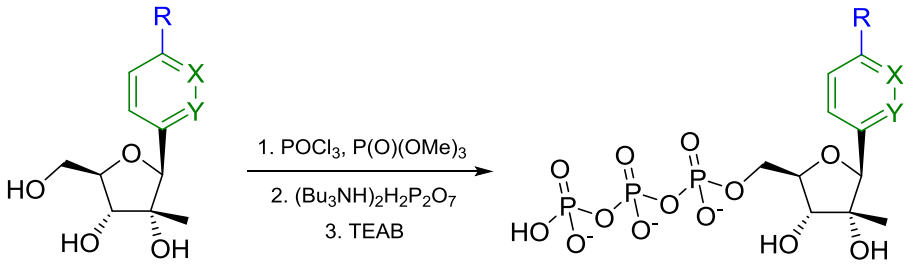


Figure 29. X-ray structure (ORTEP[226] drawing) of the final phenol nucleoside **27d** with the atom numbering scheme. Thermal ellipsoids are shown at the 50 % probability level.

3.1.4 Synthesis of the nucleoside triphosphates

It was previously shown, that the sugar-modified nucleosides may be poor substrates for nucleoside kinases and may not be efficiently activated by phosphorylation,[233,234] therefore I finally needed to prepare the corresponding nucleoside triphosphates (NTPs) suitable for direct testing for inhibition of the HCV RNA polymerase. All the NTPs were synthesized similarly to a literature procedure.[235] The corresponding modified nucleoside was first dissolved in $P(O)(OMe)_3$, and then treated with $POCl_3$ at 0 °C. After 2 h, a precooled solution of tributylammonium pyrophosphate and Bu_3N in DMF was added, and the mixture was stirred for an additional 2 h. After quenching with TEAB, the reaction mixture was coevaporated several times with water followed by separation on Sephadex and HPLC affording sodium or triethylammonium salts of triphosphates **30**, **31a–e**, **32a–e** and **33a–e** in good to excellent yields (51–96 %) as hygroscopic white solids after lyophilization (**Table 3**, **Scheme 29**).

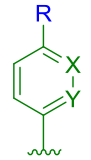
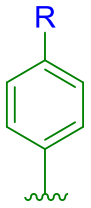
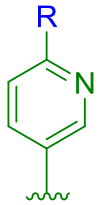
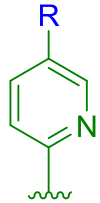
All final free *C*-nucleosides **21**, **27a–e**, **28a–e** and **29a–e** were tested at Gilead Sciences for their anti-HCV activity in Huh-7 cells harboring sub-genomic reporter replicons derived from HCV subtypes 1B and 2A.[236] At 10 μM concentration, the compounds did not show any inhibitory activity. At IOCB, Jaroslav Kozák screened all the NTPs **30**, **31a–e**, **32a–e** and **33a–e** for the inhibition of HCV NS5B polymerase,[176,178] and at 10 μM concentration no significant activity was observed. I also performed in vitro transcription reaction, and no NTP was found to be a substrate for T7 RNA polymerase.



21, **27a–e**, **28a–e**, **29a–e** → **30**, **31a–e**, **32a–e**, **33a–e**

Scheme 29. Synthesis of nucleoside triphosphates.

Table 3. Synthesis of nucleoside triphosphates.

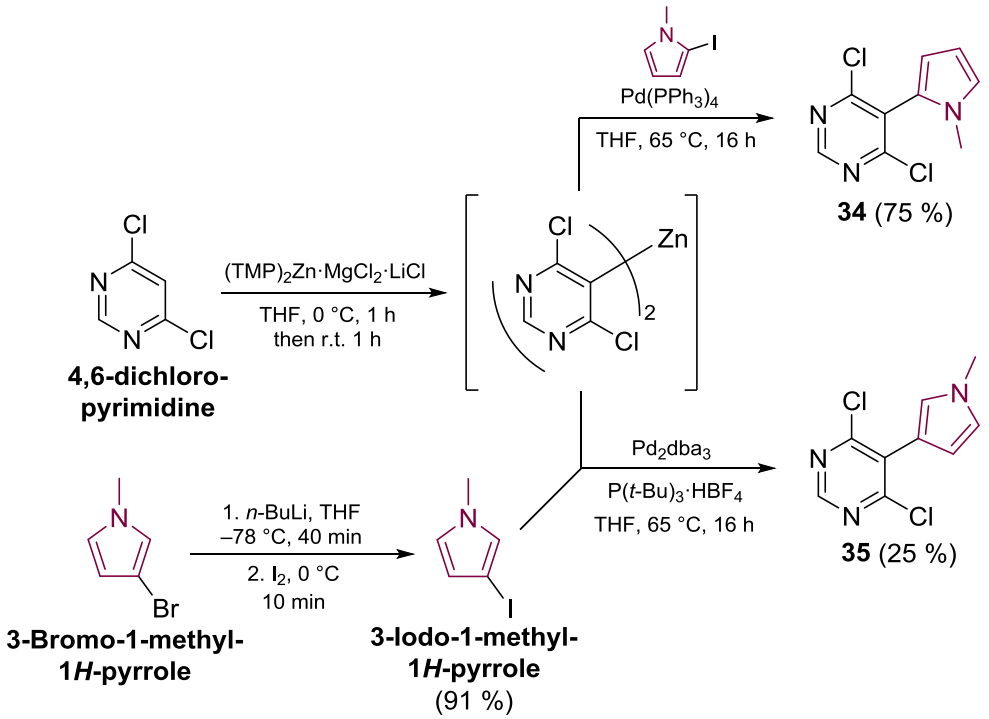
	R	Compd.	Yield, %
	H	**30**	93
	Me	**31a**	60
	NH_2	**31b**	64
	NMe_2	**31c**	82
	OH	**31d**	70
	OMe	**31e**	77
	Me	**32a**	89
	NH_2	**32b**	88
	NMe_2	**32c**	87
	OH	**32d**	75
	OMe	**32e**	96
	Me	**33a**	93
	NH_2	**33b**	51
	NMe_2	**33c**	91
	OH	**33d**	63
	OMe	**33e**	96

3.2 Pyrrolo-fused 7-deazapurine ribonucleosides

In order to extend the class of novel promising thieno-fused 7-deazapurine nucleosides recently developed in our laboratory,[105] we decided to prepare several types of analogous compounds containing other five-membered heterocyclic rings, and the aim of my work was to synthesize series of isomeric pyrrolopyrrolopyrimidine nucleosides to study their biological effects.

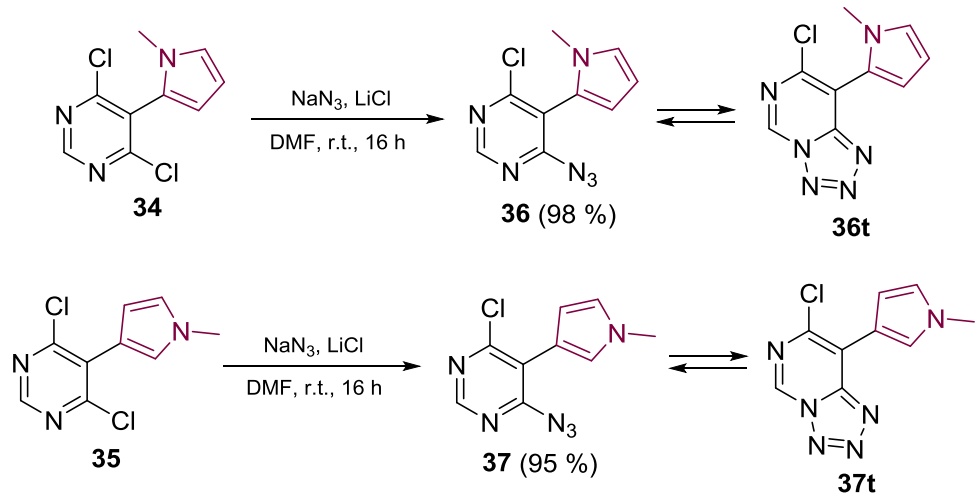
3.2.1 Synthesis of pyrrolopyrrolopyrimidines

Novel tricyclic nucleobases were synthesized similarly to the previously reported thieno-fused analogues[105] utilizing commercially available 4,6-dichloropyrimidine as a starting material (**Scheme 30**). The first step was zincation in position 5 by treatment with $(TMP)_2Zn{\cdot}MgCl_2{\cdot}LiCl$ according to the published procedure,[124] followed by Negishi cross-coupling reaction[125] with the isomeric iodopyrroles to form the corresponding 4,6-dichloro-5-pyrrolopyrimidines **34** and **35**. Standard conditions with $Pd(PPh_3)_4$ as a catalyst were suitable for the coupling with 2-iodo-1-methyl-1*H*-pyrrole affording desired pyrrolyl pyrimidine **34** in good 75 % yield. However, in the case of 3-iodo-1-methyl-1*H*-pyrrole, this reaction was much less efficient and gave isomeric derivative **35** in a very low 10 % yield. To optimize this transformation, I screened various catalysts ($Pd(OAc)_2$, Pd_2dba_3) as well as ligands (CyJohnPhos, P(*t*-Bu)$_3{\cdot}HBF_4$, Me_4(*t*-Bu)$_2$XPhos), and the best results were achieved when a combination of Pd_2dba_3 and P(*t*-Bu)$_3{\cdot}HBF_4$ was used. Under these conditions, the desired 3-pyrrolyl product **35** was obtained in the highest yield of 25 %. The starting 2-iodo-1-methyl-1*H*-pyrrole was prepared by lithiation and subsequent iodination of 1-methyl-1*H*-pyrrole according to the literature procedure,[237] whereas isomeric 3-iodo-1-methyl-1*H*-pyrrole was synthesized via lithiation/iodination sequence starting from the corresponding bromo analogue. 3-Bromo-1-methyl-1*H*-pyrrole, prepared by bromination of 1-methyl-1*H*-pyrrole with NBS in the presence of PBr_3,[238] was first treated with *n*-BuLi in THF at −78 °C, and then reacted with iodine to afford 3-iodo-1-methyl-1*H*-pyrrole in a very good 91 % yield.



Scheme 30. Synthesis of isomeric pyrrolyl pyrimidines.

The next step in the synthesis of the fused nucleobases involved nucleophilic aromatic substitution of one chlorine atom with sodium azide in DMF in the presence of LiCl to obtain pyrimidine azides **36** (98 %) and **37** (95 %), which exist in equilibrium with the corresponding tetrazoles **36t** and **37t** (**Scheme 31**). This equilibrium was studied using NMR spectroscopy, and **Table 4** shows the percentage populations of the azido and tetrazolyl tautomers of compounds **36** and **37** in various deuterated solvents. As a neat compound, derivative **37** exists in a tetrazole form (**37t**), and its structure was confirmed by X-ray analysis (**Figure 30**).



Scheme 31. Introduction of the azido group.

Table 4. Population of the azido (**A**) and tetrazolyl (**T**) tautomers of **36** and **37** in various solvents.

Compound	$CDCl_3$		DMSO-d_6	
	A	**T**	**A**	**T**
36	95 %	5 %	14 %	86 %
37	22 %	78 %	0 %	100 %

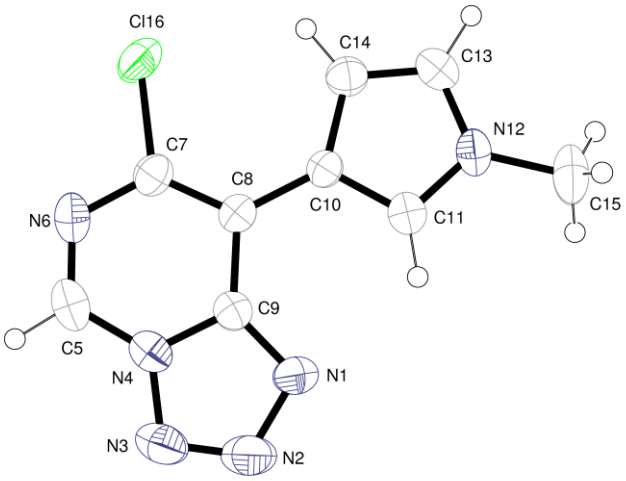
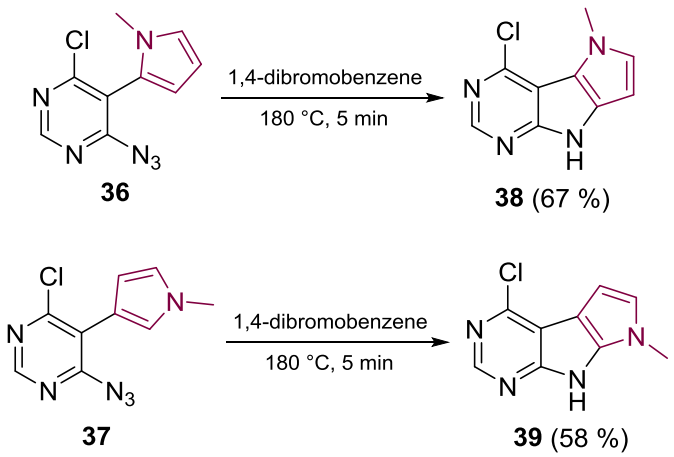


Figure 30. X-ray structure (ORTEP[226] view) of **37t** with the atom numbering scheme. Displacement ellipsoids are drawn with 50 % probability.

Compounds **36** and **37** were then subjected to cyclization reactions similarly to our previously published results.[105] I tried to apply both types of this transformation – under thermal or photochemical conditions[126] – and only the first approach was suitable for the transformation of the pyrrolyl azidopyrimidines, since UV-cyclization reactions either did not proceed or resulted only in degradation of starting material. Thermal cyclizations of compounds **36** and **37** were carried out in 1,4-dibromobenzene at 180 °C for 5 minutes yielding isomeric pyrrolopyrrolopyrimidines **38** and **39** in 67 and 58 % yields, respectively (**Scheme 32**). In theory, cyclization of 3-pyrrolyl derivative **37** could give two possible isomers, but formation of only one of them was observed. Structure of the tricyclic nucleobase **38** was confirmed by a single crystal X-ray analysis (**Figure 31**).



Scheme 32. Synthesis of the tricyclic nucleobases by thermal cyclization reaction.

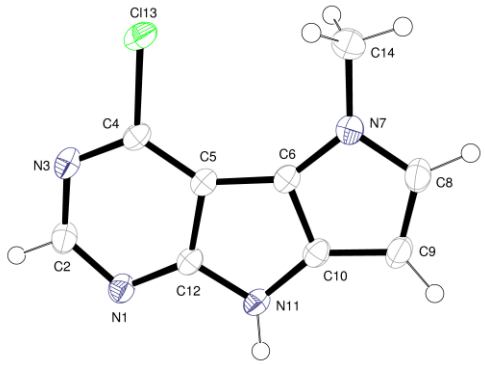
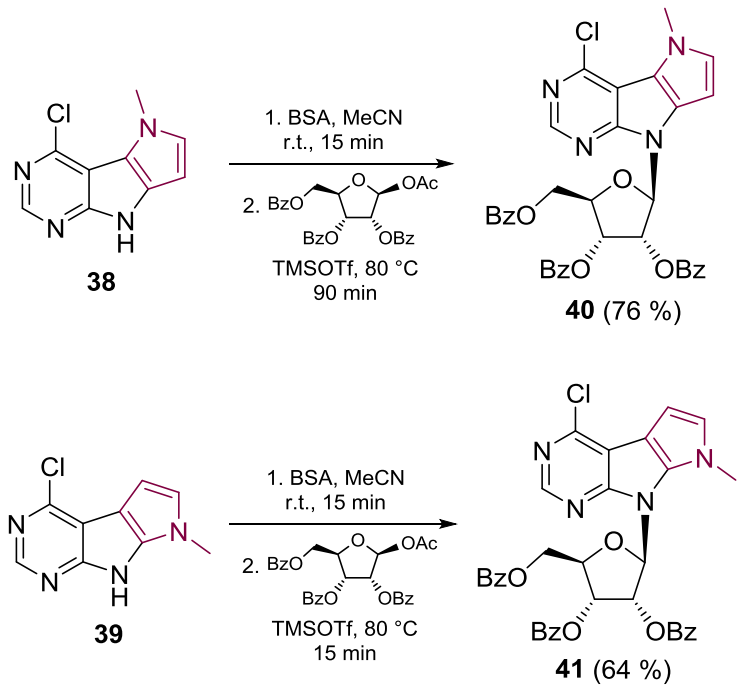


Figure 31. X-ray structure (ORTEP[226] drawing) of compound **38** with the atom numbering scheme. Displacement ellipsoids are shown with 50 % probability.

3.2.2 Synthesis of substituted pyrrolopyrrolopyrimidine ribonucleosides

Having the desired tricyclic chloro nucleobases **38** and **39** in hand, I proceeded with the attachment of a sugar moiety via modified Vorbrüggen conditions analogously to our previous publication[105] (**Scheme 33**). Since the gradual degradation of the pyrrolo-fused 7-deazapurine nucleobases was observed under acidic conditions required for the glycosylation, I tried to shorten reaction time, on one hand, and to lower the amount of Lewis acid that is added during the second step, on the other hand. Thus, treatment with only one equivalent of TMSOTf and stirring at 80 °C for 15 to 90 min allowed synthesis of the key benzoylated chloro nucleosides **40** and **41** in 76 and 64 % yields, respectively, as pure β-anomers. Anomeric configuration of compounds **40** and **41** was confirmed using two-dimensional ROESY experiments.

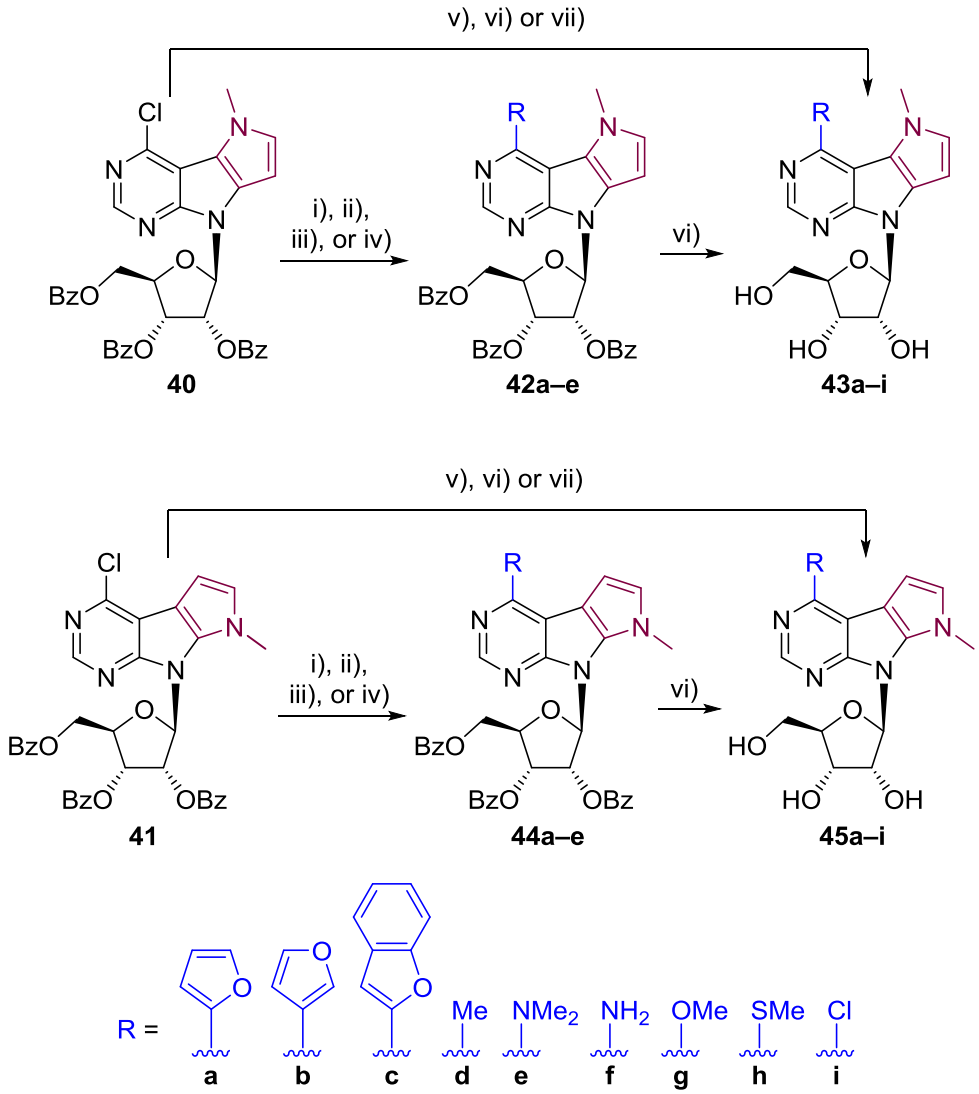


Scheme 33. Glycosylation of the pyrrolopyrrolopyrimidine nucleobases.

These key halogenated intermediates (**40** and **41**) were then further modified at position 4 by the means of various cross-coupling and nucleophilic substitution reactions to give two series of target nucleosides bearing diverse substituents, such as heteroaryls, methyl, dimethylamino, amino, methoxy and methylsulfanyl groups.

Furan-2-yl derivatives **42a** and **44a** were prepared via Stille cross-coupling reaction[239] with tributyl(furan-2-yl)stannane in the presence of $PdCl_2(PPh_3)_2$ in DMF. Furan-2-yl and benzofuran-2-yl substituents, in turn, were introduced by the means of Suzuki–Miyaura cross-coupling[240] reaction with a suitable hetarylboronic acid using $Pd(PPh_3)_4$ as a catalyst and potassium carbonate as a base, affording benzoylated furan-3-yl and benzofuran-2-yl nucleosides **42b**, **42c**, **44b** and **44c** in very good yields. Synthesis of 4-methyl derivatives **42d** and **44d** was achieved by Pd-catalyzed methylation of the starting chloro nucleosides **40** and **41** with trimethylaluminium. Dimethylamino group was introduced by nucleophilic substitution with dimethylamine, and the desired nucleosides **42e** and **44e** were obtained in 84 and 94 % yield, respectively. All benzoylated derivatives **42a–e** and **44a–e** were deprotected under Zemplén conditions (treatment with MeONa in MeOH) to afford the final nucleosides **43a–e** and **45a–e** in good to excellent yields. To introduce amino, methoxy and methylsulfanyl groups, the key-intermediate chloro nucleosides **40** and **41** were subjected to nucleophilic substitutions using aq. NH_3, MeONa or MeSNa, respectively. In case of these derivatization reactions, debenzoylation occurred simultaneously with nucleophilic substitution at the nucleobase moiety affording free nucleosides **43f–h** and **45f–h** in good

yields (68–83 %). Unlike in case of other similar benzoylated chloro nucleosides (benzo-,[103,104] naphtho-,[107] thieno-[105] and furo-fused[106] analogues), the deprotection of the more electron rich pyrrolopyrrolopyrimidine nucleosides **40** and **41** using MeONa in methanol proceeded much faster than the nucleophilic substitution of the chlorine atom. This allowed me to synthesize, under carefully optimized conditions, free pyrrolofused 7-deazapurine nucleosides **43i** and **45i** bearing chlorine at position 6 (**Scheme 34**, **Table 5**).



Scheme 34. Synthesis of 4-substituted pyrrolopyrrolopyrimidine nucleosides.

Reagents and conditions: i) tributyl(furan-2-yl)stannane, $PdCl_2(PPh_3)_2$, DMF, 100 °C, 3 h; ii) R-$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, 100 °C, 1–10 h; iii) Me_3Al, $Pd(PPh_3)_4$, THF, 70 °C, 3–24 h; iv) Me_2NH in THF, propan-2-ol/THF 2:1, 50 °C, 24–48 h; v) aq. NH_3, 1,4-dioxane, 120 °C, 16–24 h; vi) MeONa, MeOH, 25–60 °C, 2–24 h; vii) MeSNa, MeOH, 60 °C, 16–24 h.

Table 5. Synthesis of 4-substituted nucleosides **42a–e**, **43a–i**, **44a–e** and **45a–i**.

Conditions	R	Protected nucleoside	Yield, %	Final nucleoside	Yield, %
i)	furan-2-yl	**42a**	95	**43a**	92
ii)	furan-3-yl	**42b**	85	**43b**	83
ii)	benzofuran-2-yl	**42c**	86	**43c**	90
iii)	Me	**42d**	91	**43d**	89
iv)	NMe_2	**42e**	84	**43e**	87
v)	NH_2	-	-	**43f**	71
vi)	OMe	-	-	**43g**	83
vii)	SMe	-	-	**43h**	77
vi)	Cl	-	-	**43i**	82
i)	furan-2-yl	**44a**	94	**45a**	89
ii)	furan-3-yl	**44b**	86	**45b**	92
ii)	benzofuran-2-yl	**44c**	81	**45c**	86
iii)	Me	**44d**	78	**45d**	87
iv)	NMe_2	**44e**	94	**45e**	93
v)	NH_2	-	-	**45f**	68
vi)	OMe	-	-	**45g**	78
vii)	SMe	-	-	**45h**	72
vi)	Cl	-	-	**45i**	90

Since some previously prepared fused 7-deazapurine nucleosides showed strong fluorescence and were used to prepare fluorescent DNA probes,[105,107,241-243] I studied the photophysical properties of the final unprotected pyrrolopyrrolopyrimidine nucleosides **43a–i** and **45a–i**. Most of the derivatives did not show any fluorescence, with only two exceptions – methyl and amino derivatives **43d** and **43f** with low quantum yields 0.10 and 0.06, respectively (**Table 6**).

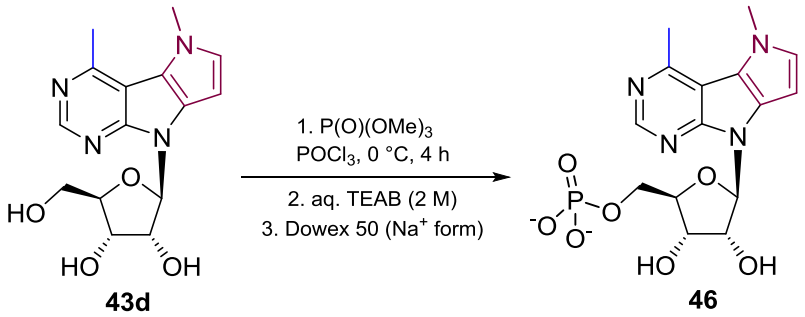
Table 6. UV and fluorescence spectra of the nucleosides **43d** and **43f**.[a]

Compd.	Absorption	Emission	
	λ_{max} [nm] ($\varepsilon \cdot 10^{-4}$ [L·mol^{-1}·cm^{-1}])	λ_{max} [nm]	φ
43d	264 (2.0), 312 (0.7)	442	0.10
43f	307 (1.2)	412	0.06

[a]UV spectra were measured in ethanol at 25 °C. Fluorescence quantum yields were measured in ethanol using quinine sulfate in 0.5 M H_2SO_4 (Φ_f = 0.55) as a reference. Excitation wavelength was 326 nm.

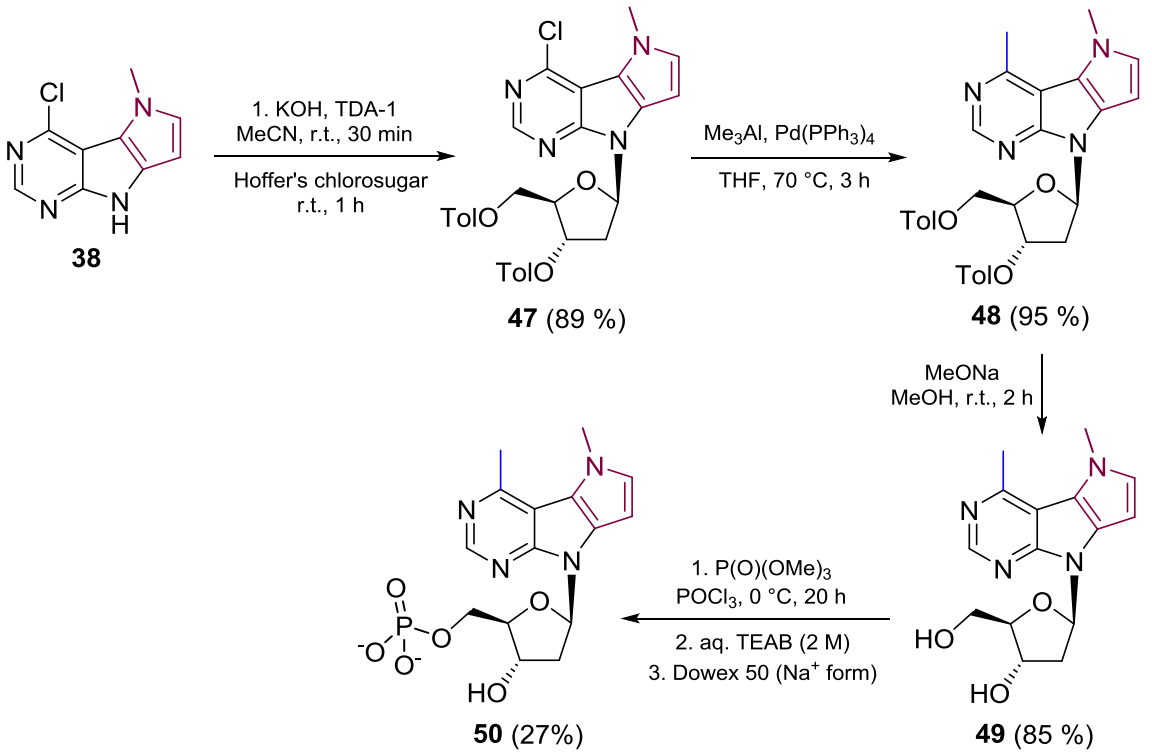
3.2.3 Synthesis of selected monophosphates and deoxyribonucleosides

For the investigation of the intracellular metabolism and mechanism of action of this type of nucleosides, I also synthesized a 5′-*O*-monophosphate **46** (derived from the methyl nucleoside **43d** selected from a group of the most cytotoxic compounds within the series) by treatment with $POCl_3$ in $P(O)(OMe)_3$ followed by addition of aq. TEAB and ion exchange on Dowex (**Scheme 35**).[244]



Scheme 35. Synthesis of the monophosphate **46**.

Since I also needed a standard for in cellulo DNA incorporation studies,[95] it was necessary to synthesize a monophosphate derived from the corresponding 2′-deoxy analogue. To obtain the key β-anomeric chlorodeoxyribonucleoside **47**, the corresponding pyrrolo-fused nucleobase **38** was deprotonated, and then subjected to a stereoselective glycosylation by Hoffer's chlorosugar. Next, Pd-catalyzed alkylation of **47** using trimethylaluminium afforded protected methylnucleoside **48**, which was subsequently deprotected under Zemplén conditions to yield the final free deoxyribonucleoside **49** in a very good yield (85 %). Synthesis of the corresponding 2′-deoxy-5′-*O*-monophosphate **50** was carried out in the same manner as for the ribo analogue **46** (**Scheme 36**).



Scheme 36. Synthesis of the deoxyribonucleoside **49** and its monophosphate **50**.

3.2.4 Biological activity profiling

All final nucleosides **43a–i** and **45a–i** were tested for their cytotoxic and antiviral activities.

3.2.4.1 Antiviral activity

The screening of antiviral activities was done against HCV, respiratory syncytial virus (RSV), influenza, dengue viruses, coxsackie and herpes simplex at Gilead Sciences and in the group of Dr. Weber at IOCB. All final nucleosides did not show any activity against RSV, influenza, coxsackie, herpes simplex and dengue viruses.

The screening of anti-HCV activities[245] showed that most of the nucleosides are active against HCV replicons 1B and 2A in sub-micromolar concentrations with some compounds being more active than a nucleoside inhibitor of viral RNA polymerase mericitabine[246,247] (**Table 7**). The most active compounds were methyl, methoxy and methylsulfanyl derivatives **43d**, **43g** and **43h**, and the isomeric nucleosides **45** were much less active or inactive, which may be caused by a different conformation and, as a result, inefficient binding.

Table 7. Anti-HCV activities of final nucleosides

Compd	HCV replicon 1B		HCV replicon 2A	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
43a	0.22	>44.44	>44.44	>44.44
43b	20.21	>44.44	3.45	>44.44
43c	1.60	26.91	32.5	>44.44
43d	0.19	>44.44	0.12	>44.44
43e	>44.44	>44.44	>44.44	>44.44
43f	0.46	40.86	0.22	>44.44
43g	0.09	>44.44	0.28	>44.44
43h	0.04	>44.44	0.13	>44.44
43i	0.37	>44.44	0.06	>44.44
45a	0.34	16.35	0.77	2.63
45b	16.53	>44.44	>44.44	>44.44
45c	7.83	27.46	15.49	29.06
45d	>44.44	>44.44	>44.44	>44.44
45e	>44.44	>44.44	>44.44	>44.44
45f	27.34	34.62	33.86	>44.44
45g	>44.44	>44.44	>44.44	>44.44
45h	5.98	43.98	17.00	36.83
45i	36.44	>44.44	>44.44	>44.44
Mericitabine	1.20	>44.44	0.99	>44.44

3.2.4.2 Cytotoxic activity

Study of cytotoxic activity of nucleosides **43a–I** and **45a–I** was performed in vitro on eight cancer (lung – A549, colon – HCT116 and HCT116p53–/– carcinomas and bone osteosarcoma – U2OS) and leukemia cell lines (CCRF-CEM, CEM-DNR, K562 and K562-TAX) at the Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc and by Eva Tloušťová at IOCB. Non-malignant fibroblasts BJ and MRC were used for comparison. IC_{50} were determined using a quantitative metabolic staining MTS[248] following a 3 day treatment, and compared with the activity of clinical drug gemcitabine.[249] The anti-proliferative effect was also tested against a promyelocytic hepatocellular carcinoma HepG2, leukemia HL-60, and cervical carcinoma HeLa S3. In this case, cell viability was determined using XTT assay after a 3 day incubation[250] (**Table 8**).

Activity profiles of nucleosides **43** and their isomeric analogues **45** were found to be completely different. All hetaryl, amino and dimethylamino derivatives from the pyrrolo-fused series **43** were either inactive or poorly active, while the methylsulfanyl, methoxy and methyl nucleosides **43d**, **43g** and **43h** were the most active (similarly to the analogous furo-fused nucleosides[106]), and displayed sub-micromolar activities against all cancer cell lines except for multidrug resistant lines CEM-DNR and K562-TAX, and lower toxicity to BJ and no toxicity to MRC-5 fibroblasts. These compounds also showed better selectivity index in comparison with the corresponding thienopyrrolopyrimidine analogues.[105] Interestingly, the 6-chloro derivative **43i** was, on one hand, the most active, but, on the other hand, the most toxic to BJ cells, and it is unclear whether this derivative undergoes hydrolysis (to provide hypoxanthine analogue) or other nucleophilic substitution of the reactive chlorine in the cell.

The isomeric series of pyrrolopyrrolopyrimidines **45** were completely inactive, with the only exceptions of benzofuran-2-yl derivative **45c** and furan-2-yl nucleoside **45a** possessing low or moderate cytotoxicity. Probably, the *N*-methyl group in position 7 is too bulky to bind to the same molecular targets as nucleosides **43**, and thus derivatives **45a** and **45c** might have a different mechanism of action.

Table 8. Cytotoxic activity of final nucleosides.

Compd.	MTS, IC_{50} (μM)										XTT, IC_{50} (μM)		
	BJ	MRC-5	A549	CCRF-CEM	CEM-DNR	HCT116	HCT116p53-/-	K562	K562-TAX	U2OS	HL60	HepG2	HeLaS3
43a	>50	>50	>50	>50	>50	>50	>50	>50	>50	7.37	>50	>50	>50
43b	>50	>50	>50	>50	>50	8.16	9.00	0.94	>50	2.41	>50	>50	>50
43c	>50	>50	>50	16.48	29.84	46.20	47.53	>50	29.33	43.89	>50	>50	>50
43d	3.91	50.0	2.08	0.21	>50	0.33	0.33	0.40	>50	0.61	1.51	1.13	1.19
43e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
43f	>50	>50	>50	50.0	>50	>50	>50	>50	>50	50.0	2.29	>50	>50
43g	4.50	50.0	>50	0.44	>50	0.36	0.62	0.51	>50	1.30	1.44	2.59	1.53
43h	5.16	>50	>50	0.34	>50	0.30	0.38	0.39	50.0	1.41	0.58	1.53	1.36
43i	1.2	50.0	2.99	0.06	50.0	0.17	0.15	0.18	50.0	0.62	0.06	0.16	0.57
45a	5.36	5.89	5.73	1.36	2.17	3.82	4.01	4.03	2.40	3.49	3.00	>50	2.97
45b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
45c	44.74	45.19	35.43	9.48	30.57	36.45	36.37	22.14	22.98	28.88	>50	>50	>50
45d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
45e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
45f	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
45g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
45h	>50	>50	>50	25.46	28.24	35.93	>50	18.03	29.98	27.45	>50	>50	>50
45i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
AB61	>50	>50	13.14	0.02	0.06	0.02	0.02	0.02	0.04	0.51	0.95	nd	0.02
gemcitabine	>50	>50	0.05	0.02	0.10	0.03	0.41	0.10	0.05	0.18	nd	nd	nd

3.2.4.3 Cell-cycle studies

The most active compounds **43d**, **43g** and **43h** were submitted for the cell-cycle study in CCRF-CEM T-lymphoblastic leukemia cells at Palacky University and University Hospital in Olomouc. Nucleosides were evaluated at $1\times IC_{50}$ and $5\times IC_{50}$ concentrations after 24 hours after the treatment (**Table 9**). Treatment with all tested derivatives led to increase in S-phase fraction of cellular population and, at higher concentrations, it induced apoptosis. Prolonged S-phase transition and/or block could be typically caused by interference with RNA synthesis and/or DNA replication. For all tested compounds, inhibition of RNA synthesis was observed, and derivatives **43g** and **43h** also inhibited synthesis of DNA. Their effect on cell cycle was similar to the results previously reported for cytostatic 7-deazapurine nucleoside AB61,[67] even though the changes were less profound than those caused by AB61. Still, these pyrrolo-fused nucleosides (similarly to analogous furo-fused derivatives[106]) are likely to show similar mechanism of action as AB61 does.

Table 9. Cell cycle analyses: proportion of apoptotic and mitotic cells ($pH3^{Ser10}$ positive), DNA and RNA synthesis in CCRF-CEM treated with **43d**, **43g** and **43h**

Compd.	Concentration	% of total cellular populations						
		sub G1 (apoptosis)	G0/G1	S	G2/M	$pH3^{Ser10}$ (mitosis)	DNA synthesis	RNA synthesis
control		3.45	39.70	41.08	19.22	1.36	43.39	49.89
AB61	$1xIC_{50}$	29.83	20.49	62.28	17.23	2.09	3.59	0.91
	$5xIC_{50}$	58.53	32.31	38.71	28.98	0.94	10.76	57.09
43d	$1xIC_{50}$	5.10	31.75	51.03	17.22	1.06	48.49	53.62
	$5xIC_{50}$	10.99	34.66	52.19	13.14	0.90	59.89	35.91
43g	$1xIC_{50}$	3.68	37.73	45.08	17.20	1.48	31.44	31.19
	$5xIC_{50}$	13.86	47.62	36.98	15.40	1.50	12.29	28.30
43h	$1xIC_{50}$	5.61	26.28	56.36	17.36	0.33	30.41	21.43
	$5xIC_{50}$	12.67	40.55	42.94	16.52	1.49	7.80	3.56

3.2.4.4 Intracellular phosphorylation

Analogously to the parent 7-substituted 7-deazaadenosines **V** (**Figure 14**),[95] we assumed that pyrrolopyrrolopyrimidine nucleosides are first activated by intracellular phosphorylation to the corresponding mono-, di- and triphosphates. To prove this, intracellular phosphorylation of methyl nucleoside **43d** was studied at Palacky University and University Hospital in Olomouc. The corresponding monophosphate **46** was used as analytical standard. Intracellular phosphorylation was studied using prototype of normal (BJ) and malignant (HCT116) cells at 1 or 10 μmol/L concentrations of the nucleoside after 1 or 3 h of treatment in vitro. Phosphorylation of the nucleoside was efficient in all cases, but, unlike in previously studied thienopyrrolopyrimidine nucleosides,[105] the monophosphate level was higher in HCT116 cells than in BJ, which might explain higher selectivity of this pyrrolo-fused nucleosides. Also, the ratio of nucleoside monophosphate and nucleoside was increased at higher concentration of **43d** (10 μM), indicating rate limited phosphorylation in non-malignant cells. Similar results were obtained for the furo-fused analogue.[106]

Table 10. Intracellular phosphorylation of nucleoside **43d**

Dosing (1 μmol/L; 1 h; pmol/5 × 10^5 cells)		
Cell line	**43d**	**46**
BJ	87.10	220.92
HCT116	54.04	466.96
Dosing (1 μmol/L; 3 h; pmol/5 × 10^5 cells)		
Cell line	**43d**	**46**
BJ	173.96	332.63
HCT116	136.87	1079.19
Dosing (10 μmol/L; 1 h; pmol/5 × 10^5 cells)		
Cell line	**43d**	**46**
BJ	220.71	1531.57
HCT116	450.10	8868.83
Dosing (10 μmol/L; 3 h; pmol/5 × 10^5 cells)		
Cell line	**43d**	**46**
BJ	753.01	5041.67
HCT116	476.53	12049.83

3.2.4.5 Human ADK – phosphorylation and inhibition

Once it was proved that the title nucleosides are efficiently phosphorylated in cells, we assumed that human ADK is the most likely enzyme to do this transformation, therefore, the in vitro phosphorylation of the final pyrrolonucleosides by human ADK was assessed (**Table 11**).[97] Cloning and expression of ADK was performed by Dr. Iva Pichová at IOCB.

Table 11. Phosphorylation of the nucleosides by ADK and their inhibition of ADK

Compd.	Phosphorylation (%)	Inhibition IC_{50} (μM)
Adenosine	73	–
43a	11	7.12 ± 0.68
43b	9	>10
43c	0	0.30 ± 0.002
43d	42	>10
43e	0	>10
43f	68	>10
43g	47	>10
43h	33	>10
43i	77	>10
45a	0	3.58 ± 0.03
45b	0	>10
45c	0	0.47 ± 0.02
45d	0	>10
45e	0	>10
45f	0	>10
45g	0	>10
45h	0	>10
45i	0	>10

Phosphorylation: 50 μM solution of compound, 243 ng of enzyme, 30 min, 37 °C.
Inhibition: 50 μM solution of adenosine, 10 μM solution of compound, 97 ng of enzyme, 20 min, 37 °C.

The rate of phosphorylation correlates well with cytotoxic activities of the title nucleosides – the better phosphorylation, the higher is cytotoxicity. Importantly, all the inactive nucleosides were not phosphorylated at all, and this fact supports the hypothesis that ADK is the enzyme responsible for phosphorylation and thus activation in cell. Amino derivative **43f** was the only exception – even though it was efficiently phosphorylated, it was devoid of cytotoxic activity. This could mean, that the title nucleosides themselves are inactive, and that the corresponding nucleotides are the active species.

ADK inhibition was also tested, and in general, the nucleosides were not potent inhibitors, with only derivatives **43a**, **43c**, **45a** and **45c** showing some moderate inhibition.

3.2.4.6 Incorporation into nucleic acids of treated cells

Since it was shown that the title nucleosides get phosphorylated preferentially in cancer cells, we decided to study incorporation of the methyl derivative **43d** and **26d** into DNA and RNA in living cells. The experiments were performed by Pavla Perlíková at IOCB and analyzed at Palacky University and University Hospital in Olomouc. After incubation with the modified nukleoside, PCCRF-CEM cells were harvested, and then RNA and DNA were isolated, digested and the concentration of nucleosides in nucleic acids was determined by HPLC-MS. The results show that nucleoside **43d** is incorporated both into RNA and DNA as a ribonucleoside (similarly to previously published AB61[95]) (**Table 12**).

Table 12. Incorporation of the selected cytotoxic nucleoside into RNA and DNA in treated CCRF-CEM T-lymphoblasts

Compound	Sample	NMP content (fmol/μg of nucleic acid)	dNMP content (fmol/μg of nucleic acid)
43d	RNA	3.74 ± 0.28	-
43d	DNA	14.1 ± 6.51	n.d.[a]

[a] Not detected. Incorporation analyzed after 2.5-h treatment at 5 × IC_{50} and subsequent digestion with nuclease P1 (data from 2–4 independent experiments).

3.2.4.7 Induction of DNA damage in treated cells

Some anti-cancer nucleosides are known to induce the DNA damage, and induction of double strand (ds) breaks upon incorporation of AB61 into RNA and DNA has been previously reported.[251,252] Therefore, we decided to study the formation of ds DNA breaks in the U2OS osteosarcoma reporter cell line stably transfected with 53BP1-GFP fusion gene, which is known to translocate to DNA damage sites and form foci in nuclei of treated cells.[95] The experiments were carried out at Palacky University and University Hospital in Olomouc. Reporter cells were treated with the methyl nucleoside **43d**, and the results were compared with Etoposide[253] (a known ds breaks inducer). **Figure 32** shows, that the title nucleoside **43d** (incorporated into nucleic acids only as a ribonucleoside) was a potent inducer of DNA damage. But in comparison with Etoposide, pyrrolo-fused nucleoside **43d** induced foci were less frequent but gradually increasing over the time at the same much larger indicating

unreparable DNA damage. These results suggest that the DNA damage and the subsequent apoptosis indeed might be at least partial mode of action of these cytotoxic nucleosides.

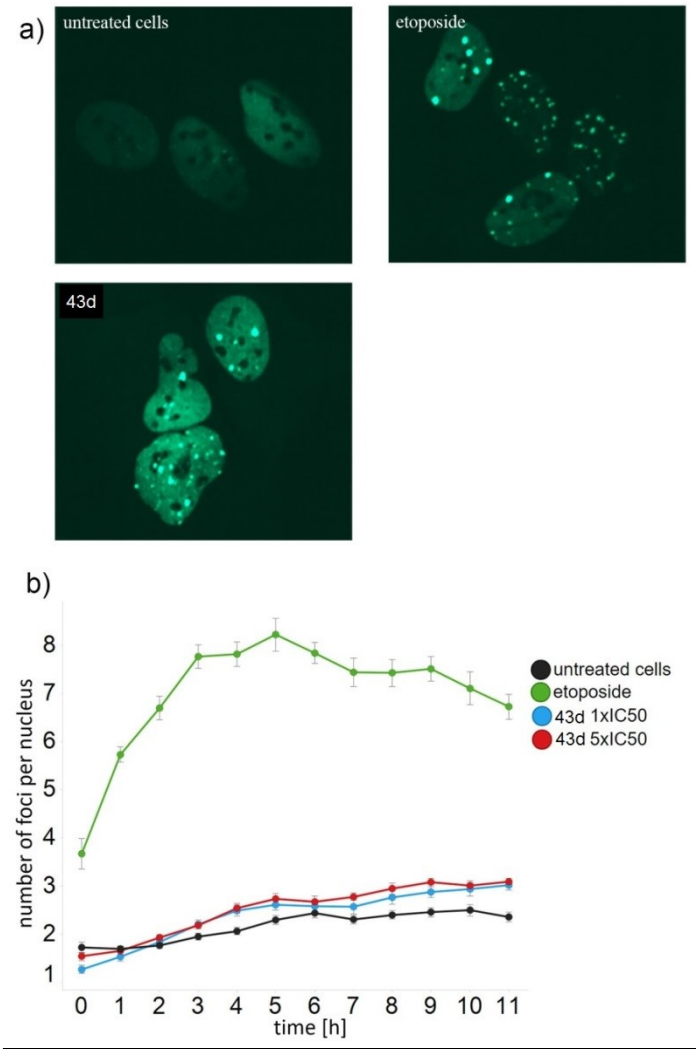


Figure 32. Induction of DNA damage.

53BP1 foci in U2OS-53BP1-GFP cells exposed to vehicle (untreated cells), Etoposide or **43d** for 11 hours. Panel a) – representative confocal microscopy images (objective magnification 40x) displaying 53-BP1 fusion protein (green) in nuclei of reporter cells. Formation of green spots (foci) in nucleus indicates for DNA damage. Panel b) time dependence of 53BP1 foci formation during treatment with Etoposide or **43d** respectively.

3.2.4.8 In vitro pharmacology

Metabolism, plasma protein binding and permeability of nucleosides **43d** and **43h** were also studied. The experiments were performed at Palacky University and University Hospital in Olomouc. The nucleosides were found to be stable in plasma (> 95 % of compound was present in plasma after 120 min, with derivative **43d** showing slightly lower stability with 79

% presence) and in microsomes (**Table 13**). Binding to proteins was significantly higher for the methylsulfanyl derivative **43h** (89 %), while methyl nucleoside **43d** was bound only from 29 %. Both nucleosides were shown to cross an artificial cellular membrane in the PAMPA, with permeability of the methyl derivative **43d** being rather low. On the other hand, permeability of methylsulfanyl nucleoside was almost one order of magnitute higher. Both nucleosides were found to be stable in human microsomal fraction (**Table 13**).

Table 13. Stability and PAMPA for nucleosides **43d** and **43h**

Compd.	Time (min)	Chemical stability	Plasma stability	Microsomal stability	Plasma protein binding	PAMPA
		Comp. remaining (%)			bound fraction (%)	log Pe
43d	0	100	100	100	29	–6.87
	15	98	98	99		
	30	97	83	98		
	60	93	81	97		
	120	91	79	-		
43h	0	100	100	100	89	–5.69
	15	100	100	99		
	30	99	99	99		
	60	98	98	97		
	120	97	98	-		

4 Conclusions

A general and modular methodology for the synthesis of 4-substituted phenyl, 2-substituted pyridin-5-yl and 5-substituted pyridin-2-yl 2′-*C*-methyl-*C*-ribonucleosides was developed. After unsuccessful attempts to form glycosidic C-C bond via stereoselective epoxide ring opening of toluoyl-protected 1,2-anhydro-2-*C*-methyl-α-D-ribofuranose with various *C*-nucleophiles, I switched to an established method that utilizes protected lactones as starting compounds. Although the strategy was similar to our previously published synthetic approaches to related *C*-ribonucleosides,[214-216] introduction of the methyl group at position 2′ of ribose leading to increased steric hindrance of the anomeric position made it necessary to search for a more suitable protecting group and to optimize every step of the synthesis.

The key protected bromo(het)aryl-*C*-nucleoside intermediates were prepared by addition of bromo(het)aryllithium species to benzylated 2-*C*-methyl-D-ribonolactone, followed by reduction of the resulting hemiketals (in the case of phenyl derivatives) or by acetylation and subsequent reduction of the resulting hemiketal acetates (in the case of pyridyl derivatives). Reduction was carried out using Et_3SiH and $BF_3 \cdot Et_2O$ in analogy to a previously developed procedure,[214] and in most cases was stereoselective affording the desired β-anomeric nucleosides.

The key halogenated intermediates were then subjected to various Pd-catalyzed cross-coupling, amination and hydroxylation reactions to introduce small hydrophilic (amino, hydroxyl) and hydrophobic (methyl, dimethylamino) substituents, and the nucleosides with hydroxyl groups were further modified by methylation giving corresponding methoxy derivatives.

The final removal of benzyl protecting groups was the most challenging step due to the fact that aryl-*C*-nucleosides inherently comprise a cyclic benzylic ether group, which could be also cleaved during deprotection resulting in a formation of unwanted side-products. Therefore, it was necessary to choose a suitable debenzylation method – either catalytic hydrogenation on Pd/C or treatment with BCl_3 – and further optimize reaction conditions for each particular derivative.

Since it is known that sugar-modified nucleosides may not be sufficiently phosphorylated by nucleoside kinases,[233,234] the corresponding nucleoside 5′-*O*-triphosphates were also synthesized. Biological tests revealed that none of the nucleosides possesses any activity in the HCV replicon assay, and none of the NTPs was found to significantly inhibit the HCV RNA polymerase.

To summarize, it was shown that the modular approach previously developed in our laboratories[214-216] can be extended to sugar-modified *C*-nucleosides allowing preparation of larger series of these rare compounds with promising potential in medicinal chemistry. Unfortunately, all synthesized nucleosides and their corresponding triphosphates were found to be devoid of significant anti-HCV activity which indicates relatively narrow specificity of the HCV RNA polymerase.

In the second part of the project, synthetic paths to two series of isomeric pyrrolo-fused 7-deazapurine ribonucleosides were developed. Novel tricyclic nucleobases were synthesized in 3 steps starting from 4,6-dichloropyrimidine and 2- or 3-iodo-1-methyl-1*H*-pyrrole, which were first connected by Negishi cross-coupling reaction, and after one chlorine atom was substituted with azido group, thermal cyclization was carried out to give the desired chlorinated pyrrolopyrrolopyrimidines. These modified nucleobases were subjected to glycosylation affording the key benzoylated β-anomeric 4-chlororibonucleosides, which were successfully derivatized at position 4 through cross-coupling or nucleophilic substitution reactions. Hetaryl (2-furyl, 3-furyl and 2-benzofuryl) substituents were introduced by Suzuki or Stille cross-coupling reactions, while methyl derivatives were prepared by Pd-catalyzed alkylation. Nucleophilic substitutions were used to introduce amino, dimethylamino, methoxy and methylsulfanyl groups. Only hetaryl, methyl and dimethylamino derivatives required final deprotection with sodium methoxide in methanol, since the other nucleosides were debenzoylated simultaneously with their derivatization. It was also found out that nucleophilic substitution of electron rich chloropyrrolopyrrolopyrimidine nucleosides using sodium methoxide proceeded much slower than their deprotection, which allowed preparation of free chloro analogues.

Biological activity screening revealed that while pyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine nucleosides are inactive, their isomeric pyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine analogues showed submicromolar cytotoxicity, with methyl, methoxy and methylsulfanyl derivatives being the most active and selective. It was also shown that the nucleosides are phosphorylated and then incorporated into RNA and DNA, which at least in case of methyl derivative results in a formation of double strand breaks and apoptosis. Some compounds within these series also exert submicromolar anti-HCV activity, but the mechanism of action, as well as biological target, still needs to be proved in further studies.

These results show that pyrrolo-fused 7-deazapurine ribonucleosides represent a novel class of potent cytotoxic compounds, and some of them are good candidates for further preclinical development.

5 Experimental section

5.1 General remarks

All reactions were carried out with magnetic stirring under an argon atmosphere, unless otherwise specified. Reactions with organometallic reagents and all Pd-catalyzed reactions were done in flame-dried glassware. Et_2O, THF and toluene were dried and distilled from sodium and benzophenone. All other solvents and reagents were purchased from commercial suppliers and used directly without further purification. Reactions were monitored using thin layer chromatography (TLC) carried out on TLC Silica gel 60 F_{254} plates (Merck) and visualized by UV light (254 nm) and by a 5% solution of 4-anisaldehyde in ethanol with 7 % of sulfuric acid and 2 % of acetic acid. Melting points were measured on Stuart SMP40 automatic melting point apparatus and are uncorrected. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research Analytical), $[\alpha]_D^{20}$ values are given in 10^{-1} deg·cm^2·g^{-1}. NMR spectra were recorded on Bruker Avance II 400 (400.0 MHz for ^{1}H, and 100.6 MHz for ^{13}C), Bruker Avance II 500 (1H at 500.0 MHz for ^{1}H, and 125.7 MHz for ^{13}C), and Bruker Avance II 600 (600.0 MHz for ^{1}H, and 150.9 MHz for ^{13}C) spectrometers in $CDCl_3$, DMSO-d_6 (referenced to the residual solvent signals) or D_2O (referenced to 1,4-dioxane as an internal or external standard). ^{31}P NMR spektra were referenced externally to the signal of H_3PO_4. Chemical shifts are given in ppm (δ scale), and coupling constants (J) in Hz. Complete assignment of all NMR signals was performed using a combination of 2D NMR (H,H-COSY, H,C-HSQC, and H,C-HMBC) experiments, and configurations were determined using 2D ROESY spektra. IR spectra were measured either on Nicolet 6700 (in CCl_4) or on Bruker Alpha FTIR (ATR) spectrometers, and the wavenumbers are given in cm^{-1}. High resolution mass spectra were measured on LTQ Orbitrap XL spectrometer (Thermo Fischer Scientific). UV spectra were recorded on CARY 100 BIO UV-visible spectrophotometer (Agilent Technologies) in Microcell 80 µL, 4×10 mm at r.t., and fluorescence spectra were recorded on Fluoromax 4 spectrophotometer. UV absorption and fluorescence measurements as well as quantum yield determination were carried out according to a published procedure.[254] High performance flash chromatography (HPFC) was carried out on Teledyne ISCO Combiflash Rf system on RediSep Rf Gold Silica Gel or Reverse-phase (C18) RediSep Rf columns. Purity of all final free nucleosides was determined by analytical HPLC on Waters assembly equipped with a model 600 Controller pump, a model 2996 Photodiode Array Detector and a model 717 plus Waters Autosampler injector, and a Phenomenex Synergi 4 µm Fusion-RP 80 C-18 250 mm × 4.6 mm column was used. X-

ray diffraction experiments of single crystals were performed on Xcalibur PX X-ray diffractometer (Oxford Diffraction) using $Cu_{K\alpha}$ radiation (λ = 1.54180 Å). Nucleosides **43d,f,g** and **45a-c** were analyzed for palladium content by energy dispersive X-ray fluorescence (ED-XRF) method. The measurements were performed on SPECTRO iQ II spectrometer (Spectro, Germany) with a detection limit of 5 ppm, and using this method, no traces of palladium were detected.

5.2 General procedures

General procedure A: reduction of hemiketals. Et_3SiH (1.5 mL, 9.2 mmol) was added to an ice-cold solution of hemiketal or hemiketal acetate (2.3 mmol) in dry CH_2Cl_2 (12 mL). After stirring for 5 min, $BF_3 \cdot Et_2O$ (0.6 mL, 4.6 mmol) was added dropwise, and the resulting mixture was warmed to r.t. over 5 min. Subsequently, Et_3N (3.2 mL, 23 mmol) was added, and volatiles were removed under reduced pressure.

General procedure B: cross-coupling with Me_3Al. Me_3Al (2 M in toluene; 2.61 mL, 5.22 mmol) was added to a solution of benzylated bromonucleoside (1.74 mmol) and $Pd(PPh_3)_4$ (101 mg, 0.087 mmol) in dry THF (11 mL) in a septum-sealed flask. After stirring at 66 °C for 1 h, the reaction mixture was quenched by pouring into sat. NaH_2PO_4, and then extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were removed in vacuo.

General procedure C: debenzylation using BCl_3. BCl_3 (1 M in CH_2Cl_2; 2 mL, 2 mmol) was added dropwise to a solution of benzyl-protected nucleoside (0.2 mmol) in dry CH_2Cl_2 (2 mL) at −78 °C. After stirring for 1 h, MeOH (2 mL) was added, and the reaction mixture was warmed to r.t., and concentrated in vacuo.

General procedure D: synthesis of nucleoside triphosphates. $POCl_3$ (22 µl, 0.24 mmol) was added to a solution of nucleoside (0.2 mmol) in $P(O)(OMe)_3$ (0.47 mL, 4 mmol) at 0 °C. Then, an ice-cold solution of Bu_3N (0.19 mL, 0.8 mmol) and $(Bu_3NH)_2H_2P_2O_7$ (548 mg, 1 mmol) in dry DMF (1.5 mL) was added dropwise, and the reaction mixture was stirred at 0 °C for an additional 2 h. Subsequently, TEAB (2 M aq.; 1.5 mL, 3 mmol) was added, and the resulting solution was warmed to r.t., and concentrated in vacuo. The residue was several times co-evaporated with water, and then it was purified on Sephadex (0 to 60 % of 2 M aq. TEAB in water) and HPLC (C18 column; 0.1 M aq. TEAB in water to 0.1 M aq. TEAB in water/MeOH 1:1) affording the desired triphosphates in good to excellent yields.

General procedure E: Zemplén deprotection. Protected nucleoside (0.2 mmol) was suspended in MeOH (4 mL), and MeONa (4.37 M in MeOH; 46 µL, 0.2 mmol) was added. The mixture was stirred at r.t. for 2 h, and then the solvent was evaporated in vacuo. Then the residue was co-evaporated several times with MeOH.

5.3 Phenyl and pyridyl 2′-*C*-methyl-*C*-ribonucleosides and nucleotides

5.3.1 Substituted protected 2′-*C*-methyl-*C*-ribonucleosides

2,3,5-Tri-*O*-(*tert*-butyldimethylsilyl)-2-*C*-methyl-D-ribono-1,4-lactone (3)

A mixture of 2-*C*-methyl-D-ribono-1,4-lactone **2** (5 g, 30.9 mmol), imidazole (15.7 g, 231.5 mmol) and TBDMSCl (21 g, 138.9 mmol) in dry DMF (125 mL) was stirred for 5 days at r.t. Then it was diluted with water and extracted with EtOAc. Combined organic layers were washed with sat. aq. NH_4Cl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude mixture was purified by flash column chromatography on silica gel (0 to 10 % of EtOAc in hexane) to give protected lactone **3** (13.7 g, 27.2 mmol, 88 %) as a white solid (m.p. 93–98 °C). ^{1}H NMR (500 MHz, $CDCl_3$): 0.05, 0.07, 0.09, 0.11, 0.11 and 0.15 (6×s, 6×3H, CH_3Si); 0.86, 0.87 and 0.91 (3×s, 3×9H, $(CH_3)_3C$), 1.40 (s, 3H, CH_3-2); 3.77 (dd, 1H, J_{gem} = 12.5, $J_{5a,4}$ = 2.4, H-5a); 3.98 (d, 1H, $J_{3,4}$ = 8.1, H-3); 4.01 (dd, 1H, J_{gem} = 12.6, $J_{5b,4}$ = 1.5, H-5b); 4.21 (ddd, 1H, $J_{4,3}$ = 8.2, $J_{4,5a}$ = 4.1, $J_{4,5b}$ = 0.5, H-4). ^{13}C NMR (125.7 MHz, $CDCl_3$): −5.32, −5.17, −4.79, −4.09, −3.99 and −2.53 (CH_3Si); 18.09, 18.39 and 18.48 ($C(CH_3)_3$); 21.28 (CH_3-2); 25.66, 25.79 and 25.93 ($(CH_3)_3C$); 59.36 (CH_2-5); 73.93 (CH-3); 75.34 (CH-2); 82.09 (CH-4); 174.82 (C-1). HRMS (ESI) *m/z* for $C_{24}H_{52}O_5Si_3Na$ $[M + Na]^+$: calcd 527.30148; found 527.30156. IR (ATR): 3032, 2936, 1768, 1741, 1498, 1265, 1277, 1141, 1035.

2,3,5-Tri-*O*-benzyl-2-*C*-methyl-D-ribono-1,4-lactone (4)

NaH (6.42 g, 160 mmol; 60 % in mineral oil) suspended in dry DMF (30 mL) was added to a solution of 2-*C*-methyl-D-ribono-1,4-lactone **2** (20 g, 123 mmol) in dry DMF (400 mL) at −10 °C. After stirring for 1 h, BnBr (22 mL, 185 mmol) was added, and the mixture was stirred for 30 min. This sequence – 1) addition of NaH (6.42 g, 160 mmol; 60 % in mineral oil) suspension in 30 mL of dry DMF; 2) stirring for 1 h; 3) addition of BnBr (22 mL, 185 mmol); and 4) stirring for 30 min – was repeated two more times. After that, the stirring was continued at −10 °C for 2 days, and then the reaction mixture was poured onto ice, extracted with EtOAc, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification using flash column chromatography on silica gel (14 to 50 % of EtOAc in hexane) afforded desired protected lactone **4** (47.5 g, 110 mmol, 89 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.51 (s, 3H, CH_3-2); 3.63 (dd, 1H, J_{gem} = 11.6, $J_{5a,4}$ = 5.0, H-5a); 3.75 (dd, 1H, J_{gem} = 11.6, $J_{5b,4}$ = 2.5, H-5b); 4.09 (d, 1H, $J_{3,4}$ = 7.5, H-3); 4.50 (bd,

1H, J_{gem} = 12.1, CH_2Bn-5); 4.55 (bd, 1H, J_{gem} = 12.1, CH_2Bn-5); 4.57 (ddd, 1H, $J_{4,3}$ = 7.5, $J_{4,5a}$ = 5.0, $J_{4,5b}$ = 2.5, H-4); 4.55–4.59 (m, 2H, CH_2Bn-2); 4.61 (bd, 1H, J_{gem} = 11.7, CH_2Bn-3); 4.75 (bd, 1H, J_{gem} = 11.7, CH_2Bn-3); 7.25–7.39 (m, 15H, H-*o*,*m*,*p*-Bn). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.76 (CH_3-2); 67.00 (CH_2Bn-2); 68.55 (CH_2-5); 72.72 (CH_2Bn-3); 72.85 (CH_2Bn-5); 77.27 (C-2); 80.33 and 80.48 (CH-3,4); 127.98 (CH-*o*-Bn); 127.99 and 128.07 (CH-*p*-Bn); 128.09 (CH-*o*-Bn); 128.28 (CH-*p*-Bn); 128.36 (CH-*o*-Bn); 128.65, 128.72 and 128.76 (CH-*m*-Bn); 138.10, 138.27 and 138.38 (C-*i*-Bn); 173.75 (C-1). HRMS (ESI) *m/z* for $C_{27}H_{28}O_5Na$ $[M + Na]^+$: calcd 455.18290; found 455.18281. IR (CCl_4): ν = 3034, 2943, 2868, 1787, 1732, 1603, 1497, 1454, 1277, 1111, 1028.

2,3,5-Tri-*O*-benzyl-2-*C*-methyl-1-*C*-phenyl-α,β-D-ribofuranose (5)

n-BuLi (8 mL, 12.71 mmol; 1.6 M in hexanes) was added to a solution of PhBr (1.21 mL, 11.55 mmol) in dry THF (27 mL) at −78 °C, and the mixture was stirred for 20 min. Then, a solution of lactone **4** (1 g, 2.31 mmol) in dry THF (10 mL) was added, and stirring was continued for a further 10 min. Subsequently, MeOH (2.8 mL, 69.3 mmol) was added, and the mixture was warmed to r.t., neutralized with 2 M HCl, washed with sat. aq. $NaHCO_3$, and extracted with EtOAc. Combined organic layers were dried over anhydrous $MgSO_4$, and the solvents were removed under reduced pressure. The crude mixture was purified using flash column chromatography on silica gel (0 to 6 % of EtOAc in hexane) to afford desired hemiketal **5** (1.17 g, 2.29 mmol, 99 %; α:β 51:49) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) **α-anomer**: δ = 0.89 (s, 3H, CH_3-2); 3.70–3.75 (m, 2H, H-5); 3.88 (d, 1H, $J_{3,4}$ = 4.2, H-3); 4.39 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 4.7, $J_{4,3}$ = 4.2, H-4); 4.52–4.66 (m, 6H, CH_2Bn-2,3,5); 5.58 (s, 1H, OH-1); 7.23–7.41 (m, 18 H, H-*o*,*m*,*p*-Bn, H-*m*,*p*-Ph); 7.53 (m, 2H, H-*o*-Ph); **β-anomer**: δ = 1.36 (s, 3H, CH_3-2); 3.65 (dd, 1H, J_{gem} = 10.4, $J_{5a,4}$ = 6.4, H-5a); 3.68 (dd, 1H, J_{gem} = 10.4, $J_{5b,4}$ = 4.0, H-5b); 4.07 (d, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.19 (d, 1H, $J_{3,4}$ = 8.0, H-3); 4.30 (ddd, 1H, $J_{4,3}$ = 8.0, $J_{4,5a}$ = 6.4, $J_{4,5b}$ = 4.0, H-4); 4.54–4.57 (m, 2H, CH_2Bn-5); 4.59 (d, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.67 and 4.71 (2×d, 2×1H, J_{gem} = 11.8, CH_2Bn-3); 6.65 (s, 1H, OH-1); 6.89 (m, 2H, H-*o*-Bn); 7.09–7.16 and 7.23–7.42 (m, 16H, H-*o*,*m*,*p*-Bn, H-*m*,*p*-Ph); 7.52 (m, 2H, H-*o*-Ph). ^{13}C NMR (125.7 MHz, DMSO-d_6) **α-anomer**: δ = 20.73 (CH_3-2); 66.22 (CH_2Bn-2); 70.15 (CH_2-5); 71.92 and 72.68 (CH_2Bn-3,5); 80.07 (CH-4); 82.48 (CH-3); 82.02 (C-2); 105.19 (C-1); 127.00 (CH-*o*-Ph); 127.33 (CH-*p*-Bn); 127.73 (CH-*o*-Bn); 127.76 (CH-*p*-Bn); 127.90 (CH-*o*-Bn); 128.24 and 128.25 (CH-*o*-Bn); 128.18, 128.36 and 128.51 (CH-*m*-Bn); 136.16, 138.51 and 139.46 (C-*i*-Bn); 141.07 (C-*i*-Ph); **β-anomer**: δ = 15.00 (CH_3-2); 65.57 (CH_2Bn-2); 72.55 (CH_2Bn-5); 73.19 (CH_2-5 and CH_2Bn-3); 79.39 (CH-4);

83.79 (C-2); 86.86 (CH-3); 106.68 (C-1); 126.29 (CH-*o*-Bn); 126.66 (CH-3); 126.92 and 127.66 (CH-*p*-Bn); 127.87 (CH-*o*-Bn); 127.90 (CH-*p*-Bn); 127.83, 127.93 and 127.99 (CH-*o*,*m*-Bn, CH-*m*-Ph); 128.23 (CH-*o*-Ph); 128.43 and 128.45 (CH-*m*-Bn); 138.62, 138.69 and 140.37 (C-*i*-Bn); 140.92 (C-*i*-Ph). HRMS (ESI) *m/z* for $C_{33}H_{34}O_5Na$ [*M* + Na]$^+$: calcd 533.22985; found 533.22972. IR (CCl_4): ν = 3592, 3534, 3032, 2866, 1497, 1454, 1362, 1208, 1097, 1068, 1029.

(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)benzene (6)

Compound **6** was prepared according to the general procedure A starting from hemiketal **5** (1.17 g, 2.3 mmol). Purification by flash column chromatography on silica gel (0 to 5 % of EtOAc in hexane) furnished **6** (1.07 g, 2.16 mmol, 94 %) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.92 (s, 3H, CH_3); 3.73 (dd, 1H, J_{gem} = 10.3, $J_{5'a,4'}$ = 5.2, H-5′a); 3.76 (dd, 1H, J_{gem} = 10.3, $J_{5'b,4'}$ = 4.6, H-5′b); 3.81 (d, 1H, $J_{3',4'}$ = 4.6, H-3′); 4.36 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 4.8, H-4′); 4.55, 4.58, 4.62, 4.65, 4.68 and 4.69 (6×d, 6×1H, J_{gem} = 11.5, 11.8, 11.6, 11.9, 12.0 and 11.8, CH_2-Bn); 5.09 (s, 1H, H-1′); 7.21–7.39 (m, 20H, H-2, H-3, H-4, H-5, H-6, H-*o*,*m*,*p*-Bn). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 19.21 (CH_3); 66.04 (CH_2Bn); 70.29 (CH_2-5′); 71.94 and 73.52 (CH_2Bn); 81.71 (CH-4′); 83.11 (CH-3′); 83.16 (C-2′); 84.54 (CH-1′); 126.45 (CH-2, CH-6); 127.10 (CH-*p*-Bn); 127.15 (CH-*o*-Bn); 127.28 and 127.70 (CH-*p*-Bn); 127.73, 127.77 and 128.15 (CH-*o*-Bn, CH-3, CH-5, CH-4); 128.28, 128.36 and 128.41 (CH-*m*-Bn); 137.80 and 138.12 (C-*i*-Bn); 138.33 (C-*i*-Ph); 139.28 (C-*i*-Bn). HRMS (ESI) *m/z* for $C_{33}H_{34}O_4Na$ [*M* + Na]$^+$: calcd 517.23493; found 517.23486. IR (CCl_4): ν = 3033, 2866, 1607, 1497, 1454, 1382, 1362, 1187, 1097, 1029.

2,3,5-Tri-*O*-benzyl-1-*C*-(4-bromophenyl)-2-*C*-methyl-α,β-D-ribofuranose (7)

n-BuLi (11.9 mL, 19.07 mmol; 1.6 M in hexanes) was slowly added to a solution of 1,4-dibromobenzene (4.09 g, 17.34 mmol) in dry THF (105 mL) at −78 °C. After stirring for 30 min, solution of lactone **4** (2.5 g, 5.78 mmol) in dry THF (25 mL) was added dropwise, and the reaction mixture was stirred for a further 10 min. Then, MeOH (7 mL, 173.4 mmol) was added, and the resulting solution was warmed to r.t., neutralized with 2 M HCl, washed with sat. aq. $NaHCO_3$, and extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were removed in vacuo. The crude mixture was purified using flash column chromatography on silica gel (0 to 10 % of EtOAc in hexane) affording the desired hemiketal **7** (3.3 g, 5.6 mmol, 97 %; α:β 44:56) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6) **α-anomer**: δ = 0.89 (s, 3H, CH_3-2); 3.67–3.74 (m, 2H, H-5);

3.87 (d, 1H, $J_{3,4}$ = 4.1, H-3); 4.39 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 4.8, $J_{4,3}$ = 3.1, H-4); 4.51–4.69 (m, 6H, CH_2Bn-2,3,5); 5.68 (s, 1H, OH); 7.22–7.56 (m, 19H, H-*o*,*m*,*p*-Bn, H-*o*,*m*-Ph); **β-anomer**: δ = 1.35 (s, 3H, CH_3-2); 3.64 (dd, 1H, J_{gem} = 10.5, $J_{5a,4}$ = 6.5, H-5a); 3.68 (dd, 1H, J_{gem} = 10.5, $J_{5b,4}$ = 3.8, H-5b); 4.18 (d, 1H, J_{gem} = 12.6, CH_2Bn-2); 4.18 (d, 1H, $J_{3,4}$ = 8.1, H-3); 4.30 (ddd, 1H, $J_{4,3}$ = 8.1, $J_{4,5a}$ = 6.5, $J_{4,5b}$ = 3.8, H-4); 4.50–4.67 (m, 3H, CH_2Bn-2,5); 4.67 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 4.70 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 6.79 (m, 2H, OH); 6.87–6.92 (m, 2H, H-*o*-Bn); 7.11–7.19 and 7.22–7.55 (2×m, 17H, H-*o*,*m*,*p*-Bn, H-*o*,*m*-Ph). ^{13}C NMR (125.7 MHz, DMSO-d_6) **α-anomer**: δ = 20.74 (CH_3-2); 66.27 (CH_2Bn-2); 70.06 (CH_2-5); 71.93 (CH_2Bn-3); 72.69 (CH_2Bn-5); 80.28 (CH-4); 82.35 (CH-3); 82.95 (C-2); 104.88 (C-1); 121.44 (C-*p*-Ph); 127.30 and 127.34 (CH-*p*-Bn); 127.38 and 127.73 (CH-*o*-Bn); 127.74 (CH-*p*-Bn); 128.24 and 127.25 (CH-*o*,*m*-Bn); 128.37 and 128.52 (CH-*m*-Bn); 129.28 and 130.55 (CH-*o*,*m*-Ph); 138.13, 138.46 and 139.38 (C-*i*-Bn); 140.53 (C-*i*-Ph); **β-anomer**: δ = 14.83 (CH_3-2); 65.77 (CH_2Bn-2); 72.56 (CH_2Bn-5); 72.97 (CH_2-5); 73.27 (CH_2Bn-3); 79.50 (CH-4); 83.73 (C-2); 86.67 (CH-3); 106.41 (C-1); 121.35 (C-*p*-Ph); 126.29 (CH-*o*-Bn); 126.75 and 127.62 (CH-*p*-Bn); 127.84 (CH-*o*,*p*-Bn); 127.96 and 128.01 (CH-*o*,*m*-Bn); 128.43 and 128.46 (CH-*m*-Bn); 130.00 (CH-*m*-Ph); 130.46 (CH-*o*-Ph); 138.57, 138.62 and 140.28 (C-*i*-Bn); 140.44 (C-*i*-Ph). HRMS (ESI) *m/z* for $C_{33}H_{33}O_5{}^{79}BrNa$ [*M* + Na]$^+$: calcd 611.14036; found 611.14042. IR (ATR): ν = 3427, 3040, 2874, 1598, 1501, 1458, 1366, 1211, 1072, 1030, 1012.

1-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-4-bromobenzene (8)

Compound **8** was prepared by reduction of hemiketal **7** (1.35 g, 2.3 mmol) following the general procedure A. The crude mixture was purified by flash column chromatography on silica gel (0 to 5 % of EtOAc in hexane) to give **8** (1.21 g, 2.11 mmol, 92 %) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.87 (s, 3H, CH_3-2′); 3.69 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.9, H-5′a); 3.72 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 4.6, H-5′b); 3.84 (d, 1H, $J_{3',4'}$ = 4.5, H-3′); 4.23 (q, 1H, $J_{4',3'}$ = $J_{4',5'a}$ = $J_{4',5'b}$ = 4.6, H-4′); 4.52 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.54 and 4.58 (2×d, 2×1H, J_{gem} = 11.6, CH_2Bn-2′); 4.60 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.62 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.62 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.88 (s, 1H, H-1′); 7.22–7.35 and 7.36–7.41 (2×m, 17 H, H-*o*,*m*,*p*-Bn, H-2, H-6); 7.48–7.52 (m, 2H, H-3, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.76 (CH_3-2′); 65.63 (CH_2Bn-2′); 70.18 (CH_2-5′); 71.48 (CH_2Bn-3′); 72.68 (CH_2Bn-5′); 81.81 (CH-4′); 82.80 (C-2′); 82.84 (CH-3′); 83.32 (CH-1′); 120.68 (C-4); 127.27 (CH-*p*-Bn); 127.40 (CH-*o*-Bn); 127.74 (CH-*p*-Bn); 127.75 (CH-*o*-Bn); 127.83 (CH-*p*-Bn); 128.24 (CH-*o*-Bn); 128.32, 128.35 and 128.51 (CH-*m*-Bn); 128.62 (CH-2,

CH-6); 130.92 (CH-3, CH-5); 137.95 (C-1); 138.21, 138.47 and 139.41 (C-*i*-Bn). HRMS (ESI) *m/z* for $C_{33}H_{33}O_4{}^{79}BrNa$ $[M + Na]^+$: calcd 595.14544; found 595.14553. IR (CCl_4): ν = 3032, 2866, 1608, 1595, 1489, 1454, 1362, 1187, 1093, 1073, 1029, 1013.

2,3,5-Tri-*O*-benzyl-1-*C*-(2-bromopyridin-5-yl)-2-*C*-methyl-α,β-D-ribofuranose (9)

n-BuLi (8 mL, 12.72 mmol; 1.6 M in hexanes) was added dropwise to a solution of 2,5-dibromopyridine (2.74 g, 11.56 mmol) in dry Et_2O (140 mL) at −78 °C. After stirring for 30 min, solution of lactone **4** (1 g, 2.31 mmol) in dry Et_2O (10 mL) was slowly added, and the reaction mixture was stirred for a further 10 min. Then, MeOH (2.8 mL, 69.3 mmol) was added, and the resulting yellow solution was warmed to r.t., neutralized with 2 M HCl, washed with sat. aq. $NaHCO_3$, and extracted with Et_2O. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (9 to 16 % of EtOAc in hexane) furnishing hemiketal **9** (1.21 g, 2.06 mmol, 89 %; α:β 23:77) as a white amorphous solid. ^{1}H NMR (500 MHz, DMSO-d$_6$) **α-anomer**: δ = 0.93 (s, 3H, CH_3-2); 3.70 (dd, 1H, J_{gem} = 10.7, $J_{5a,4}$ = 4.8, H-5a); 3.72 (dd, 1H, J_{gem} = 10.7, $J_{5b,4}$ = 4.7, H-5b); 3.89 (d, 1H, $J_{3,4}$ = 4.2, H-3); 4.40 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 4.7, $J_{4,3}$ = 4.2, H-4); 4.54–4.65 (m, 6H, CH_2Bn-2,3,5); 5.97 (bs, 1H, OH-1); 7.23–7.41 (m, 15 H, H-*o*,*m*,*p*-Bn); 7.62 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',6'}$ = 0.8, H-3′); 7.79 (dd, 1H, $J_{4',3'}$ = 8.3, $J_{4',6'}$ = 2.5, H-4′); 8.46 (dd, 1H, $J_{6',4'}$ = 2.5, $J_{6',3'}$ = 0.8, H-6′); **β-anomer**: δ = 1.34 (s, 3H, CH_3-2); 3.63 (dd, 1H, J_{gem} = 10.5, $J_{5a,4}$ = 6.5, H-5a); 3.68 (dd, 1H, J_{gem} = 10.5, $J_{5b,4}$ = 3.6, H-5b); 4.20 (d, 1H, $J_{3,4}$ = 8.1, H-3); 4.27 (d, 1H, J_{gem} = 12.4, CH_2Bn-2); 4.31 (ddd, 1H, $J_{4,3}$ = 8.1, $J_{4,5a}$ = 6.5, $J_{4,5b}$ = 3.6, H-4); 4.52 and 4.55 (2×d, 2×1H, J_{gem} = 12.0, CH_2Bn-5); 4.67 and 4.72 (2×d, 2×1H, J_{gem} = 11.8, CH_2Bn-3); 4.76 (d, 1H, J_{gem} = 12.4, CH_2Bn-2); 6.89–6.93 (m, 2H, H-*o*-Bn); 7.06 (bs, 1H, OH-1); 7.13–7.22 and 7.27–7.38 (2×m, 13H, H-*o*,*m*,*p*-Bn); 7.57 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',6'}$ = 0.8, H-3′); 7.72 (dd, 1H, $J_{4',3'}$ = 8.3, $J_{4',6'}$ = 2.5, H-4′); 8.38 (dd, 1H, $J_{6',4'}$ = 2.5, $J_{6',3'}$ = 0.8, H-6′). ^{13}C NMR (125.7 MHz, DMSO-d$_6$) **α-anomer**: δ = 20.82 (CH_3-2); 66.31 (CH_2Bn-2); 69.92 (CH_2-5); 71.99 and 72.70 (CH_2Bn-3,5); 80.65 (CH-4); 82.22 (CH-3); 82.98 (C-2); 104.02 (C-1); 127.33 (CH-*p*-Bn); 127.43 (CH-3′); 127.73 (CH-*o*-Bn); 127.76 (CH-*p*-Bn); 127.90 (CH-*o*-Bn); 128.24 and 128.25 (CH-*o*,*m*-Bn); 128.37 and 128.53 (CH-*m*-Bn); 136.63 (C-*i*-Bn); 138.15 (C-5′); 138.19 (CH-4′); 138.43 and 139.31 (C-*i*-Bn); 141.18 (C-2′); 149.02 (CH-6′); **β-anomer**: δ = 14.69 (CH_3-2); 66.08 (CH_2Bn-2); 72.57 (CH_2Bn-5); 72.65 (CH_2-5); 73.36 (CH_2Bn-3); 79.75 (CH-4); 83.61 (C-2); 86.39 (CH-3); 105.57 (C-1); 126.39 (CH-*o*-Bn); 126.66 (CH-3′); 126.92 and 127.66 (CH-*p*-Bn); 127.87 (CH-*o*-Bn); 127.90 (CH-*p*-Bn); 128.06 and 128.08 (CH-*o*,*m*-Bn); 128.45 and

128.49 (CH-*m*-Bn); 136.33 (C-5′); 138.51 (C-*i*-Bn); 139.36 (CH-4′); 139.99 (C-*i*-Bn); 140.95 (C-2′); 149.96 (CH-6′). HRMS (ESI) *m/z* for $C_{32}H_{32}O_5N^{79}BrNa$ $[M + Na]^+$: calcd 612.13561; found 612.13574. IR (CCl_4): ν = 3033, 2927, 2867, 1583, 1497, 1454, 1361, 1208, 1087, 1029.

1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-1-*C*-(2-bromopyridin-5-yl)-2-*C*-methyl-α,β-D-ribofuranose (10)

LiHMDS (1 M in THF; 5.5 mL, 5.49 mmol) was added dropwise to a solution of hemiketal **9** (2.16 g, 3.66 mmol) in dry toluene (25 mL) at 0 °C. After stirring for 10 min, Ac_2O (0.53 mL, 5.49 mmol) was slowly added, and the resulting solution was stirred for a further 10 min. Then, the reaction mixture was warmed to r.t., poured into sat. aq. $NaHCO_3$, and extracted with toluene. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were removed in vacuo. The crude mixture was purified using flash column chromatography on silica gel (9 to 16 % of EtOAc in hexane) affording hemiketal acetate **10** (2.11 g, 3.33 mmol, 91 %; α:β 44:56) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6) **α-anomer**: δ = 0.90 (s, 3H, CH_3-2); 1.99 (s, 3H, CH_3CO); 3.68 (dd, 1H, J_{gem} = 10.9, $J_{5a,4}$ = 4.7, H-5a); 3.71 (dd, 1H, J_{gem} = 10.9, $J_{5b,4}$ = 4.7, H-5b); 3.94 (d, 1H, $J_{3,4}$ = 3.4, H-3); 4.42 (td, 1H, $J_{4,5a} = J_{4,5b}$ = 4.7, $J_{4,3}$ = 3.4, H-4); 4.53 (d, 1H, J_{gem} = 11.7, CH_2Bn-3); 4.56 (d, 1H, J_{gem} = 12.0, CH_2Bn-2); 4.57–4.64 (m, 2H, CH_2Bn-5); 4.61 (d, 1H, J_{gem} = 11.9, CH_2Bn-2); 4.63 (d, 1H, J_{gem} = 11.7, CH_2Bn-3); 7.20–7.42 (m, 15 H, H-*o*,*m*,*p*-Bn); 7.65 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',6'}$ = 0.8, H-3′); 7.70 (dd, 1H, $J_{4',3'}$ = 8.3, $J_{4',6'}$ = 2.5, H-4′); 8.38 (dd, 1H, $J_{6',4'}$ = 2.5, $J_{6',3'}$ = 0.8, H-6′); **β-anomer**: δ = 1.45 (s, 3H, CH_3-2); 1.77 (s, 3H, CH_3CO); 3.52 (dd, 1H, J_{gem} = 11.3, $J_{5a,4}$ = 3.3, H-5a); 3.71 (dd, 1H, J_{gem} = 11.3, $J_{5b,4}$ = 2.5, H-5b); 4.23 (d, 1H, J_{gem} = 12.3, CH_2Bn-2); 4.38 (bddd, 1H, $J_{4,3}$ = 8.4, $J_{4,5a}$ = 3.3, $J_{4,5b}$ = 2.4, H-4); 4.41 (d, 1H, $J_{3,4}$ = 8.4, H-3); 4.45 and 4.48 (2×d, 2×1H, J_{gem} = 12.0, CH_2Bn-5); 4.67 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 4.70 (d, 1H, J_{gem} = 12.3, CH_2Bn-2); 4.75 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 6.86 (m, 2H, H-*o*-Bn); 7.18–7.42 (m, 13H, H-*o*,*m*,*p*-Bn); 7.58 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',6'}$ = 0.8, H-3′); 7.65 (dd, 1H, $J_{4',3'}$ = 8.3, $J_{4',6'}$ = 2.5, H-4′); 8.31 (dd, 1H, $J_{6',4'}$ = 2.5, $J_{6',3'}$ = 0.8, H-6′). ^{13}C NMR (125.7 MHz, DMSO-d_6) **α-anomer**: δ = 20.93 (CH_3-2); 21.99 (**C**H_3CO); 66.22 (CH_2Bn-2); 69.63 (CH_2-5); 71.45 (CH_2Bn-3); 72.77 (CH_2Bn-5); 80.90 (CH-3); 83.71 (CH-4); 84.49 (C-2); 106.47 (C-1); 127.15 (CH-*o*-Bn); 127.23 (CH-*p*-Bn); 127.57 (CH-3′); 127.65 (CH-*p*-Bn); 127.75 and 127.80 (CH-*o*-Bn); 128.21, 128.31 and 128.54 (CH-*m*-Bn); 133.84 (C-5′); 136.95 (CH-4′); 138.31, 138.53 and 139.46 (C-*i*-Bn); 141.09 (C-2′); 147.74 (CH-6′); 168.40 (CH_3**C**O); **β-anomer**: δ = 14.24 (CH_3-2); 21.73 (**C**H_3CO); 66.14 (CH_2Bn-2); 68.91 (CH_2-5); 72.64

(CH_2Bn-5); 73.50 (CH_2Bn-3); 81.16 (CH-4); 83.30 (CH-3); 84.20 (C-2); 107.98 (C-1); 126.41 (CH-*o*-Bn); 127.02 and 127.07 (CH-*p*-Bn, CH-3′); 127.78 (CH-*p*-Bn); 128.00 (CH-*o*-Bn); 128.02 (CH-*p*-Bn); 128.07 and 128.11 (CH-*o*,*m*-Bn); 128.47 and 128.54 (CH-*m*-Bn); 133.12 (C-5′); 137.93 (CH-4′); 13.30, 138.37 and 139.50 (C-*i*-Bn); 140.87 (C-2′); 148.56 (CH-6′); 167.55 (CH_3**C**O). HRMS (ESI) *m/z* for $C_{34}H_{34}O_6N^{79}BrNa$ $[M + Na]^+$: calcd 654.14617; found 654.14623. IR (ATR): ν = 3040, 2934, 2868, 1755, 1589, 1457, 1368, 1221, 1087.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-bromopyridine (11)

Nucleoside **11** was prepared starting from hemiketal acetate **10** (1.45 g, 2.3 mmol) according to the general procedure A. The residue was purified using flash column chromatography on silica gel (6 to 13 % of EtOAc in hexane) affording **11** (1.06 g, 1.84 mmol, 80 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.91 (s, 3H, CH_3-2′); 3.70 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.9, H-5′a); 3.73 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.7, H-5′b); 3.88 (d, 1H, $J_{3',4'}$ = 4.4, H-3′); 4.25 (bq, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.6, H-4′); 4.53 (d, 1H, J_{gem} = 11.4, CH_2Bn-3′); 4.54–4.65 (m, 5H, CH_2Bn-2′,3′,5′); 4.95 (bs, 1H, H-1′); 7.23–7.35 and 7.36–7.41 (2×m, 15 H, H-*o*,*m*,*p*-Bn); 7.60 (dd, 1H, $J_{3,4}$ = 8.3, $J_{3,6}$ = 0.8, H-3); 7.70 (dd, 1H, $J_{4,3}$ = 8.3, $J_{4,6}$ = 2.5, H-4); 8.34 (dd, 1H, $J_{6,4}$ = 2.5, $J_{6,3}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.65 (CH_3-2′); 65.67 (CH_2Bn-2′); 70.03 (CH_2-5′); 71.46 (CH_2Bn-3′); 72.67 (CH_2Bn-5′); 81.40 (CH-1′); 82.20 (CH-4′); 82.66 (CH-3′); 82.81 (C-2′); 127.32 (CH-*p*-Bn); 127.45 (CH-*o*-Bn); 127.62 (CH-3); 127.75 (CH-*o*,*p*-Bn); 127.85 (CH-*p*-Bn); 128.25 (CH-*o*-Bn); 128.31, 128.36 and 128.52 (CH-*m*-Bn); 133.97 (C-5); 137.60 (CH-4); 138.17, 138.42 and 139.23 (C-*i*-Bn); 140.62 (C-2); 148.51 (CH-6). HRMS (ESI) *m/z* for $C_{32}H_{32}O_4N^{79}BrNa$ $[M + Na]^+$: calcd 596.14069; found 596.14079. IR (CCl_4): ν = 3033, 2867, 1584, 1562, 1497, 1455, 1384, 1188, 1089, 1024.

2,3,5-Tri-*O*-benzyl-1-*C*-(5-bromopyridin-2-yl)-2-*C*-methyl-α,β-D-ribofuranose (12)

n-BuLi (3.4 mL, 5.5 mmol; 1.6 M in hexanes) was slowly added to a cooled solution of 2,5-dibromopyridine (1.184 g, 5 mmol) in dry toluene (59 mL) at −78 °C. After stirring of the reaction mixture at −78 °C for 7 h, solution of lactone **4** (0.433 g, 1 mmol) in dry toluene (5 mL) was added, and stirring was continued for 10 min. MeOH (1.2 mL, 30 mmol) was added, and the resulting brown-yellow solution was warmed to r.t., neutralized with 2 M HCl, washed with sat. aq. $NaHCO_3$, and extracted with toluene. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were evaporated under reduced pressure.

Purification by flash column chromatography on silica gel (9 to 16 % of EtOAc in hexane) afforded hemiketal **12** (0.425 g, 0.72 mmol, 72 %; α:β 9:91) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6) **α-anomer**: δ = 0.96 (s, 3H, CH_3-2); 3.72 (dd, 1H, J_{gem} = 10.7, $J_{5a,4}$ = 4.2, H-5a); 3.74 (dd, 1H, J_{gem} = 10.7, $J_{5b,4}$ = 5.2, H-5b); 4.00 (d, 1H, $J_{3,4}$ = 6.5, H-3); 4.35 (bddd, 1H, $J_{4,3}$ = 6.5, $J_{4,5b}$ = 5.1, $J_{4,5a}$ = 4.2, H-4); 4.55 and 4.58 (2×bd, 2×1H, J_{gem} = 12.0, CH_2Bn-5); 4.58 and 4.65 (2×d, 2×1H, J_{gem} = 11.5, CH_2Bn-3); 4.71 and 4.78 (2×d, 2×1H, J_{gem} = 11.7, CH_2Bn-2); 6.01 (bs, 1H, OH-1); 7.23–7.45 (m, 15 H, H-*o*,*m*,*p*-Bn); 7.62 (dd, 1H, $J_{3',4'}$ = 8.5, $J_{3',6'}$ = 0.8, H-3′); 8.03 (dd, 1H, $J_{4',3'}$ = 8.5, $J_{4',6'}$ = 2.4, H-4′); 8.69 (dd, 1H, $J_{6',4'}$ = 2.4, $J_{6',3'}$ = 0.8, H-6′); **β-anomer**: δ = 1.53 (s, 3H, CH_3-2); 3.65 (dd, 1H, J_{gem} = 10.5, $J_{5a,4}$ = 6.4, H-5a); 3.69 (dd, 1H, J_{gem} = 10.5, $J_{5b,4}$ = 3.7, H-5b); 4.14 (d, 1H, $J_{3,4}$ = 8.1, H-3); 4.27 (d, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.29 (ddd, 1H, $J_{4,3}$ = 8.1, $J_{4,5a}$ = 6.4, $J_{4,5b}$ = 3.7, H-4); 4.55 and 4.58 (2×d, 2×1H, J_{gem} = 12.0, CH_2Bn-5); 4.68 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 4.69 (d, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.72 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 6.74 (s, 1H, OH-1); 6.78–6.84 (m, 2H, H-*o*-Bn); 7.11–7.16 and 7.26–7.39 (2×m, 13H, H-*o*,*m*,*p*-Bn); 7.58 (dd, 1H, $J_{3',4'}$ = 8.5, $J_{3',6'}$ = 0.8, H-3′); 7.95(dd, 1H, $J_{4',3'}$ = 8.5, $J_{4',6'}$ = 2.4, H-4′); 8.68 (dd, 1H, $J_{6',4'}$ = 2.4, $J_{6',3'}$ = 0.8, H-6′). ^{13}C NMR (125.7 MHz, DMSO-d_6) **α-anomer**: δ = 19.44 (CH_3-2); 66.35 (CH_2Bn-2); 70.55 (CH_2-5); 72.55 and 72.63 (CH_2Bn-3,5); 79.60 (CH-4); 82.68 (C-2); 83.93 (CH-3); 105.49 (C-1); 119.77 (C-5′); 123.74 (CH-3′); 127.24 (CH-*p*-Bn); 127.54 and 127.69 (CH-*o*-Bn); 127.80 and 127.85 (CH-*p*-Bn); 127.86 (CH-*o*-Bn); 128.15, 128.19 and 128.36 (CH-*m*-Bn); 138.40 and 138.54 (C-*i*-Bn); 139.09 (CH-4′); 139.77 (C-*i*-Bn); 149.21 (CH-6′); 158.99 (C-2′); **β-anomer**: δ = 15.57 (CH_3-2); 65.91 (CH_2Bn-2); 72.56 (CH_2Bn-5); 72.65 (CH_2-5); 73.34 (CH_2Bn-3); 79.50 (CH-4); 84.59 (C-2); 86.45 (CH-3); 105.71 (C-1); 119.61 (C-5′); 124.95 (CH-3′); 126.30 (CH-*o*-Bn); 126.81 and 127.63 (CH-*p*-Bn); 127.86 (CH-*o*,*p*-Bn); 128.94 (CH-*o*-Bn); 128.00, 128.44 and 128.47 (CH-*m*-Bn); 138.29 (CH-4′); 138.57 and 140.15 (C-*i*-Bn); 148.66 (CH-6′); 158.69 (C-2′). HRMS (ESI) *m/z* for $C_{32}H_{32}O_5N^{79}BrNa$ [*M* + Na]$^+$: calcd 612.13561; found 612.13570. IR (ATR): ν = 3039, 2945, 2867, 1581, 1562, 1500, 1457, 1366, 1261, 1211, 1181, 1094, 1030.

1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-1-*C*-(5-bromopyridin-2-yl)-2-*C*-methyl-β-D-ribofuranose (13) and 1-*O*-Acetyl-2,3,5-tri-*O*-benzyl-1-*C*-(5-bromopyridin-2-yl)-2-*C*-methyl-α-D-ribofuranose (14)

LiHMDS (13 mL, 12.98 mmol; 1 M in THF) was slowly added to a solution of **12** (5.11 g, 8.65 mmol) in dry toluene (70 mL) at r.t. After 10 min, Ac_2O (1.23 mL, 12.98 mmol) was added, and the mixture was stirred for a farther 10 min. After pouring into sat. aq. $NaHCO_3$,

the mixture was extracted with toluene, and combined organic layers were dried over anhydrous $MgSO_4$. The volatiles were removed in vacuo, and the residue was purified by flash column chromatography on silica gel (9 to 16 % of EtOAc in hexane) giving β-anomeric hemiketal acetate **13** (3.23 g, 5.1 mmol, 59 %) as a white solid (m.p. 112–113 °C) and its α-anomeric analogue **14** (1.26 g, 1.99 mmol, 23 %) as a yellowish oil.

Compound 13. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.58 (s, 3H, CH_3-2); 1.72 (s, 3H, CH_3CO); 3.55 (dd, 1H, J_{gem} = 11.1, $J_{5a,4}$ = 3.4, H-5a); 3.73 (dd, 1H, J_{gem} = 11.1, $J_{5b,4}$ = 2.3, H-5b); 4.16 (bd, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.36 (d, 1H, $J_{3,4}$ = 8.4, H-3); 4.38 (ddd, 1H, $J_{4,3}$ = 8.4, $J_{4,5a}$ = 3.4, $J_{4,5b}$ = 2.4, H-4); 4.47 and 4.50 (2×d, 2×1H, J_{gem} = 12.0, CH_2Bn-5); 4.63 (bd, 1H, J_{gem} = 12.5, CH_2Bn-2); 4.68 and 4.76 (2×d, 2×1H, J_{gem} = 11.8, CH_2Bn-3); 6.78 (m, 2H, H-*o*-Bn-2); 7.12–7.17 (m, 3H, H-*m*,*p*-Bn-2); 7.26–7.40 (m, 10H, H-*o*,*m*,*p*-Bn-3,5); 7.55 (dd, 1H, $J_{3',4'}$ = 8.5, $J_{3',6'}$ = 0.8, H-3′); 7.97 (dd, 1H, $J_{4',3'}$ = 8.5, $J_{4',6'}$ = 2.4, H-4′); 8.62 (dd, 1H, $J_{6',4'}$ = 2.4, $J_{6',3'}$ = 0.8, H-6′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 14.94 (CH_3-2); 21.50 (**C**H_3CO); 65.92 (CH_2Bn-2); 69.29 (CH_2-5); 72.57 (CH_2Bn-5); 73.47 (CH_2Bn-3); 81.08 (CH-4); 83.78 (CH-3); 84.76 (C-2); 107.41 (C-1); 119.45 (C-5′); 125.29 (CH-3′); 126.30 (CH-*o*-Bn); 126.98 and 127.71 (CH-*p*-Bn); 127.85 (CH-*o*-Bn); 127.99 (CH-*p*-Bn); 128.00 and 128.03 (CH-*o*,*m*-Bn); 128.46 and 128.52 (CH-*m*-Bn); 138.40 and 138.41 (C-*i*-Bn); 138.44 (CH-4′); 139.64 (C-*i*-Bn); 148.53 (CH-6′); 155.83 (C-2′); 167.49 (CH_3**C**O). HRMS (ESI) *m/z* for $C_{34}H_{34}O_6N^{79}BrNa$ $[M + Na]^+$: calcd 654.14617; found 654.14626. IR (ATR): ν = 3042, 2946, 2880, 1747, 1501, 1460, 1369, 1239, 1080, 1031.

Compound 14. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.92 (s, 3H, CH_3-2); 1.96 (s, 3H, CH_3CO); 3.70 (dd, 1H, J_{gem} = 11.1, $J_{5a,4}$ = 4.2, H-5a); 3.79 (dd, 1H, J_{gem} = 11.1, $J_{5b,4}$ = 3.3, H-5b); 3.83 (d, 1H, $J_{3,4}$ = 6.1, H-3); 4.37 (ddd, 1H, $J_{4,3}$ = 6.1, $J_{4,5a}$ = 4.2, $J_{4,5b}$ = 3.3, H-4); 4.54 (d, 1H, J_{gem} = 11.7, CH_2Bn-3); 4.57 (d, 1H, J_{gem} = 12.0, CH_2Bn-5); 4.59 (bd, 1H, J_{gem} = 11.9, CH_2Bn-2); 4.61 (d, 1H, J_{gem} = 12.1, CH_2Bn-5); 4.64 (d, 1H, J_{gem} = 11.8, CH_2Bn-3); 4.90 (d, 1H, J_{gem} = 11.9, CH_2Bn-2); 7.21–7.46 (m, 15H, H-*o*,*m*,*p*-Bn); 7.58 (dd, 1H, $J_{3',4'}$ = 8.5, $J_{3',6'}$ = 0.8, H-3′); 7.99 (dd, 1H, $J_{4',3'}$ = 8.5, $J_{4',6'}$ = 2.4, H-4′); 8.67 (dd, 1H, $J_{6',4'}$ = 2.4, $J_{6',3'}$ = 0.8, H-6′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 20.66 (CH_3-2); 21.84 (**C**H_3CO); 65.99 (CH_2Bn-2); 69.04 (CH_2-5); 72.16 (CH_2Bn-3); 72.68 (CH_2Bn-5); 81.43 (CH-4); 82.06 (CH-3); 84.01 (C-2); 107.68 (C-1); 119.51 (C-5′); 123.85 (CH-3′); 127.13 (CH-*p*-Bn); 127.27 (CH-*o*-Bn); 127.75 and 127.80 (CH-*p*-Bn); 127.85 and 127.98 (CH-*o*-Bn); 128.21, 128.29 and 128.55 (CH-*m*-Bn); 138.34 and 138.51 (C-*i*-Bn); 139.11 (CH-4′); 139.89 (C-*i*-Bn); 149.15 (CH-6′); 156.50 (C-2′); 168.22 (CH_3**C**O). HRMS (ESI) *m/z* for $C_{34}H_{34}O_6N^{79}BrNa$ $[M + Na]^+$: calcd

654.14617; found 654.14652. IR (CCl_4): ν = 3032, 2917, 2865, 1756, 1574, 1497, 1454, 1365, 1232, 1094, 1029.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-5-bromopyridine (15)

A mixture of hemiketal acetates **13** and **14** (4.98 g, 7.87 mmol) in dry toluene (50 mL) was treated with Et_3SiH (3.77 mL, 23.61 mmol) at 0 °C. After 5 min, $BF_3 \cdot Et_2O$ (1.46 mL, 11.81 mmol) was added dropwise, and the resulting solution was warmed to r.t. over 5 min. After addition of Et_3N (11 mL, 78.7 mmol), the reaction mixture was concentrated in vacuo. Purification using flash column chromatography on silica gel (6 to 13 % of EtOAc in hexane) to give **15** (4.02 g, 7 mmol, 89 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.94 (s, 3H, CH_3-2′); 3.72 (dd, 1H, J_{gem} = 11.0, $J_{5'a,4'}$ = 4.5, H-5′a); 3.81 (dd, 1H, J_{gem} = 11.0, $J_{5'b,4'}$ = 3.1, H-5′b); 3.88 (d, 1H, $J_{3',4'}$ = 7.6, H-3′); 4.21 (ddd, 1H, $J_{4',3'}$ = 7.6, $J_{4',5'a}$ = 4.5, $J_{4',5'b}$ = 3.1, H-4′); 4.57 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.59 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.61 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.63 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.69 (m, 2H, CH_2Bn-2′); 5.02 (s, 1H, H-1′); 7.23–7.41 (m, 15 H, H-*o*,*m*,*p*-Bn); 7.59 (dt, 1H, $J_{3,4}$ = 8.4, $J_{3,6}$ = $J_{3,1'}$ = 0.8, H-3); 7.94 (dd, 1H, $J_{4,3}$ = 8.4, $J_{4,6}$ = 2.4, H-4); 8.67 (dd, 1H, $J_{6,4}$ = 2.4, $J_{6,3}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.13 (CH_3-2′); 65.22 (CH_2Bn-2′); 69.65 (CH_2-5′); 72.45 (CH_2Bn-3′); 72.63 (CH_2Bn-5′); 80.08 (CH-4′); 83.09 (CH-3′); 83.32 (C-2′); 85.67 (CH-1′); 119.20 (C-5); 123.36 (CH-3); 127.29 (CH-*p*-Bn); 127.41 (CH-*o*-Bn); 127.73 and 127.83 (CH-*p*-Bn); 127.85 and 128.13 (CH-*o*-Bn); 128.29, 128.36 and 128.52 (CH-*m*-Bn); 138.39 and 138.45 (C-*i*-Bn); 139.27 (CH-4); 139.50 (C-*i*-Bn); 149.50 (CH-6); 158.31 (C-2). HRMS (ESI) *m/z* for $C_{32}H_{32}O_4N^{79}BrNa$ $[M + Na]^+$: calcd 596.14069; found 596.14080. IR (CCl_4): ν = 3033, 2894, 2864, 1575, 1497, 1465, 1454, 1367, 1206, 1091, 1029.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-α-D-ribofuranosyl)-5-bromopyridine (16)

α-Anomer **16** was isolated as a by-product (yellowish oil, 0.41 g, 0.71 mmol, 9 %) in the synthesis of **15**. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.52 (s, 3H, CH_3-2′); 3.61 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 5.0, H-5′a); 3.71 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 2.7, H-5′b); 4.13 (d, 1H, $J_{3',4'}$ = 8.6, H-3′); 4.27 (d, 1H, J_{gem} = 12.4, CH_2Bn-2′); 4.30 (ddd, 1H, $J_{4',3'}$ = 8.6, $J_{4',5'a}$ = 5.0, $J_{4',5'b}$ = 2.7, H-4′); 4.51 and 4.56 (2×d, 2×1H, J_{gem} = 12.2, CH_2Bn-5′); 4.65 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.70 (d, 1H, J_{gem} = 12.4, CH_2Bn-2′); 4.74 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.84 (s, 1H, H-1′); 6.85 (m, 2H, H-*o*-Bn-2′); 7.12–7.18 (m, 3H, H-*m*,*p*-Bn-2′); 7.27–7.40 (m, 10H, H-*o*,*m*,*p*-Bn-3′,5′); 7.46 (dt, 1H, $J_{3,4}$ = 8.4, $J_{3,6}$ = $J_{3,1'}$ = 0.7, H-3); 7.98 (dd, 1H, $J_{4,3}$ = 8.4, $J_{4,6}$ = 2.4, H-4); 8.63 (dd, 1H, $J_{6,4}$ = 2.4, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ =

17.92 (CH_3-2′); 65.77 (CH_2Bn-2′); 70.50 (CH_2-5′); 72.58 (CH_2Bn-5′); 73.26 (CH_2Bn-3′); 79.66 (CH-4′); 81.82 (C-2′); 86.02 (CH-3′); 86.47 (CH-1′); 118.94 (C-5); 124.42 (CH-3); 126.34 (CH-*o*-Bn-2′); 126.87 (CH-*p*-Bn-2′); 127.66, 127.82, 129.91 and 128.00 (CH-*o*,*p*-Bn-3′,5′and CH-*m*-Bn-2′); 128.46 and 128.49 (CH-*m*-Bn-3′,5′); 138.45 and 138.51 (C-*i*-Bn-3′,5′); 138.62 (CH-4); 140.06 (C-*i*-Bn-2′); 148.73 (CH-6); 157.73 (C-2). HRMS (ESI) *m/z* for $C_{32}H_{33}O_4N^{79}Br$ $[M + H]^+$: calcd 574.15875; found 574.15905. IR (CCl_4): ν = 3032, 2876, 1578, 1497, 1467, 1454, 1367, 1209, 1090, 1029.

1-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-4-methylbenzene (17a)

Nucleoside **17a** was prepared from compound **8** (1 g, 1.74 mmol) following the general procedure B. The crude mixture was purified by flash column chromatography on silica gel (0 to 5 % of EtOAc in hexane) affording **17a** (804 mg, 1.58 mmol, 91 %) as a white solid (m.p. 57–58 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.88 (s, 3H, CH_3-2′); 2.28 (s, 3H, CH_3-4); 3.70 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.9, H-5′a); 3.73 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.6, H-5′b); 3.82 (d, 1H, $J_{3',4'}$ = 4.8, H-3′); 4.20 (q, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.7, H-4′); 4.51–4.65 (m, 6H, CH_2Bn); 4.86 (s, 1H, H-1′); 7.11 (m, 2H, H-3,5); 7.24 (m, 2H, H-2,6); 7.25–7.42 (m, 15H, H-*o*,*m*,*p*-Bn). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.88 (CH_3-2′); 20.93 (CH_3-4); 65.49 (CH_2Bn-2′); 70.28 (CH_2-5′); 71.52 (CH_2Bn-3′); 72.64 (CH_2Bn-5′); 81.45 (CH-4′); 82.83 (C-2′); 83.05 (CH-3′); 84.03 (CH-1′); 126.42 (CH-2,6); 127.21 (CH-*p*-Bn); 127.35 (CH-*o*-Bn); 127.70 (CH-*o*,*p*-Bn); 127.80 (CH-*p*-Bn); 128.22 and 128.27 (CH-*o*,*m*-Bn); 128.33 and 128.49 (CH-*m*-Bn); 128.55 (CH-3,5); 135.57 (C-1); 136.53 (C-4); 138.29, 138.52 and 138.56 (C-*i*-Bn). HRMS (ESI) *m/z* for $C_{34}H_{36}O_4Na$ $[M + Na]^+$: calcd 531.25058; found 531.25072. IR (CCl_4): ν = 3032, 2866, 1608, 1497, 1454, 1382, 1362, 1180, 1095, 1029.

4-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)aniline (17b)

LiHMDS (4.2 mL, 4.18 mmol; 1 M in THF) was added to a suspension of **8** (1.2 g, 2.09 mmol), Pd_2dba_3 (48 mg, 0.05 mmol) and Cy-JohnPhos (73 mg, 0.21 mmol) in dry THF (15 mL). The resulting solution was stirred at 66 °C for 30 min, then it was cooled to r.t., treated with 2 M HCl (10 mL) over 10 min, washed with sat. aq. $NaHCO_3$ and extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$. The solvents were removed under reduced pressure, and the crude product was purified using flash column chromatography on silica gel (10 to 60 % of EtOAc in hexane) affording **17b** (0.91 g, 1.78 mmol, 85 %) as a yellowish solid (m.p. 89–91 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.89 (s, 3H, CH_3-2′); 3.68 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.9, H-5′a); 3.71 (dd, 1H, J_{gem} = 10.6,

$J_{5'b,4'}$ = 4.5, H-5′b); 3.78 (d, 1H, $J_{3',4'}$ = 5.2, H-3′); 4.13 (bq, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.9, H-4′); 4.53 (bd, 1H, J_{gem} = 11.6, CH_2Bn-2′); 4.54 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.58 (bd, 1H, J_{gem} = 11.5, CH_2Bn-2′); 4.59 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.61 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.62 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.73 (s, 1H, H-1′); 4.98 (bs, 2H, NH_2); 6.48 (m, 2H, H-2,6); 6.99 (m, 2H, H-3,5); 7.21–7.35 (m, 11H, H-*o*,*m*,*p*-Bn); 7.36–7.42 (m, 4H, H-*o*,*m*-Bn). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.97 (CH_3-2′); 65.35 (CH_2Bn-2′); 70.38 (CH_2-5′); 71.61 (CH_2Bn-3′); 72.62 (CH_2Bn-5′); 80.90 (CH-4′); 82.83 (C-2′); 83.21 (CH-3′); 84.76 (CH-1′); 113.42 (CH-2,6); 125.56 (C-4); 127.16 (CH-*p*-Bn); 127.30 (CH-3,5, CH-*o*-Bn); 127.38 (CH-*p*-Bn); 127.66 (CH-*o*-Bn); 127.77 (CH-*p*-Bn); 128.21 and 128.25 (CH-*o*,*m*-Bn); 128.33 and 128.48 (CH-*m*-Bn); 138.38, 138.58 and 138.74 (C-*i*-Bn); 148.09 (C-1). HRMS (ESI) *m/z* for $C_{33}H_{35}O_4NNa$ $[M + Na]^+$: calcd 532.24583; found 532.24566. IR (CCl_4): ν = 3479, 3394, 3032, 2866, 1623, 1519, 1497, 1454, 1382, 1362, 1275, 1177, 1096, 1029.

4-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-*N*,*N*-dimethylaniline (17c)

Me_2NH (9 mL, 17.95 mmol; 2 M in THF) was added to a suspension of **8** (2 g, 3.49 mmol), Pd_2dba_3 (80 mg, 0.087 mmol), JohnPhos (107 mg, 0.349 mmol) and *t*-BuONa (2.012 g, 20.94 mmol) in dry toluene (10 mL) in a septum-sealed flask. The resulting mixture was stirred at 70 °C for 24 h, then it was quenched by pouring into sat. aq. $NaHCO_3$, and extracted with toluene. Organic layers were combined, dried over anhydrous $MgSO_4$, and the solvents were removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (5 to 15 % of EtOAc in hexane) furnishing **17c** (1.56 g, 2.9 mmol, 83 %) as a yellowish solid (m.p. 65–66 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.90 (s, 3H, CH_3-2′); 2.86 (s, 6H, $(CH_3)_2N$); 3.69 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.9, H-5′a); 3.72 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 4.5, H-5′b); 3.81 (d, 1H, $J_{3',4'}$ = 4.9, H-3′); 4.16 (q, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.8, H-4′); 4.53 (bd, 1H, J_{gem} = 11.5, CH_2Bn-2′); 4.54 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.58 (bd, 1H, J_{gem} = 11.5, CH_2Bn-2′); 4.60 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.61 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.62 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.80 (s, 1H, H-1′); 6.65 (m, 2H, H-2,6); 7.16 (m, 2H, H-3,5); 7.22–7.36 (m, 11H, H-*o*,*m*,*p*-Bn); 7.36–7.42 (m, 4H, H-*o*,*m*-Bn). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.94 (CH_3-2′); 40.32 ($(CH_3)_2N$); 65.41 (CH_2Bn-2′); 70.36 (CH_2-5′); 71.57 (CH_2Bn-3′); 72.64 (CH_2Bn-5′); 81.15 (CH-4′); 82.85 (C-2′); 83.20 (CH-3′); 84.39 (CH-1′); 111.86 (CH-2,6); 125.91 (C-4); 128.18 (CH-*p*-Bn); 127.34 (CH-3,5, CH-*o*-Bn); 127.67 (CH-*p*-Bn); 127.69 (CH-*o*-Bn); 127.77 (CH-*p*-Bn); 128.21 and 128.23 (CH-*o*,*m*-Bn); 128.33 and 128.48 (CH-*m*-Bn); 138.36, 138.56 and 138.65 (C-*i*-Bn);

149.92 (C-1). HRMS (ESI) *m/z* for $C_{35}H_{40}O_4N$: $[M + H]^+$: calcd 538.29519; found 538.29510. IR (ATR): ν = 3072, 3040, 2876, 1621, 1528, 1457, 1359, 1190, 1092, 1065, 1030.

4-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)phenol (17d)

Mixture of **8** (1.15 g, 2 mmol), Pd_2dba_3 (46 mg, 0.05 mmol), $Me_4(t\text{-}Bu)_2XPhos$ (96 mg, 0.2 mmol) and KOH (337 mg, 6 mmol) in water (3 mL) and 1,4-dioxane (9 mL) was stirred at 80 °C for 2 h. Then it was cooled to r.t., and diluted with EtOAc. After filtration through Celite, the solution was concentrated under reduced pressure. Purification of the crude product using flash column chromatography on silica gel (9 to 14 % of EtOAc in hexane) afforded **17d** (0.972 g, 1.9 mmol, 95 %) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.88 (s, 3H, CH_3-2′); 3.69 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.8, H-5′a); 3.72 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 4.5, H-5′b); 3.81 (d, 1H, $J_{3',4'}$ = 4.9, H-3′); 4.17 (q, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.8, H-4′); 4.53 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.53 (d, 1H, J_{gem} = 11.6, CH_2Bn-2′); 4.58 (d, 1H, J_{gem} = 11.7, CH_2Bn-2′); 4.60 (d, 1H, J_{gem} = 12.0, CH_2Bn-5′); 4.61 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.62 (d, 1H, J_{gem} = 12.0, CH_2Bn-5′); 4.80 (s, 1H, H-1′); 6.68 (m, 2H, H-2,6); 7.14 (m, 2H, H-3,5); 7.22–7.34 and 7.36–7.42 (2×m, 15H, H-*o*,*m*,*p*-Bn); 9.32 (s, 1H, OH-Ph). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.89 (CH_3-2′); 65.44 (CH_2Bn-2′); 70.32 (CH_2-5′); 71.58 (CH_2Bn-3′); 72.65 (CH_2Bn-5′); 81.19 (CH-4′); 82.82 (C-2′); 83.06 (CH-3′); 84.25 (CH-1′); 114.75 (CH-2,6); 127.19 (CH-*p*-Bn); 127.33 (CH-*o*-Bn); 127.70 (CH-*o*-Bn, CH-3,5); 127.72 (CH-*p*-Bn); 127.80 (CH-*p*-Bn); 128.22 (CH-*o*-Bn); 128.27, 128.34 and 128.49 (CH-*m*-Bn); 128.81 (C-4); 138.33, 138.55 and 139.65 (C-*i*-Bn); 156.76 (C-1). HRMS (ESI) *m/z* for $C_{33}H_{34}O_5Na$ $[M + Na]^+$: calcd 533.22985; found 533.22966. IR (ATR): ν = 3368, 3039, 2875, 1620, 1521, 1457, 1366, 1268, 1172, 1078, 1029.

4-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)anisole (17e)

Mixture of **8** (140 mg, 0.244 mmol), Pd_2dba_3 (6 mg, 0.006 mmol), $Me_4(t\text{-}Bu)_2XPhos$ (12 mg, 0.024 mmol) and KOH (41 mg, 0.732 mmol) in water (0.3 mL) and 1,4-dioxane (0.9 mL) was stirred at 80 °C for 2 h. After cooling to r.t., TBAB (8 mg, 0.024 mmol), additional KOH (27 mg, 0.488 mmol) and CH_3I (0.03 mL, 0.488 mmol) were added, and resulting suspension was stirred for a farther 30 min at 80 °C. After cooling to r.t., the reaction mixture was diluted with EtOAc, filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (5 to 11 % of EtOAc in hexane) furnishing **17e** (0.972 g, 1.9 mmol, 95 %) as a white solid (m.p. 67–68 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.88 (s, 3H, CH_3-2′); 3.70 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.9, H-5′a); 3.73 (dd, 1H, J_{gem} =

10.6, $J_{5'b,4'}$ = 4.6, H-5′b); 3.73 (s, 3H, CH_3O); 3.82 (d, 1H, $J_{3',4'}$ = 4.8, H-3′); 4.19 (q, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.8, H-4′); 4.54 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.54 and 4.59 (2×bd, 2×1H, J_{gem} = 11.6, CH_2Bn-2′); 4.61 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.62 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.63 (d, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.85 (s, 1H, H-1′); 6.86 (m, 2H, H-2,6); 7.22–7.42 (m, 17H, H-*o*,*m*,*p*-Bn, CH-3,5). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.83 (CH_3-2′); 55.12 (CH_3O); 65.46 (CH_2Bn-2′); 70.27 (CH_2-5′); 71.53 (CH_2Bn-3′); 72.64 (CH_2Bn-5′); 81.36 (CH-4′); 82.79 (C-2′); 83.06 (CH-3′); 83.93 (CH-1′); 113.34 (CH-2,6); 127.16 (CH-*p*-Bn); 127.30 (CH-*o*-Bn); 127.65 and 127.70 (CH-*o*,*p*-Bn, CH-3,5); 127.74 (CH-*p*-Bn); 128.17, 128.27, 128.34 and 128.49 (CH-*o*-Bn, CH-*m*-Bn); 130.47 (C-4); 138.28, 138.50 and 139.55 (C-*i*-Bn); 158.67 (C-1). HRMS (ESI) *m/z* for $C_{34}H_{36}O_5Na$ $[M + Na]^+$: calcd 547.24550; found 547.24537. IR (CCl_4): ν = 2900, 2865, 1615, 1514, 1454, 1382, 1362, 1248, 1172, 1094, 1041, 1029.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-methylpyridine (18a)

Compound **18a** was prepared from **11** (1 g, 1.74 mmol) according to the general procedure B. Flash column chromatography on silica gel (17 to 25 % of EtOAc in hexane) afforded **18a** (839 mg, 1.65 mmol, 95 %) as a yellowish oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (s, 3H, CH_3-2′); 2.62 (s, 3H, (CH_3-2); 3.66 (dd, 1H, J_{gem} = 10.2, $J_{5'a,4'}$ = 5.6, H-5′a); 3.71 (dd, 1H, J_{gem} = 10.2, $J_{5'b,4'}$ = 4.7, H-5′b); 3.78 (d, 1H, $J_{3',4'}$ = 3.8, H-3′); 4.36 (ddd, 1H, $J_{4',5'a}$ = 5.6, $J_{4',5'b}$ = 4.7, $J_{4',3'}$ = 3.8, H-4′); 4.49 (d, 1H, J_{gem} = 11.1, CH_2Bn-2′); 4.53 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.56 (d, 1H, J_{gem} = 11.1, CH_2Bn-2′); 4.63 and 4.65 (2×d, 2×1H, J_{gem} = 11.9, CH_2Bn-5′); 4.68 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 5.05 (s, 1H, H-1′); 7.15 (bd, 1H, $J_{3,4}$ = 8.1, H-3); 7.23–7.41 (m, 15H, H-*o*,*m*,*p*-Bn); 7.75 (bd, 1H, $J_{4,3}$ = 8.1, H-4); 8.56 (bdt, 1H, $J_{6,4}$ = 2.2, $J_{6,3} = J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 19.04 (CH_3-2′); 23.2 (CH_3-2); 66.30 (CH_2Bn-2′); 70.14 (CH_2-5′); 71.70 (CH_2Bn-3′); 73.58 (CH_2Bn-5′); 82.03 (CH-1′); 82.60 (CH-4′); 82.70 (CH-3′); 83.01 (C-2′); 123.21 (CH-3); 127.30 (CH-*o*-Bn); 127.31 (CH-*p*-Bn); 127.79 (CH-*o*-Bn); 127.83 and 127.90 (CH-*p*-Bn); 128.24 (CH-*o*-Bn); 128.34, 128.43 and 128.47 (CH-*m*-Bn); 131.69 (C-5); 136.29 (CH-4); 137.47, 138.86 and 138.72 (C-*i*-Bn); 145.69 (CH-6); 156.45 (C-2). HRMS (ESI) *m/z* for $C_{33}H_{36}O_4N$ $[M + H]^+$: calcd 510.26389; found 510.26395. IR (CCl_4): ν = 3067, 3032, 2866, 1604, 1571, 1496, 1454, 1382, 1188, 1095, 1029.

2-Amino-5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)pyridine (18b)

Suspension of **11** (400 mg, 0.7 mmol), Pd_2dba_3 (16 mg, 0.018 mmol) and Cy-JohnPhos (25 mg, 0.07 mmol) in dry THF (7.5 mL) was treated with LiHMDS (1.4 mL, 1.4 mmol; 1 M in THF). The mixture was stirred at 70 °C for 30 min, then it was cooled to r.t., and stirred with 2 M HCl (4 mL) for 10 min. After washing with sat. aq. $NaHCO_3$, the mixture was extracted with EtOAc, and combined organic layers were dried over anhydrous $MgSO_4$. The volatiles were removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (33 to 67 % of EtOAc in hexane) furnishing **18b** (335 mg, 0.66 mmol, 94 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.94 (s, 3H, CH_3-2′); 3.67 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.8, H-5′a); 3.70 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 4.6, H-5′b); 3.81 (d, 1H, $J_{3',4'}$ = 4.8, H-3′); 4.16 (td, 1H, $J_{4',5'a} = J_{4',3'}$ = 4.8, $J_{4',5'b}$ = 4.6, H-4′); 4.51–4.63 (m, 6H, CH_2Bn-2′,3′,5′); 4.75 (s, 1H, H-1′); 5.86 (bs, 2H, NH_2); 6.37 (dd, 1H, $J_{3,4}$ = 8.5, $J_{3,6}$ = 0.8, H-3); 7.22–7.34 and 7.35–7.40 (2×m, 16H, H-*o*,*m*,*p*-Bn, H-4); 8.01 (dt, 1H, $J_{6,4}$ = 2.4, $J_{6,3} = J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.88 (CH_3-2′); 65.42 (CH_2Bn-2′); 70.28 (CH_2-5′); 71.53 (CH_2Bn-3′); 72.63 (CH_2Bn-5′); 81.32 (CH-4′); 82.71 (C-2′); 82.92 (CH-1′); 83.04 (CH-3′); 107.31 (CH-3); 121.43 (C-5); 127.20 (CH-*p*-Bn); 127.33 (CH-*o*-Bn); 127.67 (CH-*o*,*p*-Bn); 127.79 (CH-*p*-Bn); 128.21 (CH-*o*-Bn); 128.26, 128.33 and 128.48 (CH-*m*-Bn); 135.69 (CH-4); 138.30 (C-*i*-Bn-3′); 138.51 (C-*i*-Bn-5′); 139.57 (C-*i*-Bn-2′); 146.18 (CH-6); 159.51 (C-2). HRMS (ESI) *m/z* for $C_{32}H_{35}O_4N_2$ $[M + H]^+$: calcd 511.25913; found 511.25905. IR (ATR): ν = 3477, 3374, 3186, 3039, 2875, 1623, 1504, 1412, 1364, 1088.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-(dimethylamino)pyridine (18c)

Me_2NH (4.9 mL, 9.85 mmol; 2 M in THF) was added to a suspension of **11** (1.13 g, 1.97 mmol), Pd_2dba_3 (45 mg, 0.049 mmol), JohnPhos (59 mg, 0.197 mmol) and *t*-BuONa (1.136 g, 11.82 mmol) in dry toluene (4 mL) in a septum-sealed flask. The resulting mixture was stirred at 70 °C for 4 h, then poured into sat. aq. $NaHCO_3$ (15 mL), and extracted with toluene. Organic layers were combined and dried over anhydrous $MgSO_4$. The solvents were evaporated under reduced pressure, and the crude product was purified by flash column chromatography on silica gel (5 to 30 % of EtOAc in hexane) affording **18c** (923 mg, 1.72 mmol, 87 %) as a yellowish solid (m.p. 76–78 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.94 (s, 3H, CH_3-2′); 2.99 (s, 6H, $(CH_3)_2$N); 3.68 (dd, 1H, J_{gem} = 10.6, $J_{5'a,4'}$ = 4.9, H-5′a); 3.71 (dd, 1H, J_{gem} = 10.6, $J_{5'b,4'}$ = 4.6, H-5′b); 3.83 (d, 1H, $J_{3',4'}$ = 4.7, H-3′); 4.17 (ddd, 1H, $J_{4',5'a}$ = 4.9, $J_{4',3'}$ = 4.7, $J_{4',5'b}$ = 4.6, H-4′); 4.53 (d, 1H, J_{gem} = 11.4, CH_2Bn-3′); 4.53 and 4.57

(2×bd, 2×1H, J_{gem} = 11.6, CH_2Bn-2′); 4.60 and 4.62 (2×bd, 2×1H, J_{gem} = 12.1, CH_2Bn-5′); 4.61 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.79 (s, 1H, H-1′); 6.56 (dd, 1H, $J_{3,4}$ = 8.8, $J_{3,6}$ = 0.8, H-3); 7.21–7.33 and 7.36–7.41 (2×m, 15H, H-*o*,*m*,*p*-Bn); 7.46 (ddd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.5, $J_{4,1'}$ = 0.6, H-4); 8.01 (dt, 1H, $J_{6,4}$ = 2.5, $J_{6,3}$ = $J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.86 (CH_3-2′); 37.85 ((CH_3)$_2$N); 65.46 (CH_2Bn-2′); 70.32 (CH_2-5′); 71.52 (CH_2Bn-3′); 72.67 (CH_2Bn-5′); 81.48 (CH-4′); 82.74 (C-2′); 82.75 (CH-1′); 83.09 (CH-3′); 105.09 (CH-3); 120.93 (C-5); 127.23 (CH-*p*-Bn); 127.38 (CH-*o*-Bn); 127.71 (CH-*o*,*p*-Bn); 127.80 (CH-*p*-Bn); 128.23 (CH-*o*-Bn); 128.27, 128.35 and 128.50 (CH-*m*-Bn); 135.86 (CH-4); 138.32 (C-*i*-Bn-3′); 138.53 (C-*i*-Bn-5′); 139.53 (C-*i*-Bn-2′); 146.05 (CH-6); 158.85 (C-2). HRMS (ESI) *m/z* for $C_{34}H_{39}O_4N_2$ [M + H]$^+$: calcd 539.29043; found 539.29035. IR (ATR): ν = 3071, 3039, 2884, 1702, 1613, 1522, 1458, 1381, 1200, 1108, 1030.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-pyridone (18d)

Mixture of **11** (1.344 g, 2.34 mmol), Pd_2dba_3 (54 mg, 0.059 mmol), Me_4(*t*-Bu)$_2$XPhos (112 mg, 0.234 mmol) and KOH (394 mg, 7.02 mmol) in 1,4-dioxane (9.6 mL) and water (3.2 mL) was stirred at 80 °C for 2 h. Then it was cooled to r.t. and diluted with EtOAc. After filtering through Celite, the mixture was concentrated in vacuo. The crude residue was purified by flash column chromatography on silica gel (0 to 5 % of MeOH in EtOAc) affording **18d** (1.115 g, 2.18 mmol, 93 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.00 (s, 3H, CH_3-2′); 3.65 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.9, H-5′a); 3.69 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.4, H-5′b); 3.82 (d, 1H, $J_{3',4'}$ = 4.9, H-3′); 4.15 (bq, 1H, $J_{4',3'}$ = $J_{4',5'a}$ = $J_{4',5'b}$ = 4.6, H-4′); 4.53 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.57 (m, 2H, CH_2Bn-2′); 4.57 and 4.60 (2×d, 2×1H, J_{gem} = 12.1, CH_2Bn-5′); 4.60 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.69 (bd, 1H, $J_{1',LR}$ = 0.9, H-1′); 6.26 (dd, 1H, $J_{3,4}$ = 9.5, $J_{3,6}$ = 0.8, H-3); 7.21–7.41 (m, 16H, H-*o*,*m*,*p*-Bn, H-6); 7.40 (bdd, 1H, $J_{4,3}$ = 9.5, $J_{4,6}$ = 2.6, H-4); 11.53 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.67 (CH_3-2′); 65.48 (CH_2Bn-2′); 70.04 (CH_2-5′); 71.57 (CH_2Bn-3′); 72.61 (CH_2Bn-5′); 81.40 (CH-4′); 81.72 (CH-1′); 82.69 (C-2′); 82.84 (CH-3′); 115.29 (C-5); 119.49 (CH-3); 127.23 (CH-*p*-Bn); 127.37 (CH-*o*-Bn); 127.71 (CH-*p*-Bn); 127.73 (CH-*o*-Bn); 127.79 (CH-*p*-Bn); 128.20 (CH-*o*-Bn); 128.24, 128.32 and 128.49 (CH-*m*-Bn); 132.90 (CH-4 or 6); 138.24, 138.41 and 139.44 (C-*i*-Bn); 139.92 (CH-4 or 6); 162.29 (C-2). HRMS (ESI) *m/z* for $C_{32}H_{33}O_5NNa$ [M + Na]$^+$: calcd 534.22509; found 534.22498. IR (CCl_4): ν = 3067, 2976, 2901, 1665, 1627, 1552, 1454, 1364, 1207, 1187, 1095, 1029.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-methoxypyridine (18e)

Compound **18d** (240 mg, 0.47 mmol), CH_3I (0.09 mL, 1.41 mmol) and $Ag_2(CO)_3$ (196 mg, 0.71 mmol) were mixed in CH_2Cl_2 (4 mL), and the resulting mixture was heated at 80 °C for 2 h. After cooling to r.t., it was filtered through Celite, and the volatiles were removed under reduced pressure. The residue was purified using flash column chromatography on silica gel (0 to 50 % of EtOAc in hexane) furnishing **18e** (165 mg, 0.32 mmol, 68 %) as a colorless oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.92 (s, 3H, CH_3-2′); 3.70 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.6, H-5′a); 3.73 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.6, H-5′b); 3.83 (s, 3H, CH_3O); 3.86 (d, 1H, $J_{3',4'}$ = 4.6, H-3′); 4.22 (q, 1H, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 4.7, H-4′); 4.54 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.55 and 4.59 (2×d, 2×1H, J_{gem} = 11.5, CH_2Bn-2′); 4.60 (bd, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.62 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.63 (bd, 1H, J_{gem} = 12.1, CH_2Bn-5′); 4.90 (s, 1H, H-1′); 6.76 (dd, 1H, $J_{3,4}$ = 8.6, $J_{3,6}$ = 0.8, H-3); 7.22–7.41 (m, 15H, H-*o*,*m*,*p*-Bn); 7.66 (ddd, 1H, $J_{4,3}$ = 8.6, $J_{4,6}$ = 2.4, $J_{4,1}$ = 0.7, H-4); 8.11 (dpent, 1H, $J_{6,4}$ = 2.4, $J_{6,3} = J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.71 (CH_3-2′); 53.21 (CH_3O); 65.50 (CH_2Bn-2′); 70.17 (CH_2-5′); 71.49 (CH_2Bn-3′); 72.66 (CH_2Bn-5′); 81.72 (CH-4′); 82.11 (CH-1′); 82.72 (C-2′); 82.90 (CH-3′); 109.85 (CH-3); 126.97 (C-5); 127.18 (CH-*p*-Bn); 127.31 (CH-*o*-Bn); 127.64 (CH-*p*-Bn); 127.66 (CH-*o*-Bn); 127.74 (CH-*p*-Bn); 128.16 (CH-*o*-Bn); 128.19, 128.28 and 128.43 (CH-*m*-Bn); 137.49 (CH-4); 138.21, 138.43 and 139.37 (C-*i*-Bn); 144.93 (CH-6); 163.28 (C-2). HRMS (ESI) *m/z* for $C_{33}H_{36}O_5N$ $[M + H]^+$: calcd 526.25880; found 526.25881. IR (ATR): ν = 3039, 2874, 1613, 1579, 1498, 1458, 1287, 1093, 1028.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-5-methylpyridine (19a)

Nucleoside **19a** was prepared from compound **15** (1 g, 1.74 mmol) following the general procedure B. The crude mixture was purified using flash column chromatography on silica gel (5 to 30 % of EtOAc in hexane) to give **19a** (685 mg, 1.34 mmol, 77%) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.92 (s, 3H, CH_3-2′); 2.28 (s, 3H, CH_3-5); 3.74 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 4.6, H-5′a); 3.82 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 3.0, H-5′b); 3.89 (d, 1H, $J_{3',4'}$ = 7.8, H-3′); 4.20 (ddd, 1H, $J_{4',3'}$ = 7.8, $J_{4',5'a}$ = 4.5, $J_{4',5'b}$ = 3.0, H-4′); 4.57 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.59 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.62 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.64 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.69 and 4.72 (2×d, 2×1H, J_{gem} = 11.9, CH_2Bn-2′); 5.01 (s, 1H, H-1′); 7.23–7.41 (m, 15H, H-*o*,*m*,*p*-Bn); 7.49 (dd, 1H, $J_{3,4}$ = 8.4, $J_{3,6}$ = 1.1, H-3); 7.51 (ddq, 1H, $J_{4,3}$ = 8.0, $J_{4,6}$ = 2.1, $J_{4,CH3}$ = 0.7, H-4); 8.37 (dpent, 1H, $J_{6,4}$ = 2.1, $J_{6,3} = J_{6,CH3}$ = 0.9, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 17.83 (CH_3-5); 19.14 (CH_3-2′); 65.11 (CH_2Bn-2′); 69.84 (CH_2-5′); 72.48 (CH_2Bn-3′); 72.61 (CH_2Bn-5′); 79.76 (CH-4′); 83.27 (CH-3′);

83.34 (C-2′); 86.29 (CH-1′); 120.95 (CH-3); 127.25 (CH-*p*-Bn); 127.39 (CH-*o*-Bn); 127.68 (CH-*p*-Bn); 127.76 (CH-*o*-Bn); 127.80 (CH-*p*-Bn); 128.10 (CH-*o*-Bn); 128.28, 128.35 and 128.49 (CH-*m*-Bn); 131.87 (C-5); 136.94 (CH-4); 138.46, 138.53 and 139.66 (C-*i*-Bn); 149.01 (CH-6); 156.59 (C-2). HRMS (ESI) *m/z* for $C_{33}H_{36}O_4N$ $[M + H]^+$: calcd 510.26389; found 510.26378. IR (ATR): ν = 3072, 3039, 2875, 1607, 1578, 1500, 1458, 1385, 1079, 1030.

5-Amino-2-(2,3,5-tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)pyridine (19b)

Mixture of compound **15** (150 mg, 0.26 mmol), Pd_2dba_3 (6 mg, 0.0065 mmol) and P(*t*-Bu)$_3$·HBF_4 (15 mg, 0.052 mmol) in dry THF (3 mL) was treated with LiHMDS (0.52 mL, 0.52 mmol; 1 M in THF). The resulting solution was stirred at 66 °C for 3 h, then cooled to r.t., and treated with 2 M HCl (3 mL) for 10 min. After washing with sat. aq. $NaHCO_3$, the mixture was extracted with EtOAc, and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified using flash column chromatography on silica gel (25 to 75 % of EtOAc in hexane) furnishing **19b** (97 mg, 0.19 mmol, 73 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.93 (s, 3H, CH_3-2′); 3.71 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 4.7, H-5′a); 3.79 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 3.0, H-5′b); 3.87 (d, 1H, $J_{3',4'}$ = 7.9, H-3′); 4.14 (ddd, 1H, $J_{4',3'}$ = 7.9, $J_{4',5'a}$ = 4.7, $J_{4',5'b}$ = 3.0, H-4′); 4.56, 4.59, 4.60 and 4.64 (4×d, 4×1H, J_{gem} = 11.9, 11.7, 12.0 and 11.7, CH_2Bn-3′,5′); 4.67 (bs, 2H, CH_2Bn-2′); 4.87 (s, 1H, H-1′); 6.28 (bs, 2H, NH_2); 6.83 (dd, 1H, $J_{4,3}$ = 8.4, $J_{4,6}$ = 2.7, H-4); 7.21 (d, 1H, $J_{3,4}$ = 8.4, H-3); 7.22–7.40 (m, 15H, H-*o*,*m*,*p*-Bn); 7.87 (bd, 1H, $J_{6,4}$ = 2.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.03 (CH_3-2′); 65.00 (CH_2Bn-2′); 70.09 (CH_2-5′); 72.47 and 72.58 (CH_2Bn-3′,5′); 79.44 (CH-4′); 83.33 (C-2′); 83.45 (CH-3′); 86.62 (CH-1′); 120.20 (CH-4); 121.62 (CH-3); 127.20 (CH-*p*-Bn); 127.35 (CH-*o*-Bn); 127.63 (CH-*p*-Bn); 127.70 (CH-*o*-Bn); 127.76 (CH-*p*-Bn); 128.08 (CH-*o*-Bn); 128.26, 128.33 and 128.44 (CH-*m*-Bn); 135.22 (CH-6); 138.54, 138.59 and 139.80 (C-*i*-Bn); 144.02 (C-5); 146.30 (C-2). HRMS (ESI) *m/z* for $C_{32}H_{34}O_4N_2Na$ $[M + Na]^+$: calcd 533.24108; found 533.24098. IR (ATR): ν = 3470, 3365, 3222, 3040, 2869, 1631, 1577, 1498, 1456, 1306, 1078, 1026.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-5-(dimethylamino)pyridine (19c)

Me_2NH (3.7 mL, 7.4 mmol; 2 M in THF) was added to a suspension of compound **15** (850 mg, 1.48 mmol), Pd_2dba_3 (34 mg, 0.037 mmol), JohnPhos (44 mg, 0.148 mmol) and *t*-BuONa (855 mg, 8.88 mmol) in dry toluene (5 mL) in a septum-sealed flask. The resulting mixture was stirred at 70 °C for 2 h, quenched by pouring into sat. aq. $NaHCO_3$, and extracted

with toluene. Organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography on silica gel (5 to 40 % of EtOAc in hexane) afforded **19c** (672 mg, 1.25 mmol, 84 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.95 (s, 3H, CH_3-2′); 2.91 (s, 6H, $(CH_3)_2N$); 3.72 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 4.8, H-5′a); 3.80 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 3.1, H-5′b); 3.90 (d, 1H, $J_{3',4'}$ = 7.8, H-3′); 4.16 (ddd, 1H, $J_{4',3'}$ = 7.8, $J_{4',5'a}$ = 4.8, $J_{4',5'b}$ = 3.1, H-4′); 4.57 (d, 1H, J_{gem} = 12.0, CH_2Bn-5′); 4.59 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.61 (d, 1H, J_{gem} = 12.0, CH_2Bn-5′); 4.64 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.68 and 4.70 (2×d, 2×1H, J_{gem} = 11.7, CH_2Bn-2′); 4.94 (s, 1H, H-1′); 6.99 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 3.1, H-4); 7.23–7.39 (m, 16H, H-*o*,*m*,*p*-Bn, H-3); 8.05 (dd, 1H, $J_{6,4}$ = 3.1, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.11 (CH_3-2′); 39.83 ($(CH_3)_2N$); 65.05 (CH_2Bn-2′); 70.12 (CH_2-5′); 72.44 (CH_2Bn-3′); 72.57 (CH_2Bn-5′); 79.62 (CH-4′); 83.30 (C-2′); 83.70 (CH-3′); 86.48 (CH-1′); 118.80 (CH-4); 121.42 (CH-3); 127.15 (CH-*p*-Bn); 127.32 (CH-*o*-Bn); 127.58 (CH-*p*-Bn); 127.70 (CH-*o*,*p*-Bn); 127.98 (CH-*o*-Bn); 128.21, 128.29 and 128.40 (CH-*m*-Bn); 133.59 (CH-6); 138.51, 138.54 and 139.75 (C-*i*-Bn); 145.31 (C-5); 146.37 (C-2). HRMS (ESI) *m/z* for $C_{34}H_{39}O_4N_2$ $[M + H]^+$: calcd 539.29043; found 539.29030. IR (CCl_4): ν = 3032, 2876, 2807, 1596, 1561, 1498, 1454, 1356, 1207, 1098, 1029.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-5-hydroxypyridine (19d)

Compound **15** (800 mg, 1.39 mmol), Pd_2dba_3 (32 mg, 0.035 mmol), $Me_4(t\text{-}Bu)_2$XPhos (67 mg, 0.139 mmol) and KOH (234 mg, 4.17 mmol) were mixed in 1,4-dioxane (3.6 mL) and water (1.2 mL), and the resulting mixture was stirred at 80 °C for 4 h. After cooling to r.t., it was diluted with EtOAc, filtered through Celite and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (10 to 50 % of EtOAc in hexane) furnishing **19d** (700 mg, 1.37 mmol, 98 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.93 (s, 3H, CH_3-2′); 3.73 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 4.6, H-5′a); 3.81 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 3.0, H-5′b); 3.88 (d, 1H, $J_{3',4'}$ = 7.8, H-3′); 4.14 (ddd, 1H, $J_{4',3'}$ = 7.8, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 3.0, H-4′); 4.57 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.59 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.60 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.69 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.68 (s, 2H, CH_2Bn-2′); 4.95 (s, 1H, H-1′); 7.05 (dd, 1H, $J_{4,3}$ = 8.5, $J_{4,6}$ = 2.9, H-4); 7.23–7.39 (m, 15H, H-*o*,*m*,*p*-Bn); 7.41 (bd, 1H, $J_{3,4}$ = 8.5, H-3); 8.08 (dd, 1H, $J_{6,4}$ = 2.9, $J_{6,3}$ = 0.6, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.04 (CH_3-2′); 65.05 (CH_2Bn-2′); 69.93 (CH_2-5′); 72.47 and 72.60 (CH_2Bn-3′,5′); 79.62 (CH-4′); 83.30 (C-2′); 83.32 (CH-3′); 86.29 (CH-1′); 122.04 (CH-3); 122.39 (CH-4); 127.21 (CH-*p*-Bn); 127.34 (CH-*o*-Bn); 127.65 (CH-*p*-Bn); 127.75 (CH-*o*,*p*-Bn); 128.07 (CH-*o*-Bn); 128.25, 128.32 and 128.44 (CH-*m*-Bn); 136.86 (CH-

6); 138.49, 138.53 and 139.71 (C-*i*-Bn); 149.77 (C-2); 152.88 (C-5). HRMS (ESI) *m/z* for $C_{32}H_{34}O_5N$ $[M + H]^+$: calcd 512.24315; found 512.24315. IR (CCl_4): ν = 3600, 3032, 2864, 1600, 1577, 1497, 1454, 1361, 1283, 1097, 1029.

2-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-5-methoxypyridine (19e)

Suspension of compound **15** (140 mg, 0.244 mmol), Pd_2dba_3 (6 mg, 0.006 mmol), $Me_4(t\text{-}Bu)_2$XPhos (12 mg, 0.024 mmol) and KOH (41 mg, 0.732 mmol) in 1,4-dioxane (0.9 mL) and water (0.3 mL) was stirred at 80 °C for 4 h. After cooling to r.t., TBAB (8 mg, 0.024 mmol), KOH (27 mg, 0.488 mmol) and CH_3I (0.03 mL, 0.488 mmol) were added, and the resulting suspension was stirred for a further 30 min at 80 °C. After cooling to r.t., it was diluted with EtOAc, filtered through Celite, and the volatiles were removed in vacuo. Purification using flash column chromatography on silica gel (6 to 13 % of EtOAc in hexane) gave **19e** (84 mg, 0.16 mmol, 66 %) as a yellowish oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.94 (s, 3H, CH_3-2′); 3.73 (dd, 1H, J_{gem} = 10.9, $J_{5'a,4'}$ = 4.7, H-5′a); 3.81 (dd, 1H, J_{gem} = 10.9, $J_{5'b,4'}$ = 3.0, H-5′b); 3.81 (s, 3H, CH_3O); 3.90 (d, 1H, $J_{3',4'}$ = 7.7, H-3′); 4.19 (ddd, 1H, $J_{4',3'}$ = 7.7, $J_{4',5'a}$ = 4.7, $J_{4',5'b}$ = 3.0, H-4′); 4.58 (d, 1H, J_{gem} = 11.9, CH_2Bn-5′); 4.59 (bd, 1H, J_{gem} = 11.6, CH_2Bn-3′); 4.62 (bd, 1H, J_{gem} = 11.8, CH_2Bn-5′); 4.64 (d, 1H, J_{gem} = 11.7, CH_2Bn-3′); 4.70 (s, 2H, CH_2Bn-2′); 5.01 (s, 1H, H-1′); 7.23–7.40 (m, 16H, H-*o*,*m*,*p*-Bn, H-4); 7.52 (dm, 1H, $J_{3,4}$ = 8.6, H-3); 8.25 (dd, 1H, $J_{6,4}$ = 3.0, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.08 (CH_3-2′); 55.65 (CH_3O); 65.10 (CH_2Bn-2′); 69.90 (CH_2-5′); 72.44 (CH_2Bn-3′); 72.59 (CH_2Bn-5′); 79.78 (CH-4′); 83.27 (C-2′); 83.41 (CH-3′); 86.10 (CH-1′); 120.78 (CH-4); 121.97 (CH-3); 127.18 (CH-*p*-Bn); 127.33 (CH-*o*-Bn); 127.62 (CH-*p*-Bn); 127.74 (CH-*o*,*p*-Bn); 128.02 (CH-*o*-Bn); 128.22, 128.30 and 128.43 (CH-*m*-Bn); 136.29 (CH-6); 138.45, 138.49 and 139.65 (C-*i*-Bn); 151.20 (C-2); 154.66 (C-5). HRMS (ESI) m/z for $C_{33}H_{35}O_5NNa$ $[M + Na]^+$: calcd 548.24074; found 548.24013. IR (CCl_4): ν = 3032, 2897, 2864, 1575, 1496, 1454, 1384, 1294, 1269, 1245, 1098, 1029.

5-(2,3,5-Tri-*O*-benzyl-2-*C*-methyl-β-D-ribofuranosyl)-1-methyl-2-pyridone (20)

Nucleoside **20** was isolated as a by-product (yellowish oil, 58 mg, 0.11 mmol, 23 %) in the synthesis of **18e**. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.03 (s, 3H, CH_3-2′); 3.32 (s, 3H, CH_3N); 3.68 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 4.6, H-5′a); 3.72 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 4.3, H-5′b); 3.85 (d, 1H, $J_{3',4'}$ = 5.4, H-3′); 4.16 (dt, 1H, $J_{4',3'}$ = 5.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.4, H-4′); 4.56 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.62–4.56 (m, 4H, CH_2Bn-2′,5′); 4.62 (d, 1H, J_{gem} = 11.5, CH_2Bn-3′); 4.69 (s, 1H, H-1′); 6.31 (bdd, 1H, $J_{3,4}$ = 9.4, $J_{3,6}$ = 0.5, H-3); 7.22–7.39 (m, 15H,

H-*o*,*m*,*p*-Bn); 7.39 (dd, 1H, $J_{4,3}$ = 9.5, $J_{4,6}$ = 2.5, H-4); 7.60 (dt, 1H, $J_{6,4}$ = 2.5, $J_{6,3}$ = $J_{6,1'}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.68 (CH_3-2′); 36.95 (CH_3N); 65.45 (CH_2Bn-2′); 69.91 (CH_2-5′); 71.73 (CH_2Bn-3′); 72.64 (CH_2Bn-5′); 80.97 (CH-4′); 81.96 (CH-1′); 82.72 (CH-3′); 82.81 (C-2′); 115.64 (C-5); 118.60 (CH-3); 127.21 (CH-*p*-Bn); 127.37 (CH-*o*-Bn); 127.72 (CH-*p*-Bn); 127.78 (CH-*o*,*p*-Bn); 127.17 and 128.19 (CH-*o*,*m*-Bn); 128.31 and 128.48 (CH-*m*-Bn); 137.36 (CH-6); 138.28 and 138.40 (C-*i*-Bn); 138.90 (CH-4); 139.45 (C-*i*-Bn); 161.70 (C-2). HRMS (ESI) *m/z* for $C_{33}H_{36}O_5N$ $[M + H]^+$: calcd 526.25880; found 526.25874. IR (CCl_4): ν = 3032, 2866, 1673, 1614, 1541, 1497, 1454, 1362, 1188, 1096, 1029.

5.3.2 Debenzylation

(2-*C*-Methyl-β-D-ribofuranosyl)benzene (21)

Method 1: catalytic hydrogenation. Compound **6** (100 mg, 0.2 mmol) and 10% Pd/C (21 mg, 0.02 mmol) were suspended in acetic acid (1 mL), and the mixure was vigorously stirred under H_2 atmosphere at r.t. for 3 h. After filtering through a paper filter, solution was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (0 to 5 % of MeOH in CH_2Cl_2) furnishing **21** (18 mg, 0.08 mmol, 40 %) as a white amorphous solid. **Method 2: treatment with BCl_3.** BCl_3 (1.3 mL, 1.28 mmol; 1 M in CH_2Cl_2) was added dropwise to a solution of **6** (210 mg, 0.425 mmol) in dry CH_2Cl_2 (2 mL) −78 °C. The mixure was stirred at −78 °C for 2 h, and then it was quenched with MeOH (1 mL) and warmed to r.t. The solvents were removed in vacuo, and the crude product was purified by flash column chromatography on silica gel (0 to 5 % of MeOH in CH_2Cl_2) to afford **21** (90 mg, 0.4 mmol, 94 %) as a white amorphous solid. $[\alpha]_D^{20}$ −9.2 (*c* 0.250, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.64 (s, 3H, CH_3); 3.49 (t, 1H, $J_{3',4'}$ = $J_{3',OH}$ = 5.8, H-3′); 3.59 (dt, 1H, J_{gem} = 11.8, $J_{5'a,4'}$ = $J_{5',OH}$ = 5.4, H-5′a); 3.68 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 3.7, H-5′b); 3.73 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'a}$ = 5.2, $J_{4',5'b}$ = 3.7, H-4′); 4.63 (s, 1H, H-1′); 4.66 (s, 1H, OH-2′); 4.81 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6, OH-5′); 5.11 (d, 1H, $J_{OH,3'}$ = 5.8, OH-3′); 7.24 (m, 1H, H-*p*-Ph); 7.30 (m, 2H, H-*m*-Ph); 7.35 (m, 2H, H-*o*-Ph). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.33 (CH_3); 61.77 (CH_2-5′); 75.66 (CH-3′); 77.63 (C-2′); 84.01 (CH-4′); 86.33 (CH-1′); 126.36 (CH-*o*-Ph); 127.17 (CH-*p*-Ph); 127.82 (CH-*m*-Ph); 139.92 (C-*i*-Ph). HRMS (ESI) *m/z* for $C_{12}H_{16}O_4Na$ $[M + Na]^+$: calcd 247.09408; found 247.09402. IR (ATR): ν = 3363, 2937, 1500, 1458, 1380, 1295, 1220, 1177, 1071, 1044, 1030.

1-Deoxy-2-*C*-methyl-1-*C*-phenylribitol (22)

Compound **22** was isolated as a by-product (white amorphous solid, 24 mg, 0.11 mmol, 53 %) in the synthesis of **21** according to the method 1 (catalytic hydrogenation). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 1.06 (s, 3H, CH$_3$-2); 2.67 (d, 1H, J_{gem} = 13.3, H-1a); 2.78 (d, 1H, J_{gem} = 13.3, H-1b); 3.11 (dd, 1H, $J_{3,4}$ = 8.3, $J_{3,OH}$ = 6.5, H-3); 3.39 (bdt, 1H, J_{gem} = 11.7, $J_{5a,4}$ = $J_{5a,OH}$ = 6.2, H-5a); 3.56–3.62 (m, 2H, H-4,5b); 4.43 (bdd, 1H, $J_{OH,5a}$ = 6.0, $J_{OH,5b}$ = 5.4, OH-5); 4.82 (d, 1H, $J_{OH,3}$ = 6.5, OH-3); 4.89 (s, 1H, OH-2); 5.22 (d, 1H, $J_{OH,4}$ = 3.6, OH-4); 7.16 (m, 1H, H-*p*-Ph); 7.20–7.27 (m, 4H, H-*o*,*m*-Ph). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 21.96 (CH$_3$-2); 45.13 (CH$_2$-1); 64.17 (CH$_2$-5); 72.68 (CH-3); 73.52 (CH-4); 75.01 (C-2); 125.72 (CH-*p*-Ph); 127.50 (CH-*m*-Ph); 131.16 (CH-*o*-Ph); 138.37 (C-*i*-Ph). HRMS (ESI) *m/z* for $C_{12}H_{18}O_4Na$ $[M + Na]^+$: calcd 249.10973; found 249.10972.

***N*,*N*-Dimethyl-4-(2-*C*-methyl-α-D-ribofuranosyl)aniline (23)**

Nucleoside **23** (white amorphous solid, 63 mg, 0.237 mmol, 37 %) was isolated as a by-product in the synthesis of **27c** according to the method 2 (treatment with BCl_3). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 1.00 (s, 3H, CH$_3$-2′); 2.87 (s, 6H, (CH$_3$)$_2$N); 3.44 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 6.3, $J_{5'a,4'}$ = 4.6, H-5′a); 3.61 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.2, $J_{5'b,4'}$ = 2.4, H-5′b); 3.76 (dd, 1H, $J_{3',4'}$ = 8.4, $J_{3',OH}$ = 7.0, H-3′); 3.80 (ddd, 1H, $J_{4',3'}$ = 8.4, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 2.4, H-4′); 3.91 (d, 1H, $J_{OH,LR}$ = 0.7, OH-2′); 4.48 (s, 1H, H-1′); 4.65 (dd, 1H, $J_{OH,5'a}$ = 6.3, $J_{OH,5'b}$ = 5.2, OH-5′); 4.82 (d, 1H, $J_{OH,3'}$ = 7.1, OH-3′); 6.66 (m, 2H, H-2,6); 7.13 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 21.14 (CH$_3$-2′); 40.49 ((CH$_3$)$_2$N); 62.54 (CH$_2$-5′); 76.54 (C-2′); 76.60 (CH-3′); 81.92 (CH-4′); 85.83 (CH-1′); 111.63 (CH-2,6); 125.99 (C-4); 128.87 (CH-3,5); 150.05 (C-1). HRMS (ESI) *m/z* for $C_{13}H_{16}O_5N$ $[M - H]^-$: calcd 266.10340; found 266.10351.

***N*,*N*-Dimethyl-4-(2-*C*-methyl-α-D-ribopyranosyl)aniline (24)**

Compound **24** was isolated as a by-product (white amorphous solid, 60 mg, 0.224 mmol, 35 %) in the synthesis of **27c** according to the method 2 (treatment with BCl_3). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.77 (s, 3H, CH$_3$-2′); 2.86 (s, 6H, (CH$_3$)$_2$N); 3.34 (dd, 1H, $J_{3',OH}$ = 8.1, $J_{3',4'}$ = 3.2, H-3′); 3.58 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 1.3, H-5′a); 3.73 (dddd, 1H, $J_{4',OH}$ = 5.2, $J_{4',3'}$ = 3.2, $J_{4',5'b}$ = 1.9, $J_{4',5'a}$ = 1.3, H-4′); 3.88 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 2.0, H-5′a); 3.96 (s, 1H, H-1′); 4.39 (bs, 1H, OH-2′); 4.52 (d, 1H, $J_{OH,3'}$ = 8.1, OH-3′); 5.39 (d, 1H, $J_{OH,4'}$ = 5.2, OH-4′); 6.63 (m, 2H, H-2,6); 7.17 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 20.89 (CH$_3$-2′); 40.41 ((CH$_3$)$_2$N); 69.98 (CH-4′); 71.28 (CH$_2$-5′); 71.92 (CH-3′); 74.50 (C-2′);

85.05 (CH-1′); 111.35 (CH-2,6); 126.28 (C-4); 129.57 (CH-3,5); 149.87 (C-1). HRMS (ESI) *m/z* for $C_{13}H_{16}O_5N$ $[M - H]^-$: calcd 266.10340; found 266.10349.

4-(2-*C*-Methyl-β-D-ribofuranosyl)cyclohexanone (25)

Compound **25** (colorless oil; 340 mg, 1.39 mmol, 48 %) was isolated as a side product in the synthesis of **27c** by catalytic hydrogenation. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.10 (s, 3H, CH_3-2′); 1.32–1.48 (m, 2H, H-3b,5b); 1.83 (tdt, 1H, $J_{4,3} = J_{4,3} = 10.7$, $J_{4,1'} = 9.2$, $J_{4,3} = J_{4,3} = 3.4$, H-4); 2.08–2.23 (m, 4H, H-3a,5a,2b,6b); 2.30–2.41 (m, 2H, H-2a,6a); 3.19 (d, 1H, $J_{1',4} = 9.2$, H-1′); 3.31 (m, 1H, H-3′); 3.40–3.53 (m, 3H, H-4′,5′); 4.29 (bs, 1H, OH-2′); 4.71 (bt, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.4$, OH-5′); 5.17 (bd, 1H, $J_{3',OH} = 4.3$, OH-3′). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 21.06 (CH_3-2′); 27.98 and 30.15 (CH_2-3,5); 35.87 (CH-4); 39.94 and 40.09 (CH_2-2,6); 62.18 (CH_2-5′); 76.08 (C-2′); 77.41 (CH-3′); 84.95 (CH-4′); 85.45 (CH-1′); 211.24 (C-1). HRMS (ESI) *m/z* for $C_{12}H_{19}O_5$ $[M - H]^-$: calcd 243.12380; found 243.12350. IR (ATR): ν = 3390, 2941, 2874, 1708, 1455, 1339, 1176, 1133, 1035.

5-(5-*O*-Benzyl-2-*C*-methyl-β-D-ribofuranosyl)-2-methylpyridine (26)

BCl_3 (1.8 mL, 1.77 mmol; 1 M in CH_2Cl_2) was slowly added to a solution of **18a** (300 mg, 0.589 mmol) in dry CH_2Cl_2 (3 mL) at −78 °C. Then the mixture was stirred at this temperature for 4 h. Subsequently, MeOH (1.5 mL) was added, and solution was warmed to r.t. and concentrated in vacuo. Purification by flash column chromatography on silica gel (0 to 10 % of MeOH in CH_2Cl_2) afforded **26** (162 mg, 0.492 mmol, 84%) as a white solid (m.p. 101–102 °C). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.66 (s, 3H, CH_3-2′); 2.44 (s, 3H, CH_3-2); 3.53 (t, 1H, $J_{3',4'} = J_{3',OH} = 6.1$, H-3′); 3.67 (dd, 1H, $J_{gem} = 10.9$, $J_{5'a,4'} = 3.2$, H-5′a); 3.74 (dd, 1H, $J_{gem} = 10.9$, $J_{5'b,4'} = 3.2$, H-5′b); 3.91 (ddd, 1H, $J_{4',3'} = 6.4$, $J_{4',5'a} = 5.5$, $J_{4',5'b} = 3.2$, H-4′); 4.59 (s, 2H, CH_2Bn); 4.68 (s, 1H, H-1′); 4.81 (m, 1H, OH-2′); 5.25 (dm, 1H, $J_{OH,3'} = 5.9$, OH-3′); 7.17 (dm, 1H, $J_{3,4} = 7.9$, H-3); 7.30 (m, 1H, H-*p*-Bn); 7.34–7.40 (m, 4H, H-*o*,*m*-Bn); 7.60 (ddd, 1H, $J_{4,3} = 7.9$, $J_{4,6} = 2.3$, $J_{4,1'} = 0.8$, H-4); 8.36 (dt, 1H, $J_{6,4} = 2.3$, $J_{6,3} = J_{6,1'} = 0.8$, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.30 (CH_3-2′); 23.91 (CH_3-2); 70.33 (CH_2-5′); 72.53 (CH_2-Bn); 75.65 (CH-3′); 77.60 (C-2′); 82.20 (CH-4′); 84.81 (CH-1′); 122.47 (CH-3); 127.58 (CH-*o*-Bn); 127.60 (CH-*p*-Bn); 128.46 (CH-*m*-Bn); 132.20 (C-5); 134.27 (CH-4); 138.64 (C-*i*-Bn); 147.02 (CH-6); 156.88 (C-2). HRMS (ESI) *m/z* for $C_{19}H_{24}O_4N$ $[M + H]^+$: calcd 330.16998; found 330.16986. IR (ATR): ν = 3420, 3071, 2805, 1505, 1454, 1305, 1151, 1090, 1018.

4-Methyl-1-(2-*C*-methyl-β-D-ribofuranosyl)benzene (27a)

Mixture of **17a** (300 mg, 0.59 mmol) and 5% Pd/C (251 mg, 0.059 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (3 mL) was vigorously stirred under H_2 atmosphere for 6 h at r.t. Then it was filtered through a paper filter, and filtrate was concentrated in vacuo. The residue was purified using flash column chromatography on silica gel (0 to 5 % of MeOH in CH_2Cl_2) furnishing **27a** (131 mg, 0.55 mmol, 93 %) as a white amorphous solid. $[\alpha]_D^{20}$ −7.7 (*c* 0.222, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.63 (s, 3H, CH_3-2′); 2.28 (s, 3H, CH_3-Ph); 3.47 (t, 1H, $J_{3',4'} = J_{3',OH}$ = 5.8, H-3′); 3.57 (dt, 1H, J_{gem} = 11.7, $J_{5'a,4'} = J_{5'a,OH}$ = 5.4, H-5′a); 3.66 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 3.7, H-5′b); 3.70 (btd, 1H, $J_{4',5'a} = J_{4',3'}$ = 5.5, $J_{4',5'b}$ = 3.7, H-4′); 4.58 (s, 1H, H-1′); 4.62 (s, 1H, OH-2′); 4.80 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.7, OH-5′); 5.09 (d, 1H, $J_{3',OH}$ = 5.8, OH-3′); 7.11 (m, 2H, H-3,5); 7.22 (m, 2H, H-2,6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 21.23 (CH_3-Ph); 22.64 (CH_3-2′); 62.09 (CH_2-5′); 75.95 (CH-3′); 77.85 (C-2′); 84.19 (CH-4′); 86.62 (CH-1′); 126.58 (CH-2,6); 128.69 (CH-3,5); 136.36 (C-4); 137.20 (C-1). HRMS (ESI) *m/z* for $C_{13}H_{18}O_4Na$ $[M + Na]^+$: calcd 261.10973; found 261.10975. IR (ATR): ν = 3369, 2932, 1521, 1455, 1381, 1180, 1039, 1024.

4-(2-*C*-Methyl-β-D-ribofuranosyl)aniline (27b)

Mixture of **17b** (400 mg, 0.785 mmol) and 5% Pd/C (176 mg, 0.039 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (4 mL) was vigorously stirred under H_2 atmosphere at r.t. for 3 days. Then it was filtered through a paper filter, and filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (0 to 3 % of MeOH in EtOAc) giving **27b** (140 mg, 0.585 mmol, 75 %) as a yellowish amorphous solid. $[\alpha]_D^{20}$ −4.2 (*c* 0.283, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.65 (s, 3H, CH_3-2′); 3.43 (t, 1H, $J_{3',4'} = J_{3',OH}$ = 6.0, H-3′); 3.55 (bdt, 1H, J_{gem} = 12.3, $J_{5'a,4'} = J_{5'a,OH}$ = 6.0, H-5′a); 3.63–3.70 (m, 2H, H-4′,5′b); 4.46 (s, 1H, H-1′); 4.46 (s, 1H, OH-2′); 4.74 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.5, OH-5′); 4.92 (bs, 2H, NH_2); 4.98 (d, 1H, $J_{OH,3'}$ = 6.0, OH-3′); 6.49 (m, 2H, H-2,6); 6.96 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.53 (CH_3-2′); 61.95 (CH_2-5′); 75.63 (CH-3′); 77.60 (C-2′); 83.35 (CH-4′); 87.17 (CH-1′); 113.41 (CH-2,6); 127.11 (C-4); 127.23 (CH-3,5); 147.95 (C-1). HRMS (ESI) *m/z* for $C_{12}H_{17}O_4NNa$ $[M + Na]^+$: calcd 262.10498; found 262.10504. IR (ATR): ν = 3404, 3331, 3255, 2896, 1619, 1521, 1451, 1384, 1275, 1162, 1076, 1024.

***N*,*N*-Dimethyl-4-(2-*C*-methyl-β-D-ribofuranosyl)aniline (27c)**

Method 1: catalytic hydrogenation. Compound **17c** (1.56 g, 2.9 mmol) and 10% Pd/C (309 mg, 0.29 mmol) were mixed in acetic acid (16 mL), and then the suspension was vigorously stirred under H_2 atmosphere at r.t. for 20 h. After filtering through a paper filter, the solvent was removed in vacuo. The crude residue was purified by HPLC (0 to 100 % of MeOH in water) giving **27c** (333 mg, 1.25 mmol, 43 %) as a white solid. **Method 2: treatment with BCl_3.** BCl_3 (1.92 mL, 1.92 mmol; 1 M in CH_2Cl_2) was slowly added to a solution of **17c** (341 mg, 0.64 mmol) in dry CH_2Cl_2 (4 mL) −78 °C. After stirring at −78 °C for 1 h, MeOH (2 mL) was added, and the reaction mixture was warmed to r.t. The volatiles were removed under reduced pressure, and the crude product was purified by HPLC (0 to 100 % of MeOH in water) to afford **27c** (36 mg, 0.134 mmol, 21 %) as a white solid (m.p. 126–127 °C). $[\alpha]_D^{20}$ −9.6 (*c* 0.198, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.65 (s, 3H, CH_3-2′); 2.86 (s, 6H, $(CH_3)_2$N); 3.46 (m, 1H, H-3′); 3.57 (m, 1H, H-5′a); 3.63–3.70 (m, 2H, H-4′,5′b); 4.50 (bs, 1H, OH-2′); 4.52 (s, 1H, H-1′); 4.75 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.6, OH-5′); 5.00 (bs, 1H, OH-3′); 6.66 (m, 2H, H-2,6); 7.13 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.48 (CH_3-2′); 40.38 ($(CH_3)_2$N); 61.90 (CH_2-5′); 75.65 (CH-3′); 77.62 (C-2′); 83.51 (CH-4′); 86.82 (CH-1′); 111.86 (CH-2,6); 127.19 (C-4); 127.52 (CH-3,5); 149.74 (C-1). HRMS (ESI) *m/z* for $C_{14}H_{21}O_4NNa$ $[M + Na]^+$: calcd 290.13628; found 290.13635. IR (ATR): ν = 3503, 3340, 2894, 2810, 1621, 1530, 1452, 1357, 1233, 1118, 1074, 1049.

4-(2-*C*-Methyl-β-D-ribofuranosyl)phenol (27d)

Mixture of **17d** (570 mg, 1.12 mmol) and 5% Pd/C (477 mg, 0.112 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (6 mL) was vigorously stirred under H_2 atmosphere at r.t. for 2 h. Then, the reaction mixture was filtered through a paper filter, and filtrate was concentrated in vacuo. The residue was purified using flash column chromatography on silica gel (0 to 10 % of MeOH in CH_2Cl_2) furnishing **27d** (244 mg, 1.02 mmol, 91 %) as a white solid (m.p. 179–181 °C). $[\alpha]_D^{20}$ −6.6 (*c* 0.288, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.64 (s, 3H, CH_3-2′); 3.45 (t, 1H, $J_{3',4'} = J_{3',OH}$ = 5.9, H-3′); 3.56 (m, 1H, H-5′a); 3.63–3.70 (m, 2H, H-4′,5′b); 4.52 (s, 1H, H-1′); 5.55 (s, 1H, OH-2′); 4.78 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.6, OH-5′); 5.03 (d, 1H, $J_{3',OH}$ = 5.9, OH-3′); 6.68 (m, 2H, H-2,6); 7.11 (m, 2H, H-3,5); 9.24 (s, 1H, OH-1). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.43 (CH_3-2′); 61.85 (CH_2-5′); 75.58 (CH-3′); 77.60 (C-2′); 83.63 (CH-4′); 86.61 (CH-1′); 114.64 (CH-2,6); 127.57 (CH-3,5); 130.24 (C-4); 156.51 (C-1). HRMS (ESI) *m/z* for $C_{12}H_{15}O_5$ $[M - H]^-$: calcd 239.09250; found

239.09258. IR (ATR): ν = 3279, 3166, 2940, 2886, 1620, 1523, 1474, 1387, 1272, 1229, 1117, 1075, 1043.

4-(2-*C*-Methyl-β-D-ribofuranosyl)anisole (27e)

Mixture of **17e** (220 mg, 0.42 mmol) and 5% Pd/C (89 mg, 0.021 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (2 mL) was vigorously stirred under H_2 atmosphere at r.t. for 6 h. Then the reaction mixture was filtered through a paper filter, and solution was concentrated in vacuo. The residue was purified using flash column chromatography on silica gel (0 to 10 % of MeOH in EtOAc) furnishing **27e** (97 mg, 0.38 mmol, 91 %) as a white solid (m.p. 79–80 °C). $[\alpha]_D^{20}$ −5.2 (*c* 0.317, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.64 (s, 3H, CH_3-2′); 3.47 (d, 1H, $J_{3',4'}$ = 5.9, H-3′); 3.47 (dd, 1H, J_{gem} = 11.5, $J_{5'a,4'}$ = 5.0, H-5′a); 3.67 (dd, 1H, J_{gem} = 11.5, $J_{5'b,4'}$ = 3.6, H-5′b); 3.70 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'a}$ = 5.0, $J_{4',5'b}$ = 3.6, H-4′); 3.73 (s, 3H, CH_3O); 4.57 (s, 1H, H-1′); 6.87 (m, 2H, H-2,6); 7.25 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.37 (CH_3-2′); 55.14 (CH_3-O); 61.78 (CH_2-5′); 75.57 (CH-3′); 77.60 (C-2′); 83.81 (CH-4′); 86.25 (CH-1′); 113.24 (CH-2,6); 127.54 (CH-3,5); 131.92 (C-4); 158.47 (C-1). HRMS (ESI) *m/z* for $C_{13}H_{18}O_5Na$ $[M + Na]^+$: calcd 277.10464; found 277.10462. IR (ATR): ν = 3379, 2975, 1619, 1519, 1464, 1295, 1260, 1164, 1120, 1057.

2-Methyl-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (28a)

Mixture of **18a** (420 mg, 0.85 mmol) and 5% Pd/C (362 mg, 0.085 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (5 mL) was vigorously stirred under H_2 atmosphere at r.t. for 2 days. Then the reaction mixture was filtered through a paper filter, and the solvent was removed in vacuo. Purification of the residue by flash column chromatography on silica gel (0 to 15 % of MeOH in EtOAc) furnished **28a** (162 mg, 0.68 mmol, 80 %) as a yellowish solid (m.p. 155–158 °C). $[\alpha]_D^{20}$ −11.5 (*c* 0.253, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.66 (s, 3H, CH_3-2′); 2.44 (s, 3H, CH_3-2); 3.52 (t, 1H, $J_{3',4'} = J_{3',OH}$ = 5.7, H-3′); 3.58 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,4'}$ = 5.8, $J_{5'a,OH}$ = 5.0, H-5′a); 3.67 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 3.7, H-5′b); 3.73 (ddd, 1H, $J_{4',3'}$ = 5.8, $J_{4',5'a}$ = 5.0, $J_{4',5'b}$ = 3.7, H-4′); 4.63 (s, 1H, H-1′); 4.74 (s, 1H, OH-2′); 4.85 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.7, OH-5′); 5.17 (d, 1H, $J_{OH,3'}$ = 5.7, OH-3′); 7.20 (dm, 1H, $J_{3,4}$ = 7.9, H-3); 7.62 (ddd, 1H, $J_{4,3}$ = 7.9, $J_{4,6}$ = 2.3, $J_{4,1'}$ = 0.8, H-4); 8.39 (dt, 1H, $J_{6,4}$ = 2.3, $J_{6,3} = J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.24 (CH_3-2′); 23.93 (CH_3-2); 61.57 (CH_2-5′); 75.49 (CH-3′); 77.67 (C-2′); 84.30 and 84.34 (CH-1′,4′); 122.43 (CH-3); 132.17 (C-5); 134.40 (CH-4); 147.08 (CH-6); 156.78 (C-2). HRMS (ESI) *m/z* for

$C_{12}H_{18}O_4N$ $[M + H]^+$: calcd 240.12303; found 240.12297. IR (ATR): ν = 3464, 3350, 2943, 2886, 1610, 1501, 1451, 1308, 1246, 1132, 1028.

2-Amino-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (28b)

BCl_3 (3.9 mL, 3.9 mmol; 1 M in CH_2Cl_2) was slowly added to a solution of **18b** (200 mg, 0.39 mmol) in dry CH_2Cl_2 (2 mL) at 0 °C. After stirring for 1 h at 0 °C, the mixture was warmed to r.t., and stirring was continued for one more hour. After addition of MeOH (1 mL), the solvents were removed under reduced pressure. The crude product was purified using flash column chromatography on silica gel (0 to 30 % of MeOH in EtOAc) to furnish **28b** (89 mg, 0.37 mmol, 95 %) as a yellowish amorphous solid. $[\alpha]_D^{20}$ −6.2 (*c* 0.273, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.70 (s, 3H, CH_3-2′); 3.47 (t, 1H, $J_{3',4'} = J_{3',OH}$ = 5.7, H-3′); 3.54 (m, 1H, H-5′a); 3.62–3.68 (m, 2H, H-5′b,4′); 4.46 (s, 1H, H-1′); 4.57 (s, 1H, OH-2′); 4.78 (bt, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.5, OH-5′); 5.06 (d, 1H, $J_{OH,3'}$ = 5.7, OH-3′); 5.79 (bs, 2H, NH_2); 6.38 (d, 1H, $J_{3,4}$ = 8.5, H-3); 7.32 (dd, 1H, $J_{4,3}$ = 8.5, $J_{4,6}$ = 2.4, H-4); 7.81 (d, 1H, $J_{6,4}$ = 2.4, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.43 (CH_3-2′); 61.72 (CH_2-5′); 75.50 (CH-3′); 77.58 (C-2′); 83.69 (CH-4′); 85.18 (CH-1′); 107.32 (CH-3); 122.91 (C-5); 135.74 (CH-4); 146.00 (CH-6); 159.33 (C-2). HRMS (ESI) *m/z* for $C_{11}H_{17}O_4N_2$ $[M + H]^+$: calcd 241.11828; found 241.11829. IR (ATR): 3353, 3232, 2936, 1628, 1574, 1510, 1457, 1416, 1026.

2-(Dimethylamino)-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (28c)

Suspension of **18c** (850 mg, 1.58 mmol) and 5% Pd/C (337 mg, 0.08 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (9 mL) was vigorously stirred under H_2 atmosphere at r.t. for 3 days. Then it was filtered through a paper filter, and the solvent was removed in vacuo. The crude product was purified using flash column chromatography on silica gel (0 to 10 % of MeOH in CH_2Cl_2) furnishing **28c** (233 mg, 0.87 mmol, 55 %) as a white solid (m.p. 163–165 °C). $[\alpha]_D^{20}$ −4.5 (*c* 0.246, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.69 (s, 3H, CH_3-2′); 2.99 (s, 6H, $(CH_3)_2N$); 3.46 (d, 1H, $J_{3',4'}$ = 6.1, H-3′); 3.55 (m, 1H, H-5′a); 3.66 (m, 1H, H-5′b); 3.68 (bddd, 1H, $J_{4',3'}$ = 6.1, $J_{4',5'}$ = 4.8 and 3.5, H-4′); 4.52 (s, 1H, H-1′); 4.60 (s, 1H, OH-2′); 4.80 (m, 1H, OH-5′); 5.07 (bs, 1H, OH-3′); 6.58 (bd, 1H, $J_{3,4}$ = 8.8, H-3); 7.46 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.4, H-4); 8.00 (d, 1H, $J_{6,4}$ = 2.4, H-6). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.45 (CH_3-2′); 37.90 ($(CH_3)_2N$); 61.66 (CH_2-5′); 75.42 (CH-3′); 77.70 (C-2′); 83.67 (CH-4′); 85.11 (CH-1′); 105.09 (CH-3); 122.55 (C-5); 135.91 (CH-4); 145.92 (CH-6); 158.77 (C-2). HRMS (ESI) *m/z* for $C_{13}H_{21}O_4N_2$ $[M + H]^+$: calcd 269.14958; found 269.14957. IR (ATR): ν = 3339, 3192, 2929, 1623, 1534, 1444, 1414, 1331, 1266, 1081.

5-(2-*C*-Methyl-β-D-ribofuranosyl)-2-pyridone (28d)

Free nucleoside **28d** was prepared by deprotection of **18d** (102 mg, 0.2 mmol) according to the general procedure C. Purification by flash column chromatography on silica gel (0 to 20 % of MeOH in EtOAc) afforded **28d** (44 mg, 0.184 mmol, 92 %) as a white solid (m.p. 79–81 °C). $[\alpha]_D^{20}$ −29.1 (*c* 0.234, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.77 (s, 3H, CH$_3$-2′); 3.48 (bd, 1H, $J_{3',4'}$ = 5.9, H-3′); 3.52 (m, 1H, H-5′a); 3.61–3.68 (m, 2H, H-5′b,4′); 4.40 (s, 1H, H-1′); 4.61 (s, 1H, OH-2′); 4.81 (m, 1H, OH-5′); 5.08 (bs, 1H, OH-3′); 6.29 (dd, 1H, $J_{3,4}$ = 9.4, $J_{3,6}$ = 0.6, H-3); 7.27 (dt, 1H, $J_{6,4}$ = 2.6, $J_{6,3}$ = $J_{6,1'}$ = 0.8, H-6); 7.40 (dd, 1H, $J_{4,3}$ = 9.4, $J_{4,6}$ = 2.6, H-4); 11.49 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.10 (CH$_3$-2′); 61.32 (CH$_2$-5′); 75.13 (CH-3′); 77.57 (C-2′); 83.78 (CH-1′,4′); 116.62 (C-5); 119.34 (CH-3); 132.43 (CH-6); 140.19 (CH-4); 162.36 (C-2). HRMS (ESI) *m/z* for $C_{11}H_{15}O_5NNa$ $[M + Na]^+$: calcd 264.08424; found 264.08432. IR (ATR): ν = 3266, 3154, 2885, 1662, 1614, 1551, 1460, 1425, 1073.

2-Methoxy-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (28e)

Mixture of **18e** (500 mg, 0.95 mmol) and 5% Pd/C (404 mg, 0.095 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (5 mL) was vigorously stirred under H_2 atmosphere at r.t. for 1 day. Then it was filtered through a paper filter, and the solvent was evaporated under reduced pressure. Purification of the residue using flash column chromatography on silica gel (0 to 3 % of MeOH in EtOAc) furnished **28e** (215 mg, 0.84 mmol, 89 %) as a white amorphous solid. $[\alpha]_D^{20}$ −6.7 (*c* 0.255, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.68 (s, 3H, CH$_3$-2′); 3.53 (t, 1H, $J_{3',4'}$ = $J_{3'OH'}$ = 5.8, H-3′); 3.57 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.8, $J_{5'a,4'}$ = 4.8, H-5′a); 3.67 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.5, $J_{5'b,4'}$ = 3.6, H-5′b); 3.72 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'a}$ = 4.8, $J_{4',5'b}$ = 3.6, H-4′); 3.83 (s, 3H, CH$_3$O); 4.62 (s, 1H, H-1′); 4.69 (s, 1H, OH-2′); 4.82 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6, OH-5′); 5.11 (d, 1H, $J_{OH,3'}$ = 5.8, OH-3′); 6.77 (dd, 1H, $J_{3,4}$ = 8.5, $J_{3,6}$ = 0.9, H-3); 7.67 (ddd, 1H, $J_{4,3}$ = 8.5, $J_{4,6}$ = 2.4, $J_{4,1'}$ = 0.6, H-4); 8.10 (dt, 1H, $J_{6,4}$ = 2.4, $J_{6,3}$ = $J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.24 (CH$_3$-2′); 53.20 (CH$_3$O); 61.48 (CH$_2$-5′); 75.32 (CH-3′); 77.66 (C-2′); 84.06 (CH-4′); 84.30 (CH-1′); 109.80 (CH-3); 128.41 (C-5); 137.56 (CH-4); 144.78 (CH-6); 163.14 (C-2). HRMS (ESI) *m/z* for $C_{12}H_{18}O_5N$ $[M + H]^+$: calcd 256.11795; found 256.11797. IR (ATR): ν = 3359, 2935, 1614, 1579, 1499, 1457, 1399, 1288, 1259, 1068, 1024.

5-Methyl-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (29a)

Mixture of **19a** (430 mg, 0.844 mmol) and 5% Pd/C (360 mg, 0.0844 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (5 mL) was vigorously stirred under H_2 atmosphere at r.t. for 1 day. Then the mixture was filtered through a paper filter, and the filtrate was concentrated in vacuo. Purification of the crude residue by flash column chromatography on silica gel (0 to 5 % of MeOH in CH_2Cl_2) gave **29a** (175 mg, 0.731 mmol, 87 %) as a yellowish amorphous solid. $[\alpha]_D^{20}$ −22.0 (*c* 0.286, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.68 (s, 3H, CH_3-2′); 2.27 (s, 3H, CH_3-5); 3.60 (m, 1H, H-5′a); 3.69 (dd, 1H, $J_{3',4'}$ = 8.2, $J_{3',OH}$ = 6.9, H-3); 3.77 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 4.1, $J_{5'b,4'}$ = 2.6, H-5′b); 3.80 (ddd, 1H, $J_{4',3'}$ = 8.2, $J_{4',5'a}$ = 3.8, $J_{4',5'b}$ = 2.6, H-4′); 4.70 (s, 1H, OH-2′); 4.75 (s, 1H, H-1′); 4.93 (d, 1H, $J_{OH,3'}$ = 6.9, OH-3′); 5.20 (dd, 1H, $J_{OH,5'a}$ = 6.5, $J_{OH,5'b}$ = 4.1, OH-5′); 7.41 (dd, 1H, $J_{3,4}$ = 8.0, $J_{3,6}$ = 0.8, H-3); 7.58 (ddq, 1H, $J_{4,3}$ = 8.0, $J_{4,6}$ = 2.3, $J_{4,CH3}$ = 0.8, H-4); 8.37 (dpent, 1H, $J_{6,4}$ = 2.3, $J_{6,3}$ = $J_{6,CH3}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 17.81 (CH_3-5); 22.19 (CH_3-2′); 61.10 (CH_2-5′); 73.90 (CH-3′); 78.56 (C-2′); 82.37 (CH-4′); 88.74 (CH-1′); 121.31 (CH-3); 131.79 (C-5); 137.08 (CH-4); 148.70 (CH-6); 157.83 (C-2). HRMS (ESI) *m/z* for $C_{12}H_{18}O_4N$ $[M + H]^+$: calcd 240.12303; found 240.12296. IR (ATR): ν = 3330, 2933, 1610, 1579, 1497, 1456, 1382, 1294, 1073, 1048, 1021.

5-Amino-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (29b)

Compound **29b** was prepared from benzyl-protected nucleoside **19b** (102 mg, 0.2 mmol) following the general procedure C. The residue was purified by flash column chromatography on silica gel (0 to 25 % of MeOH in EtOAc) to furnish **29b** (47 mg, 0.196 mmol, 98 %) as a white amorphous solid. $[\alpha]_D^{20}$ −18 (*c* 0.316, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.69 (s, 3H, CH_3-2′); 3.56 (m, 1H, H-5′a); 3.72–3.79 (m, 3H, H-5′b,4′,3′); 4.56 (s, 1H, OH-2′); 4.61 (s, 1H, H-1′); 4.86 (d, 1H, $J_{OH,3'}$ = 6.3, OH-3′); 5.27 (bs, 2H NH_2); 5.43 (bs, 1H, OH-5′); 6.89 (dd, 1H, $J_{4,3}$ = 8.3, $J_{4,6}$ = 2.7, H-4); 7.08 (bd, 1H, $J_{3,4}$ = 8.3, H-3); 7.82 (dd, 1H, $J_{6,4}$ = 2.7, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.05 (CH_3-2′); 61.24 (CH_2-5′); 73.66 (CH-3′); 78.69 (C-2′); 82.14 (CH-4′); 88.61 (CH-1′); 120.49 (CH-4); 122.12 (CH-3); 134.86 (CH-6); 144.06 (C-5); 147.56 (C-2). HRMS (ESI) *m/z* for $C_{11}H_{17}O_4N_2$ $[M + H]^+$: calcd 241.11828; found 241.11811. IR (ATR): ν = 3352, 3238, 2918, 1634, 1606, 1501, 1305, 1073, 1048, 1013.

5-(Dimethylamino)-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (29c)

Free nucleoside **29c** was prepared from **19c** (108 mg, 0.2 mmol) according to the general procedure C. Purification of the crude mixture using flash column chromatography on silica gel (0 to 40 % of MeOH in EtOAc) afforded **29c** (49 mg, 0.182 mmol, 91 %) as a white solid (m.p. 143–144 °C). $[\alpha]_D^{20}$ −21.5 (*c* 0.256, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.69 (s, 3H, CH$_3$-2′); 2.91 (s, 6H, (CH$_3$)$_2$N); 3.58 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = 6.7, $J_{5',4'}$ = 3.4, H-5′a); 3.73–3.81 (m, 3H, H-5′b,4′,3′); 4.60 (s, 1H, OH-2′); 4.69 (s, 1H, H-1′); 4.88 (d, 1H, $J_{OH,3'}$ = 6.3, OH-3′); 5.35 (bdd, 1H, $J_{OH,5'a}$ = 6.7, $J_{OH,5'b}$ = 3.4, OH-5′); 7.08 (dd, 1H, $J_{4,3}$ = 8.7, $J_{4,6}$ = 3.1, H-4); 7.26 (bdt, 1H, $J_{3,4}$ = 8.7, $J_{3,6}$ = $J_{3,1'}$ = 0.6, H-3); 7.99 (dd, 1H, $J_{6,4}$ = 3.1, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.09 (CH$_3$-2′); 39.92 ((CH$_3$)$_2$N); 61.25 (CH$_2$-5′); 73.75 (CH-3′); 78.71 (C-2′); 82.20 (CH-4′); 88.57 (CH-1′); 119.20 (CH-4); 121.85 (CH-3); 133.21 (CH-6); 145.38 (C-5); 147.76 (C-2). HRMS (ESI) *m/z* for C$_{13}$H$_{21}$O$_4$N$_2$ $[M + H]^+$: calcd 269.14958; found 269.14969. IR (ATR): ν = 3410, 2887, 1603, 1562, 1508, 1452, 1365, 1311, 1218, 1104, 1060.

5-Hydroxy-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (29d)

Mixture of **19d** (170 mg, 0.33 mmol) and 5% Pd/C (72 mg, 0.017 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (2 mL) was vigorously stirred under H$_2$ atmosphere at r.t. for 1 day. Then, the mixture was filtered through a paper filter, and the volatiles were removed under reduced pressure. The crude residue was purified using flash column chromatography on silica gel (0 to 10 % of MeOH in EtOAc) furnishing **29d** (77 mg, 0.32 mmol, 97 %) as a white amorphous solid. $[\alpha]_D^{20}$ −22.3 (*c* 0.292, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.68 (s, 3H, CH$_3$-2′); 3.58 (m, 1H, H-5′a); 3.69 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',OH}$ = 6.7, H-3′); 3.75 (m, 1H, H-5′b); 3.78 (ddd, 1H, $J_{4',3'}$ = 8.3, $J_{4',5'a}$ = 3.8, $J_{4',5'b}$ = 2.5, H-3′); 4.61 (s, 1H, OH-2′); 4.69 (s, 1H, H-1′); 4.88 (d, 1H, $J_{OH,3'}$ = 6.8, OH-3′); 5.14 (bdd, 1H, $J_{OH,5'a}$ = 6.6, $J_{OH,5'b}$ = 4.1, OH-5′); 7.13 (dd, 1H, $J_{4,3}$ = 8.5, $J_{4,6}$ = 2.8, H-4); 7.31 (d, 1H, $J_{3,4}$ = 8.5, H-3); 8.03 (dd, 1H, $J_{6,4}$ = 2.8, $J_{6,3}$ = 0.7, H-6); 9.84 (bs, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.12 (CH$_3$-2′); 61.21 (CH$_2$-5′); 73.91 (CH-3′); 78.54 (C-2′); 82.27 (CH-4′); 88.59 (CH-1′); 122.36 (CH-3); 122.65 (CH-4); 136.51 (CH-6); 151.07 (C-2); 152.84 (C-5). HRMS (ESI) *m/z* for C$_{11}$H$_{15}$O$_5$NNa $[M + Na]^+$: calcd 264.08424; found 264.08431. IR (ATR): ν = 3354, 2882, 1583, 1501, 1454, 1277, 1123, 1073.

5-Methoxy-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine (29e)

Mixture of **19e** (194 mg, 0.37 mmol) and 5% Pd/C (78 mg, 0.019 mmol; "eggshell", unreduced form, 50% wet) in acetic acid (2 mL) was vigorously stirred under H_2 atmosphere at r.t. for 1 day. Then it was filtered through a paper filter, and the filtrate was concentrated in vacuo. Purification of the crude residue using flash column chromatography on silica gel (0 to 10 % of MeOH in EtOAc) gave **29e** (72 mg, 0.28 mmol, 76 %) as a white amorphous solid. $[\alpha]_D^{20}$ −18.9 (*c* 0.312, MeOH). ^{1}H NMR (500 MHz, DMSO-d$_6$): δ = 0.68 (s, 3H, CH_3-2′); 3.60 (m, 1H, H-5′a); 3.68 (dd, 1H, $J_{3',4'}$ = 8.3, $J_{3',OH}$ = 6.9, H-3′); 3.76 (m, 1H, H-5′b); 3.79 (ddd, 1H, $J_{4',3'}$ = 8.3, $J_{4',5'a}$ = 3.9, $J_{4',5'b}$ = 2.5, H-4′); 3.81 (s, 3H, CH_3O); 4.67 (s, 1H, OH-2′); 4.74 (s, 1H, H-1′); 4.92 (d, 1H, $J_{OH,3'}$ = 6.9, OH-3′); 5.09 (dd, 1H, $J_{OH,5'a}$ = 6.3, $J_{OH,5'b}$ = 4.3, OH-5′); 7.36 (dd, 1H, $J_{4,3}$ = 8.6, $J_{4,6}$ = 3.0, H-4); 7.47 (bd, 1H, $J_{3,4}$ = 8.6, H-3); 8.20 (dd, 1H, $J_{6,4}$ = 3.0, $J_{6,3}$ = 0.7, H-6). ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 22.18 (CH_3-2′); 55.72 (CH_3O); 61.12 (CH_2-5′); 73.93 (CH-3′); 78.53 (C-2′); 82.31 (CH-4′); 88.57 (CH-1′); 121.12 (CH-4); 122.21 (CH-3); 135.90 (CH-6); 152.65 (C-2); 154.64 (C-5). HRMS (ESI) *m/z* for $C_{12}H_{17}O_5NNa$ $[M + H]^+$: calcd 278.09989; found 278.09993. IR (ATR): ν = 3334, 2939, 1580, 1495, 1461, 1403, 1275, 1122, 1073, 1018.

5.3.3 Nucleoside triphosphates

(2-*C*-Methyl-β-D-ribofuranosyl)benzene 5′-*O*-triphosphate (30)

Triphosphate **30** was prepared from nucleoside **21** (45 mg, 0.2 mmol) according to the general procedure D. Then it was converted to a sodium salt form (Dowex 50WX8 in Na^+ cycle), and subsequent lyophilization from water furnished triphosphate **30** (99 mg, 0.186 mmol, 93 %; trisodium salt) as a white powder. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.87 (s, 3H, CH_3-2′); 3.99 (d, 1H, $J_{3',4'}$ = 7.3, H-3′); 4.14 (dddd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = 4.9, $J_{4',5'b}$ = 3.1, $J_{4',P}$ = 1.2, H-4′); 4.28 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,P}$ = 6.5, $J_{5'a,4'}$ = 4.9, H-5′a); 4.36 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,P}$ = 5.6, $J_{5'b,4'}$ = 3.1, H-5′b); 4.91 (s, 1H, H-1′); 7.36–7.50 (m, 5H, H-*o*,*m*,*p*-Ph). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 21.72 (CH_3-2′); 65.91 (d, $J_{C,P}$ = 5.6, CH_2-5′); 75.07 (CH-3′); 79.42 (C-2′); 81.36 (d, $J_{C,P}$ = 8.8, CH-4′); 88.69 (CH-1′); 127.44 (CH-*o*-Ph); 128.80 (CH-*p*-Ph); 129.03 (CH-*m*-Ph); 138.73 (C-*i*-Ph). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.59 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.6, P_β); −10.94 (d, 1P, $J_{\alpha,\beta}$ = 19.5, P_α); −9.45 (d, 1P, $J_{\gamma,\beta}$ = 19.7, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{16}O_{13}Na_2P_3$ $[M - Na]^-$: calcd 506.95937; found 506.95962.

4-Methyl-1-(2-*C*-methyl-β-D-ribofuranosyl)benzene 5′-*O*-triphosphate (31a)

Compound **31a** was prepared from nucleoside **27a** (48 mg, 0.2 mmol) following the general procedure D. Lyophilization from water gave triphosphate **31a** (94 mg, 0.12 mmol, 60 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.86 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, $(C\mathbf{H_3}CH_2)_3NH^+$); 2.34 (s, 3H, CH_3-Ph); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, $(CH_3C\mathbf{H_2})_3NH^+$); 3.98 (d, 1H, $J_{3',4'}$ = 7.3, H-3′); 4.12 (dddd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = 4.9, $J_{4',5'b}$ = 3.1, $J_{4',P}$ = 1.3, H-4′); 4.27 (ddd, 1H, J_{gem} = 11.6, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.9, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.6, $J_{5'b,P}$ = 5.5, $J_{5'b,4'}$ = 3.1, H-5′b); 4.87 (s, 1H, H-1′); 7.29 (m, 2H, H-3,5); 7.33 (m, 2H, H-2,6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ($(\mathbf{C}H_3CH_2)_3NH^+$); 20.80 ($CH_3$-Ph); 21.70 ($CH_3$-2′); 47.26 ($(CH_3\mathbf{C}H_2)_3NH^+$); 65.91 (d, $J_{C,P}$ = 6.0, CH_2-5′); 75.03 (CH-3′); 79.40 (C-2′); 81.19 (d, $J_{C,P}$ = 9.0, CH-4′); 88.71 (CH-1′); 127.51 (CH-2,6); 129.58 (CH-3,5); 135.78 (C-1); 139.00 (C-4). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.56 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.7, P_β); −10.49 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −10.23 (d, 1P, $J_{\gamma,\beta}$ = 19.6, P_γ). HRMS (ESI) *m/z* for $C_{13}H_{20}O_{13}P_3$ [M − 3NEt_3 − H]$^-$: calcd 477.01222; found 477.01166.

4-(2-*C*-Methyl-β-D-ribofuranosyl)aniline 5′-*O*-triphosphate (31b)

Triphosphate **31b** was prepared from **27b** (48 mg, 0.2 mmol) using the general procedure D. Lyophilization from water gave triphosphate **31b** (87 mg, 0.128 mmol, 64 %; bis(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.88 (s, 3H, CH_3-2′); 1.27 (t, 18H, $J_{CH3,CH2}$ = 7.4, $(C\mathbf{H_3}CH_2)_3NH^+$); 3.19 (q, 12H, $J_{CH2,CH3}$ = 7.4, $(CH_3C\mathbf{H_2})_3NH^+$); 3.99 (d, 1H, $J_{3',4'}$ = 7.4, H-3′); 4.10 (dddd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = 4.7, $J_{4',5'b}$ = 3.0, $J_{4',P}$ = 1.4, H-4′); 4.26 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.7, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,P}$ = 5.4, $J_{5'b,4'}$ = 3.0, H-5′b); 4.83 (s, 1H, H-1′); 6.99 (m, 2H, H-2,6); 7.31 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.85 ($(\mathbf{C}H_3CH_2)_3NH^+$); 21.68 ($CH_3$-2′); 47.27 ($(CH_3\mathbf{C}H_2)_3NH^+$); 65.78 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.87 (CH-3′); 79.44 (C-2′); 81.08 (d, $J_{C,P}$ = 8.9, CH-4′); 88.69 (CH-1′); 118.30 (CH-2,6); 128.75 (CH-3,5); 132.04 (C-4); 142.85 (C-1). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.51 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.8, P_β); −10.44 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −10.08 (d, 1P, $J_{\gamma,\beta}$ = 19.8, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{19}O_{13}NP_3$ [M − 2NEt_3 − H]$^-$: calcd 478.00747; found 478.00726.

***N*,*N*-Dimethyl-4-(2-*C*-methyl-β-D-ribofuranosyl)aniline 5′-*O*-triphosphate (31c)**

Compound **31c** was prepared from nucleoside **27c** (53 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **31c** (133 mg, 0.164 mmol, 82 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.87 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, (C**H**$_3$CH_2)$_3$NH^+); 2.97 (s, 6H, $(CH_3)_2$N); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H**$_2$)$_3$NH^+); 4.00 (d, 1H, $J_{3',4'}$ = 7.5, H-3′); 4.11 (dddd, 1H, $J_{4',3'}$ = 7.5, $J_{4',5'a}$ = 4.5, $J_{4',5'b}$ = 2.9, $J_{4',P}$ = 1.4, H-4′); 4.27 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.6, H-5′a); 4.35 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,P}$ = 5.3, $J_{5'b,4'}$ = 2.9, H-5′b); 4.87 (s, 1H, H-1′); 7.20 (m, 2H, H-2,6); 7.43 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.85 ((**C**H_3CH_2)$_3$NH^+); 21.67 (CH_3-2′); 43.17 ($(CH_3)_2$N); 47.27 ((CH_3**C**H_2)$_3$NH^+); 65.72 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.81 (CH-3′); 79.55 (C-2′); 81.13 (d, $J_{C,P}$ = 8.8, CH-4′); 88.51 (CH-1′); 117.28 (CH-2,6); 128.74 (CH-3,5); 132.87 (C-4); 149.22 (C-1). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.54 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.8, P_β); −10.48 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −9.95 (d, 1P, $J_{\gamma,\beta}$ = 19.8, P_γ). HRMS (ESI) *m/z* for $C_{14}H_{23}O_{13}NP_3$ [M − 3NEt$_3$ − H]$^-$: calcd 506.03877; found 506.03847.

4-(2-*C*-Methyl-β-D-ribofuranosyl)phenol 5′-*O*-triphosphate (31d)

Compound **31d** was prepared from nucleoside **27d** (48 mg, 0.2 mmol) using the general procedure D. Lyophilization from water furnished triphosphate **31d** (110 mg, 0.14 mmol, 70 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.88 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.4, (C**H**$_3$CH_2)$_3$NH^+); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.4, (CH_3C**H**$_2$)$_3$NH^+); 3.99 (d, 1H, $J_{3',4'}$ = 7.4, H-3′); 4.10 (dddd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = 4.6, $J_{4',5'b}$ = 3.0, $J_{4',P}$ = 1.3, H-4′); 4.26 (ddd, 1H, J_{gem} = 11.6, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.7, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.6, $J_{5'b,P}$ = 5.4, $J_{5'b,4'}$ = 3.0, H-5′b); 4.83 (s, 1H, H-1′); 6.92 (m, 2H, H-2,6); 7.31 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$NH^+); 21.69 (CH_3-2′); 47.26 ((CH_3**C**H_2)$_3$NH^+); 65.79 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.86 (CH-3′); 79.44 (C-2′); 81.07 (d, $J_{C,P}$ = 8.9, CH-4′); 88.67 (CH-1′); 115.72 (CH-2,6); 129.10 (CH-3,5); 130.82 (C-4); 155.93 (C-1). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.36 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.5, P_β); −10.44 (d, 1P, $J_{\alpha,\beta}$ = 19.7, P_α); −9.47 (bs, 1P, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{18}O_{14}P_3$ [M − 3NEt$_3$ − H]$^-$: calcd 478.99149; found 478.99063.

4-(2-*C*-Methyl-β-D-ribofuranosyl)anisole 5′-*O*-triphosphate (31e)

Triphosphate **31e** was prepared from **27e** (51 mg, 0.2 mmol) according to the general procedure D. Before lyophilization, **31e** was converted to a sodium salt form (Dowex 50WX8 in Na^+ cycle). Lyophilization from water furnished triphosphate **31e** (86 mg, 0.154 mmol, 77 %; trisodium salt) as a white powder. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.87 (s, 3H, CH_3-2′); 3.84 (s, 3H, CH_3O); 4.00 (d, 1H, $J_{3',4'}$ = 7.5, H-3′); 4.12 (dddd, 1H, $J_{4',3'}$ = 7.5, $J_{4',5'a}$ = 4.7, $J_{4',5'b}$ = 3.0, $J_{4',P}$ = 1.2, H-4′); 4.28 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,P}$ = 6.5, $J_{5'a,4'}$ = 4.8, H-5′a); 4.35 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,P}$ = 5.5, $J_{5'b,4'}$ = 3.1, H-5′b); 4.87 (s, 1H, H-1′); 6.92 (m, 2H, H-2,6); 7.27 (m, 2H, H-3,5). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 21.7 (CH_3-2′); 56.0 (CH_3O); 65.8 (d, $J_{C,P}$ = 5.8, CH_2-5′); 74.9 (CH-3′); 79.5 (C-2′); 81.2 (d, $J_{C,P}$ = 8.7, CH-4′); 88.6 (CH-1′); 114.5 (CH-2,6); 128.9 (CH-3,5); 131.5 (C-4); 159.3 (C-1). ^{31}P NMR (202.4 MHz, D_2O): δ = −20.08 (m, 1P, P_β); −10.80 (bd, 1P, $J_{\alpha,\beta}$ = 19.5, P_α); –7.54 (m, 1P, P_γ). HRMS (ESI) *m/z* for $C_{13}H_{18}O_{14}Na_2P_3$ $[M - Na]^-$: calcd 536.96993; found 536.96975.

2-Methyl-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (32a)

Triphosphate **32a** was prepared from compound **28a** (48 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **32a** (139 mg, 0.178 mmol, 89 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.93 (s, 3H, CH_3-2′); 2.65 (s, 3H, CH_3-2); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, ($C\mathbf{H_3}CH_2)_3NH^+$); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, ($CH_3C\mathbf{H_2})_3NH^+$); 4.07 (d, 1H, $J_{3',4'}$ = 7.6, H-3′); 4.16 (dddd, 1H, $J_{4',3'}$ = 7.6, $J_{4',5'a}$ = 3.6, $J_{4',5'b}$ = 2.6, $J_{4',P}$ = 2.0, H-4′); 4.28 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,P}$ = 6.0, $J_{5'a,4'}$ = 3.7, H-5′a); 4.38 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,P}$ = 4.7, $J_{5'b,4'}$ = 2.6, H-5′b); 5.02 (s, 1H, H-1′); 7.60 (d, 1H, $J_{3,4}$ = 8.3, H-3); 8.08 (bd, 1H, $J_{4,3}$ = 8.3, H-4); 8.66 (bs, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 (($\mathbf{C}H_3CH_2)_3NH^+$); 21.17 (CH_3-2); 21.48 (CH_3-2′); 47.26 (($CH_3\mathbf{C}H_2)_3NH^+$); 64.86 (d, $J_{C,P}$ = 5.1, CH_2-5′); 73.93 (CH-3′); 79.59 (C-2′); 81.50 (d, $J_{C,P}$ = 9.0, CH-4′); 85.82 (CH-1′); 126.18 (CH-3); 134.57 (C-5); 140.78 (CH-4); 142.94 (CH-6); 156.28 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.17 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.5, P_β); −10.18 (d, 1P, $J_{\alpha,\beta}$ = 18.8, P_α); −8.58 (d, 1P, $J_{\gamma,\beta}$ = 20.2, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{19}O_{13}NP_3$ $[M - 3NEt_3 - H]^-$: calcd 478.00747; found 478.00725.

2-Amino-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (32b)

Compound **32b** was prepared from **28b** (48 mg, 0.2 mmol) according to the general procedure D. Lyophilization from water furnished triphosphate **32b** (120 mg, 0.176 mmol, 88 %; bis(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 1.01 (s, 3H, CH_3-2′); 1.27 (t, 18H, $J_{CH3,CH2}$ = 7.3, (C**H**$_3$CH_2)$_3$NH^+); 3.19 (q, 12H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H**$_2$)$_3$NH^+); 4.07 (d, 1H, $J_{3',4'}$ = 8.2, H-3′); 4.11 (dq, 1H, $J_{4',3'}$ = 8.2, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',P}$ = 2.5, H-4′); 4.24 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,P}$ = 5.8, $J_{5'a,4'}$ = 2.8, H-5′a); 4.36 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,P}$ = 4.3, $J_{5'b,4'}$ = 2.2, H-5′b); 4.88 (s, 1H, H-1′); 6.79 (d, 1H, $J_{3,4}$ = 9.2, H-3); 7.83 (dd, 1H, $J_{4,3}$ = 9.2, $J_{4,6}$ = 2.0, H-4); 8.10 (bs, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$NH^+); 21.22 (CH_3-2′); 47.26 ((CH_3**C**H_2)$_3$NH^+); 64.38 (d, $J_{C,P}$ = 5.0, CH_2-5′); 73.18 (CH-3′); 79.73 (C-2′); 80.77 (d, $J_{C,P}$ = 9.1, CH-4′); 85.65 (CH-1′); 113.58 (CH-3); 125.09 (C-5); 134.71 (CH-6); 143.12 (CH-4); 154.47 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.23 (bt, 1P, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 17.7, P_β); −10.09 (d, 1P, $J_{\alpha,\beta}$ = 18.6, P_α); −8.87 (bd, 1P, $J_{\gamma,\beta}$ = 16.7, P_γ). HRMS (ESI) *m/z* for $C_{11}H_{20}O_{13}N_2P_3$ [M − 2NEt_3 + H]$^+$: calcd 481.01727; found 481.01746.

2-(Dimethylamino)-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (32c)

Compound **32c** was prepared from **28c** (54 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **32c** (124 mg, 0.174 mmol, 87 %; bis(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 1.00 (s, 3H, CH_3-2′); 1.27 (t, 18H, $J_{CH3,CH2}$ = 7.4, (C**H**$_3$CH_2)$_3$NH^+); 3.19 (q, 12H, $J_{CH2,CH3}$ = 7.4, (CH_3C**H**$_2$)$_3$NH^+); 3.22 (s, 6H, (CH_3)$_2$N); 4.08 (d, 1H, $J_{3',4'}$ = 8.2, H-3′); 4.11 (dq, 1H, $J_{4',3'}$ = 8.2, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',P}$ = 2.4, H-4′); 4.27 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,P}$ = 6.0, $J_{5'a,4'}$ = 2.7, H-5′a); 4.37 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,P}$ = 4.2, $J_{5'b,4'}$ = 2.2, H-5′b); 4.87 (s, 1H, H-1′); 7.08 (dd, 1H, $J_{3,4}$ = 9.5, $J_{3,6}$ = 0.7, H-3); 7.84 (bdd, 1H, $J_{4,3}$ = 9.5, $J_{4,6}$ = 2.2, H-4); 8.10 (dt, 1H, $J_{6,4}$ = 2.2, $J_{6,3}$ = $J_{6,1'}$ = 0.8, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$NH^+); 21.31 (CH_3-2′); 39.35 ((CH_3)$_2$N); 47.26 ((CH_3**C**H_2)$_3$NH^+); 64.59 (d, $J_{C,P}$ = 5.2, CH_2-5′); 73.21 (CH-3′); 79.82 (C-2′); 80.77 (d, $J_{C,P}$ = 9.1, CH-4′); 85.72 (CH-1′); 111.87 (CH-3); 123.59 (C-5); 135.39 (CH-6); 141.77 (CH-4); 153.60 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.16 (bm, 1P, P_β); −10.27 (d, 1P, $J_{\alpha,\beta}$ = 19.3, P_α); −8.72 (bs, 1P, P_γ). HRMS (ESI) *m/z* for $C_{13}H_{22}O_{13}N_2P_3$ [M − 2NEt_3 − H]$^-$: calcd 507.03402; found 507.03370.

5-(2-*C*-Methyl-β-D-ribofuranosyl)-2-pyridone 5′-*O*-triphosphate (32d)

Triphosphate **32d** was prepared from nucleoside **28d** (48 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **32d** (118 mg, 0.15 mmol, 75 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 1.01 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.4, (C**H$_3$**CH$_2$)$_3$NH$^+$); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.4, (CH_3C**H$_2$**)$_3$NH$^+$); 4.01 (d, 1H, $J_{3',4'}$ = 7.7, H-3′); 4.09 (bdddd, 1H, $J_{4',3'}$ = 7.7, $J_{4',5'a}$ = 3.8, $J_{4',5'b}$ = 2.7, $J_{4',P}$ = 2.0, H-4′); 4.24 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,P}$ = 6.1, $J_{5'a,4'}$ = 3.8, H-5′a); 4.33 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,P}$ = 4.8, $J_{5'b,4'}$ = 2.7, H-5′b); 4.79 (s, 1H, H-1′); 6.66 (m, 1H, H-3); 7.70–7.74 (m, 2H, H-4,6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.85 ((**C**H$_3$CH$_2$)$_3$NH$^+$); 21.40 (CH_3-2′); 47.26 ((CH$_3$**C**H$_2$)$_3$NH$^+$); 65.14 (d, $J_{C,P}$ = 5.6, CH_2-5′); 73.99 (CH-3′); 79.44 (C-2′); 81.06 (d, $J_{C,P}$ = 9.0, CH-4′); 85.69 (CH-1′); 119.15 (CH-3); 120.68 (C-5); 133.66 and 143.33 (CH-4,6); 165.08 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.47 (bs, 1P, P_β); −10.48 (bd, 1P, $J_{\alpha,\beta}$ = 19.2, P_α); −10.16 (bs, 1P, P_γ). HRMS (ESI) *m/z* for $C_{11}H_{17}O_{14}NP_3$ [M − 3NEt$_3$ − H]$^-$: calcd 479.98674; found 479.98593.

2-Methoxy-5-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (32e)

Compound **32e** was prepared from **28e** (51 mg, 0.2 mmol) according to the general procedure D. Lyophilization from water gave triphosphate **32e** (153 mg, 0.192 mmol, 96 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.91 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, (C**H$_3$**CH$_2$)$_3$NH$^+$); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H$_2$**)$_3$NH$^+$); 3.92 (s, 3H, CH_3O); 4.02 (d, 1H, $J_{3',4'}$ = 7.3, H-3′); 4.13 (dddd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = 4.4, $J_{4',5'b}$ = 3.0, $J_{4',P}$ = 1.5, H-4′); 4.27 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.4, H-5′a); 4.34 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,P}$ = 5.3, $J_{5'b,4'}$ = 3.0, H-5′b); 4.90 (s, 1H, H-1′); 6.96 (d, 1H, $J_{3,4}$ = 8.8, H-3); 7.86 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.4, H-4); 8.10 (bd, 1H, $J_{6,4}$ = 2.3, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H$_3$CH$_2$)$_3$NH$^+$); 21.64 (CH_3-2′); 47.26 ((CH$_3$**C**H$_2$)$_3$NH$^+$); 54. 80 (CH_3O); 65.54 (d, $J_{C,P}$ = 5.5, CH_2-5′); 74.67 (CH-3′); 79.37 (C-2′); 81.50 (d, $J_{C,P}$ = 8.9, CH-4′); 86.26 (CH-1′); 110.90 (CH-3); 128.11 (C-5); 139.68 (CH-4); 145.14 (CH-6); 164.49 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.35 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.7, P_β); −10.48 (d, 1P, $J_{\alpha,\beta}$ = 19.8, P_α); −9.76 (d, 1P, $J_{\gamma,\beta}$ = 19.7, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{19}O_{14}NP_3$ [M − 3NEt$_3$ − H]$^-$: calcd 494.00239; found 494.00244.

5-Methyl-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (33a)

Compound **33a** was prepared from **29a** (48 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **33a** (146 mg, 0.186 mmol, 93 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.89 (s, 3H, CH_3-2′); 2.39 (s, 3H, CH_3-5); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, (C**H**$_3$CH_2)$_3$NH^+); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H**$_2$)$_3$NH^+); 4.00 (d, 1H, $J_{3',4'}$ = 7.6, H-3′); 4.18 (dddd, 1H, $J_{4',3'}$ = 7.6, $J_{4',5'a}$ = 4.3, $J_{4',5'b}$ = 2.7, $J_{4',P}$ = 1.6, H-4′); 4.30 (ddd, 1H, J_{gem} = 11.8 , $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.3, H-5′a); 4.40 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,P}$ = 5.3, $J_{5'b,4'}$ = 2.7, H-5′b); 5.01 (s, 1H, H-1′); 7.67 (d, 1H, $J_{3,4}$ = 8.2, H-3); 7.92 (bd, 1H, $J_{4,3}$ = 8.2, H-4); 8.41 (bs, 1H, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$NH^+); 17.88 (CH_3-5); 21.20 (CH_3-2′); 47.26 ((CH_3**C**H_2)$_3$NH^+); 65.42 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.41 (CH-3′); 79.85 (C-2′); 81.56 (d, $J_{C,P}$ = 8.6, CH-4′); 87.81 (CH-1′); 122.76 (CH-3); 135.57 (C-5); 141.53 (CH-4); 147.00 (CH-6); 153.55 (C-2). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.25 (t, 1P, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 19.0, P_β); −10.40 (d, 1P, $J_{\alpha,\beta}$ = 19.5, P_α); −9.49 (d, 1P, $J_{\gamma,\beta}$ = 18.5, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{19}O_{13}NP_3$ [M − 3NEt_3 − H]$^-$: calcd 478.00747; found 478.00737.

5-Amino-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (33b)

Compound **33b** was prepared from nucleoside **29b** (48 mg, 0.2 mmol) using the general procedure D. Lyophilization from water furnished triphosphate **33b** (70 mg, 0.102 mmol, 51 %; bis(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.95 (s, 3H, CH_3-2′); 1.27 (t, 18H, $J_{CH3,CH2}$ = 7.3, (C**H**$_3$CH_2)$_3$NH^+); 3.19 (q, 12H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H**$_2$)$_3$NH^+); 3.99 (d, 1H, $J_{3',4'}$ = 7.3, H-3′); 4.18 (dddd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = 4.1, $J_{4',5'b}$ = 2.6, $J_{4',P}$ = 1.8, H-4′); 4.28 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.1, H-5′a); 4.40 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,P}$ = 5.2, $J_{5'b,4'}$ = 2.6, H-5′b); 4.99 (s, 1H, H-1′); 7.57 (dd, 1H, $J_{4,3}$ = 8.7, $J_{4,6}$ = 2.4, H-4); 7.59 (dd, 1H, $J_{3,4}$ = 8.7, $J_{3,6}$ = 0.9, H-3); 8.07 (bdd, 1H, $J_{6,4}$ = 2.3, $J_{6,3}$ = 0.9, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.85 ((**C**H_3CH_2)$_3$NH^+); 21.11 (CH_3-2′); 47.27 ((CH_3**C**H_2)$_3$NH^+); 65.32 (d, $J_{C,P}$ = 5.4, CH_2-5′); 74.36 (CH-3′); 79.86 (C-2′); 81.92 (d, $J_{C,P}$ = 8.5, CH-4′); 86.12 (CH-1′); 124.64 (CH-3); 128.74 (CH-4); 131.30 (CH-6); 143.40 (C-2); 145.66 (C-5). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.49 (t, 1P, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 19.8, P_β); −10.42 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −9.94 (d, 1P, $J_{\gamma,\beta}$ = 19.8, P_γ). HRMS (ESI) *m/z* for $C_{11}H_{18}O_{13}N_2P_3$ [M − 2NEt_3 − H]$^-$: calcd 479.00272; found 479.00163.

5-(Dimethylamino)-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (33c)

Triphosphate **33c** was prepared from compound **29c** (54 mg, 0.2 mmol) according to the general procedure D. Lyophilization from water furnished triphosphate **33c** (129 mg, 0.182 mmol, 91 %; bis(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.95 (s, 3H, CH_3-2′); 1.27 (t, 18H, $J_{CH3,CH2}$ = 7.3, (C**H$_3$**CH_2)$_3$$NH^+$); 3.02 (s, 6H, $(CH_3)_2N$); 3.19 (q, 12H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H$_2$**)$_3$$NH^+$); 4.00 (d, 1H, $J_{3',4'}$ = 7.3, H-3′); 4.18 (dddd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = 4.0, $J_{4',5'b}$ = 2.6, $J_{4',P}$ = 1.8, H-4′); 4.30 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,P}$ = 6.5, $J_{5'a,4'}$ = 4.0, H-5′a); 4.42 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,P}$ = 5.2, $J_{5'b,4'}$ = 2.6, H-5′b); 5.02 (s, 1H, H-1′); 7.62 (dd, 1H, $J_{4,3}$ = 9.2, $J_{4,6}$ = 2.7, H-4); 7.65 (dd, 1H, $J_{3,4}$ = 9.2, $J_{3,6}$ = 0.9, H-3); 8.06 (dd, 1H, $J_{6,4}$ = 2.7, $J_{6,3}$ = 0.9, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$$NH^+$); 21.11 ($CH_3$-2′); 40.16 ($(CH_3)_2N$); 47.27 (($CH_3$**C**$H_2$)$_3$$NH^+$); 65.32 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.34 (CH-3′); 79.98 (C-2′); 81.99 (d, $J_{C,P}$ = 8.4, CH-4′); 85.84 (CH-1′); 124.34 (CH-3); 126.11 (CH-4); 128.38 (CH-6); 140.65 (C-2); 148.12 (C-5). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.44 (t, 1P, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 20.0, P_β); −10.44 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −9.70 (d, 1P, $J_{\gamma,\beta}$ = 20.0, P_γ). HRMS (ESI) *m/z* for $C_{13}H_{22}O_{13}N_2P_3$ [M − 2NEt_3 − H]$^-$: calcd 507.03402; found 507.03323.

5-Hydroxy-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (33d)

Compound **33d** was prepared from **29d** (48 mg, 0.2 mmol) following the general procedure D. Lyophilization from water furnished triphosphate **33d** (99 mg, 0.126 mmol, 63 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.92 (s, 3H, CH_3-2′); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, (C**H$_3$**CH_2)$_3$$NH^+$); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, (CH_3C**H$_2$**)$_3$$NH^+$); 4.00 (d, 1H, $J_{3',4'}$ = 7.5, H-3′); 4.17 (dddd, 1H, $J_{4',3'}$ = 7.5, $J_{4',5'a}$ = 4.1, $J_{4',5'b}$ = 2.7, $J_{4',P}$ = 1.7, H-4′); 4.29 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,P}$ = 6.4, $J_{5'a,4'}$ = 4.1, H-5′a); 4.39 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,P}$ = 5.2, $J_{5'b,4'}$ = 2.7, H-5′b); 4.99 (s, 1H, H-1′); 7.58 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.8, H-4); 7.65 (bd, 1H, $J_{3,4}$ = 8.8, H-3); 8.12 (dm, 1H, $J_{6,4}$ = 2.8, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 ((**C**H_3CH_2)$_3$$NH^+$); 21.15 ($CH_3$-2′); 47.27 (($CH_3$**C**$H_2$)$_3$$NH^+$); 65.34 (d, $J_{C,P}$ = 5.5, CH_2-5′); 74.35 (CH-3′); 79.82 (C-2′); 81.69 (d, $J_{C,P}$ = 8.6, CH-4′); 86.99 (CH-1′); 124.54 (CH-3); 128.98 (CH-4); 134.25 (CH-6); 146.13 (C-2); 155.61 (C-5). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.57 (t, 1P, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 19.8, P_β); −10.46 (d, 1P, $J_{\alpha,\beta}$ = 19.8, P_α); −10.14 (d, 1P, $J_{\gamma,\beta}$ = 19.8, P_γ). HRMS (ESI) *m/z* for $C_{11}H_{17}O_{14}NP_3$ [M − 3NEt_3 − H]$^-$: calcd 479.98674; found 479.98593.

5-Methoxy-2-(2-*C*-methyl-β-D-ribofuranosyl)pyridine 5′-*O*-triphosphate (33e)

Compound **33e** was prepared from **29e** (51 mg, 0.2 mmol) according to the general procedure D. Lyophilization from water gave triphosphate **33e** (153 mg, 0.192 mmol, 96 %; tris(triethylammonium) salt) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 0.88 (s, 3H, CH_3-2′); 3.91 (s, 3H, CH_3O); 1.27 (t, 27H, $J_{CH3,CH2}$ = 7.3, (CH_3CH_2)$_3NH^+$); 3.19 (q, 18H, $J_{CH2,CH3}$ = 7.3, (CH_3CH_2)$_3NH^+$); 4.01 (d, 1H, $J_{3',4'}$ = 8.0, H-3′); 4.16 (dddd, 1H, $J_{4',3'}$ = 8.0, $J_{4',5'a}$ = 4.3, $J_{4',5'b}$ = 2.7, $J_{4',P}$ = 1.6, H-4′); 4.30 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,P}$ = 6.3, $J_{5'a,4'}$ = 4.3, H-5′a); 4.39 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,P}$ = 5.2, $J_{5'b,4'}$ = 2.7, H-5′b); 4.97 (s, 1H, H-1′); 7.61 (dd, 1H, $J_{4,3}$ = 8.8, $J_{4,6}$ = 2.9, H-4); 7.70 (bd, 1H, $J_{3,4}$ = 8.8, H-3); 8.23 (dd, 1H, $J_{6,4}$ = 2.9, $J_{6,3}$ = 0.6, H-6). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 67.19 ppm): δ = 8.84 (($\mathbf{C}H_3CH_2$)$_3NH^+$); 21.21 (CH_3-2′); 47.26 (($CH_3\mathbf{C}H_2$)$_3NH^+$); 56.58 (CH_3O); 65.40 (d, $J_{C,P}$ = 5.6, CH_2-5′); 74.29 (CH-3′); 79.85 (C-2′); 81.21 (d, $J_{C,P}$ = 8.8, CH-4′); 88.35 (CH-1′); 123.58 (CH-3); 124.41 (CH-4); 135.54 (CH-6); 149.63 (C-2); 156.24 (C-5). ^{31}P NMR (202.4 MHz, D_2O): δ = −22.39 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.6, P_β); −10.43 (d, 1P, $J_{\alpha,\beta}$ = 19.9, P_α); −9.93 (d, 1P, $J_{\gamma,\beta}$ = 19.3, P_γ). HRMS (ESI) *m/z* for $C_{12}H_{19}O_{14}NP_3$ [M − 3NEt_3 − H]$^-$: calcd 494.00239; found 494.00168.

5.3.4 HPLC purity of final nucleosides

Table 14. HPLC purity of final 2′-*C*-methyl-*C*-ribonucleosides.

Compd.	Strong solvent	t_r, min	Purity, %	Compd.	Strong solvent	t_r, min	Purity, %
21	MeOH	22.67	96.05	**28c**	MeOH	21.42	95.17
	MeCN	18.50	96.90		MeCN	17.04	95.29
27a	MeOH	25.48	98.95	**28d**	MeOH	12.41	100.00
	MeCN	18.83	98.91		MeCN	12.52	100.00
27b	MeOH	14.44	98.48	**28e**	MeOH	22.93	97.36
	MeCN	13.82	96.43		MeCN	17.41	96.23
27c	MeOH	23.77	96.53	**29a**	MeOH	20.98	100.00
	MeCN	17.94	96.06		MeCN	16.15	100.00
27d	MeOH	16.35	99.08	**29b**	MeOH	13.64	99.17
	MeCN	14.46	97.75		MeCN	13.02	99.00
27e	MeOH	22.41	98.81	**29c**	MeOH	21.97	95.64
	MeCN	18.31	98.75		MeCN	16.83	95.33
28a	MeOH	20.38	98.70	**29d**	MeOH	15.74	100.00
	MeCN	16.81	97.94		MeCN	13.91	100.00
28b	MeOH	13.01	96.51	**29e**	MeOH	20.15	100.00
	MeCN	12.88	98.46		MeCN	15.77	100.00

Linear gradient 0 to 100 % of a strong solvent in water over 30 min was used.

5.3.5 Incorporation by T7 RNA polymerase

Solution of template oligonucleotides (100 μM each) in annealing buffer [Tris-HCl (10 mM), EDTA (1 mM), NaCl (50 mM), pH 7.8] was kept at 95 °C for 5 min, and then slowly cooled to 25 °C over a period of 45 min. The resulting dsDNA (50 μM) was used as a template for transcription reactions. In vitro transcription reactions were performed in the total volume of 10 μL in Tris-HCl buffer (40 mM; pH 7.9 at 25°C) containing modified NTP (2 mM), three natural NTPs (2 mM), DTT (20 mM), $MgCl_2$ (10 mM), NaCl (10 mM), spermidine (2 mM), Ribolock RNase inhibitor (1U/μL), Triton X-100 (0.1 %), dsDNA template (0.625 μM), T7 RNA polymerase (2U/μL, Thermo Scientific) and [α-32P]-GTP (111 TBq/mmol, 370 MBq/mL, 0.4 μL). In the positive control the natural NTP (2 mM) was used instead of the solution of modified NTP, and in the negative control experiments, water was used instead. The transcription reactions were performed at 37 °C for 2 h. The samples (2 μL) were mixed with RNA loading dye (2 μL, Thermo Scientific), heated to 70 °C for 10 min, cooled on ice, and then analyzed by gel electrophoresis on 12.5 % denaturing polyacrylamide gel containing 1x TBE buffer (pH 8) and urea (7 M) at 42 mA for 45 min. The gels were dried for 75 min at 80 °C, autoradiographed and visualized by phosphoimager (Typhoon 9410, Amersham Biosciences). Three experiments were carried out: 1) with modified NTP instead of natural ATP; 2) with modified NTP instead of natural CTP; and 3) with modified NTP instead of natural UTP. In any experiment, none of the NTPs **30**, **31a**–**e**, **32a**–**e** and **33a**–**e** was found to be a substrate for T7 RNA polymerase.

Sequence of the dsDNA template:

5′-TAATACGACTCACTATAGGGCTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′

3′-ATTATGCTGAGTGATATCCCGATCGTACTCGAGTCAGGGTACGGCGGGTAC-5′

The two 5′-terminal nucleotides in the antisense strand were 2′-OMe ribonucleotides to minimize non-templated nucleotide addition.[255]

Sequence of the RNA transcript:

GGGCUAGCAUGAGCUCAGUCCCAUGCCGCCCAUG

5.4 Pyrrolo-fused 7-deazapurine nucleosides and nucleotides

5.4.1 Tricyclic nucleobases

3-Iodo-1-methyl-1*H*-pyrrole

n-BuLi (1.6 M in hexanes, 4.1 mL, 6.6 mmol) was added dropwise to a solution of 3-bromo-1-methyl-1*H*-pyrrole[237] (0.96 g, 6 mmol) in dry THF (7 mL) at −78 °C. Solution was stirred for 40 min, and subsequently, iodine (1.68 g, 6.6 mol) in dry THF (4 mL) was added, and the mixture was warmed to r.t over 30 min. After addition of sat. aq. $Na_2S_2O_3$, the reaction mixture was extracted with Et_2O, and combined organic layers were dried over anhydrous Na_2SO_4, and filtered through basic Al_2O_3. Removal of volatiles under reduced pressure afforded 3-iodo-1-methyl-1*H*-pyrrole (1.11 g, 5.4 mmol, 90 %) as a brown liquid. ^{1}H NMR (500.0 MHz, $CDCl_3$): δ = 3.65 (s, 3H, CH_3); 6.21 (dd, 1H, $J_{4,5}$ = 2.7, $J_{4,2}$ = 1.7, H-4); 6.51 (dd, 1H, $J_{5,4}$ = 2.7, $J_{5,2}$ = 2.3, H-5); 6.66 (dd, 1H, $J_{2,5}$ = 2.3, $J_{2,4}$ = 1.7, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 36.38 (CH_3); 58.53 (C-3); 115.61 (CH-4); 123.53 (CH-5); 126.31 (CH-2).

4,6-Dichloro-5-(1-methyl-1*H*-pyrrol-2-yl)pyrimidine (34)

4,6-Dichloropyrimidine (3.4 g, 23 mmol) was dissolved in dry THF (35 mL), and then slowly added into an ice-cooled solution of $(TMP)_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$ (0.35 M in THF/toluene 9:1, 33 mL, 11.6 mmol). The mixture was stirred at 0 °C for 1 h, then let to warm to r.t. over 1 h, and added to a mixture of 2-iodo-1-methyl-1*H*-pyrrole[238] (4.8 g, 23 mmol) and $Pd(PPh_3)_4$ (2.66 g, 2.3 mmol) in dry THF (10 mL). After stirring at 65 °C for 16 h, the reaction mixture was concentrated in vacuo. Purification by flash column chromatography on silica gel (0 to 5 % of EtOAc in hexane) afforded compound **34** (6.4 g, 28 mmol, 75 %) as a yellowish solid (m.p. 56–58 °C). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.43 (s, 3H, CH_3); 6.15 (dd, 1H, $J_{4',3'}$ = 3.7, $J_{4',5'}$ = 2.6, H-4′); 6.18 (dd, 1H, $J_{3',4'}$ = 3.7, $J_{3',5'}$ = 1.7, H-3′); 6.97 (dd, 1H, $J_{5',4'}$ = 2.6, $J_{5',3'}$ = 1.7, H-5′); 8.96 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 33.97 (CH_3); 108.11 (CH-4′); 110.93 (CH-3′); 123.10 (C-2′); 124.39 (CH-5′); 126.45 (C-5); 157.91 (CH-2); 162.76 (C-4,6). HRMS (EI) *m/z* for $C_9H_7{}^{35}Cl_2N_3$ $[M]^+$: calcd 227.0017; found 227.0019.

4,6-Dichloro-5-(1-methyl-1*H*-pyrrol-3-yl)pyrimidine (35)

Solution of 4,6-dichloropyrimidine (17 g, 114 mmol) in dry THF (10 mL) was slowly added to $TMP_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$ (0.35 M in THF/toluene 9:1, 184 mL, 65 mmol) at 0 °C. Then the mixture was stirred for 1 h at 0 °C, warmed to r.t. and stirred for another 1 h at r.t. The resulting solution was then added to a mixture of 3-iodo-1-methyl-1*H*-pyrrole (23.6 g,

114 mmol), Pd_2dba_3 (2.6 g, 2.9 mmol) and P(*t*-Bu)$_3$·HBF$_4$ (6.6 g, 22.8 mmol) in dry THF (30 mL) and stirred at 65 °C for 16 h. After removal of volatiles in vacuo, the crude mixture was purified using flash column chromatography on silica gel (0 to 5 % of EtOAc in hexane) to give **35** (6.5 g, 28.5 mmol, 25 %) as a yellowish oil. ^{1}H NMR (500.0 MHz, $CDCl_3$): δ = 3.74 (s, 3H, CH_3); 6.35 (dd, 1H, $J_{4',5'}$ = 2.7, $J_{4',2'}$ = 1.9, H-4′); 6.70 (dd, 1H, $J_{5',4'}$ = 2.7, $J_{5',2'}$ = 2.1, H-5′); 6.88 (dd, 1H, $J_{2',5'}$ = 2.1, $J_{2',4'}$ = 1.9, H-2′); 8.61 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 36.53 (CH_3); 110.41 (CH-4′); 113.54 (C-3′); 121.89 (CH-5′); 123.30 (CH-2′); 129.73 (C-5); 154.63 (CH-2); 161.17 (C-4,6). HRMS (EI) *m/z* for $C_9H_7{}^{35}Cl_2N_3$ $[M]^+$: calcd 227.0017; found 227.0019.

4-Azido-6-chloro-5-(1-methyl-1*H*-pyrrol-2-yl)pyrimidine (36)

Mixture of **34** (456 mg, 2 mmol), NaN_3 (130 mg, 2 mmol) and LiCl (85 mg, 2 mmol) in dry DMF (5 mL) was stirred at r.t. for 16 h. Then it was diluted with water and extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvents were removed in vacuo. Purification using flash column chromatography on silica gel (0 to 10 % of EtOAc in hexane) afforded **36** (1.01 g, 4.3 mmol, 98 %) as a yellow oil. ^{1}H NMR (500.0 MHz, $CDCl_3$) **azide 36** (the major isomer in $CDCl_3$) : δ = 3.46 (s, 3H, CH_3); 6.21 (dd, 1H, $J_{3,4}$ = 3.7, $J_{3,5}$ = 1.8, H-3-pyrr); 6.28 (dd, 1H, $J_{4,3}$ = 3.7, $J_{4,5}$ = 2.6, H-4-pyrr); 6.81 (dd, 1H, $J_{5,4}$ = 2.6, $J_{5,3}$ = 1.8, H-5-pyrr); 8.69 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 34.37 (CH_3); 108.36 (CH-4-pyrr); 111.37 (CH-3-pyrr); 116.03 (C-5); 121.92 (C-2-pyrr); 123.92 (CH-5-pyrr); 157.17 (CH-2); 162.74, 163.61 (C-4,6). ^{1}H NMR (500.0 MHz, DMSO-d_6) **tetrazole 36t** (the major isomer in DMSO-d_6): δ = 3.53 (s, 3H, CH_3); 6.25 (dd, 1H, $J_{4,3}$ = 3.7, $J_{4,5}$ = 2.7, H-4-pyrr); 6.44 (dd, 1H, $J_{3,4}$ = 3.7, $J_{3,5}$ = 1.8, H-3-pyrr); 7.09 (ddq, 1H, $J_{5,4}$ = 2.7, $J_{5,3}$ = 1.8, $J_{5,CH3}$ = 0.4, H-5-pyrr); 10.21 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.08 (CH_3); 108.51 (CH-4-pyrr); 113.13 (CH-3-pyrr); 115.21 (C-5); 121.38 (C-2-pyrr); 126.00 (CH-5-pyrr); 138.80 (CH-2); 146.84, 151.20 (C-4,6). HRMS (EI) *m/z* for $C_9H_7{}^{35}ClN_6$ $[M]^+$: calcd 234.0421; found 234.0420. IR (ATR): ν = 3088, 2956, 2145 (strong), 1597, 1529, 1401, 1316, 1153, 1083, 898, 723.

4-Azido-6-chloro-5-(1-methyl-1*H*-pyrrol-3-yl)pyrimidine (37)

Mixture of **35** (456 mg, 2 mmol), NaN_3 (130 mg, 2 mmol) and LiCl (85 mg, 2 mmol) in dry DMF (5 mL) was stirred at r.t. for 16 h. Then it was diluted with water and extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the volatiles were removed under reduced pressure. Flash column chromatography on silica gel (5 to 80 %

of EtOAc in hexane) afforded **37** (446 mg, 1.9 mmol, 95 %) as a yellowish solid (m.p. 155–158 °C, decomp.). ^{1}H NMR (500.0 MHz, $CDCl_3$) **tetrazole 37t**: δ = 3.81 (s, 3H, CH_3); 6.79 (ddq, 1H, $J_{5,4}$ = 3.0, $J_{5,2}$ = 2.2, H-5-pyrr); 7.31 (dd, 1H, $J_{4,5}$ = 3.0, $J_{4,2}$ = 1.8, H-4-pyrr); 8.09 (dd, 1H, $J_{2,5}$ = 2.2, $J_{2,4}$ = 1.8, H-2-pyrr); 9.28 (s, 1H, H-2); **azide 37**: δ = 3.73 (s, 3H, CH_3); 6.42 (dd, 1H, $J_{4,5}$ = 2.8, $J_{4,2}$ = 1.8, H-4-pyrr); 6.69 (ddq, 1H, $J_{5,4}$ = 2.8, $J_{5,2}$ = 2.2, H-5-pyrr); 6.94 (dd, 1H, $J_{2,5}$ = 2.2, $J_{2,4}$ = 1.8, H-2-pyrr); 8.53 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): **tetrazole 37t**: δ = 36.83 (CH_3); 110.96 (CH-4-pyrr); 112.22 (C-3-pyrr); 119.00 (C-5); 122.92 (CH-5-pyrr); 127.47 (CH-2-pyrr); 131.19 (CH-2); 140.73, 150.27 (C-4,6); **azide 37**: δ = 36.54 (CH_3); 110.45 (CH-4-pyrr); 111.98 (C-3-pyrr); 119.35 (C-5); 121.85 (CH-5-pyrr); 123.60 (CH-2-pyrr); 154.39 (CH-2); 159.38, 161.04 (C-4,6). ^{1}H NMR (500.0 MHz, DMSO-d_6) **tetrazole 37t**: δ = 3.78 (s, 3H, CH_3); 6.98 (dd, 1H, $J_{5,4}$ = 2.9, $J_{5,2}$ = 2.1, H-5-pyrr); 7.17 (dd, 1H, $J_{4,5}$ = 2.9, $J_{4,2}$ = 1.8, H-4-pyrr); 8.03 (dd, 1H, $J_{2,5}$ = 2.1, $J_{2,4}$ = 1.8, H-2-pyrr); 9.93 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6) **tetrazole 37t**: δ = 36.49 (CH_3); 110.49 (CH-4-pyrr); 111.98 (C-3-pyrr); 117.82 (C-5); 123.34 (CH-5-pyrr); 126.73 (CH-2-pyrr); 134.67 (CH-2); 140.00, 150.36 (C-4,6). HRMS (ESI) *m/z* for $C_9H_7{}^{35}ClN_6Na$ $[M + Na]^+$: calcd 257.03129; found 257.03110. IR (ATR): ν = 3145, 3114, 3087, 1583, 1531, 1375, 1178, 998, 817, 727, 608.

4-Chloro-5-methyl-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (38)

Mixture of azide **36** (350 mg, 1.5 mmol) and 1,4-dibromobenzene (3.54 g, 15 mmol) was stirred at 180 °C for 5 min. After cooling to r.t., it was dissolved in CH_2Cl_2 and purified by HPFC on silica gel (25 to 40 % of EtOAc in petroleum ether) to afford **38** (207 mg, 1 mmol, 67 %) as a white solid (m.p. 231–236 °C). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 4.08 (s, 3H, CH_3); 6.17 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.17 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.45 (s, 1H, H-2); 12.17 (bs, 1H, NH-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.02 (CH_3); 90.92 (CH-7); 105.86 (C-4a); 115.65 (C-4b); 130.44 (CH-6); 132.50 (C-7a); 144.03 (C-4); 148.14 (CH-2); 154.77 (C-8a). HRMS (EI) *m/z* for $C_9H_7{}^{35}ClN_4$ $[M]^+$: calcd 206.0359; found 206.0357.

4-Chloro-7-methyl-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (39)

Mixture of compound **37** (350 mg, 1.5 mmol) with 1,4-dibromobenzene (3.54 g, 15 mmol) was stirred at 180 °C for 5 min. After cooling to r.t., it was purified by HPFC on silica gel (0 to 5 % of MeOH in CH_2Cl_2) to give **39** (180 mg, 0.87 mmol, 58 %) as an orange solid. (m.p. 240–250 °C; decomp.). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.80 (s, 3H, CH_3); 6.42 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.88 (d, 1H, $J_{6,5}$ = 3.1, H-6); 8.46 (s, 1H, H-2); 12.84 (bs, 1H, NH). ^{13}C NMR

(125.7 MHz, DMSO-d_6): δ = 33.48 (CH_3); 99.23 (CH-5); 101.53 (C-4b); 111.45 (C-4a); 123.53 (CH-6); 138.65 (C-7a); 146.03 (C-4); 148.18 (CH-2); 155.06 (C-8a). HRMS (EI) *m/z* for $C_9H_7{}^{35}ClN_4$ $[M]^+$: calcd 206.0359; found 206.0357.

5.4.2 Substituted ribonucleosides

4-Chloro-5-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (40)

BSA (0.59 mL, 2.4 mmol) was added to a suspension of **38** (496 mg, 2.4 mmol) in dry acetonitrile (20 mL), and the mixture was stirred at r.t. for 15 min. Then, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.42 g, 4.8 mmol) and TMSOTf (0.43 mL, 2.4 mmol) were added, and the reaction mixture was heated at 80 °C for 90 min. After cooling down to r.t., sat. solution of $NaHCO_3$ was added, and the mixture was extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the solvent was removed in vacuo. Purification using HPFC on silica gel (0 to 30 % of EtOAc in hexane) afforded nucleoside **40** (1.19 g, 1.82 mmol, 76 %) as a yellowish amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 4.07 (s, 3H, CH_3N); 4.67 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 4.7, H-5′a); 4.77 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 3.3, H-5′b); 4.90 (td, 1H, $J_{4',5'a} = J_{4',3'}$ = 4.9, $J_{4',5'b}$ = 3.3, H-4′); 6.12 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 5.1, H-3′); 6.37 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.0, H-2′); 6.47 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.90 (d, 1H, $J_{1',2'}$ = 5.8, H-1′); 7.21 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.40 (m, 2H, H-*m*-Bz-2′); 7.50 (m, 2H, H-*m*-Bz-3′); 7.51 (m, 2H, H-*m*-Bz-5′); 7.60, 7.678 and 7.681 (m, 3×1H, H-*p*-Bz); 7.80 (m, 2H, H-*o*-Bz-2′); 7.95 (m, 2H, H-*o*-Bz-3′); 7.98 (m, 2H, H-*o*-Bz-5′); 8.52 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.33 (CH_3N); 63.71 (CH_2-5′); 70.77 (CH-3′); 71.83 (CH-2′); 78.80 (CH-4′); 85.23 (CH-1′); 91.79 (CH-7); 107.36 (C-4a); 115.71 (C-4b); 128.34 and 128.80 (C-*i*-Bz); 128.98, 129.00 and 129.06 (CH-*m*-Bz); 129.40 (C-*i*-Bz); 129.45 (CH-*o*-Bz-5′); 129.51 (CH-*o*-Bz-2′); 129.65 (CH-*o*-Bz-3′); 130.96 (CH-6); 131.52 (C-7a); 133.85 (CH-*p*-Bz-5′); 134.18 and 134.24 (CH-*p*-Bz-2′,3′); 144.88 (C-4); 148.55 (CH-2); 154.31 (C-8a); 164.64 (CO-2′); 165.10 (CO-3′); 165.64 (CO-5′). HRMS (ESI) *m/z* for $C_{35}H_{28}O_7N_4{}^{35}Cl$ $[M + H]^+$: calcd 651.16410; found 651.16402.

4-Chloro-7-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (41)

Suspension of **39** (496 mg, 2.4 mmol) in dry acetonitrile (20 mL) was treated with BSA (0.59 mL, 2.4 mmol), and the resulting mixture was stirred at r.t. for 15 min. Sabsequently, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.42 g, 4.8 mmol) and TMSOTf (0.43 mL,

2.4 mmol) were added, and the mixture was stirred at 80 °C for 15 min. After cooling to r.t., sat. aq. $NaHCO_3$ was added, and the mixture was extracted with EtOAc. Organic layers were combined and dried over anhydrous $MgSO_4$, and the volatiles were removed in vacuo. Crude reaction mixture was purified by HPFC on silica gel (5 to 25 % of EtOAc in hexane) to afford nucleoside **41** (1 g, 1.54 mmol, 64 %) as a yellowish amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.99 (s, 3H, CH_3N); 4.58 (dd, 1H, J_{gem} = 12.6, $J_{5'a,4'}$ = 4.0, H-5′a); 4.75 (dd, 1H, J_{gem} = 12.6, $J_{5'b,4'}$ = 3.1, H-5′b); 4.91 (td, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.5, H-4′); 6.44 (dd, 1H, $J_{3',4'}$ = 7.5, $J_{3',2'}$ = 6.2, H-3′); 6.46 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.81 (d, 1H, $J_{1',2'}$ = 3.4, H-1′); 6.88 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.4, H-2′); 6.94 (d, 1H, $J_{6,5}$ = 3.1, H-6); 7.39 (m, 2H, H-*m*-Bz-5′); 7.46 and 7.47 (m, 2×2H, H-*m*-Bz-2′,3′); 7.60–7.68 (m, 3H, H-*p*-Bz); 7.68 (m, 2H, H-*o*-Bz-5′); 7.91–7.95 (m, 4H, H-*o*-Bz-2′,3′); 8.50 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 34.95 (CH_3N); 62.69 (CH_2-5′); 70.17 (CH-3′); 73.30 (CH-2′); 78.88 (CH-4′); 87.30 (CH-1′); 98.90 (CH-5); 103.32 (C-4b); 112.12 (C-4a); 126.16 (CH-6); 128.82 (CH-*m*-Bz-5′); 128.84 and 128.88 (C-*i*-Bz-2′,3′); 129.03 and 129.06 (CH-*m*-Bz-2′,3′); 129.18 (CH-*o*-Bz-5′); 129.22 (C-*i*-Bz-5′); 129.64 and 129.68 (CH-*o*-Bz-2′,3′); 133.77 (CH-*p*-Bz-5′); 134.18 and 134.21 (CH-*p*-Bz-2′,3′); 136.83 (C-7a); 147.08 (C-4); 148.38 (CH-2); 153.79 (C-8a); 164.98 and 165.03 (CO-2′,3′); 165.49 (CO-5′). HRMS (ESI) *m/z* for $C_{35}H_{27}O_7N_4{}^{35}ClNa$ $[M + Na]^+$: calcd 673.14605; found 673.14605.

4-(Furan-2-yl)-5-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (42a)

Protected nucleoside **40** (195 mg, 0.3 mmol), tributyl(furan-2-yl)stannane (0.11 mL, 0.36 mmol) and $PdCl_2(PPh_3)_2$ (21 mg, 0.03 mmol) were dissolved in anhydrous DMF (3 mL) and then stirred at 100 °C for 3 h. Then the mixture was cooled to r.t., filtered through a plug of silica gel with 10 % of KF, and the volatiles were removed in vacuo. HPFC on silica gel (10 to 50 % of EtOAc in hexane) afforded **42a** (195 mg, 0.29 mmol, 95 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.85 (s, 3H, CH_3N); 4.68 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 4.7, H-5′a); 4.77 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 3.4, H-5′b); 4.89 (td, 1H, $J_{4',5'a}$ = $J_{4',3'}$ = 4.9, $J_{4',5'b}$ = 3.4, H-4′); 6.13 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 5.2, H-3′); 6.41 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.1, H-2′); 6.46 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.80 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.8, H-4-furyl); 6.94 (d, 1H, $J_{1',2'}$ = 5.9, H-1′); 7.17 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.28 (dd, 1H, $J_{3,4}$ = 3.4, $J_{3,5}$ = 0.9, H-3-furyl); 7.40 (m, 2H, H-*m*-Bz-2′); 7.50 (m, 2H, H-*m*-Bz-3′); 7.52 (m, 2H, H-*m*-Bz-5′); 7.60 (m, 1H, H-*p*-Bz); 7.65–7.71 (m, 2×1H, H-*p*-Bz); 7.81 (m, 2H, H-*o*-Bz-2′); 7.96–8.00 (m, 2×2H, H-*o*-Bz-3′,5′); 8.08 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.9, H-5-furyl); 8.65 (s, 1H, H-2).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 38.07 (CH_3N); 63.82 (CH_2-5′); 70.78 (CH-3′); 71.66 (CH-2′); 78.60 (CH-4′); 84.90 (CH-1′); 91.27 (CH-7); 103.90 (C-4a); 112.39 (CH-3-furyl); 113.09 (CH-4-furyl); 117.13 (C-4b); 128.38 (C-*i*-Bz-2′); 128.83 (C-*i*-Bz-3′); 129.00 and 129.07 (CH-*m*-Bz); 129.44 (C-*i*-Bz-5′); 129.49, 129.50 and 129.64 (CH-*o*-Bz); 131.42 (CH-6); 132.12 (C-7a); 133.85, 134.18 and 134.23 (CH-*p*-Bz); 144.14 (C-4); 145.14 (CH-5-furyl); 148.70 (CH-2); 152.27 (C-2-furyl); 155.26 (C-8a); 164.67 (CO-2′); 165.11 (CO-3′); 165.69 (CO-5′). HRMS (ESI) *m/z* for $C_{39}H_{31}O_8N_4$ $[M + H]^+$: calcd 683.21364; found 683.21375.

4-(Furan-3-yl)-5-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (42b)

Nucleoside **40** (195 mg, 0.3 mmol), furan-3-boronic boronic acid (50 mg, 0.45 mmol), K_2CO_3 (83 mg, 0.6 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) were suspended in toluene (2 mL) and heated to 100 °C for 3 h. The mixture was then filtered through a plug of Celite, and the solvents were removed in vacuo. HPFC on silica gel (0 to 50 % of EtOAc in hexane) afforded **42b** (174 mg, 0.26 mmol, 85 %) as a yellowish amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.49 (s, 3H, CH_3N); 4.68 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 4.6, H-5′a); 4.77 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 3.3, H-5′b); 4.90 (td, 1H, $J_{4',5'a} = J_{4',3'}$ = 4.7, $J_{4',5'b}$ = 3.3, H-4′); 6.12 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 4.9, H-3′); 6.41 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.2, H-2′); 6.42 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.93 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 6.94 (d, 1H, $J_{1',2'}$ = 6.2, H-1′); 7.06 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.41 (m, 2H, H-*m*-Bz-2′); 7.48–7.56 (m, 4H, H-*m*-Bz-3′,5′); 7.61 (m, 1H, H-*p*-Bz); 7.66–7.71 (m, 2×1H, H-*p*-Bz); 7.81 (m, 2H, H-*o*-Bz-2′); 7.88 (t, 1H, $J_{5,4} = J_{5,2}$ = 1.7, H-5-furyl); 7.96–8.02 (m, 2×2H, H-*o*-Bz-3′,5′); 8.26 (dd, 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl); 8.68 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.23 (CH_3N); 63.90 (CH_2-5′); 70.85 (CH-3′); 71.62 (CH-2′); 78.64 (CH-4′); 84.74 (CH-1′); 91.73 (CH-7); 107.98 (C-4a); 111.87 (CH-4-furyl); 117.35 (C-4b); 125.55 (C-3-furyl); 128.35 (C-*i*-Bz-2′); 128.83 (C-*i*-Bz-3′); 129.02, 129.03 and 129.08 (CH-*m*-Bz); 129.46 (C-*i*-Bz-5′); 129.51 and 129.65 (CH-*o*-Bz); 129.99 (CH-6); 130.90 (C-7a); 133.87, 134.19 and 134.26 (CH-*p*-Bz); 143.43 (CH-2-furyl); 143.99 (CH-5-furyl); 146.87 (C-4); 149.13 (CH-2); 154.55 (C-8a); 164.67 (CO-2′); 165.12 (CO-3′); 165.70 (CO-5′). HRMS (ESI) *m/z* for $C_{39}H_{31}O_8N_4$ $[M + H]^+$: calcd 683.21364; found 683.21379.

4-(Benzofuran-2-yl)-5-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (42c)

Nucleoside **40** (195 mg, 0.3 mmol), benzofuran-2-boronic acid (73 mg, 0.45 mmol), K_2CO_3 (83 mg, 0.6 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) were dissolved in toluene (2 mL) and heated to 100 °C for 3 h. Then, the mixture was filtered through a plug of Celite, and the volatiles were removed under reduced pressure. HPFC on silica gel (0 to 30 % of EtOAc in hexane) gave **42c** (189 mg, 0.26 mmol, 86 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.88 (s, 3H, CH_3N); 4.70 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 4.6, H-5′a); 4.79 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 3.3, H-5′b); 4.92 (btd, 1H, $J_{4',5'a} = J_{4',3'}$ = 4.8, $J_{4',5'b}$ = 3.4, H-4′); 6.15 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 5.2, H-3′); 6.43 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.0, H-2′); 6.51 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.97 (d, 1H, $J_{1',2'}$ = 5.9, H-1′); 7.21 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.36 (btd, 1H, $J_{5,6} = J_{5,4}$ = 7.5, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.40 (m, 2H, H-*m*-Bz-2′); 7.44 (ddd, 1H, $J_{6,7}$ = 8.3, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.3, H-6-benzofuryl); 7.48–7.55 (m, 4H, H-*m*-Bz-3′,5′); 7.60 (m, 1H, H-*p*-Bz); 7.65–7.71 (m, 2×1H, H-*p*-Bz); 7.72 (d, 1H, $J_{3,7}$ = 0.9, H-3-benzofuryl);); 7.78 (dm, 1H, $J_{7,6}$ = 8.3, H-7-benzofuryl); 7.79–7.84 (m, 3H, H-*o*-Bz-2′, H-4-benzofuryl); 7.97–8.01 (m, 2×2H, H-*o*-Bz-3′,5′); 8.74 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 38.05 (CH_3N); 63.83 (CH_2-5′); 70.81 (CH-3′); 71.70 (CH-2′); 78.68 (CH-4′); 84.98 (CH-1′); 91.51 (CH-7); 104.96 (C-4a); 108.26 (CH-3-benzofuryl); 111.85 (CH-7-benzofuryl); 117.10 (C-4b); 122.51 (CH-4-benzofuryl); 124.09 (CH-5-benzofuryl); 126.13 (CH-6-bnezofuryl); 128.33 and 128.38 (C-3a-benzofuryl, C-*i*-Bz-2′); 128.84 (C-*i*-Bz-3′); 129.01 and 129.07 (CH-*m*-Bz); 129.44 (C-*i*-Bz-5′); 129.50 and 129.66 (CH-*o*-Bz); 131.72 (CH-6); 132.56 (C-7a); 133.85 (CH-*p*-Bz-5′); 134.18 and 134.23 (CH-*p*-Bz-2′,3′); 142.04 (C-4); 148.69 (CH-2); 154.25 (C-2-benzofuryl); 154.75 (C-7a-benzofuryl); 155.47 (C-8a); 164.68 (CO-2′); 165.12 (CO-3′); 165.69 (CO-5′). HRMS (ESI) *m/z* for $C_{43}H_{33}O_8N_4$ $[M + H]^+$: calcd 733.22929; found 733.22946.

4,5-Dimethyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (42d)

$AlMe_3$ (2 M in toluene, 0.3 mL, 0.6 mmol) was added to a solution of **40** (195 mg, 0.3 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) in dry THF (4 mL), and the mixture was stirred at 70 °C for 3 h. Subsequently, dry MeOH (0.5 mL) was added, and the reaction mixture was filtered through a plug of Celite and concentrated in vacuo. HPFC on silica gel (50 to 90 % of EtOAc in hexane) furnished **42d** (172 mg, 0.27 mmol, 91 %) as a cream powder (m.p. 92–100 °C). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.89 (s, 3H, CH_3-4); 4.04 (s, 3H, CH_3-5); 4.66 (dd, 1H,

J_{gem} = 12.2, $J_{5'b,4'}$ = 4.5, H-5′b); 4.75 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.3, H-5′a); 4.87 (dd, 1H, $J_{4',3'}$ = 5.0, $J_{4',5'}$ = 4.5, 3.3, H-4′); 6.11 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 5.0, H-3′); 6.36 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.38 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 6.0, H-2′); 6.89 (d, 1H, $J_{1',2'}$ = 6.0, H-1′); 7.06 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.38–7.42 and 7.48–7.54 (2×m, 6H, H-*m*-Bz); 7.58–7.62 and 7.65–7.71 (2×m, 3H, H-*p*-Bz); 7.78–7.81 and 7.96–8.00 (2×m, 6H, H-*o*-Bz); 8.56 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 24.77 (CH_3-4); 36.99 (CH_3-5); 63.81 (CH_2-5′); 70.81 (CH-3′); 71.63 (CH-2′); 78.51 (CH-4′); 84.70 (CH-1′); 91.23 (CH-7); 107.92 (C-4a); 117.86 (C-4b); 128.33 and 128.80 (C-*i*-Bz); 128.93, 128.95 and 129.00 (CH-*m*-Bz); 129.19 (CH-6); 129.42 (C-*i*-Bz); 129.43, 129.45 and 129.58 (CH-*o*-Bz); 130.05 (C-7a); 133.78, 134.10 and 134.16 (CH-*p*-Bz); 149.22 (CH-2); 153.38 (C-4); 153.66 (C-8a); 164.59, 165.06 and 165.63 (CO-Bz). HRMS (ESI) *m/z* for $C_{36}H_{31}O_7N_4$ $[M + H]^+$: calcd 631.21873; found 631.21883.

_N_,_N_,5-Trimethyl-8-(2,3,5-tri-_O_-benzoyl-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (42e)

Me_2NH (2 M in THF, 0.75 mL, 1.5 mmol) was added to a solution of compound **40** (195 mg, 0.3 mmol) in propan-2-ol (8 mL) and THF (4 mL). The solution was stirred at 50 °C for 24 h, then it was concentrated in vacuo, and subjected to a HPFC (20 to 60 % of EtOAc in petroleum ether) to aford **42e** (166 mg, 0.25 mmol, 84 %) as a white amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.99 (s, 6H, $(CH_3)_2N$); 3.97 (s, 3H, CH_3N); 4.64 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 4.6, H-5′a); 4.74 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 3.3, H-5′b); 4.84 (td, 1H, $J_{4',5'a} = J_{4',3'}$ = 4.8, $J_{4',5'b}$ = 3.3, H-4′); 6.11 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 5.0, H-3′); 6.38 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.1, H-2′); 6.40 (d, 1H, $J_{7,6}$ = 3.0, H-7); 6.84 (d, 1H, $J_{1',2'}$ = 6.0, H-1′); 6.99 (d, 1H, $J_{6,7}$ = 3.0, H-6); 7.41 (m, 2H, H-*m*-Bz-2′); 7.49 (m, 2H, H-*m*-Bz-3′); 7.52 (m, 2H, H-*m*-Bz-5′); 7.61 and 7.49 (2×m, 2×1H, H-*p*-Bz-2′,3′); 7.69 (m, 1H, H-*p*-Bz-5′); 7.82 (m, 2H, H-*o*-Bz-2′); 7.96 (m, 2H, H-*o*-Bz-3′); 7.99 (m, 2H, H-*o*-Bz-5′); 8.27 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 36.25 (CH_3N); 41.20 ($(CH_3)_2N$); 63.91 (CH_2-5′); 70.81 (CH-3′); 71.70 (CH-2′); 78.40 (CH-4′); 84.77 (CH-1′); 92.32 (CH-7); 97.02 (C-4a); 118.82 (C-4b); 127.95 (C-7a); 128.40 (C-*i*-Bz-2′); 128.66 (CH-6); 128.83 (C-*i*-Bz-3′); 129.01, 129.02 and 129.05 (CH-*m*-Bz); 129.46 (C-*i*-Bz-5′); 129.51 and 129.62 (CH-*o*-Bz); 133.85 (CH-*p*-Bz-5′); 134.15 and 134.23 (CH-*p*-Bz-2′,3′); 148.74 (CH-2); 154.74 (C-8a); 158.81 (C-4); 164.69 (CO-2′); 165.09 (CO-3′); 165.70 (CO-5′). HRMS (ESI) *m/z* for $C_{37}H_{34}O_7N_5$ $[M + H]^+$: calcd 660.24527; found 660.24537.

4-(Furan-2-yl)-5-methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43a)

Nucleoside **43a** was prepared from **42a** (137 mg, 0.2 mmol) following the general procedure E. The crude mixture was subjected to a reverse-phase HPFC (0 to 100 % of MeOH in water) to afford **43a** (68 mg, 0.18 mmol, 92 %) as a yellow powder (m.p. 245–253 °C, decomp.). $[\alpha]_D^{20}$ +19.3 (*c* 0.192, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.58 and 3.62 (2×ddd, 2×1H, J_{gem} = 11.7, $J_{5',OH}$ = 5.3, $J_{5',4'}$ = 4.3, H-5′); 3.85 (s, 3H, CH_3); 3.92 (td, 1H, $J_{4',5'}$ = 4.3, $J_{4',3'}$ = 2.7, H-4′); 4.13 (dt, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = $J_{3',OH}$ = 2.7, H-3′); 4.62 (ddd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.5, $J_{2',OH}$ = 5.0, H-3′); 5.01 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.19 (d, 1H, $J_{OH,3'}$ = 2.7, OH-3′); 5.25 (d, 1H, $J_{OH,2'}$ = 5.0, OH-2′); 6.43 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.44 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.94 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.8, H-4-furyl); 7.17 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.26 (dd, 1H, $J_{3.4}$ = 3.4, $J_{3,5}$ = 0.9, H-3-furyl); 8.07 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.9, H-5-furyl); 8.65 (s, 1H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.88 (CH_3); 62.08 (CH_2-5′); 70.67 (CH-3′); 71.22 (CH-2′); 85.07 (CH-4′); 86.01 (CH-1′); 92.42 (CH-7); 103.54 (C-4a); 111.98 (CH-3-furyl); 112.97 (CH-4-furyl); 117.13 (C-4b); 131.04 (CH-6); 132.50 (C-7a); 142.08 (C-4); 144.83 (CH-5-furyl); 148.44 (CH-2); 152.53 (C-2-furyl); 155.41 (C-8a). HRMS (ESI) *m/z* for $C_{18}H_{19}O_5N_4$ $[M + H]^+$: calcd 371.13500; found 371.13503. IR (ATR): ν = 3289, 1610, 1436, 1246, 1112, 635.

4-(Furan-3-yl)-5-methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43b)

Compound **43b** was prepared from **42b** (137 mg, 0.2 mmol) using to the general procedure E. The mixture was purified by a reverse-phase HPFC (0 to 100 % of MeOH in water) to give **43b** (62 mg, 0.17 mmol, 83 %) as a yellowish powder (m.p. 202–207 °C). $[\alpha]_D^{20}$ −7.4 (*c* 0.215, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.51 (s, 3H, CH_3); 3.57 and 3.61 (2×bdd, 2×1H, J_{gem} = 11.7, $J_{5',4'}$ = 4.3, H-5′); 3.91 (td, 1H, $J_{4',5'}$ = 4.3, $J_{4',3'}$ = 2.7, H-4′); 4.13 (dd, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.7, H-3′); 4.61 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.5, H-3′); 5.00 (bs, 1H, OH-5′); 5.23 (bs, 2H, OH-2′,3′); 6.40 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.41 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.94 (dd, 1H, $J_{4,5}$ = 1.7, $J_{4,2}$ = 0.9, H-4-furyl); 7.08 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.89 (t, 1H, $J_{5,2}$ = $J_{5,4}$ = 1.7, H-5-furyl); 8.25 (dd, 1H, $J_{2,5}$ = 1.7, $J_{2,4}$ = 0.9, H-2-furyl); 8.67 (s, 1H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.14 (CH_3); 62.12 (CH_2-5′); 70.72 (CH-3′); 71.35 (CH-2′); 85.04 (CH-4′); 86.01 (CH-1′); 92.86 (CH-7); 107.47 (C-4a); 111.87 (CH-4-furyl); 117.31 (C-4b); 125.74 (C-3-furyl); 129.64 (CH-6); 131.35 (C-7a); 143.18 (CH-5-furyl); 143.90 (CH-2-furyl); 146.27 (C-4); 148.84 (CH-2); 154.67 (C-8a). HRMS (ESI) *m/z* for $C_{18}H_{19}O_5N_4$ [*M* +

H]$^+$: calcd 371.13500; found 371.13507. IR (ATR): ν = 3086, 1563, 1482, 1342, 1256, 1053, 795, 620.

4-(Benzofuran-2-yl)-5-methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43c)

Nucleoside **43c** was prepared from **42c** (147 mg, 0.2 mmol) following the general procedure E. The mixture was purified by a reverse-phase HPFC on (0 to 100 % of MeOH in water) to afford **43c** (76 mg, 0.18 mmol, 90 %) as a yellow powder (m.p. 145–154 °C). $[\alpha]_D^{20}$ +56.8 (*c* 0.190, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.60 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.2, $J_{5'b,4'}$ = 4.3, H-5′b); 3.64 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 4.3, H-5′a); 3.88 (s, 3H, CH_3); 3.94 (td, 1H, $J_{4',5'}$ = 4.3, $J_{4',3'}$ = 2.7, H-4′); 4.15 (ddd, 1H, $J_{3',2'}$ = 5.3, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.7, H-3′); 4.64 (ddd, 1H, $J_{2',1'}$ = 7.2, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.3, H-3′); 5.01 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5′); 5.19 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3′); 5.27 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 6.46 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.49 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.22 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.38 (ddd, 1H, $J_{5,4}$ = 7.7, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.45 (ddd, 1H, $J_{6,7}$ = 8.3, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.3, H-6-benzofuryl); 7.71 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.80 (dq, 1H, $J_{7,6}$ = 8.3, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 1.0, H-7-benzofuryl); 7.83 (ddd, 1H, $J_{4,5}$ = 7.7, $J_{4,6}$ = 1.3, $J_{4,7}$ = 1.0, H-4-benzofuryl); 8.75 (s, 1H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.87 (CH_3); 62.06 (CH_2-5′); 70.66 (CH-3′); 71.29 (CH-2′); 85.13 (CH-4′); 86.07 (CH-1′); 92.64 (CH-7); 104.54 (C-4a); 107.92 (CH-3-benzofuryl); 111.79 (CH-7-benzofuryl); 117.08 (C-4b); 122.39 (CH-4-benzofuryl); 123.99 (CH-5-benzofuryl); 125.94 (CH-6-benzofuryl); 128.34 (C-3a-benzofuryl); 131.36 (CH-6); 132.94 (C-7a); 141.54 (C-4); 148.44 (CH-2); 154.52 (C-2-benzofuryl); 154.65 (C-7a-benzofuryl); 155.62 (C-8a). HRMS (ESI) *m/z* for $C_{22}H_{21}O_5N_4$ [*M* + H]$^+$: calcd 421.15065; found 421.15071. IR (ATR): ν = 3342, 3065, 2943, 1520, 1485, 982, 742, 602.

4,5-Dimethyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43d)

Compound **43d** was prepared from **42d** (126 mg, 0.2 mmol) using to the general procedure E. The crude mixture was purified using reverse-phase HPFC (0 to 100 % of MeOH in water) to furnish **43d** (57 mg, 0.18 mmol, 89 %) as a white powder (m.p. 237–241 °C). $[\alpha]_D^{20}$ −48.5 (*c* 0.237, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.91 (s, 3H, CH_3-4); 3.54 (bdd, 1H, J_{gem} = 11.7, $J_{5'a,4'}$ = 4.4, H-5′a); 3.60 (bdd, 1H, J_{gem} = 11.7, $J_{5'b,4'}$ = 4.2, H-5′b); 3.88 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.4, $J_{4',3'}$ = 2.8, H-4′); 4.06 (s, 3H, CH_3N); 4.10 (dd, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.8, H-3′); 4.58 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.5, H-2′); 5.00 (bs, 1H, OH-5′); 5.20 (bs, 2H, OH-

2′,3′); 6.34 (d, 1H, $J_{7,6}$ = 2.8, H-7); 6.35 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.08 (d, 1H, $J_{6,7}$ = 2.8, H-6); 8.54 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 24.82 (CH_3-4); 37.04 (CH_3N); 62.16 (CH_2-5′); 70.75 (CH-3′); 71.33 (CH-2′); 84.93 (CH-4′); 86.02 (CH-1′); 92.34 (CH-7); 107.44 (C-4a); 117.82 (C-4b); 128.93 (CH-6); 130.60 (C-7a); 149.00 (CH-2); 152.74 (C-4); 153.84 (C-8a). HRMS (ESI) *m/z* for $C_{15}H_{19}O_4N_4$ [M + H]$^+$: calcd 319.14008; found 319.14051. IR (ATR): ν = 3256, 3120, 1612, 1546, 1486, 1132, 986, 675.

_N_,_N_,5-Trimethyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (43e)

Nucleoside **43e** was prepared from **42e** (132 mg, 0.2 mmol) following the general procedure E. The reaction mixture was subjected to a reverse-phase HPFC (0 to 100 % of MeOH in water) to furnish **43e** (60 mg, 0.17 mmol, 87 %) as a yellowish powder (m.p. 99–106 °C). $[\alpha]_D^{20}$ +14.6 (*c* 0.195, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.01 (s, 6H, $(CH_3)_2$N); 3.53 (bdd, 1H, J_{gem} = 11.6, $J_{5'a,4'}$ = 4.4, H-5′a); 3.59 (bdd, 1H, J_{gem} = 11.6, $J_{5'b,4'}$ = 4.4, H-5′b); 3.87 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.4, $J_{4',3'}$ = 2.8, H-4′); 3.99 (s, 3H, CH_3N); 4.09 (dd, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.8, H-3′); 4.59 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.6, H-2′); 5.03 (bs, 1H, OH-5′); 5.10–5.29 (m, 2H, OH-2′,3′); 6.28 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.35 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.02 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.27 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 36.13 (CH_3N); 41.29 ((CH_3)$_2$N); 62.23 (CH_2-5′); 70.78 (CH-3′); 71.36 (CH-2′); 84.87 (CH-4′); 86.21 (CH-1′); 93.43 (CH-7); 96.71 (C-4a); 118.75 (C-4b); 128.40 (CH-6); 128.50 (C-7a); 148.49 (CH-2); 154.81 (C-8a); 158.71 (C-4). HRMS (ESI) *m/z* for $C_{16}H_{22}O_4N_5$ [M + H]$^+$: calcd 348.16663; found 348.16617. IR (ATR): ν = 3265, 2896, 1563, 1503, 1409, 1126, 1036, 715.

5-Methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (43f)

Solution of **40** (130 mg, 0.2 mmol) in 1,4-dioxane (5 mL) and 30% aq. NH_3 (10 mL) was stirred in a pressure glass vial at 120 °C for 16 h. Then the solvents were removed in vacuo, and the crude mixture was purified by a reverse-phase HPFC (0 to 100 % of MeOH in water) to give nucleoside **43f** (45 mg, 0.14 mmol, 71 %) as a white powder (m.p. 240–245 °C, decomp.). $[\alpha]_D^{20}$ −41.6 (*c* 0.243, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.52 (ddd, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = 5.8, $J_{5'a,4'}$ = 4.4, H-5′a); 3.58 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 4.4, H-5′b); 3.85 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.4, $J_{4',3'}$ = 2.9, H-4′); 4.02 (s, 3H, CH_3N); 4.07 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 5.1, $J_{3',4'}$ = 2.9, H-3′); 4.56 (td, 1H, $J_{2',1'}$ = $J_{2',OH}$ = 7.0, $J_{2',3'}$ = 5.5, H-2′); 5.08 (bt, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6, OH-5′); 5.12 (bd, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.16 (bd,

1H, $J_{OH,2'}$ = 6.8, OH-2′); 6.20 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.22 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 6.33 (bs, 2H, NH_2); 6.88 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.07 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 36.42 (CH_3N); 62.30 (CH_2-5′); 70.85 (CH-3′); 71.38 (CH-2′); 84.78 (CH-4′); 86.38 (CH-1′); 92.22 (CH-7); 92.69 (C-4a); 118.41 (C-4b); 126.44 (CH-6); 127.97 (C-7a); 149.92 (CH-2); 154.08 (C-8a); 154.62 (C-4). HRMS (ESI) *m/z* for $C_{14}H_{17}O_4N_5Na$ [M + Na]$^+$: calcd 342.11728; found 342.11693. IR (ATR): ν = 3335, 3256, 2896, 1562, 1396, 1086, 726.

4-Methoxy-5-methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43g)

Suspension of **40** (130 mg, 0.2 mmol) in MeOH (4 mL) was treated with MeONa (4.37 M in MeOH, 0.46 mL, 2 mmol) and stirred at 60 °C for 12 h. Then, the volatiles were evaporated in vacuo, and the crude mixture was co-evaporated several times with MeOH. Purification by a reverse-phase HPFC (0 to 100 % of MeOH in water) afforded nucleoside **43g** (55 mg, 0.17 mmol, 83 %) as a white powder (m.p. 231–234 °C). $[\alpha]_D^{20}$ −39.7 (*c* 0.244, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.54 (bdt, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 4.7, H-5′a); 3.59 (bdt, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = $J_{5'b,4'}$ = 4.8, H-5′b); 3.88 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.4, $J_{4',3'}$ = 2.7, H-4′); 3.98 (s, 3H, CH_3N); 4.10 (m, 1H, H-3′); 4.12 (s, 3H, CH_3O); 4.58 (bt, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.2, H-2′); 4.99 (bt, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.4, OH-5′); 5.17 (bs, 1H, OH-3′); 5.23 (bs, 1H, OH-2′); 6.30 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.32 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.03 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.36 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.67 (CH_3N); 54.01 (CH_3O); 62.18 (CH_2-5′); 70.78 (CH-3′); 71.50 (CH-2′); 84.95 (CH-4′); 86.32 (CH-1′); 93.07 (CH-7); 93.93 (C-4a); 117.78 (C-4b); 127.09 (CH-6); 128.56 (C-7a); 148.84 (CH-2); 155.12 (C-8a); 159.34 (C-4). HRMS (ESI) *m/z* for $C_{15}H_{18}O_5N_4Na$ [M + Na]$^+$: calcd 357.11694; found 357.11718. IR (ATR): ν = 3302, 2965, 1596, 1545, 1440, 1356, 1056, 756.

5-Methyl-4-(methylsulfanyl)-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo-[2,3-*d*]-pyrimidine (43h)

Suspension of **40** (130 mg, 0.2 mmol) and MeSNa (70 mg, 1 mmol) in MeOH was heated to 60 °C for 16 h. The solvents were removed in vacuo, and the crude reaction mixture was co-evaporated several times with MeOH, and purified by a reverse-phase HPFC (0 to 100 % of MeOH in water) to furnish nucleoside **43h** (54 mg, 0.15 mmol, 77 %) as a white powder (m.p. 212–214 °C). $[\alpha]_D^{20}$ −35.6 (*c* 0.194, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.72 (s, 3H, CH_3S); 3.55 and 3.60 (2×ddd, 2×1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.4, $J_{5',4'}$ = 4.3, H-5′); 3.89 (td, 1H, $J_{4',5'}$ = 4.3, $J_{4',3'}$ = 2.7, H-4′); 4.10 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.6, $J_{3',4'}$ = 2.7, H-

3′); 4.14 (s, 3H, CH_3N); 4.57 (ddd, 1H, $J_{2',1'}$ = 7.2, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.5, H-3′); 4.99 (t, 1H, $J_{OH,5'}$ = 5.4, OH-5′); 5.17 (d, 1H, $J_{OH,3'}$ = 4.6, OH-3′); 5.23 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2′); 6.33 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.37 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.11 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.56 (s, 1H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 12.44 (CH_3S); 37.25 (CH_3N); 62.11 (CH_2-5′); 70.71 (CH-3′); 71.35 (CH-2′); 85.04 (CH-4′); 86.05 (CH-1′); 92.68 (CH-7); 105.19 (C-4a); 117.25 (C-4b); 129.32 (CH-6); 130.32 (C-7a); 148.60 (CH-2); 152.03 (C-8a); 153.98 (C-4). HRMS (ESI) *m/z* for $C_{15}H_{18}O_4N_4SNa$ [M + Na]$^+$: calcd 373.09410; found 373.09408. IR (ATR): ν = 3205, 3102, 2965, 1569, 1429, 1096, 985, 542.

4-Chloro-5-methyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (43i)

Nucleoside **43i** was prepared from **40** (130 mg, 0.2 mmol) following the general procedure E. The crude mixture was subjected to a reverse-phase HPFC on (0 to 100 % of MeOH in water) to give **43i** (56 mg, 0.16 mmol, 82 %) as a white powder (m.p. 217–219 °C). $[\alpha]_D^{20}$ −42.5 (*c* 0.219, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.57 (dt, 1H, J_{gem} = 11.7, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 4.7, H-5′a); 3.61 (ddd, 1H, J_{gem} = 11.7, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 4.4, H-5′b); 3.92 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.3, $J_{4',3'}$ = 2.6, H-4′); 4.10 (s, 3H, CH_3N); 4.12 (m, 1H, H-3′); 4.58 (m, 1H, H-2′); 5.03 (bt, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.24 (bd, 1H, $J_{OH,3'}$ = 3.4, OH-3′); 5.24 (d, 1H, $J_{OH,2'}$ = 5.6, OH-2′); 6.37 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 6.46 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.22 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.54 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.36 (CH_3N); 62.07 (CH_2-5′); 70.74 (CH-3′); 71.52 (CH-2′); 85.39 (CH-4′); 86.32 (CH-1′); 93.04 (CH-7); 106.77 (C-4a); 115.77 (C-4b); 130.71 (CH-6); 131.92 (C-7a); 144.50 (C-4); 148.36 (CH-2); 154.66 (C-8a). HRMS (ESI) *m/z* for $C_{14}H_{15}O_4N_4{}^{35}ClNa$ [M + Na]$^+$: calcd 361.06740; found 361.06744. IR (ATR): ν = 3305, 2985, 1570, 1450, 1075, 966, 556.

4-(Furan-2-yl)-7-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (44a)

Nucleoside **41** (195 mg, 0.3 mmol), tributyl(furan-2-yl)stannane (0.11 mL, 0.36 mmol) and $PdCl_2(PPh_3)_2$ (21 mg, 0.03 mmol) were dissolved in anhydrous DMF (3 mL), and the resulting solution was stirred at 100 °C for 3 h. After cooling to r.t., the reaction mixture was filtered through a plug of silica gel with 10 % of KF, and the volatiles were removed under reduced pressure. HPFC on silica gel (10 to 50 % of EtOAc in hexane) furnished **44a** (193 mg, 0.28 mmol, 94 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 4.00 (s, 3H, CH_3N); 4.60 (dd, 1H, J_{gem} = 12.6, $J_{5'a,4'}$ = 4.1, H-5′a); 4.73 (dd, 1H, J_{gem} =

12.6, $J_{5'b,4'}$ = 2.9, H-5′b); 4.90 (btd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.5, H-4′); 6.48 (dd, 1H, $J_{3',4'}$ = 7.4, $J_{3',2'}$ = 6.3, H-3′); 6.79 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.80 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 6.82 (d, 1H, $J_{1',2'}$ = 3.6, H-1′); 6.89 (d, 1H, $J_{6,5}$ = 3.1, H-6); 6.91 (dd, 1H, $J_{2',3'}$ = 6.3, $J_{2',1'}$ = 3.6, H-2′); 7.37 (m, 2H, H-*m*-Bz-5′); 7.40 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl); 7.43–7.50 (m, 4H, H-*m*-Bz-2′,3′); 7.57 (m, 1H, H-*p*-Bz-5′); 7.65 and 7.66 (m, 2×1H, H-*p*-Bz-2′,3′); 7.73 (m, 2H, H-*o*-Bz-5′); 7.91–7.95 (m, 4H, H-*o*-Bz-2′,3′); 8.16 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.64 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 34.96 (CH_3N); 62.87 (CH_2-5′); 70.17 (CH-3′); 73.26 (CH-2′); 78.57 (CH-4′); 87.06 (CH-1′); 101.96 (CH-5); 104.85 (C-4b); 107.58 (C-4a); 111.92 (CH-3-furyl); 112.80 (CH-4-furyl); 124.73 (CH-6); 128.80 (CH-*m*-Bz-5′); 128.85 and 128.90 (C-*i*-Bz-2′,3′); 128.98 and 129.00 (CH-*m*-Bz-2′,3′); 129.25 (CH-*o*-Bz-5′); 129.29 (C-*i*-Bz-5′); 129.60 and 129.64 (CH-*o*-Bz-2′,3′); 133.65 (CH-*p*-Bz-5′); 134.13 (CH-*p*-Bz-2′,3′); 137.10 (C-7a); 143.21 (C-4); 146.00 (CH-5-furyl); 148.96 (CH-2); 152.97 (C-2-furyl); 155.33 (C-8a); 164.95 and 165.01 (CO-2′,3′); 165.33 (CO-5′). HRMS (ESI) *m/z* for $C_{39}H_{31}O_8N_4$ $[M + H]^+$: calcd 683.21364; found 683.21407.

4-(Furan-3-yl)-7-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (44b)

Nucleoside **41** (195 mg, 0.3 mmol), furan-3-boronic acid (50 mg, 0.45 mmol), K_2CO_3 (83 mg, 0.6 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) were dissolved in toluene (2 mL) and stirred at 100 °C for 1 h. Then the mixture was filtered through a plug of Celite, and solvents were removed in vacuo. HPFC on silica gel (0 to 50 % of EtOAc in hexane) gave **44b** (176 mg, 0.26 mmol, 86 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 4.00 (s, 3H, CH_3N); 4.60 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 4.1, H-5′a); 4.73 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 3.1, H-5′b); 4.90 (btd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.6, H-4′); 6.49 (dd, 1H, $J_{3',4'}$ = 7.4, $J_{3',2'}$ = 6.3, H-3′); 6.65 (d, 1H, $J_{5,6}$ = 3.3, H-5); 6.83 (d, 1H, $J_{1',2'}$ = 3.6, H-1′); 6.90–6.94 (m, 2H, H-6,2′); 7.24 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.38 (m, 2H, H-*m*-Bz-5′); 7.43–7.50 (m, 4H, H-*m*-Bz-2′,3′); 7.59 (m, 1H, H-*p*-Bz-5′); 7.65 and 7.66 (m, 2×1H, H-*p*-Bz-2′,3′); 7.74 (m, 2H, H-*o*-Bz-5′); 7.90–7.97 (m, 5H, H-*o*-Bz-2′,3′, H-5-furyl); 8.55 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 8.68 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 34.98 (CH_3N); 62.88 (CH_2-5′); 70.18 (CH-3′); 73.27 (CH-2′); 78.56 (CH-4′); 87.08 (CH-1′); 100.53 (CH-5); 103.84 (C-4b); 109.74 (C-4a, C-4-furyl); 124.98 (CH-6); 125.74 (C-3-furyl); 128.82 (CH-*m*-Bz-5′); 128.86 and 128.90 (C-*i*-Bz-2′,3′); 128.99 and 129.01 (CH-*m*-Bz-2′,3′); 129.26 (CH-*o*-Bz-5′); 129.29 (C-*i*-Bz-5′); 129.61 and 129.64 (CH-*o*-Bz-2′,3′); 133.69 (CH-*p*-Bz-5′); 134.12

and 134.13 (CH-*p*-Bz-2′,3′); 136.71 (C-7a); 143.86 (CH-2-furyl); 144.75 (CH-5-furyl); 146.85 (C-4); 149.02 (CH-2); 154.79 (C-8a); 164.96 and 165.03 (CO-2′,3′); 165.53 (CO-5′). HRMS (ESI) *m/z* for $C_{39}H_{31}O_8N_4$ $[M + H]^+$: calcd 683.21364; found 683.21392.

4-(Benzofuran-2-yl)-7-methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (44c)

Nucleoside **41** (195 mg, 0.3 mmol), benzofuran-2-boronic acid (73 mg, 0.45 mmol), K_2CO_3 (83 mg, 0.6 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) were dissolved in toluene (2 mL), and the resulting solution was stirred at 100 °C for 10 h. Then, the mixture was filtered through a plug of Celite, and solvents were removed in vacuo. HPFC on silica gel (0 to 40 % of EtOAc in hexane) gave **44c** (178 mg, 0.24 mmol, 81 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 4.03 (s, 3H, CH_3N); 4.61 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 4.0, H-5′a); 4.76 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 3.0, H-5′b); 4.92 (btd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.5, H-4′); 6.50 (dd, 1H, $J_{3',4'}$ = 7.3, $J_{3',2'}$ = 6.2, H-3′); 6.86 (d, 1H, $J_{1',2'}$ = 3.6, H-1′); 6.93 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.5, H-2′); 6.97 (m, 2H, H-5,6); 7.34–7.40 (m, 3H, H-*m*-Bz-5′, H-5-benzofuryl); 7.44–7.50 (m, 5H, H-*m*-Bz-2′,3′, H-6-benzofuryl); 7.55, 7.65 and 7.66 (m, 3×1H, H-*p*-Bz); 7.74 (m, 2H, H-*o*-Bz-5′); 7.83 (m, 1H, H-4-benzofuryl); 7.84 (m, 1H, H-3-benzofuryl); 7.91–7.98 (m, 5H, H-*o*-Bz-2′,3′, H-7-benzofuryl); 8.74 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.01 (CH_3N); 62.85 (CH_2-5′); 70.19 (CH-3′); 73.28 (CH-2′); 78.65 (CH-4′); 87.09 (CH-1′); 102.33 (CH-5); 104.76 (C-4b); 107.61 (CH-3-benzofuryl); 108.90 (C-4a); 111.95 (CH-7-benzofuryl); 122.48 (CH-4-benzofuryl); 124.01 (CH-5-benzofuryl); 125.25 (CH-6); 126.32 (CH-6-benzofuryl); 128.14 (C-3a-benzofuryl); 128.78 (CH-*m*-Bz-5′); 128.86 and 128.90 (C-*i*-Bz-2′,3′); 128.99 and 129.01 (CH-*m*-Bz-2′,3′); 129.23 (CH-*o*-Bz-5′); 129.27 (C-*i*-Bz-5′); 129.61 and 129.65 (CH-*o*-Bz-2′,3′); 133.64 (CH-*p*-Bz-5′); 134.14 (CH-*p*-Bz-2′,3′); 137.61 (C-7a); 142.62 (C-4); 148.92 (CH-2); 154.73 (C-2-benzofuryl); 155.40 (C-7a-benzofuryl); 155.66 (C-8a); 164.96 and 165.03 (CO-2′,3′); 165.51 (CO-5′). HRMS (ESI) *m/z* for $C_{43}H_{33}O_8N_4$ $[M + H]^+$: calcd 733.22929; found 733.22957.

4,7-Dimethyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (44d)

$AlMe_3$ (2 M in toluene, 0.3 mL, 0.6 mmol) was added to a solution of **41** (195 mg, 0.3 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) in dry THF (4 mL), and the resulting mixture was stirred at 70 °C for 24 h. Subsequently, dry MeOH (0.5 mL) was added, and the reaction mixture was filtered through a plug of Celite, and the volatiles were removed in vacuo. HPFC on silica gel

(30 to 60 % of EtOAc in hexane) afforded **44d** (148 mg, 0.23 mmol, 78%) as a yellowish amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.69 (s, 3H, CH_3-4); 3.97 (s, 3H, CH_3N); 4.58 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 4.1, H-5′a); 4.72 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 3.1, H-5′b); 4.88 (btd, 1H, $J_{4',3'}$ = 7.3, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.6, H-4′); 6.45 (dd, 1H, $J_{3',4'}$ = 7.3, $J_{3',2'}$ = 6.2, H-3′); 6.48 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.78 (d, 1H, $J_{1',2'}$ = 3.7, H-1′); 6.85 (d, 1H, $J_{6,5}$ = 3.1, H-6); 6.89 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.7, H-2′); 7.40 (m, 2H, H-*m*-Bz-5′); 7.43–7.49 (m, 4H, H-*m*-Bz-2′,3′); 7.60–7.68 (m, 3H, H-*p*-Bz); 7.73 (m, 2H, H-*o*-Bz-5′); 7.90–7.95 (m, 4H, H-*o*-Bz-2′,3′); 8.55 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.14 (CH_3-4); 34.84 (CH_3N); 62.89 (CH_2-5′); 70.19 (CH-3′); 73.15 (CH-2′); 78.56 (CH-4′); 86.98 (CH-1′); 99.20 (CH-5); 104.43 (C-4b); 112.56 (C-4a); 124.84 (CH-6); 128.82 (CH-*m*-Bz-5′); 128.85 and 128.87 (C-*i*-Bz-2′,3′); 128.98 and 129.00 (CH-*m*-Bz-2′,3′); 129.27 (CH-*o*-Bz-5′); 129.28 (C-*i*-Bz-5′); 129.60 and 129.62 (CH-*o*-Bz-2′,3′); 133.70 (CH-*p*-Bz-5′); 134.11 and 134.13 (CH-*p*-Bz-2′,3′); 135.87 (C-7a); 149.10 (CH-2); 153.50 (C-8a); 155.03 (C-4); 164.95 and 164.99 (CO-2′,3′); 165.52 (CO-5′). HRMS (ESI) *m/z* for $C_{36}H_{31}O_7N_4$ $[M + H]^+$: calcd 631.21873; found 631.21807.

_N_,_N_,7-Trimethyl-8-(2,3,5-tri-_O_-benzoyl-β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (44e)

Solution of **41** (195 mg, 0.3 mmol) and Me_2NH (2 M in THF, 0.75 mL, 1.5 mmol) in propan-2-ol (8 mL) and THF (4 mL) was heated to 50 °C for 48 h. After the solvents were removed in vacuo, the crude mixture was purified by HPFC (0 to 30 % of EtOAc in hexane) to furnish **44e** (186 mg, 0.28 mmol, 94 %) as a yellow amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.34 (s, 6H, $(CH_3)_2N$); 3.91 (s, 3H, CH_3N); 4.58 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 4.3, H-5′a); 4.70 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 3.2, H-5′b); 4.84 (btd, 1H, $J_{4',3'}$ = 7.4, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.7, H-4′); 6.27 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.46 (dd, 1H, $J_{3',4'}$ = 7.4, $J_{3',2'}$ = 6.4, H-3′); 6.69–6.79 (m, 2H, H-1′,6); 6.82 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 3.6, H-2′); 7.38–7.49 (m, 6H, H-*m*-Bz); 7.60–7.68 (m, 3H, H-*p*-Bz); 7.80 (m, 2H, H-*o*-Bz-5′); 7.88–7.95 (m, 4H, H-*o*-Bz-2′,3′); 8.14 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 34.92 (CH_3N); 38.66 ($(CH_3)_2N$); 63.06 (CH_2-5′); 70.23 (CH-3′); 73.38 (CH-2′); 78.35 (CH-4′); 87.07 (CH-1′); 97.46 (C-4a); 100.75 (CH-5); 105.37 (C-4b); 123.33 (CH-6); 128.85 (CH-*m*-Bz-5′); 128.87 and 128.94 (C-*i*-Bz-2′,3′); 129.95 and 128.98 (CH-*m*-Bz-2′,3′); 129.36 (C-*i*-Bz-5′); 129.38 (CH-*o*-Bz-5′); 129.56 and 129.61 (CH-*o*-Bz-2′,3′); 133.51 (C-7a); 133.68 (CH-*p*-Bz-5′); 134.06 and 134.08 (CH-*p*-Bz-2′,3′); 149.30 (CH-2); 153.48 (C-8a); 156.51 (C-4); 164.93 and 165.02 (CO-2′,3′); 165.58 (CO-5′). HRMS (ESI) *m/z* for $C_{37}H_{34}O_7N_5$ $[M + H]^+$: calcd 660.24527; found 660.24445.

4-(Furan-2-yl)-7-methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45a)

Nucleoside **45a** was prepared from **44a** (137 mg, 0.2 mmol) following the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) afforded **45a** (66 mg, 0.18 mmol, 89 %) as a yellow powder (m.p. 199–202 °C). $[\alpha]_D^{20}$ −2.8 (*c* 0.288, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.60 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 6.6, $J_{5'a,4'}$ = 4.6, H-5′a); 3.73 (ddd, 1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 5.0, $J_{5'b,4'}$ = 3.6, H-5′b); 3.93 (q, 1H, $J_{4',5'a}$ = $J_{4',3'}$ = $J_{4',5'b}$ = 4.1, H-4′); 3.94 (s, 3H, CH_3N); 4.21 (bdt, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 6.0, $J_{3',4'}$= 4.3, H-3′); 4.80 (bq, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ = 6.6, H-2′); 5.21 (bt, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.8, OH-5′); 5.23 (bd, 1H, $J_{OH,3'}$ = 5.6, OH-3′); 5.38 (bd, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 6.32 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 6.79 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.8, H-4-furyl); 6.81 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.88 (d, 1H, $J_{6,5}$ = 3.1, H-6); 7.37 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl); 8.16 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.9, H-5-furyl); 8.66 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.64 (CH_3N); 61.59 (CH_2-5′); 69.33 (CH-3′); 72.02 (CH-2′); 85.59 (CH-4′); 87.96 (CH-1′); 102.02 (CH-5); 105.02 (C-4b); 107.26 (C-4a); 111.76 (CH-3-furyl); 112.76 (CH-4-furyl); 124.67 (CH-6); 137.46 (C-7a); 142.96 (C-4); 145.88 (CH-5-furyl); 148.77 (CH-2); 153.09 (C-2-furyl); 155.65 (C-8a). HRMS (ESI) *m/z* for $C_{18}H_{19}O_5N_4$ $[M + H]^+$: calcd 371.13500; found 371.13464. IR (ATR): ν = 3256, 2956, 1586, 1532, 1498, 1423, 1336, 1029, 752.

4-(Furan-3-yl)-7-methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45b)

Nucleoside **45b** was prepared from **44b** (137 mg, 0.2 mmol) using the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) afforded **45b** (68 mg, 0.18 mmol, 92 %) as a yellowish solid (m.p. 221–224 °C, decomp.). $[\alpha]_D^{20}$ +0.5 (*c* 0.227, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.60 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 6.7, $J_{5'a,4'}$ = 4.6, H-5′a); 3.73 (ddd, 1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 4.9, $J_{5'b,4'}$ = 3.6, H-5′b); 3.93 (btd, 1H, $J_{4',5'a}$ = $J_{4',3'}$ = 4.5, $J_{4',5'b}$ = 3.7, H-4′); 3.95 (s, 3H, CH_3N); 4.22 (dt, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = $J_{3',OH}$ = 4.7, H-3′); 4.81 (q, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ = 6.4, H-2′); 5.21 (bt, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.9, OH-5′); 5.24 (bd, 1H, $J_{OH,3'}$ = 4.9, OH-3′); 5.38 (bd, 1H, $J_{OH,2'}$ = 6.1, OH-2′); 6.33 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 6.66 (d, 1H, $J_{5,6}$ = 3.2, H-5); 6.91 (d, 1H, $J_{6,5}$ = 3.2, H-6); 7.23 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.93 (t, 1H, $J_{5,4}$ = $J_{5,2}$ = 1.7, H-5-furyl); 8.54 (dd, 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl); 8.69 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.65 (CH_3N); 61.60 (CH_2-5′); 69.36 (CH-3′); 72.00 (CH-2′); 85.59 (CH-4′); 88.02 (CH-1′); 100.57 (CH-5); 104.05 (C-4b);

109.42 (C-4a); 109.78 (CH-4-furyl); 124.92 (CH-6); 125.83 (C-3-furyl); 137.09 (C-7a); 143.73 (CH-2-furyl); 144.68 (CH-5-furyl); 146.58 (C-4); 148.82 (CH-2); 155.11 (C-8a). HRMS (ESI) *m/z* for $C_{18}H_{19}O_5N_4$ $[M + H]^+$: calcd 371.13500; found 371.13527. IR (ATR): ν = 3356, 3156, 1563, 1499, 1306, 1256, 1132, 1026, 825.

4-(Benzofuran-2-yl)-7-methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45c)

Compound **45c** was prepared from **44c** (147 mg, 0.2 mmol) following the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) furnished **45c** (72 mg, 0.17 mmol, 86 %) as a yellow powder (m.p. 198–202 °C). $[\alpha]_D^{20}$ −6.6 (*c* 0.227, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.62 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 6.5, $J_{5'a,4'}$ = 4.7, H-5′a); 3.75 (ddd, 1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 5.1, $J_{5'b,4'}$ = 3.7, H-5′b); 3.94 (bq, 1H, $J_{4',5'a} = J_{4',3'} = J_{4',5'b}$ = 4.2, H-4′); 3.97 (s, 3H, CH_3N); 4.24 (td, 1H, $J_{3',2'} = J_{3',OH}$ = 6.0, $J_{3',4'}$ = 4.5, H-3′); 4.82 (q, 1H, $J_{2',1'} = J_{2',3'} = J_{2',OH}$ = 6.6, H-2′); 5.18 (dd, 1H, $J_{OH,5'a}$ = 6.3, $J_{OH,5'b}$ = 5.2, OH-5′); 5.25 (d, 1H, $J_{OH,3'}$ = 5.7, OH-3′); 5.41 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 6.36 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 6.96 (bd, 1H, $J_{6,5}$ = 3.1, H-6); 7.00 (bd, 1H, $J_{5,6}$ = 3.1, H-5); 7.37 (m, 1H, H-5-benzofuryl); 7.47 (bddd, 1H, $J_{6,7}$ = 8.3, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.3, H-6-benzofuryl); 7.82 (s, 1H, H-3-benzofuryl); 7.83 (m, 1H, H-4-benzofuryl); 7.94 (dm, 1H, $J_{7,6}$ = 8.3, H-7-benzofuryl); 8.76 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.71 (CH_3N); 61.55 (CH_2-5′); 69.28 (CH-3′); 72.03 (CH-2′); 85.59 (CH-4′); 87.99 (CH-1′); 102.40 (CH-5); 104.96 (C-4b); 107.45 (CH-3-benzofuryl); 108.59 (C-4a); 111.93 (CH-7-benzofuryl); 122.45 (CH-4-benzofuryl); 123.99 (CH-5-benzofuryl); 125.19 (CH-6); 126.25 (CH-6-benzofuryl); 128.17 (C-3a-benzofuryl); 137.99 (C-7a); 142.36 (C-4); 148.80 (CH-2); 154.88 (C-2-benzofuryl); 155.38 (C-7a-benzofuryl); 156.06 (C-8a). HRMS (ESI) *m/z* for $C_{22}H_{21}O_5N_4$ $[M + H]^+$: calcd 421.15065; found 421.15104. IR (ATR): ν = 3325, 3289, 1560, 1426, 1305, 1086, 802.

4,7-Dimethyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45d)

Nucleoside **45d** was prepared from **44d** (126 mg, 0.2 mmol) using the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) furnished **45c** (55 mg, 0.17 mmol, 87 %) as a white powder (m.p. 214–217 °C). $[\alpha]_D^{20}$ −8.4 (*c* 0.239, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.71 (s, 3H, CH_3-4); 3.58 (bdm, 1H, J_{gem} = 12.0, H-5′a); 3.71 (bdm, 1H, J_{gem} = 12.0, H-5′b); 3.91 (s, 3H, CH_3N); 3.92 (m, 1H, H-4′); 4.19 (dd, 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 4.2, H-3′); 4.78 (dd, 1H, $J_{2',1'} = J_{2',3'}$ = 6.6, H-2′); 5.16–5.43 (m, 3H, OH-2′,3′,5′);

6.25 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 6.50 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.84 (d, 1H, $J_{6,5}$ = 3.1, H-6); 8.55 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.11 (CH_3-4); 35.46 (CH_3N); 61.66 (CH_2-5′); 69.46 (CH-3′); 72.04 (CH-2′); 85.58 (CH-4′); 88.02 (CH-1′); 99.24 (CH-5); 104.57 (C-4b); 112.24 (C-4a); 124.76 (CH-6); 136.25 (C-7a); 148.83 (CH-2); 153.72 (C-8a); 154.76 (C-4). HRMS (ESI) *m/z* for $C_{15}H_{19}O_4N_4$ $[M + H]^+$: calcd 319.14008; found 319.13983. IR (ATR): ν = 3489, 3352, 2912, 1523, 1423, 1126, 1056, 702.

_N_,_N_,7-Trimethyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (45e)

Nucleoside **45e** was prepared from **44e** (132 mg, 0.2 mmol) following to the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) gave **45e** (65 mg, 0.19 mmol, 93 %) as a reddish amorphous solid. $[\alpha]_D^{20}$ −12.9 (*c* 0.193, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.34 (s, 6H, $(CH_3)_2$N); 3.57 (ddd, 1H, J_{gem} = 12.2, $J_{5'a,OH}$ = 7.8, $J_{5'a,4'}$ = 4.0, H-5′a); 3.70 (ddd, 1H, J_{gem} = 12.3, $J_{5'b,OH}$ = 4.1, $J_{5'b,4'}$ = 3.3, H-5′b); 3.85 (s, 3H, CH_3N); 3.91 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.5, H-4′); 4.16 (m, 1H, H-3′); 4.76 (q, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ = 6.5, H-2′); 5.18 (bd, 1H, $J_{OH,3'}$ = 4.6, OH-3′); 5.31 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2′); 5.64 (dd, 1H, $J_{OH,5'a}$ = 7.8, $J_{OH,5'b}$ = 4.1, OH-5′); 6.21 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 6.28 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.70 (d, 1H, $J_{6,5}$ = 3.1, H-6); 8.09 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.50 (CH_3N); 38.68 ($(CH_3)_2$N); 61.99 (CH_2-5′); 69.86 (CH-3′); 72.01 (CH-2′); 85.81 (CH-4′); 88.34 (CH-1′); 97.24 (C-4a); 100.73 (CH-5); 105.30 (C-4b); 123.32 (CH-6); 134.00 (C-7a); 148.75 (CH-2); 153.26 (C-8a); 156.56 (C-4). HRMS (ESI) *m/z* for $C_{16}H_{22}O_4N_5$ $[M + H]^+$: calcd 348.16663; found 348.16634. IR (ATR): ν = 3320, 2903, 1463, 1415, 1096, 1045, 802.

7-Methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-_d_]pyrimidin-4-amine (45f)

Solution of compound **41** (130 mg, 0.2 mmol) in a mixture of 1,4-dioxane (5 mL) and 30% aq. NH_3 (10 mL) was heated in a pressure glass tube to 120 °C for 24 h. Then, the reaction mixture was cooled to r.t. and concentrated in vacuo. Purification by reverse-phase HPFC (0 to 100 % of MeOH in water) afforded nucleoside **45f** (43 mg, 0.14 mmol, 68 %) as a pink powder (m.p. 220–225 °C, decomp.). $[\alpha]_D^{20}$ −22.4 (*c* 0.246, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.56 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 8.2, $J_{5'a,4'}$ = 3.8, H-5′a); 3.70 (dt, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = $J_{5'b,OH}$ = 3.5, H-5′b); 3.85 (s, 3H, CH_3N); 3.92 (q, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.4, H-4′); 4.14 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 5.6, $J_{3',4'}$ = 3.4, H-3′); 4.76 (q, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ =

6.7, H-2′); 5.17 (bd, 1H, $J_{OH,3'}$ = 5.1, OH-3′); 5.31 (bd, 1H, $J_{OH,2'}$ = 6.9, OH-2′); 5.78 (m, 1H, OH-5′); 6.14 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 6.63 (d, 1H, $J_{5,6}$ = 3.0, H-5); 6.66 (d, 1H, $J_{6,5}$ = 3.0, H-6); 6.81 (bs, 2H, NH_2-4); 8.01 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.23 (CH_3N); 62.10 (CH_2-5′); 70.10 (CH-3′); 72.21 (CH-2′); 85.93 (CH-4′); 88.43 (CH-1′); 96.53 (C-4a); 99.14 (CH-5); 104.50 (C-4b); 123.09 (CH-6); 134.12 (C-7a); 149.60 (CH-2); 152.63 (C-8a); 155.98 (C-4). HRMS (ESI) *m/z* for $C_{14}H_{17}O_4N_5Na$ [M + Na]$^+$: calcd 342.11728; found 342.11705. IR (ATR): ν = 3501, 3205, 2905, 1506, 1463, 1402, 1323, 1050, 720.

4-Methoxy-7-methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45g)

MeONa (4.37 M in MeOH, 0.46 mL, 2 mmol) was added to a suspension of compound **41** (130 mg, 0.2 mmol) in a MeOH (4 mL), and the mixture was stirred at 60 °C for 24 h. After the solvents were removed under reduced pressure, the crude mixture was co-evaporated several times with MeOH and purified by reverse-phase HPFC (0 to 100 % of MeOH in water) affording **45g** (52 mg, 0.16 mmol, 78 %) as a white powder (m.p. 188–195 °C, decomp.). $[\alpha]_D^{20}$ −11.6 (*c* 0.233, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.58 (dd, 1H, J_{gem} = 12.1, $J_{5'a,4'}$ = 4.4, H-5′a); 3.71 (dd, 1H, J_{gem} = 12.1, $J_{5'b,4'}$ = 3.6, H-5′b); 3.89 (s, 3H, CH_3N); 3.91 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 4.1, H-4′); 4.07 (s, 3H, CH_3O); 4.18 (dd, 1H, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 4.1, H-3′); 4.76 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.6, H-2′); 5.18–5.48 (m, 3H, OH-2′,3′,5′); 6.24 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 6.31 (d, 1H, $J_{5,6}$ = 3.0, H-5); 6.80 (d, 1H, $J_{6,5}$ = 3.0, H-6); 8.36 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 35.44 (CH_3N); 53.71 (CH_3O); 61.72 (CH_2-5′); 69.51 (CH-3′); 72.24 (CH-2′); 85.64 (CH-4′); 88.25 (CH-1′); 98.70 (CH-5); 99.06 (C-4a); 103.71 (C-4b); 124.66 (CH-6); 135.36 (C-7a); 148.40 (CH-2); 154.53 (C-8a); 160.85 (C-4). HRMS (ESI) *m/z* for $C_{15}H_{18}O_5N_4Na$ [M + Na]$^+$: calcd 357.11694; found 357.11658. IR (ATR): ν = 3598, 3465, 2963, 1623, 1420, 1105, 986, 680.

7-Methyl-4-(methylsulfanyl)-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45h)

Suspension of **41** (130 mg, 0.2 mmol) and MeSNa (70 mg, 1 mmol) in MeOH was heated to 60 °C for 24 h. Then the solvents were removed under reduced pressure, and the crude mixture was several times co-evaporated with MeOH and purified by a reverse-phase HPFC (0 to 100 % of MeOH in water) to give nucleoside **45h** (50 mg, 0.14 mmol, 72 %) as a white powder (m.p. 180–183 °C). $[\alpha]_D^{20}$ −14.3 (*c* 0.231, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.68 (s, 3H, CH_3S); 3.58 (ddd, 1H, J_{gem} = 12.1, $J_{5'a,OH}$ = 6.7, $J_{5'a,4'}$ = 4.6, H-5′a); 3.71 (ddd,

1H, J_{gem} = 12.1, $J_{5'b,OH}$ = 4.9, $J_{5'b,4'}$ = 3.7, H-5′b); 3.91 (s, 3H, CH_3N); 3.91 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 4.2, H-4′); 4.19 (btd, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 5.9, $J_{3',4'}$ = 4.2, H-3′); 4.76 (q, 1H, $J_{2',1'}$ = $J_{2',3'}$ = $J_{2',OH}$ = 6.6, H-2′); 5.18–5.24 (m, 2H, OH-3′,5′); 5.36 (bd, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 6.24 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 6.35 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.86 (d, 1H, $J_{6,5}$ = 3.1, H-6); 8.57 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 11.47 (CH_3S); 35.50 (CH_3N); 61.62 (CH_2-5′); 69.41 (CH-3′); 72.18 (CH-2′); 85.62 (CH-4′); 88.08 (CH-1′); 98.92 (CH-5); 104.21 (C-4b); 110.13 (C-4a); 125.17 (CH-6); 135.81 (C-7a); 148.69 (CH-2); 151.37 (C-8a); 156.31 (C-4). HRMS (ESI) *m/z* for $C_{15}H_{18}O_4N_4SNa$ $[M + Na]^+$: calcd 373.09410; found 373.09371. IR (ATR): ν = 3325, 3305, 1502, 1469, 1305, 1265, 1132, 915, 805.

4-Chloro-7-methyl-8-(β-D-ribofuranosyl)-7,8-dihydropyrrolo[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (45i)

Compound **45i** was prepared from **41** (130 mg, 0.2 mmol) following the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) afforded **45i** (61 mg, 0.18 mmol, 90 %) as a white powder (m.p. 207–210 °C, decomp.). $[\alpha]_D^{20}$ −14.6 (*c* 0.240, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 3.59 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 6.2, $J_{5'a,4'}$ = 4.9, H-5′a); 3.71 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 5.7, $J_{5'b,4'}$ = 3.8, H-5′b); 3.92 (bq, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 4.3, H-4′); 3.94 (s, 3H, CH_3N); 4.22 (td, 1H, $J_{3',2'}$ = $J_{3',OH}$ = 5.9, $J_{3',4'}$ = 4.5, H-3′); 4.77 (q, 1H, $J_{2',1'}$ = $J_{2',OH}$ = $J_{2',3'}$ = 6.5, H-2′); 5.06 (t, 1H, $J_{OH,5'}$ = 5.7, OH-5′); 5.25 (bd, 1H, $J_{OH,3'}$ = 5.6, OH-3′); 5.40 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2′); 6.28 (d, 1H, $J_{1',2'}$ = 6.7, H-1′); 6.49 (d, 1H, $J_{5,6}$ = 3.1, H-5); 6.95 (d, 1H, $J_{6,5}$ = 3.1, H-6); 8.55 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 37.61 (CH_3N); 61.42 (CH_2-5′); 69.20 (CH-3′); 72.15 (CH-2′); 85.66 (CH-4′); 88.17 (CH-1′); 98.95 (CH-5); 103.59 (C-4b); 111.66 (C-4a); 126.07 (CH-6); 137.10 (C-7a); 146.83 (C-4); 148.33 (CH-2); 154.40 (C-8a). HRMS (ESI) *m/z* for $C_{14}H_{15}O_4N_4{}^{35}ClNa$ $[M + Na]^+$: calcd 361.06740; found 361.06768. IR (ATR): ν = 3336, 3296, 1523, 1495, 1302, 1252, 1156, 956.

5.4.3 Monophosphates and deoxyribonucleosides

4,5-Dimethyl-8-(β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine 5'-*O*-monophosphate sodium salt (46)

Nucleoside **43d** (48 mg, 0.15 mmol) was dried under vacuum at 75 °C for 1 h, and then it was suspended in trimethyl phosphate (0.58 mL). The mixture was cooled to 0 °C, and treated with $POCl_3$ (21 μL, 0.23 mmol). After stirring at 0 °C for 4 h, an aq. solution of TEAB (2 M, 2.5 mL, 5 mmol) was added, and the solvents where removed in vacuo. The residue was co-evaporated several times with water, then it was subjected to a purification on DEAE-

Sephadex column (0 to 1.2 M aq. TEAB) and to ion exchange on Dowex 50 (Na^+ form). Lyophilization from water furnished **46** as a pink powder (24 mg, 0.054 mmol, 36 %). ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 2.77 (s, 3H, CH_3-4); 3.91 (s, 3H, CH_3-5); 4.02 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 4.3, $J_{5'b,4'}$ = 3.8, H-5′b); 4.06 (ddd, 1H, J_{gem} = 11.5, $J_{H,P}$ = 5.7, $J_{5'a,4'}$ = 4.1, H-5′a); 4.32 (dddd, 1H, $J_{4',5'}$ = 4.1, 3.8, $J_{4',3'}$ = 2.6, $J_{H,P}$ = 1.3, H-4′); 4.48 (dd, 1H, $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 2.6, H-3′); 4.90 (dd, 1H, $J_{2',1'}$ = 7.6, $J_{2',3'}$ = 5.6, H-2′); 6.45 (d, 1H, $J_{1',2'}$ = 7.6, H-1′); 6.53 (d, 1H, $J_{7,6}$ = 2.9, H-7); 7.03 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.49 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 69.3 ppm): δ = 25.66 (CH_3-4); 39.48 (CH_3-5); 67.06 (d, $J_{C,P}$ = 4.6, CH_2-5′); 73.19 (CH-3′); 73.70 (CH-2′); 86.33 (d, $J_{C,P}$ = 8.9, CH-4′); 88.13 (CH-1′); 94.95 (CH-7); 110.64 (C-4a); 120.90 (C-4b); 132.63 (C-7a); 132.80 (CH-6); 150.24 (CH-2); 155.64 (C-4); 156.10 (C-8a). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): δ = 2.85. HRMS (ESI) *m/z* for $C_{15}H_{18}O_7N_4P$ $[M - H]^-$: calcd 397.09186; found 397.09167.

4-Chloro-5-methyl-8-[2'-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-ribofuranosyl]-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (47)

Mixture of TDA-1 (42 μL; 0.13 mmol) and KOH (293 mg, 5.22 mmol) in dry MeCN (10 mL) was stirred at r.t for 15 min, and then the nucleobase **38** (300 mg, 1.45 mmol) was added. The resulting solution was stirred at r.t for 30 min. Subsequently, Hoffer's chlorosugar (853 mg, 2.18 mmol) was added, and the mixture was stirred at r.t. for 1 h, then it was filtered through Celite, and concentrated in vacuo. HPFC (5 to 30 % of EtOAc in hexane) afforded nucleoside **47** (720 mg, 1.29 mmol, 89 %) as a white powder (m.p. 186–190 °C). ^{1}H NMR (500.0 MHz, $CDCl_3$): δ = 2.41 (s, 3H, CH_3-Tol-5′); 2.44 (s, 3H, CH_3-Tol-3′); 2.65 (ddd, 1H, J_{gem} = 14.1, $J_{2'b,1'}$ = 6.0, $J_{2'b,3'}$ = 3.0, H-2′b); 3.25 (ddd, 1H, J_{gem} = 14.1, $J_{2'a,1'}$ = 8.2, $J_{2'a,3'}$ = 6.9, H-2′a); 4.13 (s, 3H, CH_3-5); 4.57–4.63 (m, 2H, H-4′,5′b); 4.70 (m, 1H, H-5′a); 5.81 (dt, 1H, $J_{3',2'}$ = 6.9, 3.0, $J_{3',4'}$ = 3.0, H-3′); 6.28 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.82 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.02 (dd, 1H, $J_{1',2'}$ = 8.2, 6.0, H-1′); 7.17–7.21 (m, 2H, H-*m*-Tol-5′); 7.27–7.30 (m, 2H, H-*m*-Tol-3′); 7.86–7.89 (m, 2H, H-*o*-Tol-5′); 7.97–8.01 (m, 2H, H-*o*-Tol-3′); 8.56 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 21.68 (CH_3-Tol-5′); 21.72 (CH_3-Tol-3′); 35.52 (CH_2-2′); 37.54 (CH_3-5); 63.99 (CH_2-5′); 74.60 (CH-3′); 81.31 (CH-4′); 83.36 (CH-1′); 92.18 (CH-7); 107.69 (C-4a); 116.74 (C-4b); 126.54 (C-*i*-Tol-3′); 126.88 (C-*i*-Tol-5′); 129.03 (CH-*m*-Tol-5′); 129.24 (CH-*m*-Tol-3′); 129.35 (CH-6); 129.73 (CH-*o*-Tol-5′); 129.82 (CH-*o*-Tol-3′); 131.13 (C-7a); 143.84 (C-*p*-Tol-5′); 144.39 (C-*p*-Tol-3′); 145.43 (C-4); 148.52 (CH-2); 154.55 (C-8a); 166.12 (CO-Tol-3′); 166.24 (CO-Tol-5′). HRMS (ESI) *m/z* for $C_{30}H_{27}O_5N_4{}^{35}ClNa$ $[M + Na]^+$: calcd 581.15622; found 581.15639.

4,5-Dimethyl-[2'-deoxy-3,5-bis-*O*-(4-methylbenzoyl)-β-D-ribofuranosyl]-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (48)

$AlMe_3$ (2 M in toluene, 0.3 mL, 0.6 mmol) was added to a solution of **47** (168 mg, 0.3 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) in dry THF (4 mL), and the resulting mixture was stirred at 70 °C for 3 h. Subsequently, dry MeOH (0.5 mL) was added, and the mixture was filtered through a plug of Celite, and concentrated in vacuo. HPFC on silica gel (20 to 100 % of EtOAc in hexane) afforded **48** (154 mg, 0.29 mmol, 95 %) as a white amorphous solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.37 and 2.41 (2×s, 2×3H, CH_3-Tol); 2.63 (ddd, 1H, J_{gem} = 14.2, $J_{2'a,1'}$ = 6.0, $J_{2'a,3'}$ = 2.6, H-2′a); 2.90 (s, 3H, CH_3-4); 3.19 (ddd, 1H, J_{gem} = 14.2, $J_{2'b,1'}$ = 8.6, $J_{2'b,3'}$ = 6.9, H-2′b); 4.05 (s, 3H, CH_3N); 4.46–4.53 (m, 2H, H-5′a, H-4′); 4.60 (m, 1H, H-5′b); 5.77 (dt, 1H, $J_{3',2'b}$ = 6.9, $J_{3',2'a}$ = $J_{3',4'}$ = 2.7, H-3′); 6.34 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.95 (dd, 1H, $J_{1',2'b}$ = 8.6, $J_{1',2'a}$ = 6.0, H-1′); 7.08 (d, 1H, $J_{6,7}$ = 2.9, H-6); 7.30 and 7.38 (2×m, 2×2H, H-*m*-Tol); 7.84 and 7.97 (2×m, 2×2H, H-*o*-Tol); 8.57 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 21.43 and 21.48 (CH_3-Tol); 24.83 (CH_3-4); 34.40 (CH_2-2′); 37.04 (CH_3N); 64.33 (CH_2-5′); 74.88 (CH-3′); 80.54 (CH-4′); 82.38 (CH-1′); 91.76 (CH-7); 107.56 (C-4a); 117.99 (C-4b); 126.77 and 126.82 (C-*i*-Tol); 129.13 (CH-6); 129.51, 129.54, 129.61 and 129.75 (CH-*o*,*m*-Tol); 129.85 (C-7a); 144.06 and 144.32 (C-*p*-Tol); 149.17 (CH-2); 153.09 (C-4); 153.47 (C-8a); 165.64 and 165.73 (CO). HRMS (ESI) *m/z* for $C_{31}H_{31}O_5N_4$ $[M + H]^+$: calcd 539.22890; found 539.22911.

4,5-Dimethyl-8-(2'-deoxy-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (49)

Nucleoside **49** was prepared from **48** (108 mg, 0.2 mmol) using the general procedure E. Reverse-phase HPFC (0 to 100 % of MeOH in water) afforded **49** (51 mg, 0.17 mmol, 85 %) as a white powder (m.p. 181–185 °C). $[\alpha]_D^{20}$ −37.2 (*c* 0.304, DMSO). ^{1}H NMR (500.0 MHz, DMSO-d_6): δ = 2.09 (ddd, 1H, J_{gem} = 13.1, $J_{2'a,1'}$ = 5.9, $J_{2'a,3'}$ = 2.4, H-2′a); 2.68 (ddd, 1H, J_{gem} = 13.1, $J_{2'b,1'}$ = 8.9, $J_{2'b,3'}$ = 6.1, H-2′b); 2.90 (s, 3H, CH_3-4); 3.49 (dd, 1H, J_{gem} = 11.4, $J_{5'a,4'}$ = 5.0, H-5′a); 3.55 (dd, 1H, J_{gem} = 11.4, $J_{5'b,4'}$ = 5.2, H-5′b); 3.81 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 5.1, $J_{4',3'}$ = 2.6, H-4′); 4.05 (s, 3H, CH_3N); 4.37 (dt, 1H, $J_{3',2'b}$ = 6.1, $J_{3',2'a}$ = $J_{3',4'}$ = 2.5, H-3′); 4.91 (bs, 1H, OH-5′); 5.33 (bs, 1H, OH-3′); 6.31 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.81 (d, 1H, $J_{1',2'b}$ = 8.9, $J_{1',2'a}$ = 5.8, H-1′); 7.09 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.55 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 24.84 (CH_3-4); 37.04 (CH_3N); 37.40 (CH_2-2′); 62.30 (CH_2-5′); 71.20 (CH-3′); 82.26 (CH-4′); 86.94 (CH-1′); 92.22 (CH-7); 107.32 (C-4a); 117.86 (C-4b); 128.99 (CH-6); 130.25 (C-7a); 149.02 (CH-2); 152.81 (C-4); 153.20 (C-8a). HRMS (ESI) *m/z* for

$C_{15}H_{18}O_3N_4Na$ [M + Na]$^+$: calcd 325.12711; found 325.12689. IR (ATR): ν = 3324, 3286, 2975, 1620, 1563, 1302, 1125, 978.

4,5-Dimethyl-8-(2'-deoxy-β-D-ribofuranosyl)-5,8-dihydropyrrolo[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine 5'-*O*-monophosphate sodium salt (50)

Compound **49** (45 mg, 0.15 mmol) was dried under vacuum at 75 °C for 1 h, and then suspended in trimethyl phosphate (0.58 mL). The mixture was cooled to 0 °C, and treated with $POCl_3$ (21 µL, 0.23 mmol). After stirring at 0 °C for 20 h, aq. TEAB (2 M, 2.5 mL, 5 mmol) was added, and the volatiles were removed in vacuo. The residue was co-evaporated several times with water, and purified on DEAE-Sephadex column (0 to 1.2 M aq. TEAB) and to ion exchange on Dowex 50 (Na^+ form). Lyophilization from water gave **50** (16 mg, 0.041 mmol, 27 %) as a white powder. ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane): δ = 3.75 ppm): δ = 2.36 (ddd, 1H J_{gem} = 14.0, $J_{2'b,1'}$ = 6.3, $J_{2'b,1'}$ = 3.3, H-2′b); 2.69 (s, 3H, CH_3-4); 2.91 (ddd, 1H J_{gem} = 14.0, $J_{2'a,1'}$ = 8.4, $J_{2'a,1'}$ = 6.7, H-2′a); 3.84 and 3.88 (2×dt, 2×1H, J_{gem} = 11.0, $J_{H,P}$ = $J_{5',4'}$ = 5.3, H-5′); 3.89 (s, 3H, CH_3-5); 4.13 (td, 1H, $J_{4',5'}$ = 5.3, $J_{4',3'}$ = 3.3, H-4′); 4.69 (dd, 1H, $J_{3',2'}$ = 6.7, 3.3, $J_{3',4'}$ = 3.3, H-3′); 6.40 (d, 1H, $J_{7,6}$ = 2.9, H-7); 6.69 (d, 1H, $J_{1',2'}$ = 8.4, 6.3, H-1′); 7.01 (d, 1H, $J_{6,7}$ = 2.9, H-6); 8.41 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane): δ = 69.3 ppm): δ = 25.92 (CH_3-4); 38.69 (CH_2-2′); 39.48 (CH_3-5); 66.72 (d, $J_{C,P}$ = 4.5, CH_2-5′); 74.19 (CH-3′); 85.16 (CH-1′); 87.47 (d, $J_{C,P}$ = 8.6, CH-4′); 94.71 (CH-7); 110.33 (C-4a); 120.72 (C-4b); 132.29 (C-7a); 132.49 (CH-6); 150.38 (CH-2); 155.26 (C-4); 155.75 (C-8a). ^{31}P{^{1}H} NMR (202.4 MHz, D_2O): δ = 3.85. HRMS (ESI) *m/z* for $C_{15}H_{18}O_6N_4P$ [M − H]$^-$: calcd 381.09694; found 381.09711.

5.4.4 HPLC purity of final nucleosides

Table 15. HPLC purity of final pyrrolo-fused 7-deazapurine nucleosides.

Compd.	Strong solvent	t_r, min	Purity, %	Compd.	Strong solvent	t_r, min	Purity, %
43a	MeOH	25.67	99.12	**45b**	MeOH	28.36	99.48
	MeCN	19.03	98.33		MeCN	20.30	98.85
43b	MeOH	24.52	98.99	**45c**	MeOH	32.87	98.15
	MeCN	18.46	98.40		MeCN	23.83	97.72
43c	MeOH	30.12	98.98	**45d**	MeOH	25.27	99.83
	MeCN	22.42	99.37		MeCN	17.89	99.23
43d	MeOH	21.97	99.47	**45e**	MeOH	26.58	97.78
	MeCN	16.52	99.91		MeCN	19.09	97.09
43e	MeOH	24.07	99.24	**45f**	MeOH	21.36	99.22
	MeCN	18.04	99.22		MeCN	16.23	97.18
43f	MeOH	19.51	99.40	**45g**	MeOH	26.86	99.07
	MeCN	15.39	99.62		MeCN	19.38	99.07
43g	MeOH	24.93	99.44	**45h**	MeOH	28.96	98.66
	MeCN	18.40	99.34		MeCN	20.75	97.95
43h	MeOH	25.56	99.60	**45i**	MeOH	27.37	99.63
	MeCN	19.72	99.83		MeCN	19.83	98.27
43i	MeOH	24.59	98.65	**49**	MeOH	23.94	99.80
	MeCN	18.37	98.38		MeCN	17.48	99.70
45a	MeOH	28.13	99.17				
	MeCN	20.24	99.09				

Linear gradient 0 to 100 % of a strong solvent in water over 30 min was used, with subsequent washing with 100 % of a strong solvent for 15 min.

5.5 Single-crystal X-ray diffraction analysis

The single-crystal diffraction data were collected on Xcalibur X-ray diffractometr either with $Cu_{K\alpha}$ (λ = 1.54180 Å) (for compounds **13** and **27d**) or with $Mo_{K\alpha}$ (λ= 0.71073 Å) (for compounds **37t** and **38**) radiation at 180 K. CrysAlisProCCD was used for data collection, cell refinement and data reduction, and the structures were solved by direct methods with SIR92[256] and refined by full-matrix least-squares on F with CRYSTALS[257]. The hydrogen atoms were found from a Fourier difference map, but those that are attached to carbon atoms were recalculated into idealized positions and refined with riding constraints. All non-hydrogen atoms were refined with anisotropic displacement parameters.

Crystal data for 13 (colourless block, 0.19 × 0.36 × 0.74 mm): $C_{34}H_{34}Br_1N_1O_6$, monoclinic, space group $P2_1$, a = 7.91533(7) Å, b = 18.37168(13) Å, c = 10.53591(8) Å, β = 95.3841(7)°, V = 1525.35(2) Å^3, Z = 2, M = 632.55, 19330 reflections measured, 6149 independent reflections. Final R = 0.032, wR = 0.039, GoF = 1.010 for 6078 reflections with $I > 2\sigma$(I) and 381 parameters, Flack parameter x = −0.026(10).

Crystal data for 27d (colourless block, 0.18 × 0.37 × 0.53 mm): $C_{12}H_{16}O_5$, orthorhombic, space group $P2_12_12_1$, a = 6.90623(14) Å, b = 9.06533(16) Å, c = 19.1665(3) Å, V = 1199.96(4) Å^3, Z = 4, M = 240.26, 6593 reflections measured, 2423 independent reflections. Final R = 0.044, wR = 0.052, GoF = 1.063 for 2291 reflections with $I > 2\sigma$(I) and 155 parameters, Flack parameter x = 0.10(19).

Crystal data for 37t (0.09 × 0.12 × 0.89 mm): $C_9H_7Cl_1N_6$, orthorhombic, space group $P2_12_12_1$, a = 5.436(4) Å, b = 9.003(6) Å, c = 21.664(12) Å, V = 1060.2(12) Å^3, Z = 4, M = 234.65, 31055 reflections measured, 1880 independent reflections. Final R = 0.030, wR = 0.030, GoF = 1.125 for 1565 reflections with $I > 2\sigma(I)$ and 147 parameters. Flack parameter x = 0.05(7).

Crystal data for 38 (0.28 × 0.46 × 0.76 mm): $C_9H_7Cl_1N_4$, monoclinic, space group $P2_1/n$, a = 8.2465(4) Å, b = 6.6263(4) Å, c = 16.3920(7) Å, β = 97.969(2)°, V = 887.07(8) Å^3, Z = 4, M = 206.63, 35530 reflections measured, 2040 independent reflections. Final R = 0.030, wR = 0.032, GoF = 1.066 for 1947 reflections with $I > 2\sigma(I)$ and 128 parameters.

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