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Syntéza nových typů anelovaných deazapurinových nukleosidů s potenciální biologickou aktivitou

Synthesis of novel types of annulated deazapurine nucleosides with potential biological activity

Dizertační práce

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

Prohlášení:

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

V Praze, 09.02.2015

Podpis

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All the synthetic experiments were performed by me. I have also performed most of the spectroscopic measurements (IR, UV, fluorescence), but the measurement and interpretation of NMR spectra of some intermediates and all final compounds were done by Dr. Radek Pohl. Crystal structure analyses were done by Dr. Blanka Klepetářová. All cytostatic/cytotoxic activity screening and antiviral screening (Chapters 3.2, 3.4) were performed by our collaborators from Novartis Institute for Tropical Diseases (Singapore), Gilead Sciences (California) and by the group of Dr. Weber and Dr. Helena Mertlíková-Kaiserová from IOCB. The parts that were done by our collaborating groups are always distinctly denoted in the thesis.

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Abstract

This thesis reports the syntheses and biological activities of benzo- and thieno-fused 7-deazapurine ribonucleosides, which were designed as extended analogues of potent cytostatic 6-hetaryl-7-deazapurine or 6-amino-7-hetaryl-7-deazapurine ribonucleosides. First of all, multigram syntheses of (di)chloro-9*H*-pyrimido[4,5-*b*]indoles from simple chloro-nitrobenzenes were developed. Pyrimidoindoles were successfully glycosylated and used for the synthesis of 4-hetaryl-6-chloro-, 4,6-bis(hetaryl)-, 4-amino-6-hetaryl-, 4-amino-5-hetaryl- and 4-susbtituted pyrimido[4,5-*b*]indole ribonucleosides. Hetaryl groups were introduced by Suzuki or Stille cross-coupling reaction. Standard catalysts and conditions were used for reaction in position 4. To observe some reactivity of unreactive chlorine in position 6, modification of standard protocol was necessary. Screening of several ligands had been done and Buchwald ligand X-Phos was found to be optimal. As chlorine in position 4 is activated for nucleophilic substitution, amino and dimethylamino derivatives were prepared by reaction with aqueous ammonia and dimethylamine, respectively. 4-Alkyl derivatives were synthesized by palladium-catalyzed alkylation with trialkylaluminium or by Negishi coupling in case of cyclopropyl derivative. Desired free nucleosides were obtained directly from reaction with nucleophiles or by Zemplén deprotection. The whole series of new ribonucleosides were screened for cytotoxic and antiviral (HCV and dengue) activity. 4-Amino-5(6)-hetaryl- as well as 4,6-disubstituted nucleosides were completely inactive, whereas several compounds from 4-hetaryl-6-chloro series showed interesting anti-dengue activities and 4-methylpyrimidoindole nucleoside displayed sub-micromolar activity against HCV.

The syntheses of two series of thienopyrrolopyrimidine ribonuclesides were developed. Tricyclic bases were synthesized from simple dichloropyrimidine and iodothiophene by three-step methodology involving thermally or photochemically induced cyclization of tetrazoles. Target nucleosides bearing hetaryl, amino, dimethylamino, methyl, methoxy and methylsulfanyl groups in position 4 were synthesized by the same methodology as pyrimidoindole derivatives. Thieno-fused nucleosides are also completely new, so they are screened for cytotoxic, antiviral (HCV, dengue, influenza, coxsackie, herpes simplex virus) and antimicrobial activity. Methyl, methoxy and methylsulfanyl derivatives from both series showed submicromolar activities accompanied by cytotoxicity in micromolar range.

Abstrakt

Tato práce popisuje syntézy a biologické aktivity dvou typů kondenzovaných 7-deazapurinových nukleosidů, které byly navrženy jako prodloužená analoga 6-hetaryl-7-deazapurinových a 6-amino-7-hetaryl-7-deazapurinových nukleosidů, známých účinných cytostatik. Nejprve byla vyvinuta multigramová syntéza (di)chlor-9*H*-[4,5-*b*]pyrimidoindolů, vycházející ze snadno dostupných chlornitrobenzenů. Pyrimidoindoly byly úspěšně glykosylovány a použity na syntézu 4-hetaryl-6-chlor-, 4,6-bis(hetaryl)-, 4-amino-6-hetaryl-, 4-amino-5-hetaryl- pyrimidoindolových ribonukleosidů a také pyrimidoindolových nukleosidů substituovaných v poloze 4. Hetarylové skupiny byly zavedeny do polohy 4 pomocí Suzukiho nebo Stilleho cross-couplingové reakce. Pro úspěšné provedení reakce s nereaktivním chlorem v poloze 6 bylo nutné najít vhodný katalytický systém a upravit standardní reakční podmínky. Bylo vyzkoušeno několik ligandů, přičemž nejlepší výsledky byly získány při použití ligandu X-Phos. Protože chlor v poloze 4 je aktivovaný pro nukleofilní substituci, amino a dimethylamino deriváty byly připraveny reakcí s vodným amoniakem, respektive s dimethylaminem. Látky nesoucí alkylovou skupinu v poloze 4 byly získány palladiem katalyzovanou alkylací pomocí trialkylhliníku nebo Negishiho reakcí v případě cyklopropyl derivátu. Cílové volné nukleosidy byly získány přímo z reakcí s nukleofily, případně Zemplénovou metodou. Protože všechny připravené nukleosidy jsou úplně novým typem látek, byla studována jejich cytotoxická a protivirová (HCV a dengue) aktivita. Zatímco všechny 4-amino-5(6)-hetaryl- stejně jako 4,6-disubstituované pyrimidoindolové nukleosidy byly zcela neaktivní, několik sloučenin ze série 4-hetaryl-6-chlorpyrimidoindolových nukleosidů vykázalo zajímavou aktivitu proti dengue viru a 4-methyl derivát dokonce sub-mikromolární aktivitu proti HCV. Dále byla vyvinuta syntéza dvou izomerních thienopyrrolopyrimidinových nukleosidů. Tricyklické báze byly připraveny z dichlorpyrimidinu a jodthiofenu tříkrokovou syntézou využívající termické nebo fotochemické cyklizace tetrazolů. Cílové nukleosidy nesoucí hetaryl, amino, dimethylamino, methyl, methoxy a methylsulfanylovou skupinu v poloze 4 byly připraveny pomocí stejných reakcí jako v případě pyrimidoindolů. Nukleosidy anelované s thiofenem jsou testovány na cytotoxické, protivirové a antimikrobiální aktivity. Prozatím jsou dostupné jen výsledky testování anti-HCV aktivit, kde několik sloučenin disponuje sub-mikromolární aktivitou, zároveň ovšem i výraznou (mikromolární) cytotoxicitou.

List of abbreviations

AIDS	acquired immune deficiency syndrome
APCI	atmospheric-pressure chemical ionization
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
CCRF-CEM	human T-lymphoblastoid cell line
CMD	concerted metalation-deprotonation
Cy	cyclohexyl
dba	dibenzylideneacetone
DCM	dichloromethane
DMF	*N*,*N*-dimethylformamide
DMA	*N*,*N*-dimethylacetamide
DME	1,2-dimethoxyethane
eq.	equivalent
ESI	electrospray ionization
FDA	U.S. Food and Drug Administration
HCV	hepatitis C virus
HeLa S3	human cervic carcinoma
HepG2	hepatocelular carcinoma cells
HIV	human immunodeficiency virus
HPFC	high performance flash chromatography
Huh7	hepatocellular carcinoma cells
L1210	mouse lymphocytic leukemia cell line
LiHMDS	lithium hexamethyldisilazane
m.p.	melting point
NCS	*N*-chlorsuccinimide
PyBroP	Bromotripyrrolidinophosphonium hexafluorophosphate

Refl.	reflux
r.t.	room temperature
SAR	structure-activity relationship
TBAB	tetrabutylammonium bromide
TEA	triethylamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
THP-1	acute monocytic leukemia cell line
TMP	2,2,6,6-tetramethylpiperidin
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPPTS	tris(3-sulfophenyl)phosphine trisodium salt
WHO	World Health Organization

List of publications of the author related to the thesis

1) Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y. L.; Yokokawa, F.; Shi, P.-Y.; Hocek, M.: "Synthesis and antiviral activity of 4,6-disubstituted pyrimido[4,5-*b*]indole ribonucleosides" *Bioorg. Med. Chem*. **2012**, *20*, 6123-6133.
2) Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Lee, Y.-J.; Hocek, M.: "Synthesis and biological activity of benzo-fused 7-deazaadenosine analogues. 5- and 6-substituted 4-amino- or 4-alkylpyrimido[4,5-*b*]indole ribonucleosides" *Bioorg. Med. Chem*. **2013**, *21*, 5362-5372.

Contents

1. Introduction

1.1 Purine nucleosides and their biological activities and functions

Nucleosides and nucleotides are an important class of biomolecules. They are basic building blocks of nucleic acids, so they play main role in storage and expression of genetic information. Adenosine triphosphate (ATP) is also an energy rich compound involved in most metabolic energy-releasing or energy-requiring pathways. Nucleotide derivatives, such as NAD, FAD and coenzyme A are necessary parts of many enzymatic reactions, NAD and FAD are involved in cellular redox processes, acetyl-coenzyme A transfers acyl groups. Around 4-7 % of all proteins encoded by genome depend on purine nucleotides (ATP, GTP, cAMP, cGMP, NAD, FAD) as co-factors or co-substrates for their functions.[1] Purine receptors are present in all organs of human body and purine derivatives can serve as agonist or antagonist of these receptors, which are important for regulation of pain signals, blood pressure regulation etc.[2] All these facts put purine nucleosides to the centre of interest, because even a small modification in their structure can affect important cellular mechanisms. This thesis will focus mainly on cytostatic and antiviral effects of selected new base-modified purine nucleosides.

Although purine is the most widely distributed *N*-heterocycle in nature, unsubstituted purine itself has not been found there.[3] However, there are many natural compounds containing purine core, for example purine alkaloids like caffeine and theophylline,[4] plant-growth hormons called cytokinins like zeatin and kinetin and,[5] of course, nucleosides. 2-Methoxyadenosine (spongosine)[6] was isolated from Australian sponge and its corresponding triphosphate inhibits DNA polymerases.3 Nebularine, the simplest purine nucleoside isolated from *Agaricus nebuleris*, is higly toxic nucleoside, it inhibits adenosine deaminase.[7] In addition to many natural purine derivatives, thousands of synthetic purine derivatives and nalogues have been prepared and many of them excerted important biological activity.

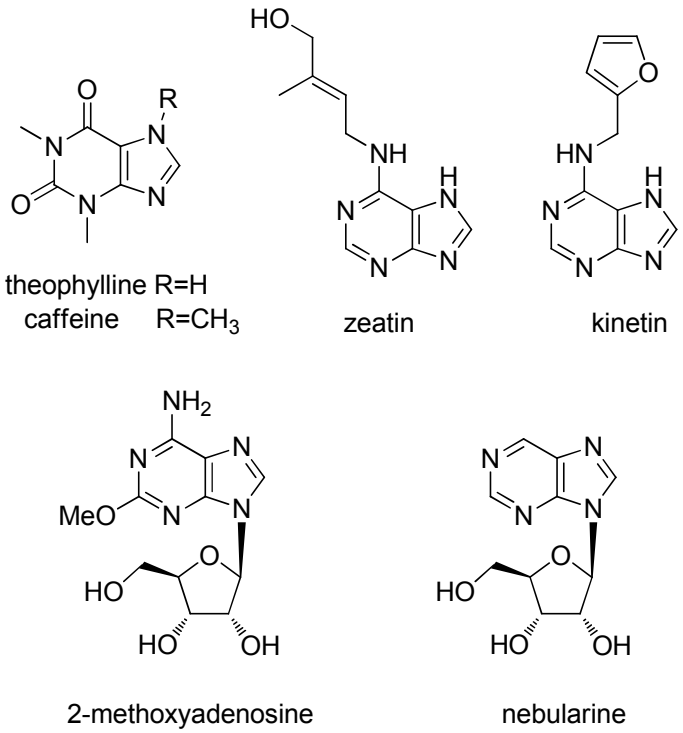


Figure 1 Natural purine derivatives

1.1.1 Purine nucleosides as antivirals

Viral infections are responsible for millions of deaths every year and are caused by DNA or RNA viruses from 21 different families. One of the most famous and dangerous threats is HIV virus causing Acquired Immunodeficiency Syndrome (AIDS). There were around 35 million people infected with HIV worldwide at the end of 2013, and 2 million people became newly infected with HIV in 2013.[8] Since the beginning of AIDS spreading in 1970s and discovery of HIV virus in 1980s, treatment of HIV became a big challenge for scientists, who succeeded during past decades.

Anti-HIV drugs are divided into 6 classes – nucleoside reverse transriptase inhibitors (NRTI), non-nucleoside reverse transriptase inhibitors (NNRTI), protease inhibitors (PI), CCR5 inhibitors, fusion inhibitors and integrase strand transfer inhibitors (ISTI). Reverse transcriptase is a key enzyme in virus life cycle, it is responsible for synthesis of double stranded DNA from viral RNA. It is obvious that nucleosides are potencial inhibitors of reverse transcriptase, so the first FDA approved anti-HIV drug was AZT (Zidovudine, azathymidine) in 1987. AZT was far from being optimal drug against HIV, further studies showed that benefits of AZT do not last long and resistance was being developed over time. For this reason, combination of AZT with dideoxynucleoside reverse transcriptase inhibitors was tested. First tested compounds were didanosine and zalcitabine (Figure 2) and it was shown that their combination with AZT reduced the mortality by 42 % and 32 %

respectively.[9] This was the beginning of combined antiretroviral therapy, also known as HAART (higly active antiretroviral therapy), which originaly consisted of 1-2 nucleoside reverse transcriptase inhibitors, 1 non-nucleoside reverse transcriptase inhibitor and 1-2 protease inhibitors.[10] In 2013, update to the Guidelines for the use of antiretroviral therapy was released.[11] All recommended combinations contain Tenofovir disoproxyl fumarate, purine derivative developed by Antonín Holý and marketed by Gilead Sciences under the trade name Viread.[12] Viread is one of the most succesful drugs against HIV and is also used in HBV therapy.

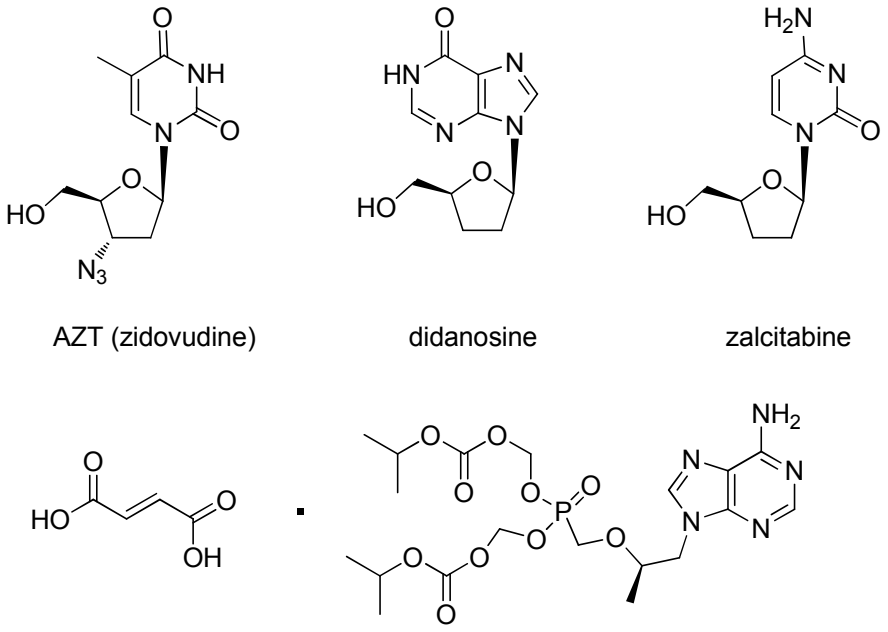


Figure 2 Anti-HIV nucleoside analogues

Another dangerous virus is hepatitis C virus (HCV), which occurs in 6 genotypes and which is now one of the leading causes of death among the people with HIV. Up to 1 from 7 people with HIV are co-infected with HCV[13] and 130-150 million people worldwide are infected by HCV itself. Up to 500 000 people die every year for HCV related diseases.[14] HCV was identified in 1989,[15] 15 years after first suggestion that it exists. Since that time, scientists started with development of HCV treatment.

Pegylated interferon alpha in combination with ribavirin has been used for HCV therapy since 1998,[16] but the clinical benefit of this therapy is limited. Efficacy of this therapy is just around 50 % and has a duration of the treatment of 48 days. This is why there was still need for better therapy. Several nucleosides were identified as HCV replication inhibitors, all of them modified in position 2´ of the ribose part, for example specific inhibitors of RNA-

dependent RNA polymerase 2´-C-methyladenosine and 2´-C-methylguanosine[17,18] or 2´-C-methylcytidine (Fig. 3).[18] Revolution in HCV therapy in last decade led to development of Sofosbuvir (trade mark Sovaldi), which was approved in 2013 by FDA as anti-HCV drug and has become part of all available cures of HCV against all six genotypes. Currently available treatment has effectivity around 90 - 95 % and it takes just 12 weeks.[19] However, there are other viruses, which threaten millions of people and there is no available treatment and no vaccination against them.

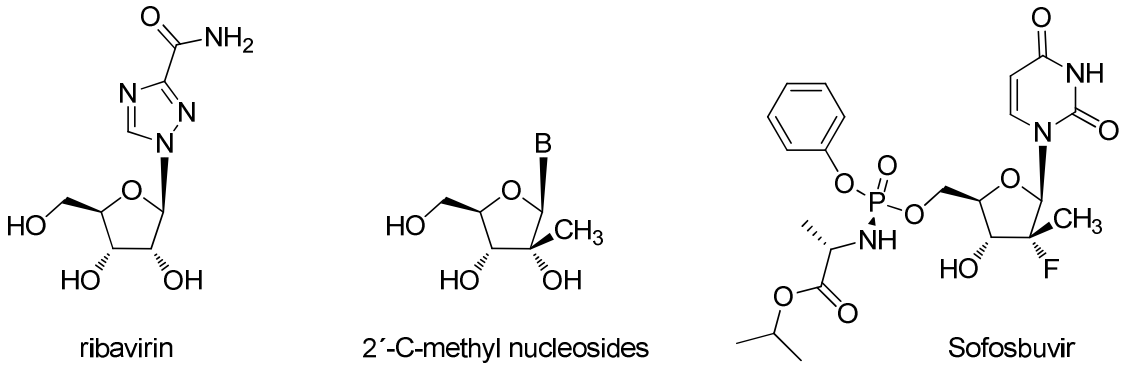
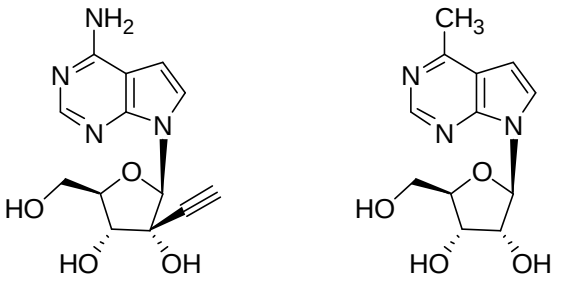


Figure 3 Anti-HCV nucleoside analogues

One of such viruses is Dengue virus, mosquito-born RNA virus with 4 serotypes (DEN-1 – DEN-4) which causes dengue fever and in some cases dengue hemorrhagic fever. Whereas dengue fever is characterized by fever, headache, pain behind the eyes and usually by skin rush and is not life threatening, dengue hemorrhagic fever is assosiated with thrombocytopenia and capillary plasma leakage and can be fatal.[20] There may be 50-100 million of dengue infections worldwide every year, 500 000 of them are dengue hemorrhagic fever with mortality about 2-3 %.[21] Even there is an intensive research on development of dengue treatment, no antivirotics or vaccine are available yet. Although nucleosides have great potential to inhibit dengue virus, there are just few examples of nucleosides with *in vitro* activity, one of them is an adenosine analog NITD-008, which is potent inhibitor of dengue replication and did not show any adverse effect in mice in one week treatment.[22] However, after 2 weeks of oral dosing, severe side-effects were observed in both rats and dogs. These results led to the termination of NITD-008 for further development for DENV treatment.[23] Another example is 6-methyl-7-deazapurine ribonucleoside with IC_{50} = 0.88 μM against DENV-2 virus. This compound was more active against Polio virus in nanomolar concentration and surprisingly, during biological assays of corresponding triphosphates was found, that this compound is not RNA chain terminator, it does not inhibit viral polymerase and is not lethal mutagen, which suggest a different mechanism of action.[24]



NITD-008

6-methyl-7-deazapurine ribonucleoside

Figure 4 Anti-dengue nucleosides

1.1.2 Purine nucleosides in cancer treatment

Cancer is one of the leading causes of death with 14.1 million new cancer cases and 8.2 million cancer death in 2012 worldwide. In 2012, there were almost 33 million people living with cancer worldwide.[25] There are currently 9 nucleoside analogues approved by FDA as cytotoxic agents – cytarabine, fludarabine, cladribine, gemcitabine, clofarabine, nelarabine, capecitabine, floxuridine and pentostatin (Fig. 5).[26]

Mechanism of action of these nucleoside derivatives is based on same metabolic pathways as endogenous nucleosides. Compounds enter cells through specific nucleoside transporters,[27, 28] are phosphorylated inside the cell by nucleoside kinases to mono-, di- and triphosphorylated nucleosides,[29] which can inhibit intracellular enzymes (polymerases, ribonucleotide reductase) or they can be incorporated into newly synthesised nucleic acid and induce termination of chain elongation.[30] But all these nucleosides suffer by severe adverse effects like neurotoxicity, immunosupresion, hepatotoxicity etc., so there is still need for new, more selective nucleosides.

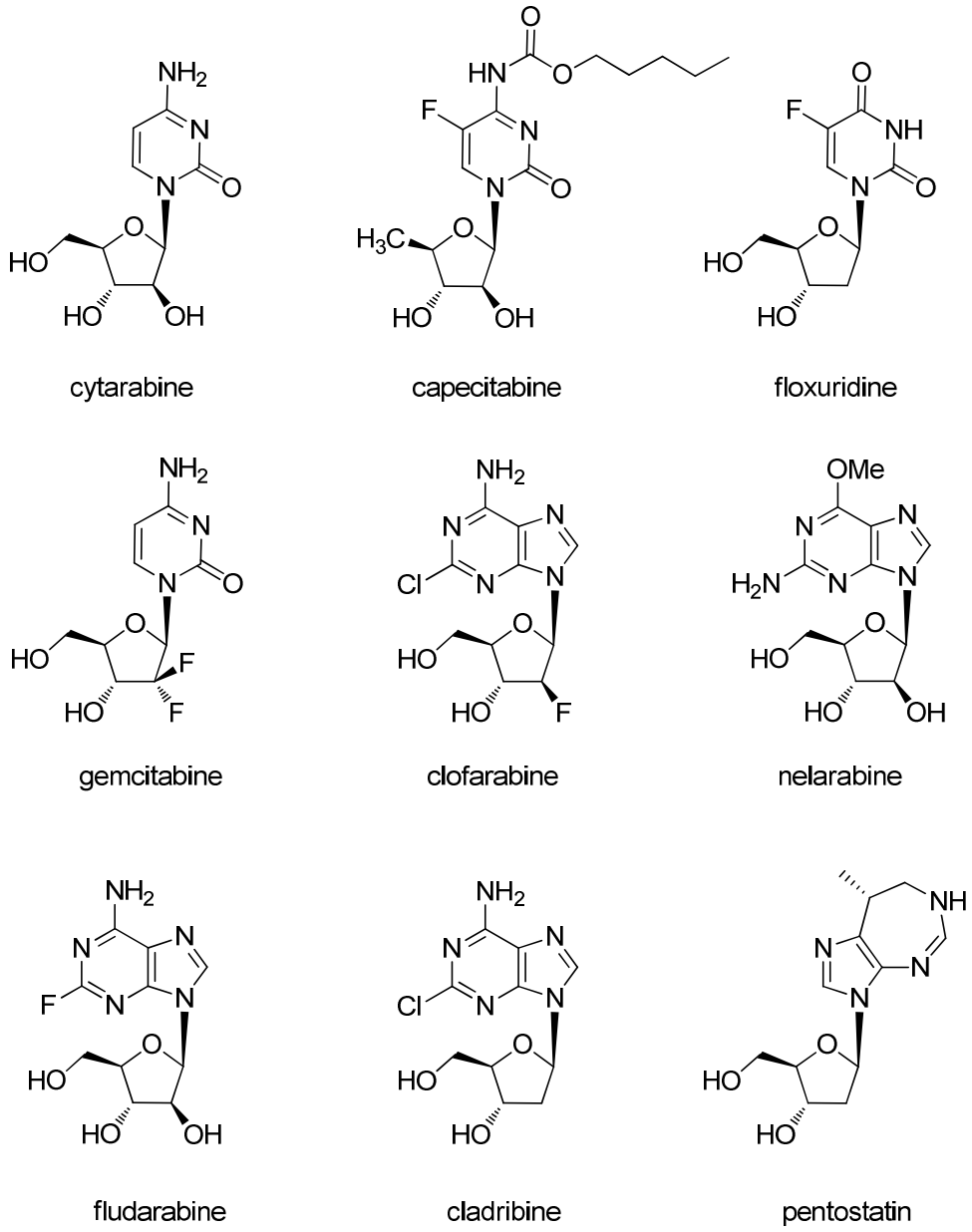


Figure 5 Approved cytotoxic nucleosides

1.1.3 Purine and deazapurine nucleosides previously developed in our group

During a long-term investigation of biological activities (especially cytotoxic and anti-HCV) of modified purine nucleosides in our group, many different types of substituted purine nucleosides have been prepared. In principle, it is possible to modify nucleosides on the heterocyclic base or sugar moiety. Purine base can be substituted in position 2, 6 or 8, another approach can be substitution of one of nitrogen atoms by carbon leading to deazapurines and giving us space for further modification at new carbon atom.

At the beginning of our groups research, many diverse substituted 6-phenyl purine ribonucleosides **I** were discovered as micromolar cytostatics in 2000.[31] Only 4-substituted

phenyl derivatives were active against L1210, HeLa and CCRF-CEM cancer cell lines, whereas 3- or 2-substituted phenyl derivatives as well as 2-aminopurine derivatives were inactive. In order to improve the activity, enlarge SAR study and understand the mechanism of action, 5´-deoxyribo- **II**, 2´-deoxyribonucleosides **III** and 9-(2,3-dihydroxypropyl) acyclic nucleoside analogues **IV** were prepared, but none of these compounds showed any significant cytostatic activity.[32] Also 3´-deoxy analogues **V** were inactive.[33] Next option was the preparation of L-nucleosides **VI**, enantiomers of naturally occuring D-nucleosides. A series of derivatives bearing aryl, hetaryl or alkyl groups in position 6 were synthesized, but all of them were inactive against cancer cell lines.[34]

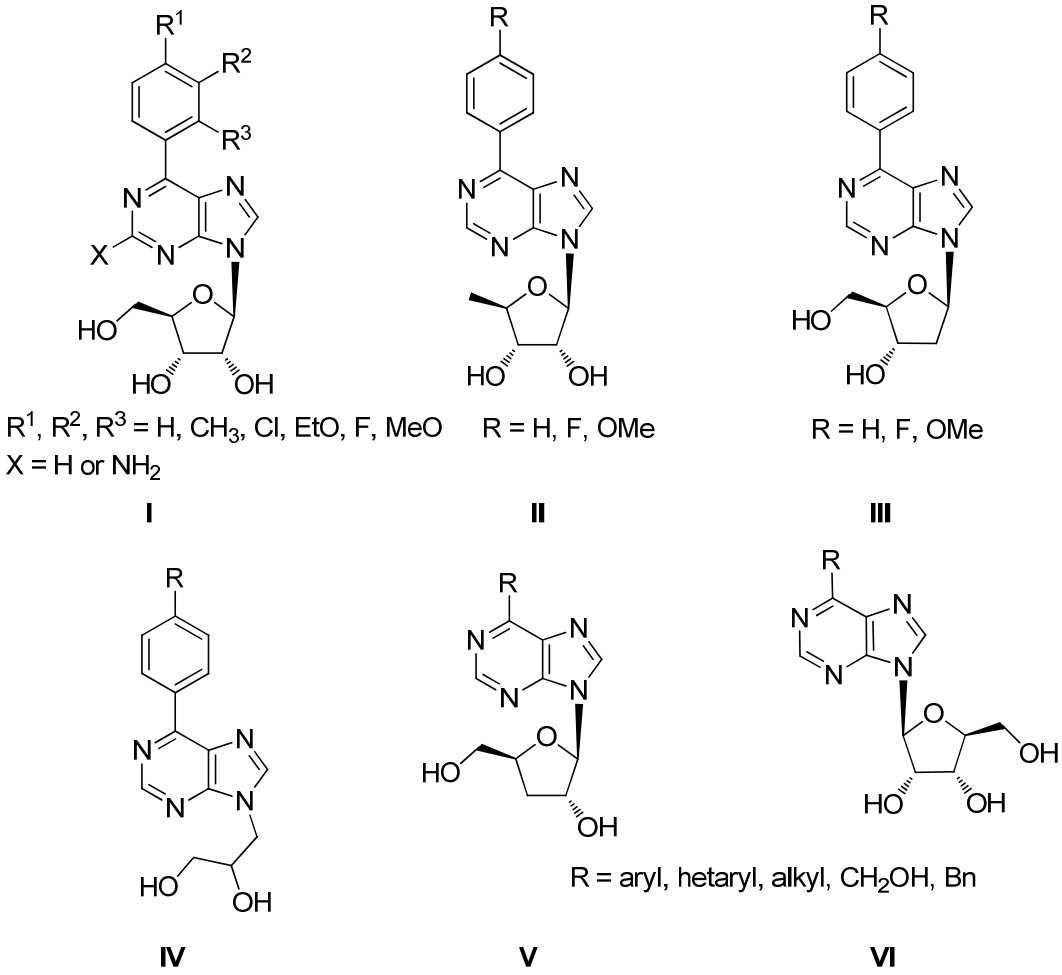
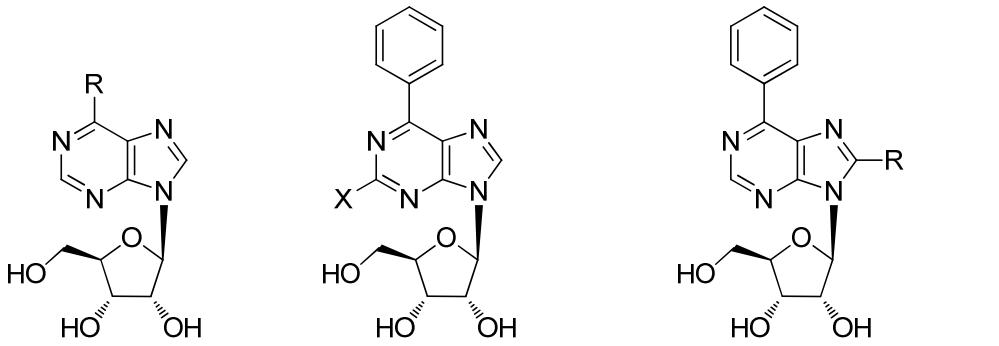


Figure 6 6-Substituted purine nucleosides

In another study, various aryl and hetaryl groups were introduced into position 6 of purine nucleosides **VII**. Since derivatives containing bulky naphtyl groups or 4-trifluoromethylphenyl and 4-hydroxyphenyl in position 6 were completely inactive, nucleosides bearing 2-thienyl and 2-furyl were more active against CCRF-CEM and L1210 cancer cell lines than the parent 6-phenylpurine nucleoside.[35] Series of 6-hetaryl derivatives was later extended and all compounds were also tested for anti-HCV activity with promising

results of furyl, thienyl and pyrazolyl derivatives with sub-micromolar activity against HCV, unfortunately accompanied by effect on cellular RNA.[36]

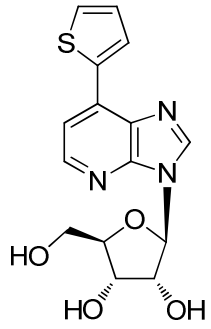
The replacement of a hydrogen atom in position 2 of 6-phenylpurine ribonucleoside by small groups like amino, methyl, chloro, bromo or iodo **VIII** led to complete loss of activity.[37] Substituents in position 2 can interfere with hydrogen-bonding of complementary nucleobase, which is not a case of 6-phenyl ribonucleosides substituted in position 8 by Ph, Me, Et, Bn, OH, OMe, NH_2, NMe_2 and SH groups **IX**. But also all these compounds were inactive.[38]



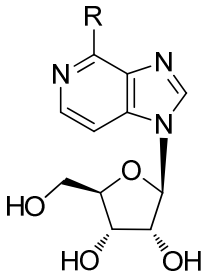
VII **VIII** **IX**

Figure 7 2-, 6- and 8- Substituted purine nucleosides

Last option how to modify purine nucleosides was the replacement of one of the nitrogen atoms by carbon, which leads to deazapurine derivatives. 6-(2-Thienyl)-1-deazapurine nucleoside **X** was tested for cytostatic activity against CCRF-CEM, HT-1080 cells and human WI-38 cells and showed lower activity than corresponding purine analogue. This suggests, that 1-N is not essential for biological activity.[39] On the other hand, 3-deazapurine derivatives **XI** substituted with various aryl, hetaryl, alkyl and cyclopropyl groups at position 6 were all completely inactive, which leads to conclusion, that 3-N is crucial for interaction of these compounds with biomolecular targets.[40]



X



R = aryl, hetaryl

XI

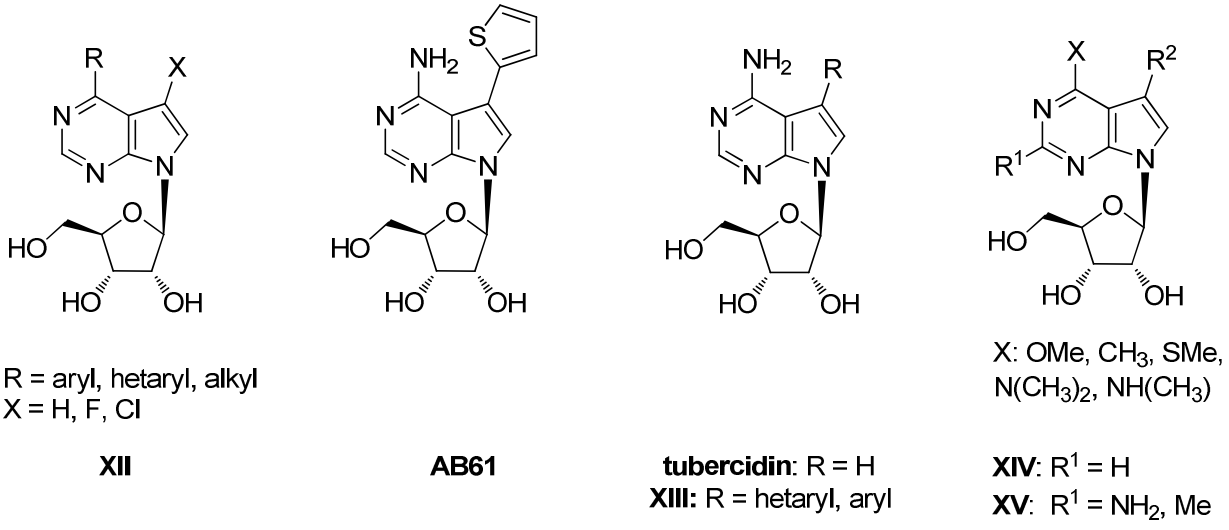
Figure 8 Deazapurine nucleosides

Last type of deazapurines are 7-deaza analogues. A series of 7-deazapurine nucleosides **XII** bearing H, F or Cl atom in position 7 and many different heteroaryl as well as alkyl and hydroxymethyl groups was prepared and cytostatic and anti-HCV activity was explored.[41] Whereas all derivatives bearing 6-membered ring in position 6 were almost inactive or inactive, nucleosides substituted by 5-membered ring displayed interesting activities. Nucleosides bearing *N*-heterocycles were micromolar cytostatics, whilst furyl and thienyl analogues showed nanomolar cytostatic activity against several cancer cell lines, comparable to commercially used cytostatic clofarabine. The most active compounds were 2-furyl and 2-thienyl derivatives non-substituted in position 7. Their 7-fluoro analogues were similarly potent, whilst 7-chloro analogues were less active. Mechanism of action of these compounds is unknown, but they are probably phosphorylated by cellular kinases and inhibit synthesis of RNA.[41] 6-Substituted 7-deazapurine ribonucleosides were also tested as human or mycobacterial adenosine kinase inhibitors. Although 6-hetaryl nucleosides are strong and mostly selective inhibitors of mycobacterial kinase, they showed only limited potential to inhibit growth of mycobacteria, probably because of their poor penetration through bacterial cell wall.[42]

Another class of 7-deazapurine nucleosides are 7-substituted 7-deazapurines **XIII**, analogues of tubercidin (7-deazaadenosine), natural cytostatic antibiotic.[43] 7-Aryl derivatives were almost inactive or totally inactive, whereas 7-hetaryl tubercidins displayed nanomolar activity against human T-lymphoblastic leukemia cell line CCRF-CEM, promyelocytic leukemia HL-60, cervical carcinoma HeLa S3, lung (A-549 cells and NCI-H23), prostate (Du145 and PC3), colon (HCT116 and HCT15) and breast (Hs578 and BT549) carcinomas. The most active compounds from this series were 7-deazaadenosines bearing 2- or 3-thienyl and 2- or 3-furyl groups in position 7 (Fig. 9).[44] AB61 was selected as the most promising compound for further testing and also for investigation of mechanism of action, which is still

underway. What is known so far is that AB61 inhibits RNA synthesis and also DNA synthesis at higher concentration. Inhibition of RNA synthesis is main target for AB61, but it is not caused by direct inhibition of RNA polymerase II, even if AB61 is efficiently phosphorylated.[44] Triphosphate form is incorporated to RNA by T7 RNA polymerase and to DNA by Klenow fragment of DNA polymerase I *in vitro* and also *in vivo* in living CCRF-CEM cells. After incorporation into mRNA, it completely blocks translation. In DNA, AB61 nucleotide leads to DNA damage.[45]

Later on, an extended series of derivatives of 7-deaza-7-hetarylpurine nucleosides was prepared, including 6-methoxy, 6-methylsulfanyl, 6-methylamino, 6-dimethylamino, 6-methyl or 6-oxo nucleosides **XIV** as well as 2,6-disubstituted derivatives **XV**. All 7-deazahypoxanthine derivatives and also all the 2-substituted nucleosides did not show any significant cytotoxic effect. In 6-substituted series, 2-furyl derivatives were always the most active ones in whole series with nanomolar activities. Unfortunately, these compounds were also the most cytotoxic to fibroblasts, which suggest non-specific cytostatic effect. On the other hand, other 7-hetaryl (2-thienyl, 3-thienyl, 3-furyl) nucleosides were less active or inactive. But 6-methoxy-7-(2-thienyl) nucleoside displayed significant activities and no toxicity to fibroblasts, showing promissing therapeutic index. And also 6-methylsulfanyl derivative bearing small ethynyl group in position 7 was shown to be nanomolar cytostatic against most tumour cells with low effect on fibroblasts. This study showed that H-bond donating NH_2 group at position 6 can be in some cases replaced by isosteric nonpolar methyl group or H-bond acceptor groups retaining cytotoxic activity.[46]

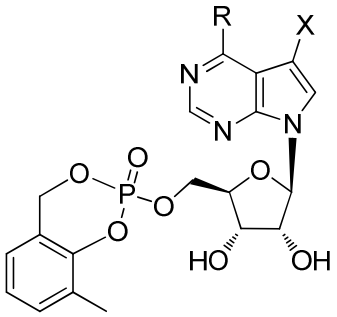


XII **AB61** **tubercidin**: R = H **XIII:** R = hetaryl, aryl **XIV**: R^1 = H **XV**: R^1 = NH_2, Me

Figure 9 7-Deazapurine nucleoside cytostatics

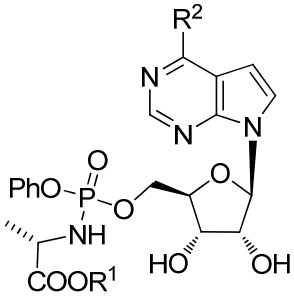
Discovery of these promising new types of cytostatics led to their deeper studies. To overcome the first phosphorylation, which can be crucial step of their activation, two types of prodrugs were designed and synthesised. First type of pronucleotides were *cyclo*Sal prodrugs **XVI** derived from 7-deazapurines bearing hetaryl groups in position 6 and hydrogen or fluorine atom in position 7. *Cyclo*Sal analogues of 7-deaza-7-hetaryl purines showed similar or lower cytostatic activity than the parent nucleosides against the same panel of cell lines - (lung NCI-H23 cells), prostate (DU145 cells), colon (HCT116 cells), and breast (HS578 cells) carcinomas and also human T-lymphoid (CCRF-CEM), promyelocytic leukemia (HL60) and cervical carcinoma (HeLa) cell lines. This means that *cyclo*Sal pronucleotides are probably not suitable type of prodrugs for cytostatic nucleosides and also the first phosphorylation to 5´-*O*-monophosphate is not a limiting step of their activation.[47]

Another class of prodrugs were the phosphoramidate pronucleotides **XVII**. Four series of *O*-phenyl, methyl-, ethyl- and benzylalanyl phosphoramidate pronucleotides derived from 6-hetaryl-7-deazapurines were prepared and tested for cytostatic activity against same cell lines as mentioned above. Although most of the ProTides showed *in vitro* cytostatic activity, these activities were lower than those of parent nucleosides. This leads to the conclusion, that pronucleotide approach is not the way to improve potent *in vitro* cytostatic activity of 6-hetaryl-7-deazapurine nucleosides.[48]



R = hetaryl
X = H, F

XVI



R^1 = Me, Et, Bn
R^2 = hetaryl

XVII

Figure 10 *Cyclo*Sal and phosphoramidate nucleotide prodrugs

In analogy to commercially used cytostatics fludarabine and clofarabine, arabinosides **XVIII**, 2´-deoxy-2´-fluoroarabinonucleosides **XIX** and 2′-C-methylribonucleosides **XX** of 6-hetaryl-7-deazapurines were prepared and tested for cytostatic and anti-HCV activities, but none of them showed any significant activity.[49] Also sugar modified (2´-deoxy-2´-fluororibo **XXI** and 2´-deoxy-2´,2´-difluororibonucleosides **XXII**) derivatives were inactive.[50]

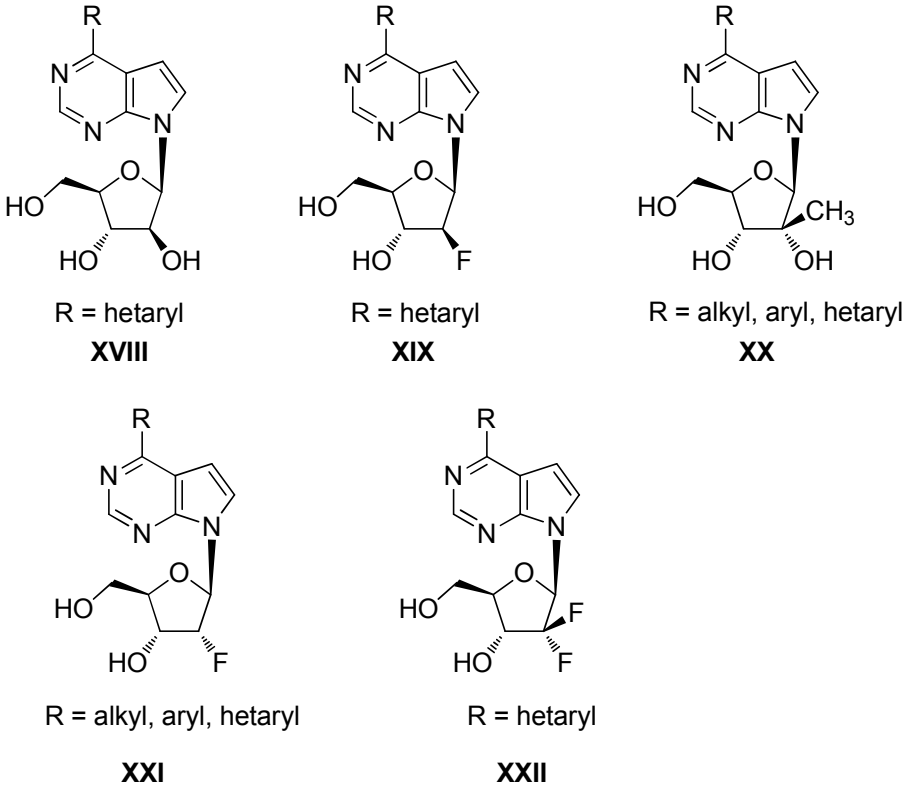
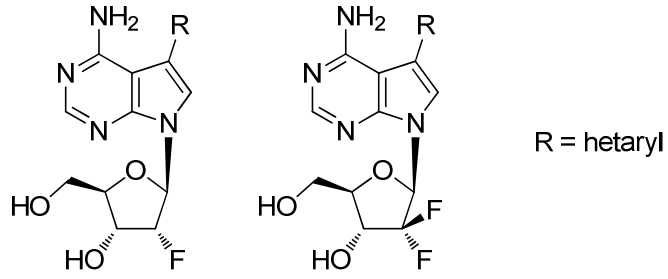


Figure 11 Sugar modified 6-hetaryl-7-deazapurine nucleosides

Sugar modified (2´-deoxy-2´-fluororibo **XXIII** and 2´-deoxy-2´,2´-difluororibonucleosides **XXIV**) analogues of 7-hetaryl-7-deazaadenines were also prepared and their biological activity was studied. All compounds were tested against human T-lymphoid (CCRF-CEM), cervical carcinoma (HeLa), human pro-myelocytic leukemia HL60 and hepatocellular carcinoma HepG2 cell lines. The most active (submicromolar) compounds were fluoro **XXIII** and difluoro **XXIV** 7-iodo-7-deazaadenosines. 2-Furyl, 2-thienyl and surprisingly also bulky naphtyl and benzofuryl 2´,2´-difluoro nucleosides displayed micromolar activity, whereas 3-furyl and 3-thienyl derivatives were completely inactive. In 2´-fluororiboseries, just 7-iodo and 7-ethynyl derivatives were active, none of the 7-hetaryl substituted nucleosides showed any cytostatic activity. This means that the mechanism of action is probably different in each series and most likely differs from parent ribonucleosides, which are more active in all cases.[51]



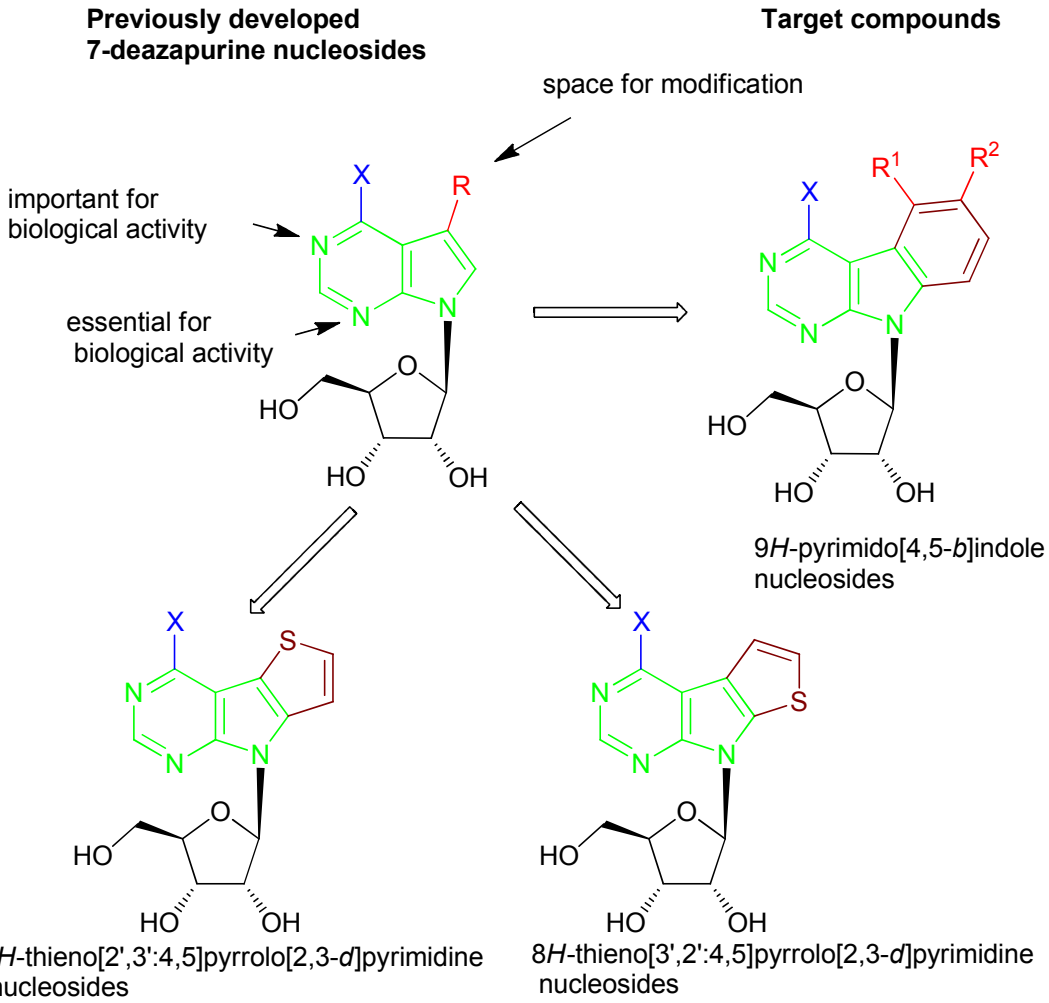
XXIII **XXIV**

Figure 12 7-Hetaryl analogues of tubercidin

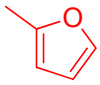
Some of substituted 7-deazapurine nucleosides **XIV** and **XV** (Fig. 9) showed also submicromolar activities against HCV virus. Unfortunately, these activities in most cases correlate with cytotoxicities suggesting that antiviral activity was observed in replicon assays due to interference of such compounds with host targets.[46]

Few 7-substituted analogues of tubercidin **XIII** (Fig. 9) were found to be good inhibitors of mycobacterial adenosine kinase. Whilst nucleosides bearing small hetaryl groups in position 7 were inhibitors of both mycobacterial and human adenosine kinase and above that were cytotoxic, nucleosides bearing bulky groups (dibenzofurane, phenanthrene) were non-toxic and specific inhibitors of mycobacterial adenosine kinase.[52]

The previous studies showed that any modification at the Watson-Crick edge or minor-groove parts of purine are not allowed, whereas there is a space for even bulky modifications in the major groove part (position 7). Therefore, the aim of my project was to further modify this part of the structure by benzo- and thieno-fused extended analogues.



$X = NH_2$, MeNH, MeO, MeS, CH_3; R =

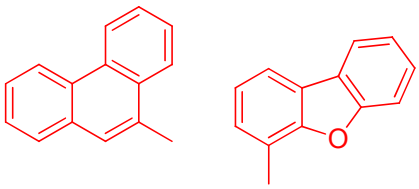


nanomolar non-selective cytostatics

$X = NH_2$; R =

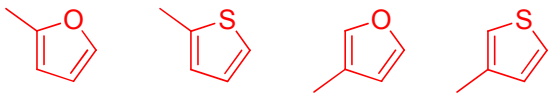
inhibitor of both human and mycobacterial kinase cytotoxic

$X = NH_2$; R =



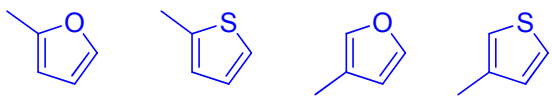
selective inhibitors of mycobacterial kinase non-cytotoxic

$X = NH_2$; R =



nanomolar cytostatics

X =



R = H, F

nanomolar cytostatics

1.2 Pyrimidoindoles – synthesis and biological activities

Fused-7-dezapurines are compounds containing basic 7*H*-pyrrolo[2,3-*d*]pyrimidine annulated with another aromatic system. This thesis will be only about 7-deazapurines fused with 6- and 5-membered (hetero)aryls. One type of such compounds are pyrimido[4,5-*b*]-indoles, which are heterocycles composed of fused pyrimidine and indole ring (Figure 13). From a perspective of a nucleoside chemists it is 7-deazapurine base fused with benzene.

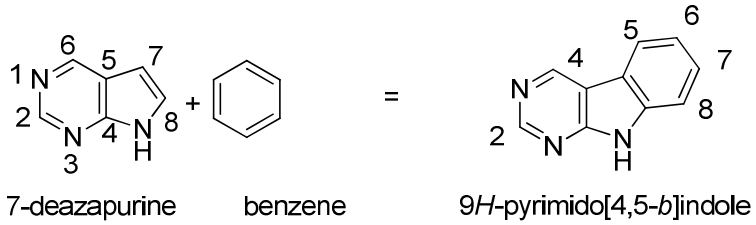
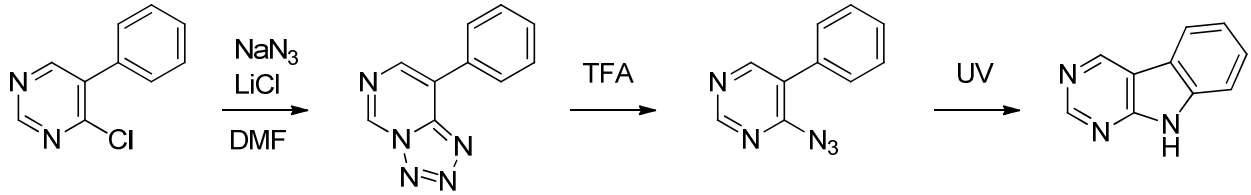


Figure 13 Numbering of 9*H*-pyrimido[4,5-*b*]indole

1.2.1 Synthesis of pyrimido[4,5-b]indoles

Pyrimidoindole bases are well known, the first synthesis was described in 1972.[53] Unsubstituted pyrimidoindole **XXVIII** was prepared by photolysis of easily available tetrazole **XXVI** in TFA (Scheme 1). The reaction was also tried in tetrahydrofuran or acetonirile, but the yield was just about 20 %. TFA is necessary for shifting the equilibrium to azide **XXVII**,[54] which is under irradiation cleaved to N_2 and reactive nitrene species,[55] which then attacks the phenyl ring to form a tricyclic product. Thermolysis of tetrazoles was also studied and no advantage of thermolysis over photolysis was found.[56] Starting tetrazole can be easily prepared by nucleophilic substitution of 4-chloro-5-arylpyrimidine **XXV** with sodium azide and lithium chloride in DMF.

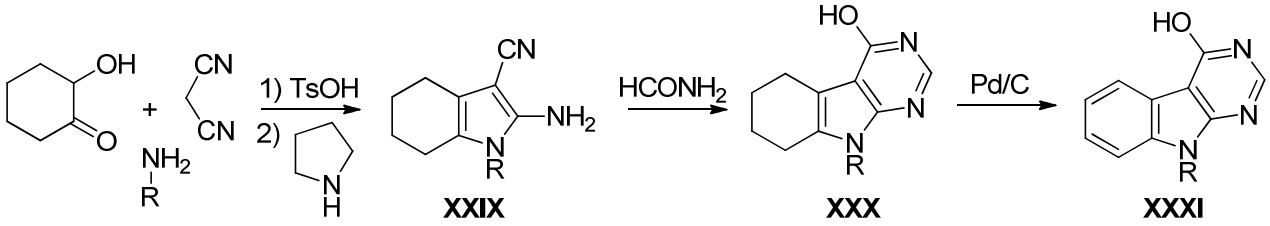


XXV **XXVI** **XXVII** **XXVIII**

Scheme 1 Synthesis of 9*H*-pyrimido[4,5-*b*]indole via photocyclization

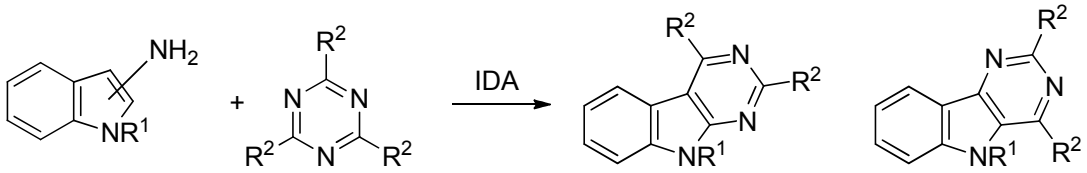
Another approach to pyrimidoindoles is based on dehydrogenation of corresponding 5,6,7,8-tetrahydropyrimidoindoles **XXX** by DDQ or by palladium on charcoal, which was shown to be more efficient and general, but on the other hand, it is not compatible with halogen atoms.[57] 5,6,7,8-tetrahydropyrimidoindoles **XXX** can be synthesised from

2-hydroxycyclohexanone, amine and malononitrile, resulting 2-aminotetrahydroindole-3-carbonitrile **XXIX** is than cyclized with formamide (Scheme 2).



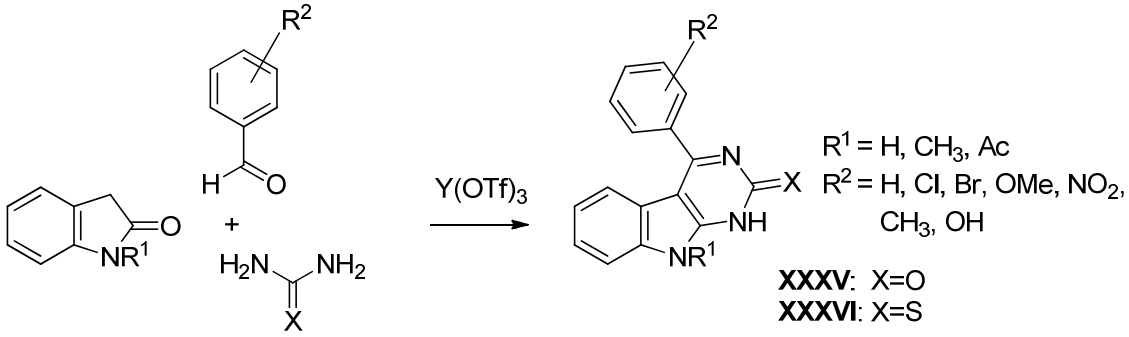
Scheme 2 Synthesis of 9*H*-pyrimido[4,5-*b*]indole via dehydrogenation

Recently, a new method for synthesis of pyrimidoindoles was developed. This method is based on new inverse-electron-demand Diels–Alder (IDA) reaction of aminoindoles as dienophiles with 1,3,5-triazines. Depending on aminoindole, 2,4-disubstituted pyrimido-[5,4-*b*]indoles **XXXIVb** or pyrimido[4,5-*b*]indoles **XXXIVa** can be prepared from 3-aminoindole (**XXXIIb**) or 2-aminoindole (**XXXIIa**), respectively. The reaction can be performed in methanol or DMF at r.t. and it allows introduction of groups like COOR, CF_3, OMe etc. into positions 2 and 4 (Scheme 3).[58,59]



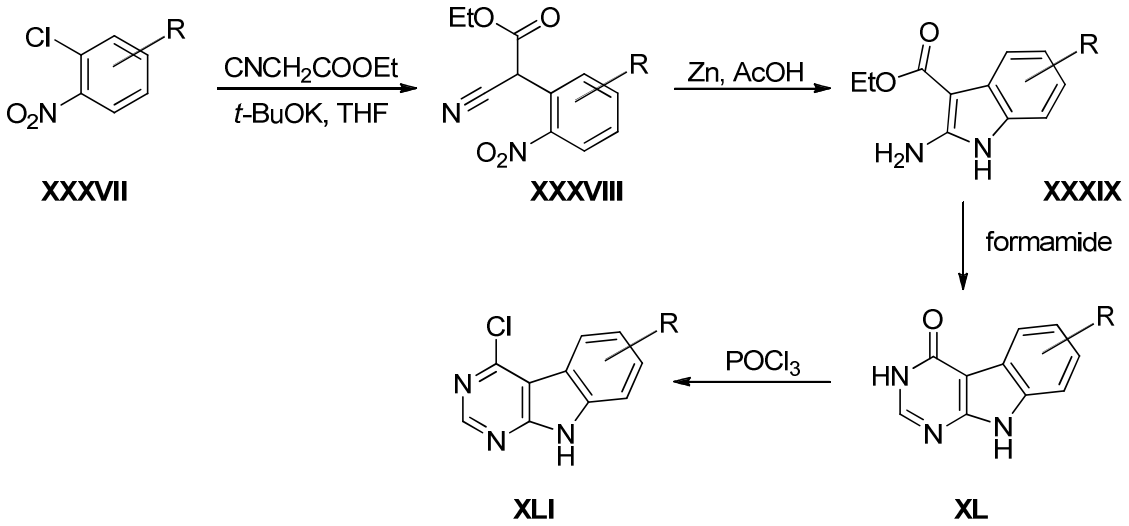
Scheme 3 Synthesis of 9*H*-pyrimido[4,5-*b*]indole via inverse-electron-demand Diels-Alder reaction

In 2014, a new one pot multi-component solvent-free synthesis of pyrimidoindoles was published. An aromatic aldehyde reacts with oxindole and urea or thiourea in solvent free conditions using $Y(OTf)_3$ as a catalyst to furnish 4-arylpyrimido[4,5-*b*]indol-2-ones **XXXV** or corresponding 4-arylpyrimido[4,5-*b*]indol-2-thiones **XXXVI** in good yields. This reaction works also with acetic acid instead of $Y(OTf)_3$, but yield is slightly lower. Both EWG and EDG *o*- and *p*-substituted benzaldehydes can be used as substrates, *p*- and EWG substituted ones gave better yields. But this methodology is limited just to 4-substituted derivatives bearing oxo- or thio- groups in position 2.[60] This three component synthesis can be also performed in ethanol and catalyzed by KF/alumina (Scheme 4).[61]



Scheme 4 Solvent-free multicomponent synthesis of 9*H*-pyrimido[4,5-*b*]indoles

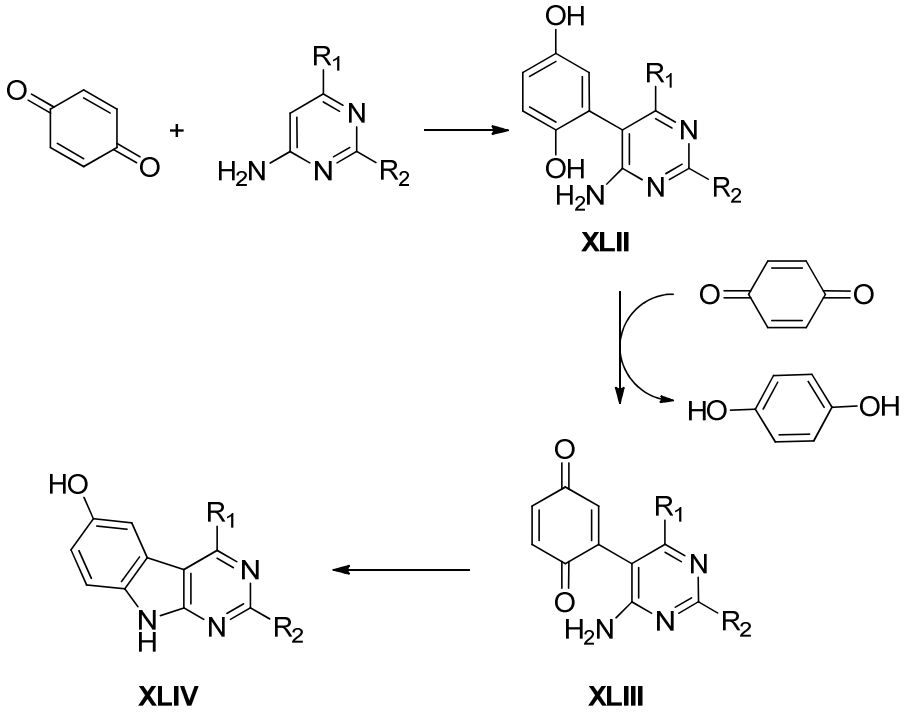
Pyrimidoindoles can be also built up from simple and commercially easily available *o*-chloronitrobenzenes **XXXVII**. In the first step, chlorine is replaced by ethyl-cyanoacetate salt, this nucleophilic aromatic substitutiton is followed by reduction of nitro group and spontaneous cyclization to ethyl 2-aminoindole-3-carboxylate **XXXIX**. This useful intermediate can be than cyclized with different reagents to pyrimidoindolones **XL**, substituted or unsubstituted in position 2. Reagents like carbamidic chloride hydrochloride,[85] triethyl orthoformate,[62] formamide[63] or formamidine acetate[64] can be used for cyclization to provide pyrimidoindolones which can be converted to chloro derivatives **XLI** by treatment with $POCl_3$ (Scheme 5). Availability of variously substituted *o*-chloronitrobenzenes makes this approach quite general for synthesis of halogenated pyrimidoindoles as intermediates for further derivatization.



Scheme 5 Synthesis of pyrimidoindoles from *o*-chloronitrobenzenes

2,4-Diaminopyrimidoindoles **XLIV** can be also synthesised by extended Nenitzescu reaction. The mechanism of this reaction was investigated in 1966.[65] The first step is an

addition of enamine to benzoquinone to produce intermediate **XLII**, which is than oxidized to benzophenone derivative **XLIII**, next step is intramolecular cyclization followed by reduction by hydroquionone formed during the reaction. (Scheme 6). Originally, Nenitzescu reaction was a reaction between 1,4-benzoquinone and ethyl β-aminocrotonate which produces 2,3-disubstituted 5-hydroxyindoles.[65] An extended version of this reaction uses aromatic triamines instead of β-enaminoesters. Reaction between 1,4-benzoquinone and pyrimidine-1,3,6-triamine in boiling glacial acetic acid leads to 2,4-substituted pyrimido[4,5-*b*]indol-6-oles **XLIV**. There are several limitations of this reaction, this method allows introduction of hydroxy group just into one position at benzene ring (position 6) and substituent in position 2 is usually an amine. The limitation is caused by the fact, that ^{13}C NMR shift of C-5 of pyrimidine component must be lower than 77 ppm, otherwise there is no reaction.[66]



Scheme 6 Synthesis of pyrimidoindoles via Nenitzescu reaction

1.2.2 Synthesis of pyrimidoindoles via C-H arylation

Previously mentioned methods are all based on classical synthetic reactions. During the past two decades, there was a big boom of palladium catalyzed C-H activations including C-H arylations. Cross-coupling reactions are usually the first choice for making aryl-aryl bond, but they require two functionalized molecules, aryl halide or pseudohalide and molecule bearing some metal containing group, which is not always easily available or stable.

An advantage of direct C-H funcionalization over cross-coupling is in saving the number of synthetic steps, on the other hand, it can be difficult to perform the reaction regioselectively.

C-H arylations are usually sensitive to all possible factors like Pd catalyst, ligand, base and also solvent and temperature, optimization of reaction conditions is always necessary.[67] Although there are some ligand-free examples,[68] trialkyl phosphine, tricyclohexylphosphine or mainly phosphines bearing electron-withdrawing fluorine atoms are usually used as ligands and $Pd(OAc)_2$ as a palladium source.[69] Best yields are usually obtained with KOAc or Cs_2CO_3 in DMF, DMA or toluene at temperatures above 100 °C. In some cases, other additives can be used. In case of aryl iodides, Ag_2CO_3 is added to protect catalyst from poisoning by iodine anions.[70] Usually, some other additives like TBAB,[71] or pivalic acid[72,73] are used. Pivalate anion acts as a base in arylation reaction and was found to be the best from several other carboxylates (acetate, propanoate, 2-methylpropanoate, trifluoroacetate and adamantan carboxylate).[74]

Several mechanisms were originally suggested for C-H arylation, but only electrophilic aromatic substitution in case of some electron-rich compounds[75] and concerted metalation-deprotonation (CMD) mechanisms are generally accepted today. CMD mechanism, sometimes called proton-transfer pathway, was supported by experimental and computational studies and is shown in Figure 14.[76] Pivalate anion acts as proton shuttle in this catalytic cycle.[77,78]

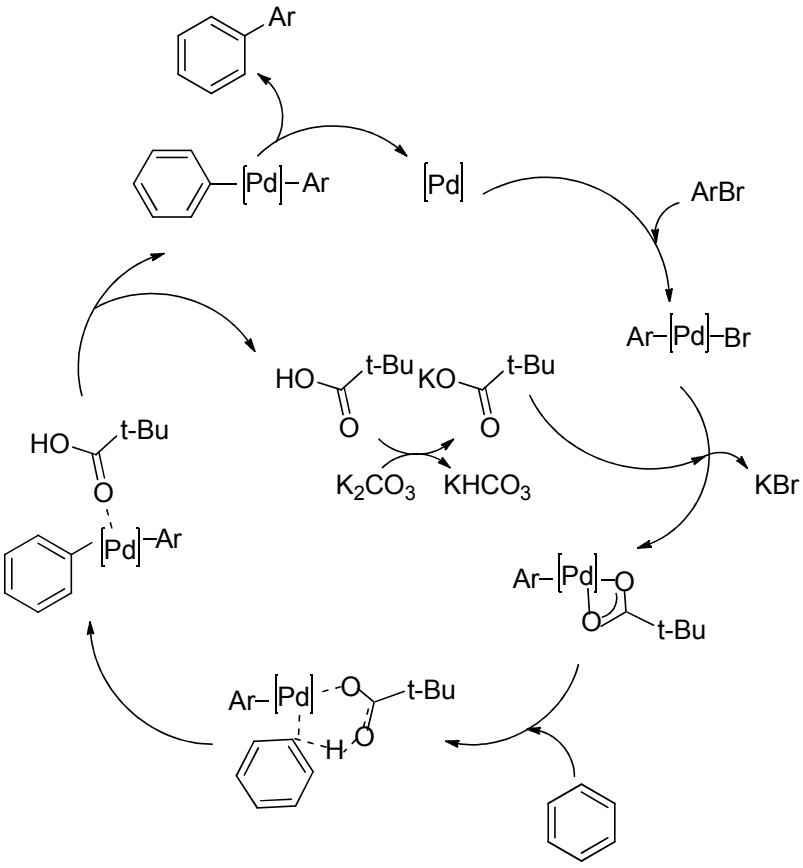
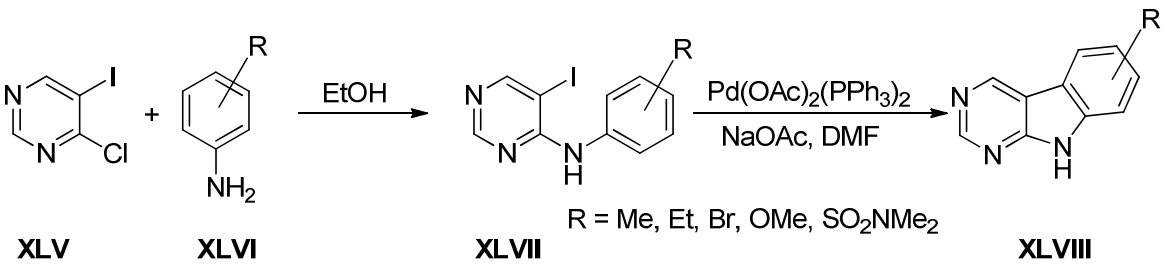


Figure 14 Concerted metalation-deprotonation mechanism (CMD)[79]

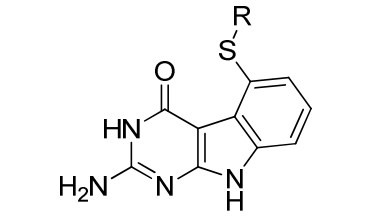
C-H arylation approach was applied for synthesis of series of pyrimidoindoles in 2002. Pyrimidoindoles **XLVIII** were synthesised by palladium catalyzed intramolecular arylation of variously substituted 5-iodo-4-anilinopyrimidines **XLVII**, which were easily prepared by nucleophilic substitution of 4-chloro-5-iodopyrimidine **XLV** with different anilines **XLVI**. $Pd(OAc)_2(PPh_3)_2$ in combination with 1.5 eq. of NaOAc in DMF at 85 °C were found to be the best catalytic system for the intramolecular arylation (Scheme 7). [80]



Scheme 7 Synthesis of pyrimidoindoles via C-H arylation

1.2.3 Biological activities of pyrimidoindoles

In last decade, the biological activities of pyrimidoindoles have been intensively studied. 2-Aminopyrimido[4,5-*b*]indol-4-ones bearing arylthio substituents in position 5 **XLIX** were reported as strong and selective inhibitors of thymidylate synthase-dihydrofolate reductase with nanomolar K_i. This enzyme is crucial for *Toxoplasma gondii* parasite, which has infected around one third of world´s population and is potential threat for immunosuppressed people, like patients with AIDS, after organ transplantation or people on chemotheraphy.[81]

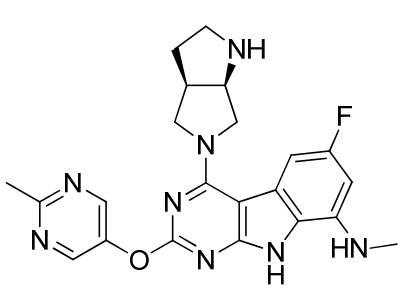


R = 1-naphtyl, 2-naphtyl, phenyl

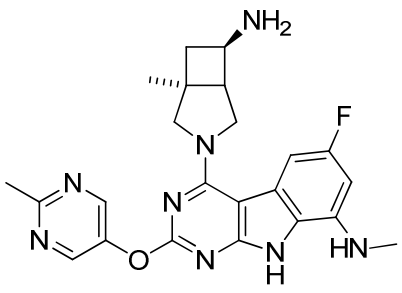
XLIX

Figure 15 Toxoplasma gondii enzyme inhibitors

Higly substituted pyrimido[4,5-*b*]indoles **L** and **LI** are also a new class of dual-targeting antibacterial agents with broad spectrum of nanomolar activities against both gram-positive and gram-negative bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis, Escherichia coli* etc.) including highly drug resistant strains like *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. These compounds are inhibitors of both bacterial topoisomerases DNA gyrase and topoisomerase IV, enzymes necessary for DNA replication. This dual action effectively suppress the emergence of resistance.[82] The most active compound (cyclobutyl) was further tested against 303 nonduplicate *Pseudomonas aeruginosa* strains with MIC 1μg/ml.[83]



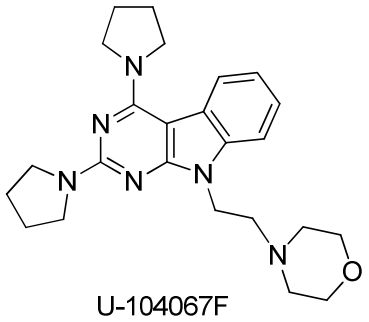
L



LI

Figure 16 Antibacterial pyrimidoindoles

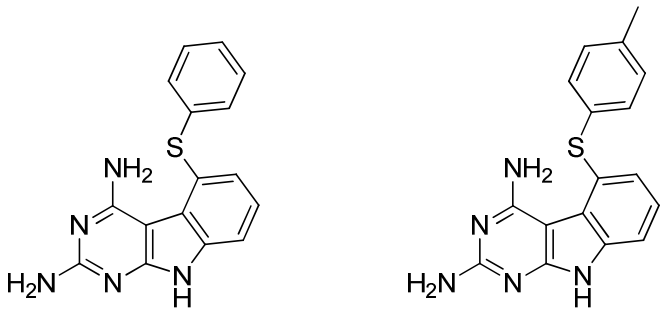
Other pyrimidoindole derivative U-104067F, a brain-penetrating compound, was shown to have neuroprotective effect on nigrostriadal neurons, which if lost leads to Parkinson disease. U-104067F significantly reduces the loss of neurons to just 20 % at 30mg/kg dose in gerbils, which were insulted with induced forebrain ischemia.[84]



U-104067F

Figure 17 Neuroprotective pyrimidoindole derivative U-104067F

2,4-Diaminopyrimido[4,5-*b*]indoles with phenylthio **LII** and *p*-tolyl **LIII** substitutents in position 5 were also evaluated as receptor tyrosine kinase and thymidylate synthase inhibitors and as antitumor agents. Both derivatives inhibit vascular endothelial growth factor receptor (VEGFR-2) and platelet-derived growth factor receptor (PDGFR-β) in concentrations comparable with commercially used compounds and human dihydrofolate reductase (hTS) better than clinicaly used PMX, which is probably caused by better C-ring interaction with active site. Derivative **LII** also decreased tumor growth, significantly reduced liver metastasis and decreased primary tumor blood vessels in *in vivo* mice metastatic colon cancer COLO-205 at 25 mg/kg dose.[85]

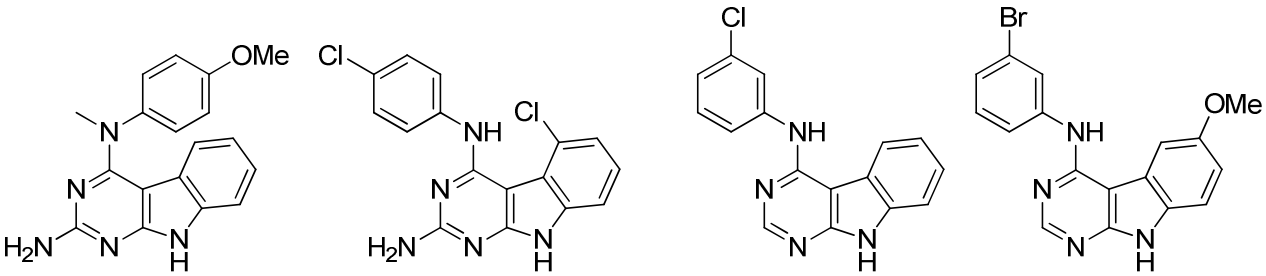


LII **LIII**

Figure 18 Structures of kinase inhibitors

N^4-(4-methoxyphenyl)-N^4-methyl-*9H*-pyrimido[4,5-*b*]indole-2,4-diamine (**LIV**) was found to be very effective microtubule disrupting compound and became a lead compound in development of antimitotic agents.[86] Very similar compounds with additional chlorine substituent in position 5 were tested as vascular endothelial growth factor receptor-2

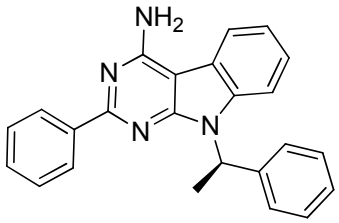
inhibitors and 4-(*p*-chlorophenylamino) derivative **LV** showed comparable activity and selectivity with approved drugs sunitibib and semaxinib.[87] Pyrimidoindole bases **LVI** and **LVII** were also reported as tyrosine kinase inhibitors[88] and inhibitors of the epidermal growth factor receptor tyrosine kinase, respectively.[89]



LIV **LV** **LVI** **LVII**

Figure 19 Biologically active aminopyrimidoindoles

Several pyrrolo[2,3-*d*]pyrimidine and pyrimido[4,5-*b*]indole derivatives were tested as adenosine receptor antagonists, which are divided into 4 subtypes A_1, A_{2a}, A_{2b}, A_3. A_1-antagonists are currently developed for the treatment of cognitive diseases, renal failure, and cardiac arrhythmias. The most active compound from whole 7-deazapurine series was 4-amino-9-(*R*-1-phenylethyl)-2-phenylpyrimido[4,5-*b*]indole (**LVIII**) with inhibition constant K_i 2 nM with more than 2000 fold selectivity for A_1 than A_{2b}.[90]



LVIII

Figure 20 Adenosine receptor antagonist

1.2.4 Pyrimidoindole nucleosides and their application

Even if the pyrimidoindole bases are well known and extensively studied compounds, no pyrimidoindole ribonucleosides have been published (besides those prepared in this thesis) in February 2015. On the other hand, several 2´-deoxyribonucleosides have been prepared and used in biochemical applications by Saito group.[91,92,93]

6-Methoxybenzodeazaadenosine (^{MD}A) was phosphorylated to its triphosphate and incorporated into DNA by several polymerases, KOD Dash in presence of manganese ions proved to be the most effective one. Such DNA containing ^{MD}A bases has high hole-transport

efficiency.[91] Also DNA containing benzodeazaadenosine (^{BD}A) and naphtalene-fused deazaadenosine (NDA) showed high hole-transporting ability and can be used for preparation of DNA nanowires.[92]

^{MD}A and ^{MD}I are base-discriminating fluorescent nucleotides, which emit strong fluorescence only if the base on complementary strand is C or T, respectively, so these nucleotides can be used for detection of single-nucleotide alteration like single-nucleotide polymorphism typing.[93]

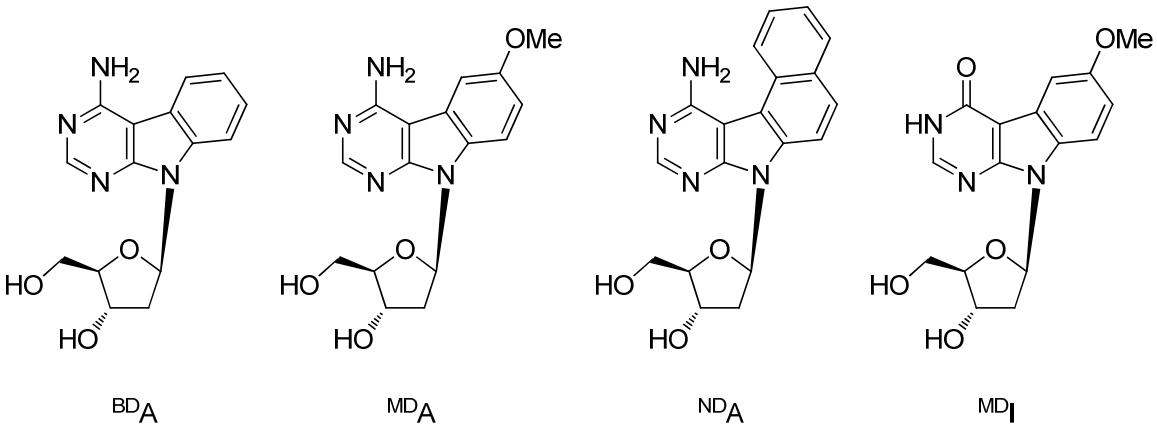
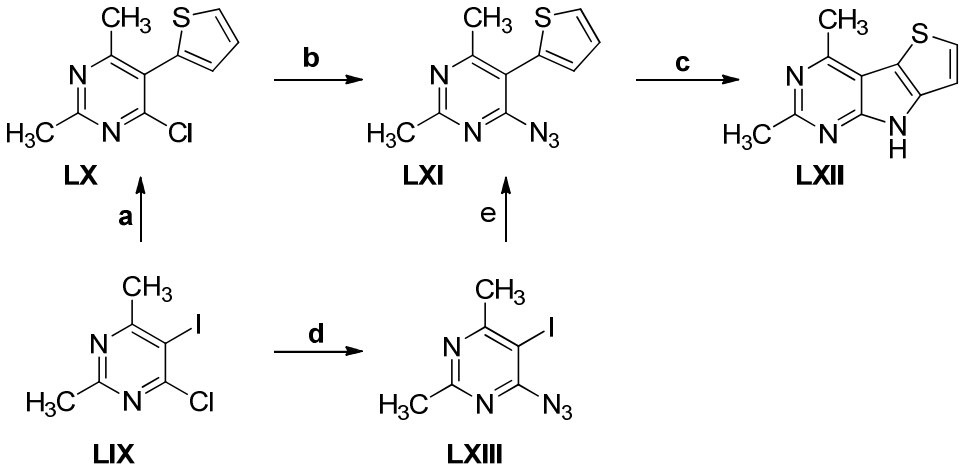


Figure 21 Saito´s fluorescent pyrimidoindole deoxyribonucleosides

1.3 Thiophene-fused 7-deazapurines

Thiophene-fused deazapurines are an unexplored class of compounds. There is just one published compound with such tricyclic motif, 2,4-dimethyl-8*H*-thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (**LXII**), which was prepared by photochemical cyclization of azide **LXI** in TFA in 73 % yield. The whole synthesis started from 2,4-dimethyl-5-iodo-6-chloropyrimidine (**LIX**), thienyl group was introduced into position 5 by Stille coupling using $PdCl_2(PPh_3)_2$, azide **LXI** was prepared by direct nucleophilic substitution with sodium azide. It is possible to do first nucleophilic substitution and then cross-coupling, but better yields were obtained with reverse way.[94] To the best of my knowledge, no other thiophene-fused deazapurine base or even nucleoside is known at this moment.

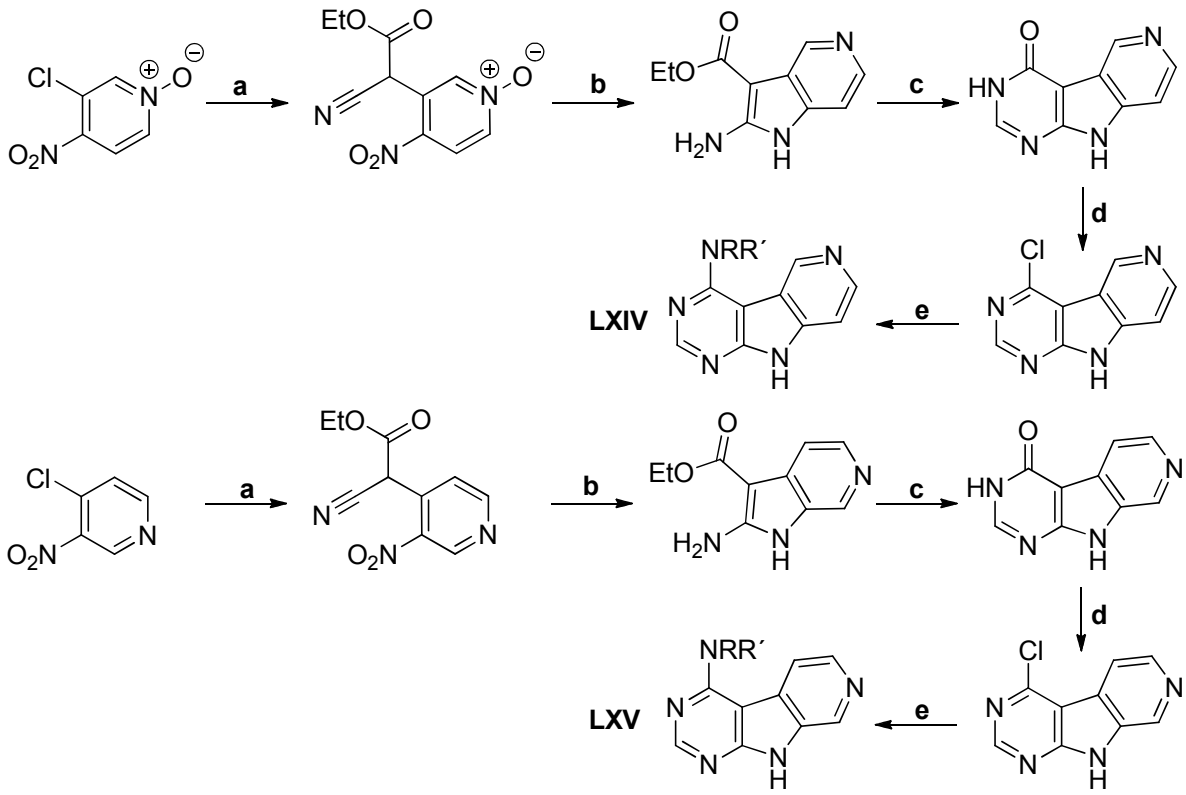


a, e: 2-(tributylstannyl)thiophene (1.5 eq.), $PdCl_2$ (0.1 eq.), Et_3N (1 eq.), DMF, 120 °C;
b, d: NaN_3, EtOH, refl., 12 h; **c**: TFA, UV (mercury lamp), r.t., 2 h.

Scheme 8 Synthesis of thiophene-fused 7-deazapurine

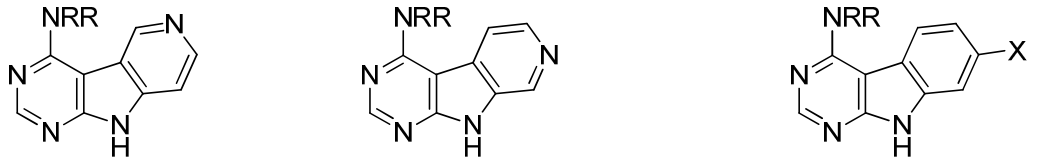
1.4 Pyridine-fused 7-deazapurines

There are several other types of hypothetical 7-deazapurines annulated with 5- or 6-membered heteroaryl ring. Only few of them have already been synthesized. One class of such compounds are for example pyridine-fused deazapurines. 9*H*-pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidines **LXIV** bearing various amines were prepared as selective micromolar checkpoint kinase (CHK1) inhibitors.[95] Pyridopyrrolopyrimidines **LXIV, LXV** were synthesized by classical heterocyclization reactions (Scheme 9) and together with substituted pyrimido[4,5-*b*]indoles **LXVI** patented as CHK1 kinase function inhibitors with potential use for cancer treatment.



a: $CNCH_2COOEt$, *t*-BuOK, THF, 60 °C; **b**: Zn, AcOH, r.t.; **c**: formamide, ammonium formate, 170 °C, 16 h; **d**: $POCl_3$, 75 °C, 18 h; **e**: amine, TEA, DMF, 120 °C.

Scheme 9 Synthesis of pyridopyrrolopyrimidines



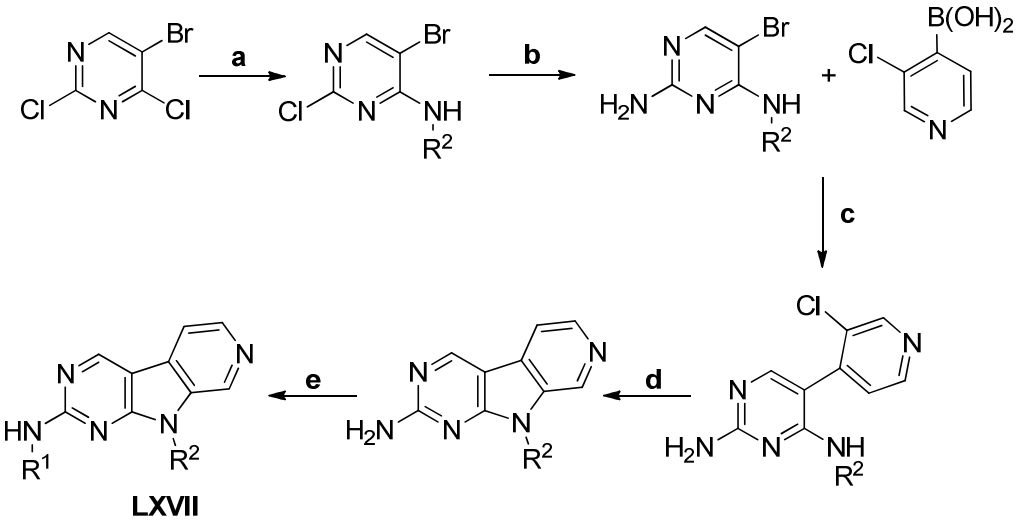
R = morfoline and subst. morfoline, alkyldiamines etc.

X = H, CN, COOMe, $CONH_2$

LXIV **LXV** **LXVI**

Figure 22 Tricyclic heterocyles as CHK1 inhibitors

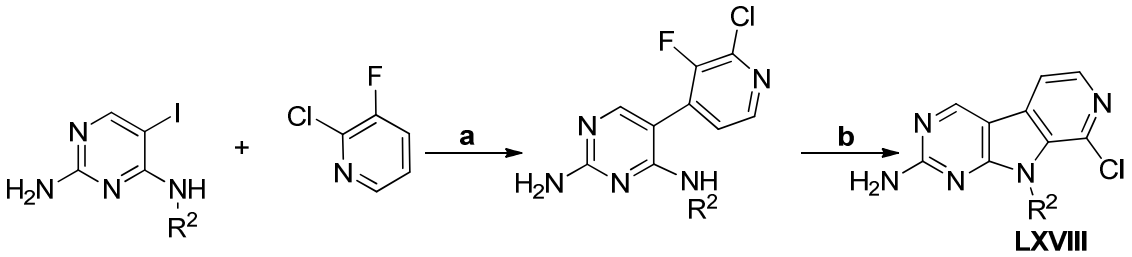
Three types of tricyclic heterocycles (pyridine-fused **LXVII**, *o*-substitutedpyridine-fused **LXVIII**) were studied as dual kinase inhibitors - FMS-like tyrosine kinase (FLT3) and cyclin-dependent kinase 4 (CDK4) for treatment of acute myeloid leukemia. Tricyclic core was synthesized by Suzuki (Scheme 10) or Negishi (Scheme 11) coupling between pyrimidine and pyridine ring, central pyrrol ring was formed by Buchwald-Hartwig amination.[96,97,98]



R^1: 2-pyridinyl (subst. in position 4 or fused with cylohexane, morfoline, piperazine, piperidine)
R^2: cyclopentyl, cyclohexyl

a: R^2-NH_2, dioxane, r.t.; **b**: NH_4OH, 120°C; **c**: $PdCl_2(PPh)_3$
Na_2CO_3, dioxane, 120 °C; **d**: Xantphos, $Pd_2(dba)_3$, *t*-BuONa, dioxane, 150 °C;
e: chloraryl, Xantphos, $Pd_2(dba)_3$, *t*-BuONa, dioxane, 120 °C .

Scheme 10 Synthesis of pyridine-fused compounds **LXVII** via Suzuki coupling



a: 1) 3-fluoro-2-chloropyridin, BuLi, *i*-Pr_2NH, THF, -78 °C, 20 min then $ZnCl_2$, -78 °C to r.t., 30 min;
2) pyrimidine, $Pd(PPh_3)_4$,THF, refl.;
b: LiHMDS. dioxane/THF, 85 °C.

Scheme 11 Synthesis of pyridine-fused compound **LXVIII** via Negishi coupling

Structure-based drug design led to the discovery of pyridine-fused derivative **AMG925** with potent *in vitro* activities against FLT3 and CDK4 kinases (3 nM and 1 nM, respectively) and *in vivo* antitumor efficacy.[96] In preclinical trials, **AMG925** inhibited xenograft tumor growth by 96 % in 37.5 mg/kg dose without significant body weight loss.[99]

Another pyridine-fused compound, PNU-107484A was shown to be unique $GABA_A$ receptor ligand and it could be used for investigation of the physiological roles of various isoforms of α subtypes.[100]

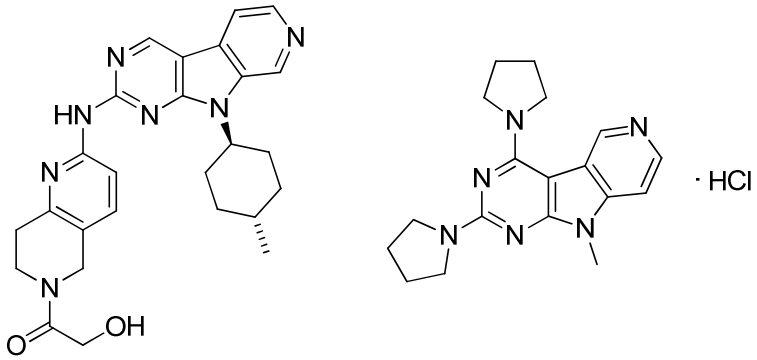
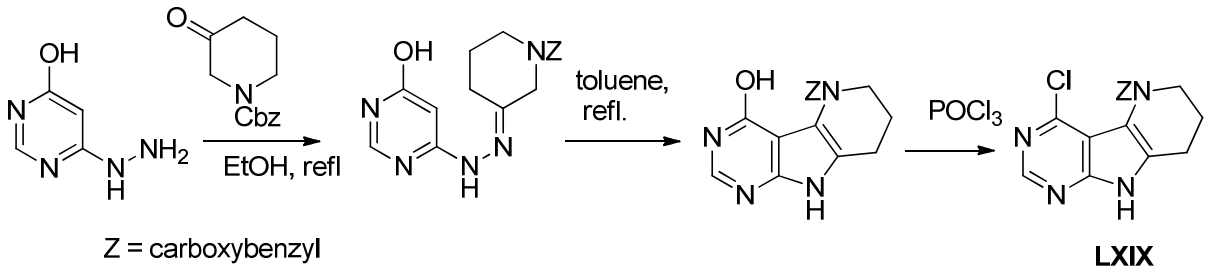


Figure 23 Structures of **AMG925** and **PNU-107484A**

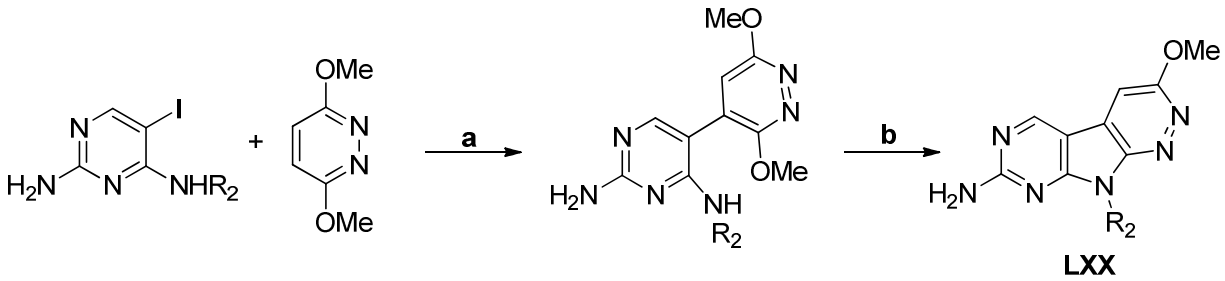
The last type of pyridine-fused 7-dezapurine bases are 9*H*-pyrido[2',3':4,5]pyrrolo[2,3-*d*]pyrimidines. These compounds have not been reported yet, but synthesis of partially saturated tricyclic base **LXIX** (Scheme 12) was patented among synthesis of other compounds as potential inhibitors of cardiac troponin l-interacting kinase (TNNI3K), also known as CARK (cardiac ankyrin repeat kinase), which exhibits highly selective expression in cardiac tissues.[101] These compounds could be in principle dehydrogenated to aromatic analogues by Pd/C or DDQ.[57]



Scheme 12 Synthesis of potential 9*H*-pyrido[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine precursors

1.5 Pyridazine and pyrimidine-fused deazapurines

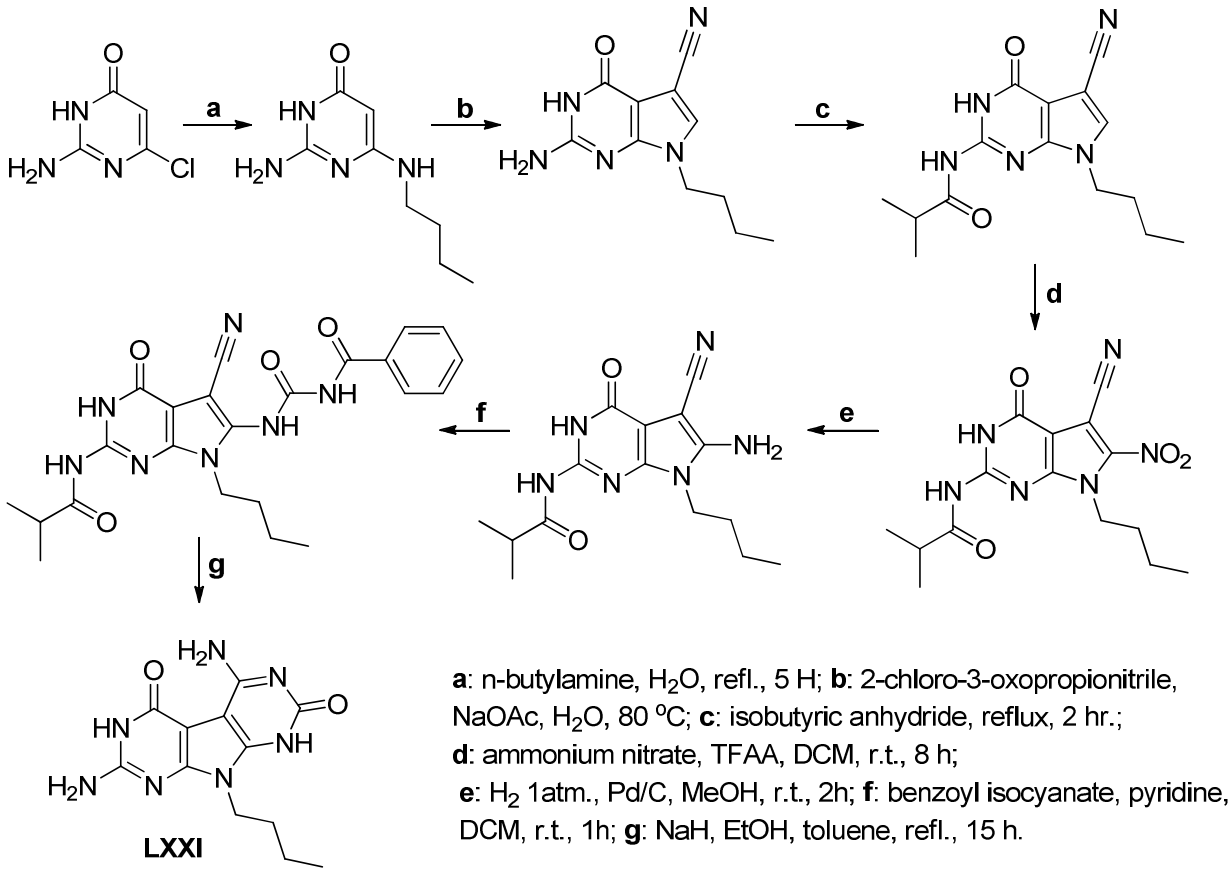
Tricyclic bases can contain even more nitrogen atoms. One type of such compounds are pyridazine-fused derivatives **LXX**, which were found to be dual kinase inhibitors.[96]



a) 1) pyridazine, LiTMP, $ZnCl_2$, 2) pyrimidine, $Pd(PPh_3)_4$. THF, refl.; **b**) NaH, THF, 150 °C

Scheme 13 Pyridazine-fused deazapurines **LXX**

Pyrrolo[2,3-*d*:5,4-*d'*]dipyrimidine **LXXI**, also called Janus type base, was synthesized (Scheme 14) as G-C self-complementary heterocycle from 2-amino-6-chloro-4-hydroxy pyrimidine and used for study of its organization into tetrameric structures.[102]

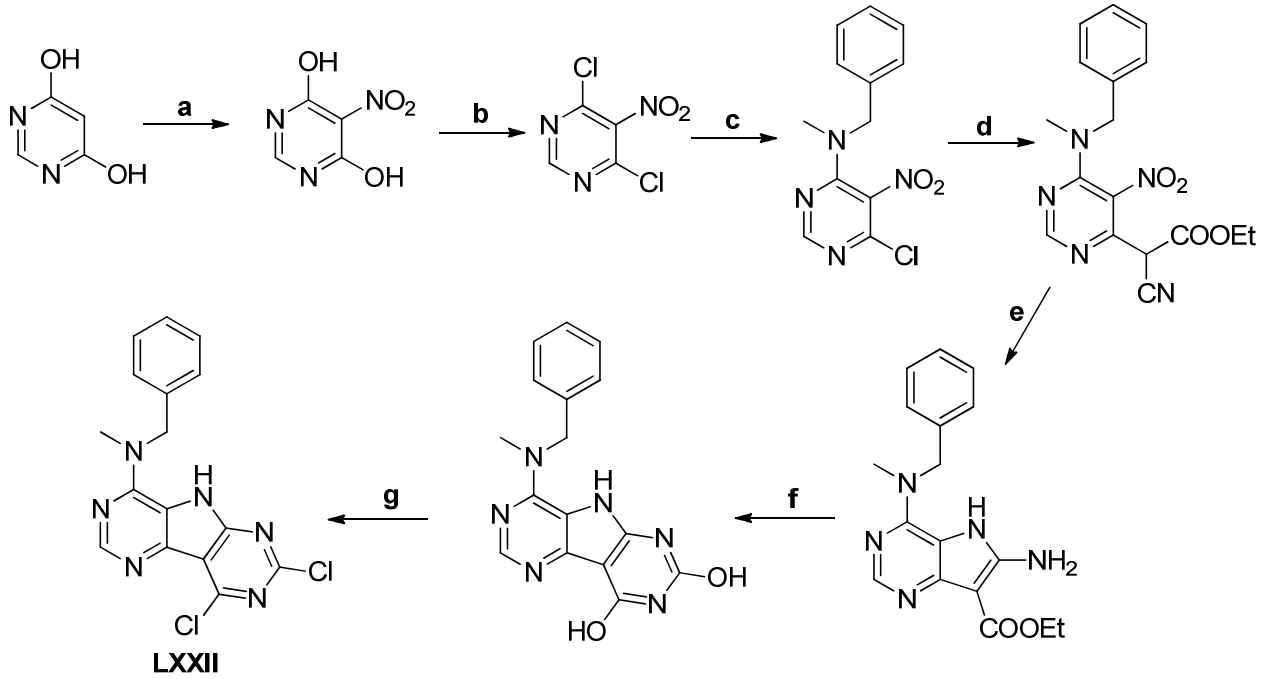


a: n-butylamine, H_2O, refl., 5 H; **b**: 2-chloro-3-oxopropionitrile, NaOAc, H_2O, 80 °C; **c**: isobutyric anhydride, reflux, 2 hr.; **d**: ammonium nitrate, TFAA, DCM, r.t., 8 h; **e**: H_2 1atm., Pd/C, MeOH, r.t., 2h; **f**: benzoyl isocyanate, pyridine, DCM, r.t., 1h; **g**: NaH, EtOH, toluene, refl., 15 h.

Scheme 14 Synthesis of Janus type base **LXXI**

Substituted tricyclic heterocycles (5*H*-pyrrolo[2,3-*d*:4,5-*d'*]dipyrimidines **LXXII**, pyrimido[4,5-*b*]indoles, and 9*H*-pyrido[4',3':4,5]pyrrolo[2,3-*d*]pyrimidines) were also prepared and patented as DNA gyrase inhibitors for treating bacterial infections. Several

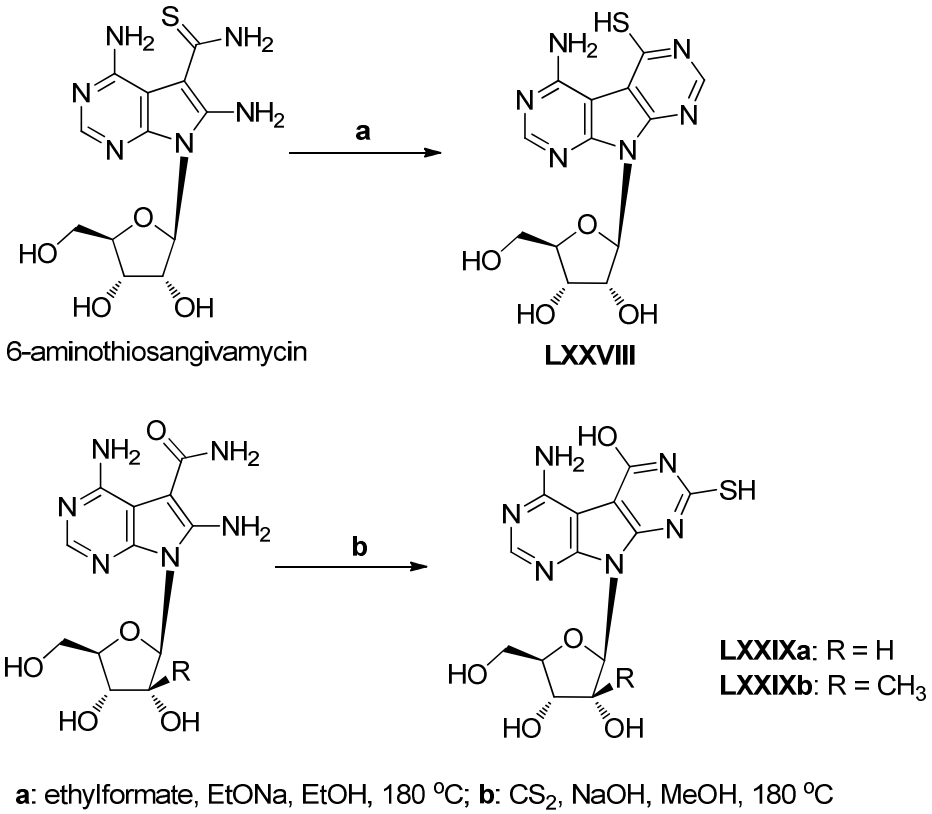
compounds inhibited *Staphylococcus aureus, Staphylococcus pneumoniae, Escherichia coli, Acinetobacter baumanii, Burkholderia thailandesis, Francissela tularensis* and *Yersinia pestis* with MIC lower than 0.5 μg/ml. All compounds were synthesized by cyclization reactions from *o*-chloronitroaromates. Synthesis of dipyrimidine derivative **LXII** is shown (Scheme 15).[103]



a: fuming HNO_3; **b**: $POCl_3$; **c**: BnNHMe, THF, TEA; **d**: ethyl-cyanoacetate, K_2CO_3, DMF; **e**: Zn, AcOH; **f**: urea; **g**: $POCl_3$

Scheme 15 Synthesis of Janus type dipyrimidine derivative **LXXII**

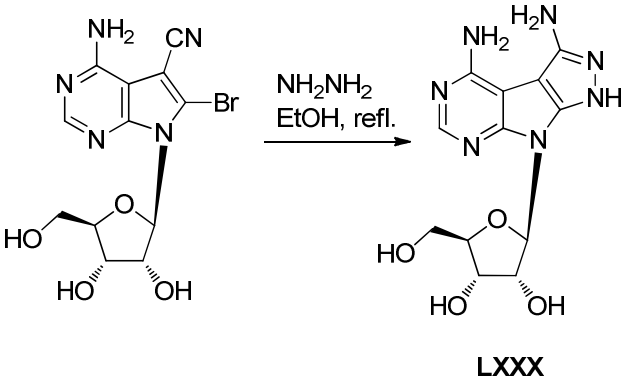
Janus type bases are also rare examples of tricyclic heterocycles used for synthesis of nucleosides. Janus type nucleosides **LXXIII** prepared by Townsend in 1980 (Scheme 16)[104] were tested for cytotoxic activities on L1210 cell line with micromolar activities.[105] Later on, series of ribonucleosides **LXXIXa** and 2´-C-methylribonucleosides **LXXIXb** with the same Janus type bases were prepared and tested for anti-HCV and anti-HIV activity, but only low activity accompanied by cytotoxicity was observed.[106]



Scheme 16 Synthesis of Janus type nucleosides

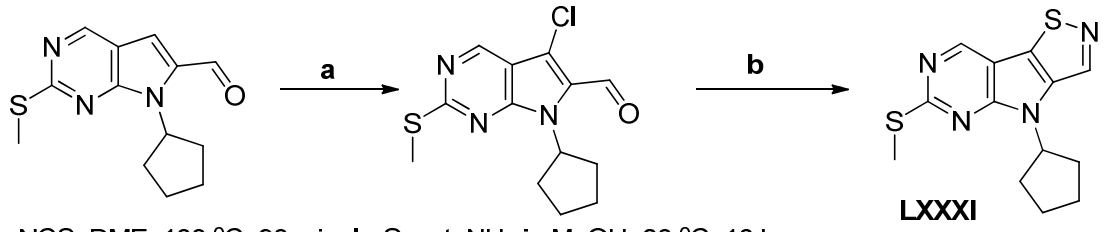
1.6 Deazapurines fused with 5-membered heteroaryl ring

7-Deazapurines fused with 5-membered heterocycles are quite rare, just a very few examples can be found in literature. First tricyclic nucleoside, analogue of pyrazole-fused 7-deazaadenine **LXXX**, was synthesized in 1968 from 5-cyano-6-bromo-7-deazaadenine and hydrazine.[107]



Scheme 17 Synthesis of pyrazine-fused nucleoside **LXXX**

Besides thiophene-fused deazapurine **LXII** mentioned above, only isothiazole-fused derivatives **LXXXI** are known (Scheme 18), their synthesis can be found in a patent about cell cycle inhibitors.[98]



a: NCS, DMF, 100 °C, 90 min; **b**: S, sat. NH_3 in MeOH, 80 °C, 16 h

Scheme 18 Synthesis of isothiazole base **LXXXI**

2 Specific aims of the thesis

1) Development of multi-gram synthesis of pyrimido[4,5-*b*]indoles bearing additional halogen atoms in positions 4, 5 or 6 and synthesis of their nucleosides.
2) Synthesis of 4-(het)aryl-6-chloropyrimido[4,5-*b*]indole nucleosides; 4,6-bis(het)aryl pyrimido[4,5-*b*]indole nucleosides and nucleosides bearing small alkyl, (het)aryl and amino groups in position 4
3) Synthesis of 4-aminopyrimido[4,5-*b*]indole nucleosides bearing (het)aryl groups in position 5 or 6
4) Synthesis of thiophene-fused 7-deazapurine ribonucleosides substituted in position 4

Rationale of the specific aims:

Two novel types of nanomolar cytostatic 7-deazapurine nucleosides bearing hetaryl group in position 6 and H or F atoms in position 7 or 7-amino-6-hetaryl-7-deazapurine nucleosides were recently discovered in our group.[41,44] Further attempts to improve their ativities or pharmacokinetic properties by synthesis of *Cyclo*-Sal and ProTides prodrugs derived from parent cytostatic nucleosides or by preparation of sugar-modified analogues were not successful, all compounds were less active or inactive. All 7-deazapurine nucleosides disposing anti-HCV activity are also toxic to host cells. Activity of 7-hetaryl-7-deazapurine nucleosides shows that there is a space for modification in this „major groove“ part of the molecule, so the question is how bulky can the modification be and if it is possible to reach some selectivity for viral RNA-dependent RNA polymerase. This led us to design of new base-modified nucleosides with modification in position 7 and 8, specifically annulated 7-deazapurine nucleosides.

My goal was to develop and optimize synthesis of desired pyrimido[4,5-*b*] indole bases and synthesize 5 series of variously substituted pyrimido[4,5-*b*]indole ribonucleosides for structure-activity relationship studies. Based on these SAR studies, we further optimized and designed new class of fused 7-deazapurine nucleosides, and my next task was to develop synthesis of new thiophene-fused 7-deazapurine bases and prepare 2 series of their ribonucleosides.

As these types of ribonucleosides are novel, broader biological activity screening needs to be done, all compounds will be tested for cytostatic activity on several cancer cell lines, for antibacterial activity and also for antiviral activity against several viruses.

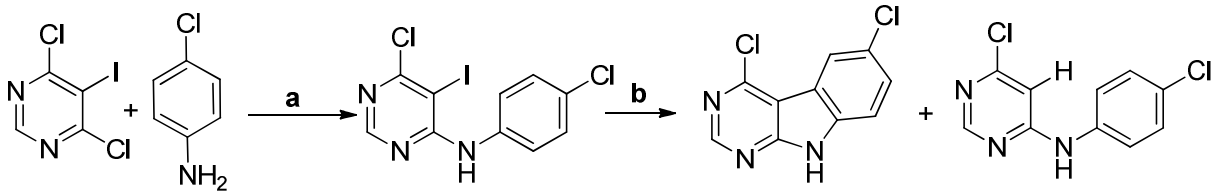
3 Results and discussion

3.1 Pyrimido[4,5-*b*]indole ribonucleosides

3.1.1 Synthesis of pyrimido[4,5-b]indoles via C-H activation

My first goal was to synthesize sufficient amounts of pyrimidoindole bases for the synthesis of several series of desired nucleosides, so I was looking for an appropriate synthesis, which should be short, compatible with halogen substituents and should have a potential for scale-up. In 2002, pyrimido[4,5-*b*]indoles were prepared by direct palladium catalyzed C-H arylation.[80] In principle, it should be possible to apply this approach also for synthesis of 4,6-dichloropyrimido[4,5-*b*]indole (**4**), even though there is one more chlorine atom in position 4 of pyrimidine ring, which is quite reactive in palladium catalyzed reactions and can cause problems with regioselectivity.

The synthesis started from commercially available 4,6-dichloropyrimidine, in the first step, iodine was introduced into position 5 by Knochel´s procedure,[108] which means reaction with *in situ* generated tetramethylpiperidinylzinc complex with magnesium chloride and lithium chloride followed by the quenching with iodine solution in THF. 4,6-Dichloro-5-iodopyrimidine **(1**) was then subjected to nucleophilic substitution with 4-chloroaniline (**2**), this reaction furnished desired iodinated intermediate **3**, which was used for palladium catalyzed C-H arylation (Scheme 19). I first tried the published procedure,[80] $Pd(OAc)_2(PPh_3)_2$ and NaOAc in DMF at 85 °C, but there was no reaction (Table 1). Firstly the temperature was optimized. At 100 °C, there was still the starting material in the mixture even after 24 hours, but part of it was decomposed to unidentified black precipitate. Increasing temperature to 130 °C led to faster decomposition of the starting material and isolation of 2 % of deioninated compound **5**. Desired pyrimidoindole **4** was not isolated, just a small peak was found in mass spectra, but with low intensity. Addition of pivalic acid[77] did not change anything, only black precipitate was isolated again. Then the base was changed from acetate to carbonates, which are also often used in C-H activation reactions. [74, 109] But in this case, just more dehalogenated product **5** was obtained, 40 % in case of K_2CO_3 and 60 % in case of Cs_2CO_3 (Table 1, Entries 6-8).



1 **2** **3** 80 % **4** **5**

a: *i*-PrOH, r.t., 16 h; **b**: $Pd(OAc)_2(PPh_3)_2$ 10 %; base, additive; DMF, 18 h.

Scheme 19 Attempts on intramolecular C-H arylation

Table 1 Conditions for C-H arylation of **3**

Entry	Base [eq.]	Additive	Temp. [°C]	**3** [%]	**4** [%]	**5** [%]
1	NaOAc 1.5	-	80	100	0	0
2	NaOAc 1.5	-	100	60	0	0
3[a]	NaOAc 1.5	-	130	0	traces[b]	2
4	NaOAc 1.5	-	150	0	0	2
5	NaOAc 1.5	PivOH 30%	130	0	0	0
6	K_2CO_3 2	-	80	90	0	2
7[a]	K_2CO_3 2	-	100	40	0	40
8	Cs_2CO_3 2	-	100	60	0	40

isolated yields; [a]: reaction was performed also in a microwave reactor with the same result; b: peak of product detected in mass spectra

Ligand has usually a crucial effect on C-H arylation reactions, so I tested tricyclohexyl-phosphine,[78,70] tri(*tert*-butyl) phosphine[73] and $(4\text{-FPh})_3P$,[74] ligands which wese succesfully used in intramolecular C-H arylations, results are summarized in Table 2. According to literature conditions, $(4\text{-FPh})_3P$ was always used with 30 % of pivalic acid and 3 eq. of K_2CO_3. Results were similar to previous experiments, at lower temperature (80 °C), only starting material was recovered, at higher temperatures 120 °C and 130 °C, dehalogenated product **5** was obtained in 30 % and 40 % yield, respectively. Microwave irradiation did not have any significant effect on the reaction, results were the same as from thermal reactions (Entries 3-4). Perfluorinated triphenylphosphine with cesium carbonate and addition of copper (I) iodide led to decomposition of the starting material as well as all other experiments with CuI (Entries 6-8). Using of $PCy_3 \cdot HBF_4$ in combination with K_2CO_3 and pivalic acid led again just to decomposition of the starting material. Substitution of pivalic acid by silver carbonate, which is supposed to protect palladium catalyst from poisoning by iodine anions generated during the reaction,[70] led to dehalogenation, deiodinated product **5** was isolated in 60 % yield. Palladium acetate with $t\text{-}Bu_3P \cdot HBF_4$ in refluxing dioxane and

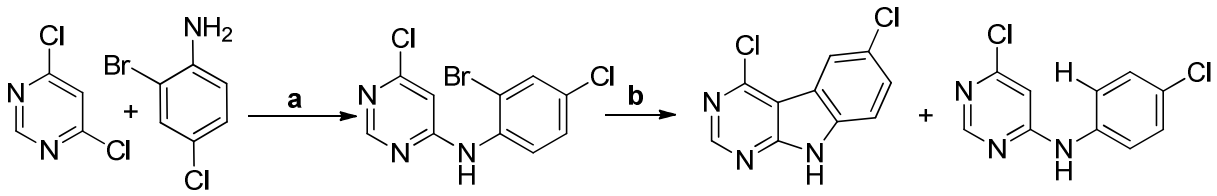
sodium *tert*-butoxide gave only 40 % conversion to dehalogenated product **5** (Table 2, Entry 10). Ligand free conditions also led to decomposition of the starting material (Entry 9).

Table 2 Conditions for C-H arylation of **3** (Scheme 19)

Entry	Ligand [20 %]	Base [eq.]	Additive	Temp. [°C]	**3** [%]	**4** [%]	**5** [%]
1	$PCy_3 \cdot HBF_4$	K_2CO_3 2	-	130	0	0	0
2	$PCy_3 \cdot HBF_4$	K_2CO_3 2	Ag_2CO_3 0.5 eq.	130	0	0	60
3[a]	$(4\text{-FPh})_3P$	K_2CO_3 3	PivOH 30 %	80	90	0	0
4[a]	$(4\text{-FPh})_3P$	K_2CO_3 3	PivOH 30 %	120	0	traces[d]	10
5[c]	$(4\text{-FPh})_3P$	K_2CO_3 3	PivOH 30 %	100	40	0	40
6	$(perFPh)_3P$	Cs_2CO_3 2.5	CuI 3 eq.	130	0	0	0
7	$(perFPh)_3P$	Cs_2CO_3 2.5	CuI+PivOH	130	0	0	0
8[b]	-	Cs_2CO_3 2.5	CuI 3 eq.	130	0	0	0
9	-	KOAc	TBAB 1eq.	150	0	0	0
10	*t*-$Bu_3P \cdot HBF_4$	*t*-BuONa 5	-	refl.	40	0	60

Reaction conditions: **a**: $Pd(OAc)_2$ 10 %; ligand 20 %; base; additive; DMF, 18 h. Isolated yields. [a]: reaction was performed also in microwave reactor with the same result; [b]: reaction without $Pd(OAc)_2$; [c]: reaction time 48 h; d: peak of product detected in mass spectra

From the experiments mentioned above, it can be concluded, that 4,6-dichloro-[4,5-*b*]pyrimidoindole **4** can not be prepared by intramolecular C-H arylation of **3**. As dehalogenated product **5** was isolated from several reactions, oxidative addition proceeded succesfully, but the tricky step was the actual C-H activation of hydrogen at benzene ring. Therefore, I decided to try different substrate, brominated derivative **7**, which was prepared in analogous manner as **3** (Scheme 20). Bromine is still supposed to be more reactive in oxidative addition to palladium than chlorine and H5 on pyrimidine should be more reactive in C-H activation step. Several different reaction conditions were tried, results are summarized in Table 3. All reactions led just to decomposition of starting material (Table 3, Entries 3, 5), no reaction (Entry 2) or to debromination (Entries 1, 4).



6 **7** 82 % **4** **5**

a: *i*-PrOH, r.t., 16 h; **b**: $Pd(OAc)_2$ 10 %; ligand 20 %; base; additive; DMF, 18 h.

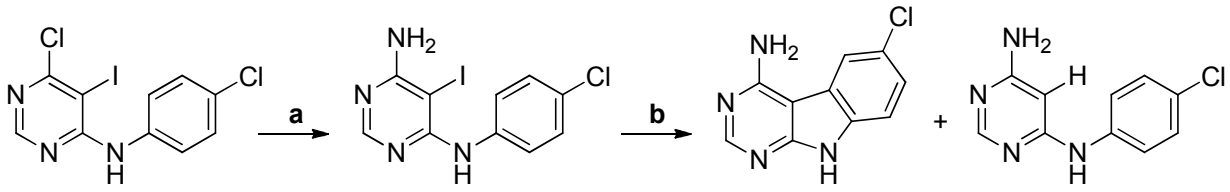
Scheme 20 Attempts on C-H arylation of **7**

Table 3 Conditions for C-H arylation of **7**

Entry	Ligand [20 %]	Base [eq.]	Additive	Temp. [°C]	**7** [%]	**4** [%]	**5** [%]
1[b]	-	NaOAc 1.5	-	100	0	0	40
2[a]	*t*-$Bu_3P{\cdot}HBF_4$	*t*-BuONa 5	-	160	100	0	0
3	-	KOAc 1.5	TBAB 1eq.	150	0	0	0
4[a]	$(perFPh)_3P$	Cs_2CO_3 2.5	CuI 3 eq.	150	0	0	90
5	$(4\text{-}FPh)_3P$	K_2CO_3 3	PivOH 30 %	120	0	0	0

Isolated yields; [a]: reaction was performed also in microwave reactor with the same result; [b]: $Pd(OAc)_2(PPh_3)_2$ used instead of $Pd(OAc)_2$

Another possible substrate for C-H arylation is the amino derivative **8**, which can be prepared by amination of **3** in liquid ammonia. The presence of amino group in position 4 of pyrimidine ring can change the reactivity and can also coordinate palladium catalyst. Palladium acetate as a palladium source in a combination with 3 different ligands was tested. The reaction with *t*-$Bu_3P{\cdot}HBF_4$ gave only partially decomposed starting material; with $(perFPh)_3P$, cesium carbonate and copper (I) iodide, decomposition was completed (Entry 2) and the same result was obtained from reaction without palladium acetate (Entry 3). On the other hand, reaction with palladium acetate without any ligand in the presence of potassium acetate in DMF with TBAB furnished 90 % of dehalogenated product and also 10 % of desired cyclized pyrimidoindole **9** (Entry 4). Reaction using $(4\text{-}FPh)_3P$, 3 equivalents of potassium carbonate in DMA with 30 % of pivalic acid gave also a mixture of dehalogenated product **10** (5 %) and desired amino base **9** (10 %). Unfortunately, the rest was decomposed starting material.



3 **8** 78 % **9** **10**

a: NH_3 (l), r.t., 8h; **b**: $Pd(OAc)_2$ 10 %; ligand 20 %; base; additive; DMF, 18 h.

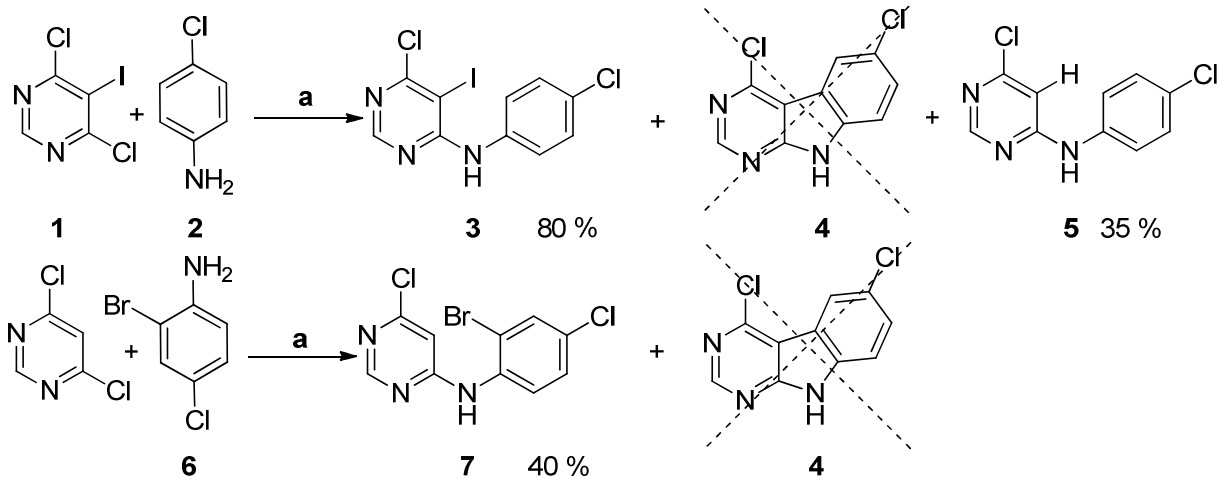
Scheme 21 Synthesis of 4-amino-6-chloropyrimidoindole **9** via C-H arylation

Table 4 Conditions for C-H arylation of **8**

Entry	Ligand [20 %]	Base [eq.]	Additive	Temp. [°C]	**8** [%]	**9** [%]	**10** [%]
1[c]	*t*-$Bu_3P{\cdot}HBF_4$	*t*-BuONa 5	-	80	70	0	0
2[a]	$(perFPh)_3P$	Cs_2CO_3 2.5	CuI 3 eq.	150	0	0	trace[d]
3[b]	-	Cs_2CO_3 2.5	CuI 3 eq.	150	0	0	0
4	-	KOAc 2	TBAB 1eq.	150	0	10	90
5	$(4\text{-}FPh)_3P$	K_2CO_3 3	PivOH 30 %	130	0	10	5

Isolated yields; [a]: reaction was done also in microwave reactor with the same result; [b]: reaction without $Pd(OAc)_2$; [c]: reaction time 48 h, dioxane as a solvent; d: peak of product detected in mass spectra

In recent years, tandem Buchwald-Hartwig amination/C-H arylation reactions were used for synthesis of carbazoles,[110] pyrido[2,3-*b*]indoles and pyrazino[2,3-*b*]indoles.[111] This approach can be in principle applied also to the synthesis of pyrimido[4,5-*b*]indoles. 4,6-Dichloro-5-iodopyrimidine (**1**) was submitted to reaction with 4-chloroaniline (**2**) or 2-bromo-4-chloroaniline (**6**) under literature conditions.[110] Firstly, the amination step was succesful in both cases, but this reaction works even without palladium. Deiodinated product **5** was isolated from reaction with 4-chloroaniline (**2**). Reaction with bromoaniline **6** gave just product of amination **7** (Scheme 22).



a: $Pd(OAc)_2$ 10 %, $[HPt\text{-}Bu_3][BF_4]$ 15 %, NaO*t*-Bu 5 eq., toluene, 160 °C, μw, 3 hours

Scheme 22 Attempts on Pd-catalyzed tandem amination/C-H arylation

C-H activation reactions including intramolecular C-H arylations are rapidly evolving and many different compounds prepared by various conditions are published every year. Although I have tried many combinations of palladium catalysts, ligands, bases, solvents and other additives, I have not been able to prepare desired 4,6-dichloro-9*H*-pyrimido[4,5-*b*]indole (**4**) by C-H arylation approach. The best result obtained so far was isolation of 10 % of 6-chloro-9*H*-pyrimido[4,5-*b*]indole-4-amine (**9**).

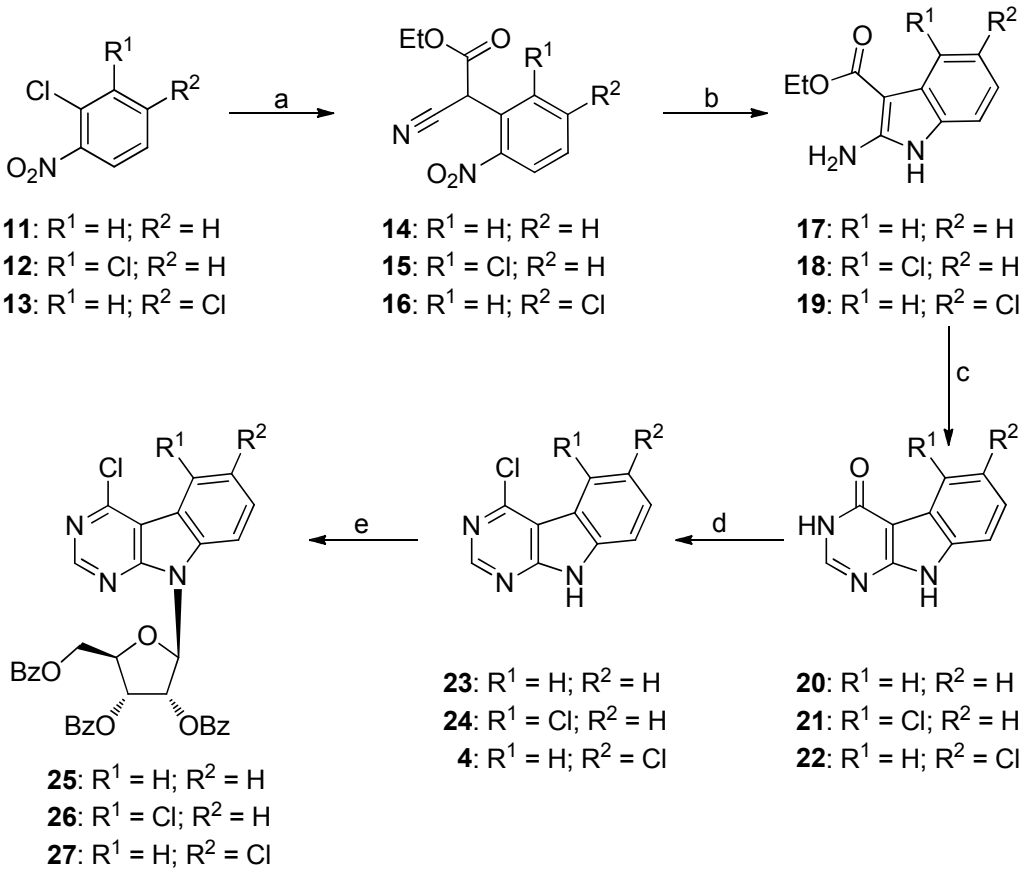
Although there was some more space for further improvement, the C-H activation approach was abandoned, because in a parallel study, I found that the target compounds can be efficiently prepared by heterocyclization approach (see below).

3.1.2 Synthesis of pyrimidoindole bases from chloronitrobenzenes

Another synthetical approach to pyrimido[4,5-*b*]indoles is based on heterocyclization reactions[85] and it starts from commercially available *o*-chloronitrobenzenes. In order to achieve my goals, I needed to start from 2-chloronitrobenzene (**11**), 2,3-dichloronitrobenzene (**12**) and 2,4-dichloronitrobenzene (**13**). Synthesis was optimized for 2,4-dichloronitrobenzene (**13**) and the same conditions were then applied also for other chloronitrobenzenes **11** and **12** (Scheme 21). First step was aromatic nucleophilic substitution of chlorine atom in position 2 by potassium salt of ethyl cyanoacetate according to literature procedure.[112] Although there are two chlorine atoms in positions activated for nucleophilic substitution, the reaction is completely regioselective and proceeds to yield only desired product with substituion at position 2. The reactions with the other isomers **11**, **12** worked without any problems and yields were around 80 – 90 % in all cases. Obtained ethyl-2-(2-nitrophenyl)cyanoacetates **14-16** were reduced by zinc dust in acetic acid and spontaneously cyclized to indole derivatives **17-19** with 70-90 % yields. This reaction worked at 30 g scale, but unlike the reported procedure,[85] it is not necessary to heat the reaction mixture, because reaction itself is exothermic enough to reach 60 °C in the flask. Another practical aspect of this reduction is a necessity of portionwise addition of zinc dust into vigorously stirred reaction mixture, otherwise zinc creates solid crust in the flask and reduction can not proceed.

In the next step, pyrimidoindole motif was formed by cyclization with formamide at 190 °C for 18 hours, all three isomers **20-22** were obtained in ~ 90 % yields. The best way was to use 3.3 ml of formamide to 1 g of indole derivative to reach full conversion and precipitation of desired product **22** from solution. With lower amount of formamide, conversion was not quantitative and difficult column chromatography was needed, with a larger amount of formamide, the yield was lower because part of the product stays in formamide solution and can not be isolated by simple filtration. Formamidine acetate is also often used for such cyclization, [64] but in this case, there was no reaction even after 2 days in refluxing ethanol. Final chloropyrimido[4,5-*b*]indoles **23, 24** and **4** were prepared by treatment of pyrimidoindolones **20-22** with $POCl_3$ under reflux. $POCl_3$ was used also as a solvent and its amount had to be optimized. The ratio of 13 ml of $POCl_3$ to 1 g of starting pyrimidoindolone **22** was found to be the best and it is necessary for the full conversion. Full conversion was especially important in this case, because starting material and product are both quite polar compounds and it is really difficult to separate them on colmun, in amounts over 1-2 grams it is almost impossible. Reaction is finished in two days of heating at 120 °C,

$POCl_3$ is then evaporated and solid material is very carefully neutralized, washed well with water and after drying can be used directly into next step. Synthesis of chlorinated pyrimidoindole heterocycles was optimized for 30 g scale without any chromatography and with excellent ~ 50 % overall yield over 4 steps. Structure of 4,6-dichloro-9*H*-pyrimido[4,5-*b*]indole (**4**) was confirmed by X-Ray analysis (Figure 24).



a: $CNCH_2COOEt$, *t*-BuOK, THF, reflux, 48 h; **b**: Zn, AcOH, 55 °C, 150 min;
c: formamide, 190 °C, 12 h; **d**: $POCl_3$, reflux, 2 days;
e: 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose, BSA, TMSOTf, 60 °C, 8 h.

Scheme 23 Synthesis of pyrimidoindole bases **23,24,4** and protected nucleosides **25-27**

The key chlorinated ribonucleoside intermediates **25-27** were prepared by Vorbrüggen glycosylation in analogy to published procedure used for synthesis of deazapurine nucleosides (Scheme 23).[41] Nucleosides **25-27** were isolated in about 50 % yields, which is very good for this type of glycosylation. Structure of 4,5-dichloro-pyrimidoindole ribonucleoside **26** was also confirmed by X-Ray (Figure 24). Overall yields from chloronitrobenzenes **11-13** to protected chloronucleosides **25-27** are about 25 % over 5 steps, which is pretty good. This optimized process allows synthesis of 30 grams of pyrimidoindole bases starting from 30 g of chloronitrobenzenes in less than two weeks.

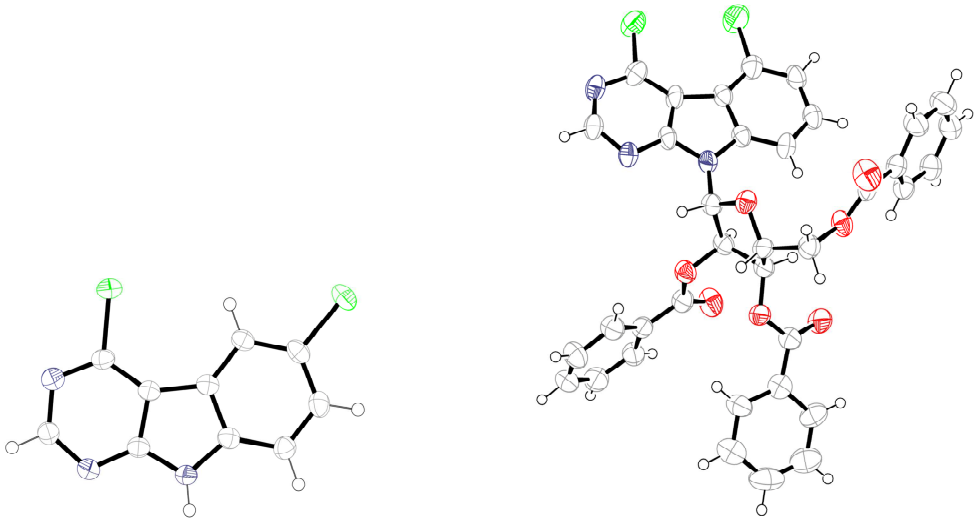
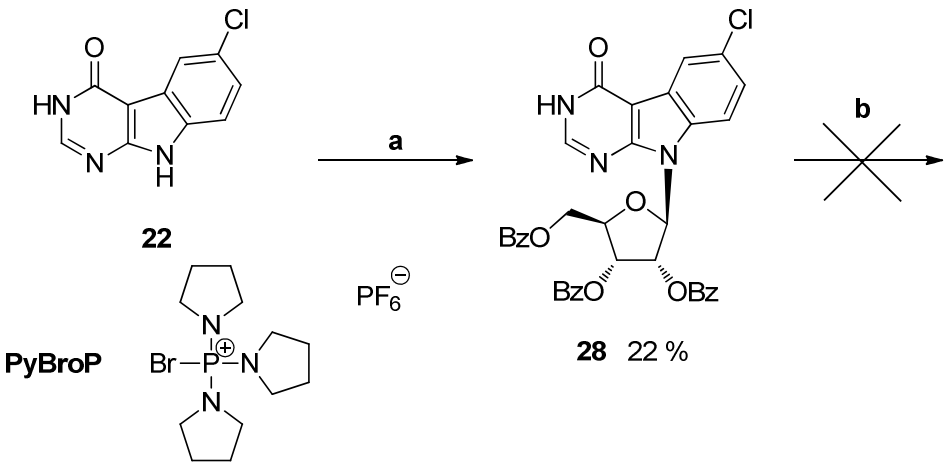


Figure 24 An ORTEP[4] view of base **4** and protected nucleoside **26**, displacement ellipsoids shown with 50 % probability.

Recently, palladium catalyzed cross-coupling reaction of tautomerizable heterocycles via C-OH bond activation was published[113] and this approach could in principle be used for synthesis of 4-subsituted pyrimidoindole ribonucleosides directly from benzo-fused 7-deazainosine analogue **28**. However, Vorbrüggen glycosylation of pyrimidoindolone **22** gave only 22 % yield of desired crude nucleoside **28**. The set of the optimized conditions, which were successfully applied for cross-coupling reaction of tautomerizable heterocycles as well as unprotected inosine with various boronic acids, were tested.[113] Combination of $PdCl_2(PPh_3)_2$ and sodium carbonate in a mixture of dioxane and water was used for reaction of nucleoside **28** with phenylboronic acid. The nucleoside was first preactivated with PyBroP, but only the starting material was recovered from reaction mixture (Scheme 24).

As the yields of glycosylation of 4-chloropyrimidoindole bases **4**, **23, 24** were higher (50 %) than the yield of glycosylation of **22** (22 %) and also because cross-coupling reactions of deazapurine nucleosides bearing chlorine atom in position 4 of pyrimidine ring were optimized and well established, all target nucleosides were synthesized from 4-chloropyrimidoindole nucleosides.

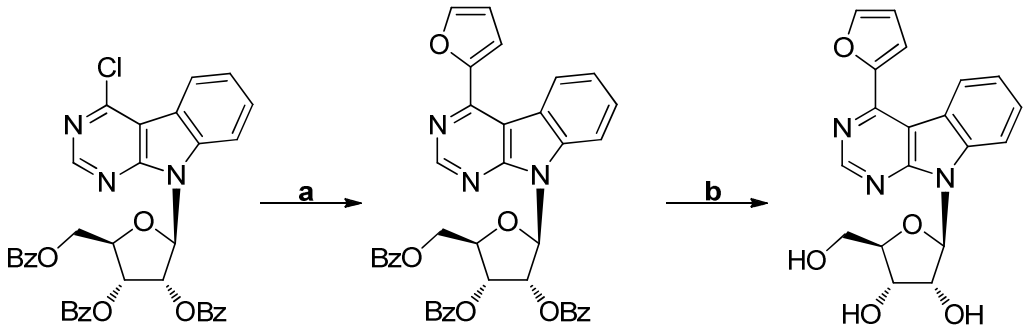


a: 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose 2 eq., BSA 1 eq., TMSOTf 2 eq., 60 °C, 8 h.
b: PyBroP 1.2 eq., Et_3N 3 eq., dioxane, r.t., 2 h, then $PdCl_2(PPh_3)_2$ 0.05 eq., Na_2CO_3 5 eq., $PhB(OH)_2$ 2 eq., water, 100 °C.

Scheme 24 Attempt on direct Pd-catalyzed coupling of **28** with phenylboronic acid

3.1.3 Synthesis of 4-(het)aryl-6-chloropyrimido[4,5-b]indole nucleosides

The first synthesized series of target nucleosides was the series of 4-(het)aryl-6-chloropyrimidoindole ribonucleosides **32**. Reactivity of chlorine atom in position 4 was first tested on 4-chloro-9*H*-pyrimido[4,5-*b*]indole ribonucleoside **25**. The Stille cross-coupling was performed under standard conditions[41] with 2-furyl(tributyl)stannane in the presence of $PdCl_2(PPh_3)_2$ in DMF at 100 °C to give the desired 4-(2-furyl)pyrimido[4,5-*b*]indole ribonucleoside **29** in excellent 82 % yield. Its deprotection under the Zemplén conditions using NaOMe in methanol afforded the target free ribonucleoside **30** (Scheme 25).



25 **29** 82 % **30** 80 %

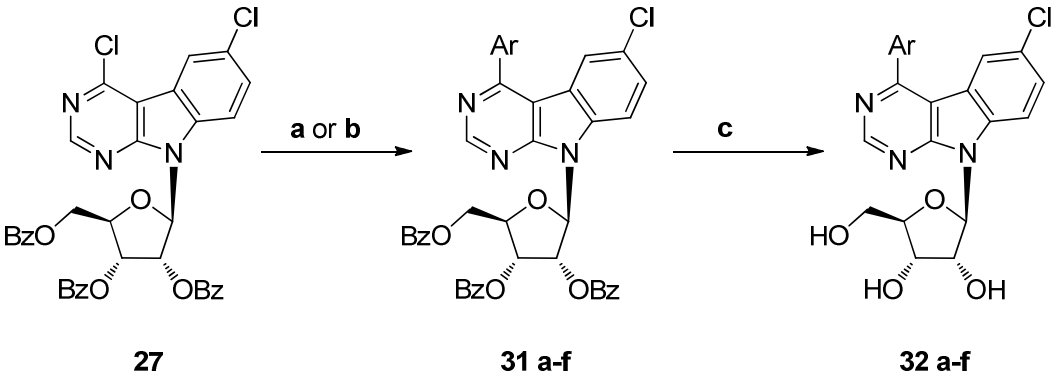
a: (furan-2-yl)$SnBu_3$ (1.2 eq.); $PdCl_2(PPh_3)_2$ (0.05 eq.), DMF, 100 °C, 8 h;
b: 1M MeONa in MeOH (0.3 eq.), MeOH, r.t., 24 h.

Scheme 25 Synthesis of 2-furyl nucleoside **30**

As we were even more interested in compounds with additional substituent in position 6, regioselectivity of palladium catalyzed Suzuki or Stille cross-coupling reactions on 4,6-dichloropyrimido[4,5-*b*]indole ribonucleoside **27** was tested. Suzuki reaction was used for synthesis of all nucleosides bearing 3-furyl, 3-thienyl or 2-benzofuryl groups. 2-Furyl and 2-thienyl analogues were prepared by Stille reaction. 2-Furyl and 2-thienyl boronic acids are known to be unstable[120,114] and their decomposition during the reaction was faster than cross-coupling reaction of protected nucleosides in organic solvents.[49] As 2-tributylstannylfuran and 2-tributylstannylthiophene are stable, Stille reaction gives better yields than Suzuki coupling, even if the purification of the desired nucleosides from tin residues is more complicated. Tin residues can be removed by flash chromatography on silica with 10 % of potassium fluoride as a stationary phase.[115]

Chlorine atom in position 4 is supposed to be much more reactive due to electron-poor nature of a pyrimidine ring. Both types of cross-coupling reactions were performed under standard conditions, which means $PdCl_2(PPh_3)_2$ in DMF at 100 °C for the Stille

coupling and $Pd(PPh_3)_4$ and K_2CO_3 in toluene at 100 °C for the Suzuki coupling. All reactions worked well and fully regioselectively; not even trace amounts of 6-substituted product was observed. Desired 4-(het)aryl-6-chloropyrimidoindole nucleosides **31a-f** (Scheme 26) were isolated in good yields (64 - 79 %) (Table 5). Zemplén deprotection using sodium methoxide in methanol furnished series of final free nucleosides **32a-f** in excellent yields (78-91 %).



27 **31 a-f** **32 a-f**

a) $ArB(OH)_2$ (1.5 eq.): $Pd(PPh_3)_4$ (0.05 eq.), K_2CO_3 (2 eq.), toluene, 100 °C, 8h;
b) $ArSnBu_3$ (1.2 eq.): $PdCl_2(PPh_3)_2$ (0.05 eq.), DMF, 100 °C, 8 h;
c) 1M MeONa in MeOH (0.3 eq.), MeOH, r.t., 24 h.

Scheme 26 Synthesis of 4-(het)arylpyrimidoindole nucleosides **32a-f**

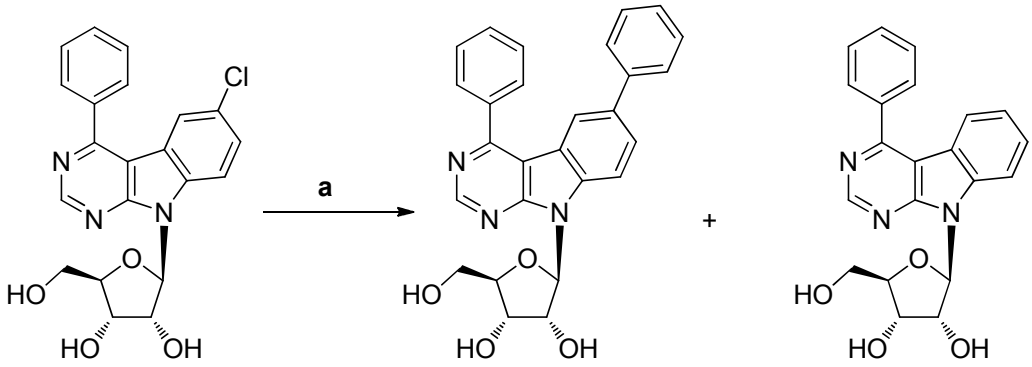
Table 5 Cross-coupling reactions of **27** followed by deprotection

Entry	Ar	M	Coupling product	Yield [%]	Free nucleoside	Yield [%]
1	2-furyl	$SnBu_3$	**31a**	79	**32a**	86
2	3-furyl	$B(OH)_2$	**31b**	69	**32b**	91
3	2-thienyl	$SnBu_3$	**31c**	78	**32c**	93
4	3-thienyl	$B(OH)_2$	**31d**	70	**32d**	86
5	2-benzofuryl	$B(OH)_2$	**31e**	64	**32e**	78
6	phenyl	$B(OH)_2$	**31f**	72	**32f**	89

3.1.4 Synthesis of 4,6-disusbtituted pyrimidoindole nucleosides

To achieve the next goal, synthesis of 4,6-disubstituted pyrimidoindole nucleosides, conditions for cross-coupling reaction at unreactive chlorine atom in position 6 had to be found. As there are many successful Suzuki reactions in aqueous media,[116] both protected or deprotected nucleosides can be used. It is also more elegant and faster to perform cross-coupling on a free nucleoside and get directly a final compound, previously prepared free 4-phenylpyrimidoindole nucleoside **32f** was chosen as a substrate and several catalytic systems and sets of conditions including water soluble ligands in aqueous mixtures or

Buchwald phosphine ligands were tested for Suzuki coupling with phenylboronic acid. Results are summarized in Table 6, Scheme 27. TPPTS ligand in acetonitrile/water mixture was tried first, because these conditions are used in our group for modification of 7-deazapurines in position 7,[117] howewer, there was no reaction. Other water soluble ligand, CataXCium F in butanol/water mixture[118] gave 60 % conversion of starting material to mixture of desired nucleoside **33f** and dehalogenated nucleoside **34** in ratio 0.7 to 1. Buchwald ligands, which were successfully used for cross-coupling reaction with various arylchlorides, were also tested.[119] Reaction with DavePhos and S-Phos in DMF led to only 30 % and 20 % conversion, respectively (Table 6, Entries 3,4). Although X-Phos ligand was the best with 60 % conversion of starting nucleoside to product (Table 6), these conditions are not suitable for synthesis of larger series of nucleosides, because of small conversion and complicated separation of product from unreacted starting material.



32f **33f** **34**

a) $Pd(OAc)_2$ (0.05 eq.), ligand (0.1 eq.), phenylboronic acid (1.5 eq.), K_2CO_3 (3 eq.), solvent, 100 ° 8h.

Scheme 27 Optimization of Suzuki coupling of free nucleoside **33f**

Table 6. Optimization of cross-coupling reaction of free nucleoside **32f** with phenylboronic acid

Entry	Ligand	Solvent	Conversion[a]
1	CataXCium F	*n*-BuOH/H_2O, 2.5:1	60[b]
2	TPPTS	MeCN/H_2O, 2:1	0
3	S-Phos	DMF	30
4	DavePhos	DMF	20
5	X-Phos	MeCN/H_2O, 2:1	35
6	X-Phos	DMF	60

[a]Determined by NMR from crude reaction mixture; [b] Reaction mixture contained a mixture of compounds **34** and **33f** in ratio 0.7:1.

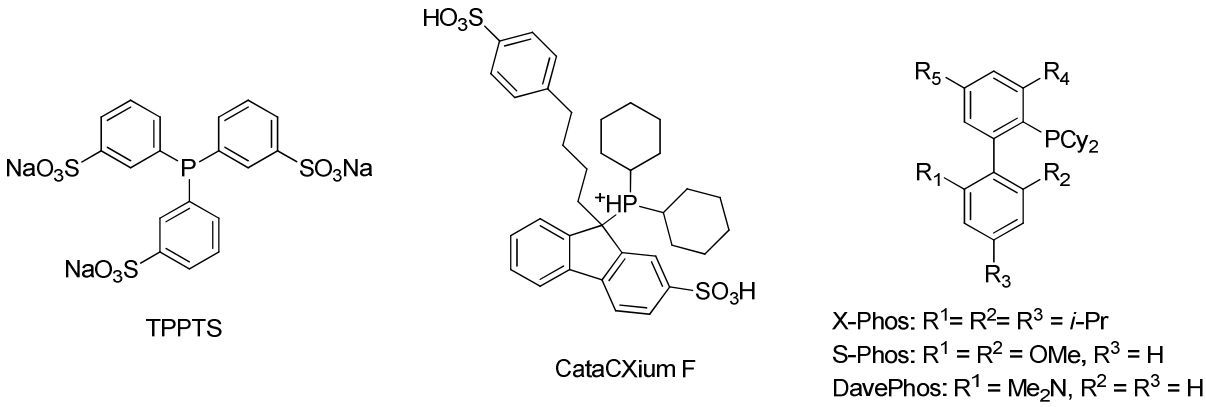
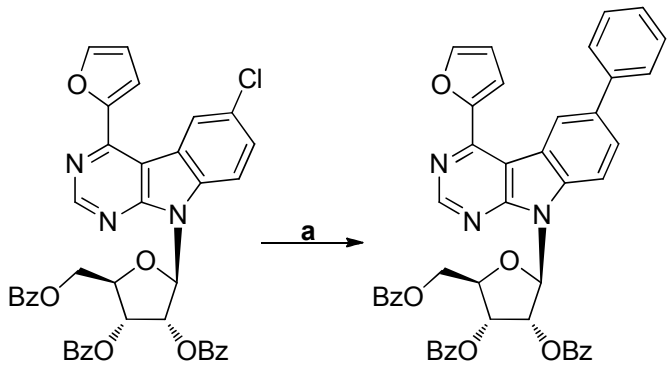


Figure 25 Structures of ligands used during optimization

Therefore I turned my attention to protected nucleoside **31a** and classical cross-couplings in organic solvents. Again, three Buchwald ligands (Table 7, Scheme 28) in DMF were tested, and whilst conversions with DavePhos and S-Phos were still moderate, X-Phos ligand gave almost quantitative conversion to desired product **35f** in 8 hours.



31a **35f**

a: $Pd(OAc)_2$ (0.05 eq.), ligand (0.1 eq.), phenylboronic acid (1.5 eq.), K_2CO_3 (3 eq.), DMF, 100 °C, 8h.

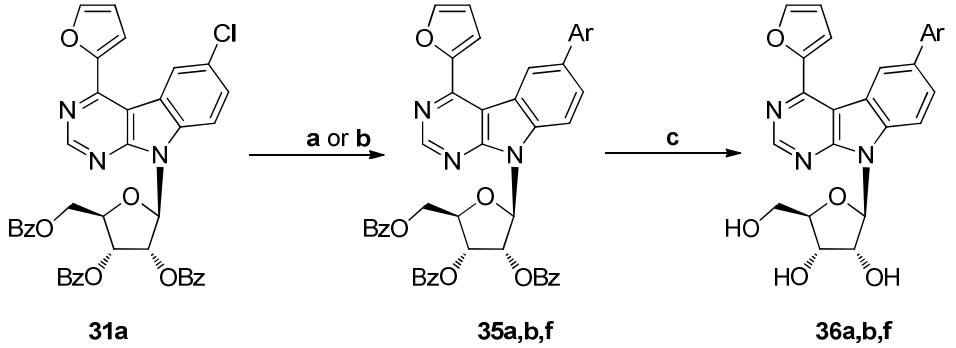
Scheme 28 Synthesis of 6-phenyl nucleoside **35f**

Table 7. Optimization of cross-coupling reaction of nucleoside **31a** with phenylboronic acid

Entry	Ligand	Conversion[a]
1	S-Phos	40
2	DavePhos	20
3	X-Phos	100

[a]Determined by NMR from crude reaction mixture

With these optimized conditions in hand, a small series of 3 nucleosides was synthesized (Scheme 29). Reaction with phenylboronic acid furnished desired nucleoside **35f** in good yield (64 %). However, only 46 % of product **35a** was isolated from cross-coupling reaction with 3-furylboronic acid. It is known, that boronic acids derived from 5-membered heterocycles are rather unstable under higher temperature and their decomposition can cause lower yields.[120] Therefore this reaction was repeated and boronic acid was added into the mixture in three portions (at the beginning and after 1 and 2 h), which led to increased isolated yield 75 %. On the other hand, the portions-wise addition of phenylboronic acid did not improve the yield significantly (Table 8, Entries 3, 4). The same catalyst/ligand system was used for Stille coupling with 2-furyl(tributyl)stannane, target compound **35a** was isolated in 79 % yield. All benzoylated derivatives **35 a,b,f** were successfully deprotected with sodium methanolate to final free nucleosides **36 a,b,f** in good yields (Table 8, Scheme 29).



31a **35a,b,f** **36a,b,f**

a) $ArB(OH)_2$ (1.5 eq.): $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), K_2CO_3 (3 eq.), DMF, 95 °C 3h;
b) $ArSnBu_3$ (1.2 eq.): $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), DMF, 95 °C, 3 h;
c) 1M MeONa in MeOH (0.3 eq.), MeOH, r.t., 24 h.

Scheme 29 Synthesis of 4,6-disubstituted derivatives **36**

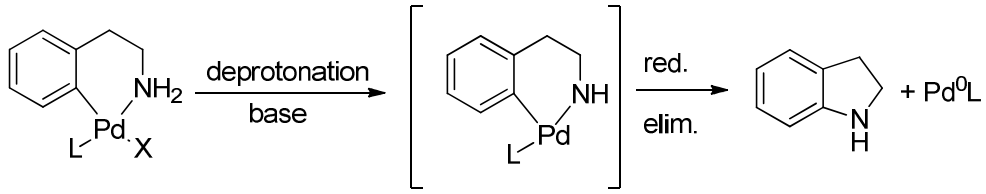
Table 8 Synthesis of 4,6-disubstituted derivatives

Entry	Ar	Conditions	Coupling product	Yield [%]	Free nucleoside	Yield [%]
1	3-furyl	a	**35b**	46	**36b**	90
2[a]	3-furyl	a	**35b**	75		
3	phenyl	a	**35f**	62	**36f**	81
4[a]	phenyl	a	**35f**	64		
5	2-furyl	b	**35a**	79	**36a**	89

[a]Boronic acid was added in three parts within 2 h.

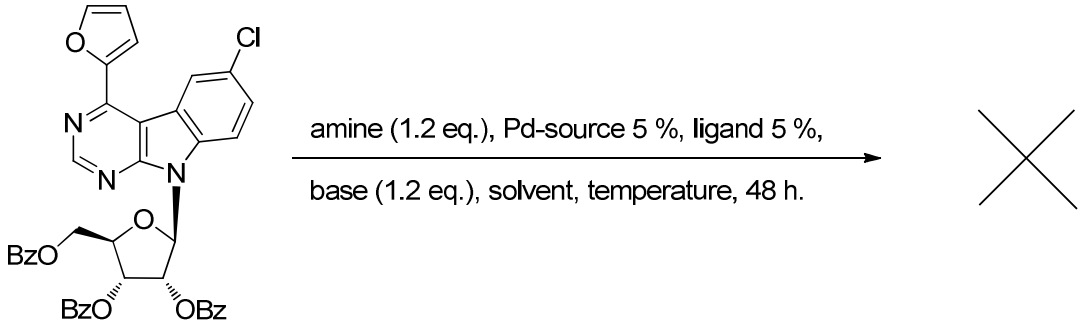
3.1.5 Buchwald-Hartwig aminations

In order to get an extended library of compounds, palladium catalyzed Buchwald-Hartwig aminations were attempted. In past years, various catalysts for amination of even unreactive chloroarenes have been developed and used for synthesis of broad scope of aromatic amines.[121] Several experiments were done under various conditions with Buchwald biarylphosphine ligands or precatalysts, results are summarized in Table 9. Reaction conditions and catalysts were selected according to excellent Buchwald´s user´s guide to Pd-catalyzed aminations.[122] $Pd_2(dba)_3$ was first employed as a palladium source, because it is air stable Pd^0 catalyst and it does not need the reduction step like $Pd(OAc)_2$ does.[123] In combination with JohnPhos or DavePhos ligands and sodium *tert*-butoxide, reactions of nucleoside **31a** with dimethylamine were unsuccessful even at temperatures above 100 °C (Table 9, Entries 1,2). As the coordination of dibenzylidineacetone to the metal can reduce the activity of catalyst,[124] palladium precatalysts were synthesised and used for aminations of complicated substrates. Advantage of such precatalysts is in their simple activation, because under reaction conditions, reactive ligand-Pd complex is formed in few minutes by deprotonation of amino nitrogen and rapid reductive elimination of formed Pd-amide (Scheme 30).[125] Strong base such a sodium *tert*-butoxide, LiHMDS or carbonates can be used to generate the active complex. These precatalysts are also air and moisture stable and commercially available.



Scheme 30 Pd-precatalysts and their activation by base

All tested reactions were tried at temperatures used in relevant publications and then also at elevated temperatures, a new reaction mixture was always used for another experiment. But all those reactions were unsuccessful, not even a trace of desired amination product was observed, only the starting material was recovered. This shows that the reactivity of the chlorine at the position 6 of pyrimido[4,5-*b*]indole moiety is extremely low (Scheme 31, Table 9).



Scheme 31 Attempts on Buchwald-Hartwig aminations

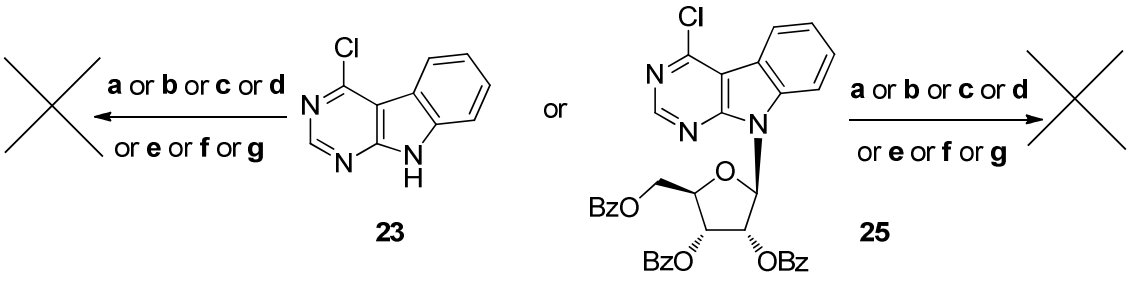
Table 9 Conditions for Buchwald-Hartwig aminations

Entry	Pd cat.	ligand	base	amine	solvent	Temp. [°]
1[126]	$Pd_2(dba)_3$	JohnPhos	t-BuONa	Me_2NH	toluene	60-150
2	$Pd_2(dba)_3$	DavePhos	t-BuONa	Me_2NH	DME	100-120
3[127]	t-BuX-Phos precatalyst		LiHMDS	Me_2NH	THF	50-120
4	X-Phos precatalyst		$K_3PO_4 \cdot 3H_2O$	Me_2NH	THF	110
5[128]	RuPhos prec. + ligand		t-BuONa	Me_2NH	THF	85-120
6[129]	RuPhos prec. + ligand		t-BuONa	piperidin	THF	85-120
7[129]	RuPhos prec. + ligand		Cs_2CO_3	piperidin	t-BuOH	85-120
8	BrettPhos prec.+ligand		LiHMDS	piperidin	THF	65-120
9	BrettPhos prec. +ligand		LiHMDS	$CyNH_2$	THF	65-120

3.1.6 Electrophilic aromatic substitution

Another option, how to introduce substituent into a benzene ring of pyrimidoindole system is a classical electrophilic aromatic substitution (Scheme 32). All reactions were tested on nucleoside **25** and base **23**. Simple sulfonation in sulphuric acid was tried as an initial attempt on 4-chloropyrimidoindole base **23** and also on protected nucleoside **25**. If the starting material was stirred with sulphuric acid at r.t. overnight, nucleoside **25** decomposed and base **23** did not react. Heating of the base **23** to 40 °C did not help, just the starting material was recovered. Also nitration with classical nitration mixture was tested at room temperature overnight. Althought this procedure was succesfully used for nitration of a 7-deazapurine nucleoside,[130] just a decomposed starting material was observed in case of pyrimidoindole nucleoside **25**. The same result was obtained from the reaction at 0 °C. Base **23** did not react at 0° and was decomposed at r.t. Nitronium salts are known to be reactive nitrating agents,[131] so nitronium tetrafluoroborate in sulfolane was used for nitration of nucleoside **25** and base **23**, but no reaction was observed.

Another classical electrophilic substitution reaction is bromination, but reaction of nucleoside **25** or base **23** with bromine in DCM did not work.[132] Also Friedel-Crafts acylation with acetyl chloride or bromide in the presence of $AlCl_3$ was not succesfull. Reaction with Eschenmoser´s salt, *N*,*N*-dimethylmethyleniminium chloride, did not work either.[133] Mannich reaction using formaldehyde and piperidin in isopropanol did not furnish desired product.[134] Vilsmeyer-Haack formylation was attempted on chloropyrimidoindole **23** and also on nucleoside **25**, but without any success.[135] Few reactions (Friedel-Crafts acylation, bromination and Vilsmeyer-Haack reaction) were also tried with 4-aminopyrimido-[4,5-*b*]indole nucleoside **37**, but no product of electrophilic aromatic substitution was observed even if excess of Lewis acid was used.



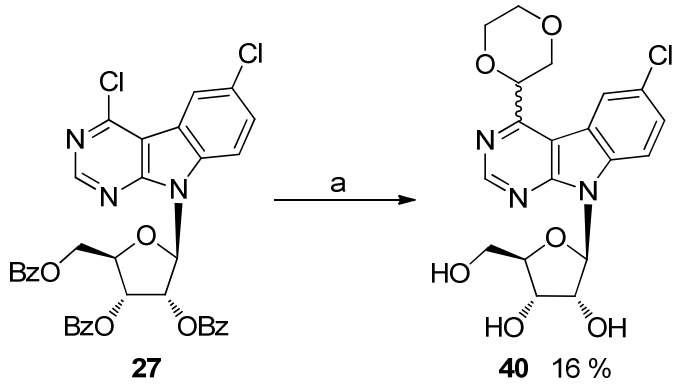
a: H_2SO_4. r.t., overnight; **b**: H_2SO_4/HNO_3, 0 °C- r.t.; **c**: $NO_2^+BF_4^-$, sulfolane, 0 - 70 °C; **d**: Br_2, DCM, r.t., **e**: AcCl or AcBr, $AlCl_3$, DCM, r.t.; **f**: $POCl_3$, DMF, r.t.; **g**: piperidine 1 eq., paraformaldehyde 1eq., isopropanol, 60 °C.

Scheme 32 Attempts on electrophilic aromatic substitution

Based on those results, it seems that pyrimido[4,5-*b*]indole system is unreactive under electrophilic aromatic substitution conditions and because during the time it was found, that any substitution on benzene ring leads to inactive nucleosides, no more attempts on these reactions had been done.

3.1.7 Synthesis of 4-amino-6-hetaryl pyrimidoindole nucleosides

4-Amino-6-chloropyrimidoindole ribonucleoside **39** was first prepared as a key-intermediate for a synthesis of final 6-hetarylpyrimidoindole nucleosides. Aqueous ammonia in dioxane was used for amination and deprotection of **27**, but only unexpected product **40** was isolated (Scheme 33). Formation of this product was probably caused by the presence of trace amount of peroxides in dioxane and therefore radical reaction instead of nucleophilic substitution took place. In next attempts, a new bottle of peroxide-free dioxane was used and product **40** was never observed again.

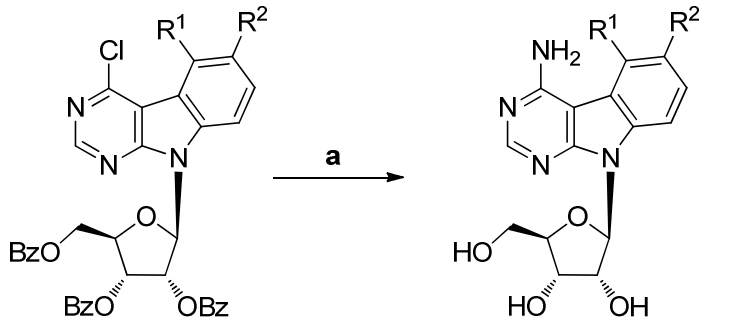


27 **40** 16 %

a: aq. NH_3, dioxane, 100 °C, 12 h.

Scheme **33** Formation of unexpected product **40**

Amination reaction was then tested in liquid ammonia, but even after heating to 70 °C for 24 hours, just mixture of aminonucleoside **39** with mono and dibenzoylated products was obtained. Methanolic ammonia can not be used, because during preparation of 4-amino-6-chloropyrimidoindole **9** was found out, that cca 20 % of 4-methoxyderivative was formed. The best way to aminonucleoside **39** is using aqueous ammonia in peroxide-free dioxane at 120 °C for 24 hours, after that time and cooling to room temperature, product precipitates from the reaction mixture and can be isolated just by filtration (Scheme 34). Lower temperature or lower amount of ammonia leads to partial deprotection and 5´-O-benzoylated product can be isolated. This methodology was successfully applied for synthesis of 4-amino-, 4-amino-5-chloro- and 4-amino-6-chloropyrimidoindole nucleosides **37-39**. Yields of aminopyrimidoindole nucleosides **37, 38** are lower (72 and 76 %, respectively) than in case of **39** (96 %), because nucleosides **37, 38** are more soluble in the reaction mixture and only 30-40 % of the products precipitate from the cold reaction mixture, the rest of the product can be obtained by crystallization from methanol/water mixture or by reverse-phase HPFC, which is much faster. I tried to change the ratio between aqueous ammonia and dioxane as well as concentration of nucleoside in reaction mixture, but it did not help.



25: R^1 = H; R^2 = H
26: R^1 = Cl; R^2 = H
27: R^1 = H; R^2 = Cl

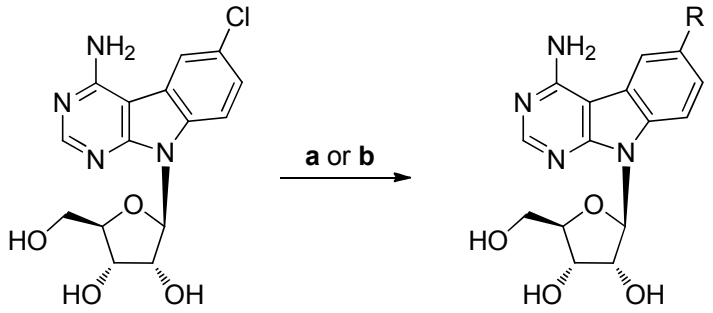
37: R^1 = H; R^2 = H 72 %
38: R^1 = Cl; R^2 = H 76 %
39: R^1 = H; R^2 = Cl 96 %

a: aq. NH_3, dioxane, 120 °C, 24 h.

Scheme 34 Synthesis of 4-aminopyrimidoindole nucleosides

Key step in the synthesis of 4-amino-6-hetarylpyrimidoindole nucleosides **41** was a cross-coupling reaction of unreactive 4-amino-6-chloropyrimidoindole nucleoside **39** and heteroaryl boronic acids or stannanes (Scheme 35). Several sets of catalytic systems and conditions were again tested for reaction with phenylboronic acid, including water soluble ligand TPPTS and $Pd(OAc)_2$ in acetonitrile/water, Cataxcium F in mixtures of water and acetonitrile and Buchwald-type ligands (X-Phos, S-Phos and RuPhos) in aqueous mixtures or in DMF. Whilst TPPTS gave no reaction, all other reactions were at least partially successful. X-Phos ligand was the best, coupling worked even in the mixture of acetonitrile/water, but full conversion was observed only from reaction with X-Phos ligand in DMF at 100 °C. Under these conditions, phenyl derivative **41f** was isolated in excellent 93 % yield. Reaction with 3-furylboronic acid in aqueous media gave only trace amount of desired product **41b**, probably because of decomposition of the boronic acid. Reaction was therefore done in DMF and the boronic acid was added in three parts, which led to the desired product **41b** in a good 72 % yield. The same procedure was applied also on the synthesis of 3-thienyl **41d** and 2-benzofuryl **41e** derivatives. Stille reaction using the same catalyst/ligand system was successfully used for synthesis of 2-furyl **41a** and 2-thienyl **41c** derivatives. Results are summarized in Table 10. Although all reactions are quantitative according to TLC, isolated yields of all nucleosides **41** except phenyl derivative **41a** are about 70 %, which is probably caused by loses during chromatography.

In conclusion, efficient catalyst/ligand system ($Pd(OAc)_2$/X-Phos ligand) was found for Suzuki and Stille cross-couplings of unreactive chlorine in position 6 of pyrimidoindoles and was used for synthesis of series of 4-amino-6-hetaryl-9*H*-pyrimido[4,5-*b*]indole nucleosides, which were isolated in good yields.



39 **41a-f**

a: $RB(OH)_2$ (1.5 eq.), $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), K_2CO_3 (3 eq.), DMF, 100 °C, 12 h;
b: $RSnBu_3$ (1.2 eq.), $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), DMF, 100 °C, 12 h.

Scheme 35 Synthesis of final 4-amino-6-hetarylpyrimidoindole nucleosides **41**

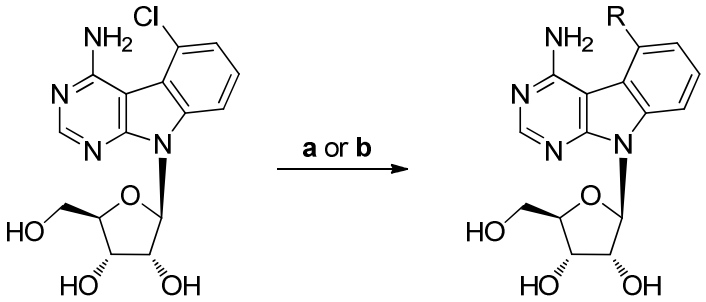
Table 10 Yields of 4-amino-6-hetarylpyrimidoindole nucleosides

Comp.	Reagent	R-	Yield [%]
41a	phenylboronic acid	phenyl	93
41b	(furan-2-yl)$SnBu_3$	2-furyl	68
41c	(furan-3-yl)$B(OH)_2$	3-furyl	72
41d	(thiophen-2-yl)$SnBu_3$	2-thienyl	73
41e	(thiophen-3-yl)$B(OH)_2$	3-thienyl	77
41f	(benzofuran-2-yl)$B(OH)_2$	2-benzofuryl	75

3.1.8 Synthesis of 4-amino-5-substituted pyrimidoindole nucleosides

The previously optimized conditions were applied also on the synthesis of 4-amino-5-hetarylpyrimidoindole nucleosides **42**. Suzuki cross-coupling reaction with phenylboronic acid furnished only 28 % of the target nucleoside **42f**. Increased temperature to 120 °C and 2 or even 3 equivalents of boronic acid did not improve the yield significantly. Suzuki reaction with thiophene-3-boronic acid gave just 5 % of product **42d**, which was probably caused by decomposition of boronic acid under reaction conditions. Portionwise addition of 2 equivalents of boronic acid into reaction mixture improved the yield to 24 %, which is still quite low and was not further improved by using 3 equivalents of boronic acid. Stille reaction with 2-tributylstannylthiophene was even more surprising, only nucleoside **42g** containing butyl group in position 5 was isolated in 42 % yield, not even trace of desired 2-thienyl nucleoside was isolated. A better result was obtained from reaction with 2-tributylstannylfuran, in this case, product of butyl transfer **42g** was isolated again together with desired 2-furyl derivative **42a**. However, target nucleosides **44** were obtained in

sufficient amount for characterization and biological testing (Scheme 36, Table 11). Much lower reactivity of 5-chloro nucleoside **38** in comparison with 6-chloro derivative **39** is probably caused by steric hindrance of the chlorine atom by the amino group in position 4.



38 **42 a,d,f,g**

a: $RB(OH)_2$ (1.5 eq.), $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), K_2CO_3 (3 eq.), DMF, 100 °C, 12 h;
b: $RSnBu_3$ (1.2 eq.), $Pd(OAc)_2$ (0.05 eq.), X-Phos (0.1 eq.), DMF, 100 °C, 12 h.

Scheme 36 Synthesis of 4-amino-5-substituted nucleosides **42**

Table 11 Yields of 4-amino-5-susbtituted pyrimidoindole nucleosides **42**

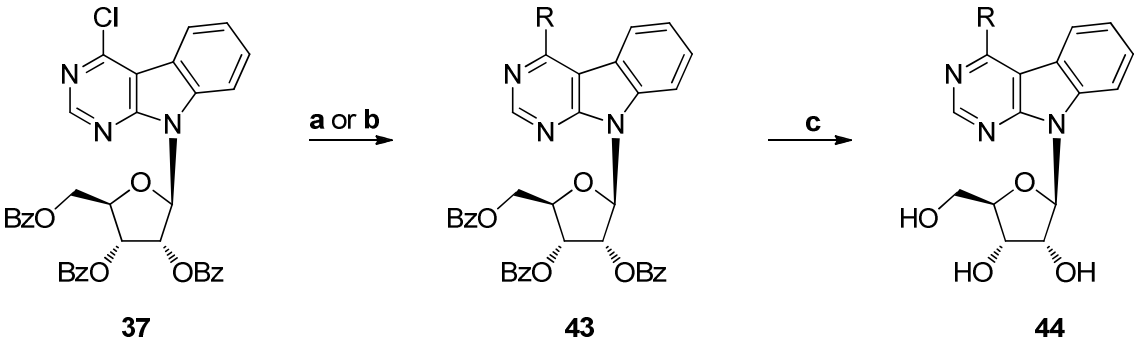
Entry	Comp.	Reagent	R-	Yield [%]
1	**42a**	phenylboronic acid	phenyl	28
2	**42b**	(furan-2-yl)$SnBu_3$	2-furyl	33
	42g		butyl	34
3	**42g**	(thiophen-2-yl)$SnBu_3$	butyl	42
4	**42d**	(thiophen-3-yl)$B(OH)_2$	3-thienyl	24

3.1.9 4-Substituted pyrimidoindole nucleosides

To investigate the influence of the amino group in position 4 on biological activity, a series of 4-substituted pyrimidoindole nucleosides was prepared. Amino group was replaced by methylamino, dimethylamino and also by methyl, ethyl and cyclopropyl groups. Inspiration for synthesis of methyl and ethyl derivatives was 6-methyldeazapurine ribonucleoside[24] with interesting anti-dengue activity. 6-Cyclopropylpurine ribonucleoside showed micromolar cytostatic activity against several cancer cell lines.[136]

Methyl **43a**, ethyl **43b** and cyclopropyl **43c** derivatives were synthesized by Pd-catalyzed cross-coupling reaction of protected 4-chloropyrimidoindole nucleoside **37** with trimethylaluminium, triethylaluminium or by Negishi reaction with cyclopropylzinc chloride under standard conditions in the presence of $Pd(PPh_3)_4$ in THF at 70 °C. All reactions proceeded smoothly and gave benzoylated nucleosides in good yields.[46,136]

N,*N*-Dimethylamino derivative **43d** was prepared from 4-chloropyrimidoindole nucleoside **25** by a simple nucleophilic substitution with dimethylamine in THF/isopropanol at r.t. in 74 % yield. Zemplén deprotection furnished target free nucleosides **44** in good yields (88-92 %, Scheme 37).



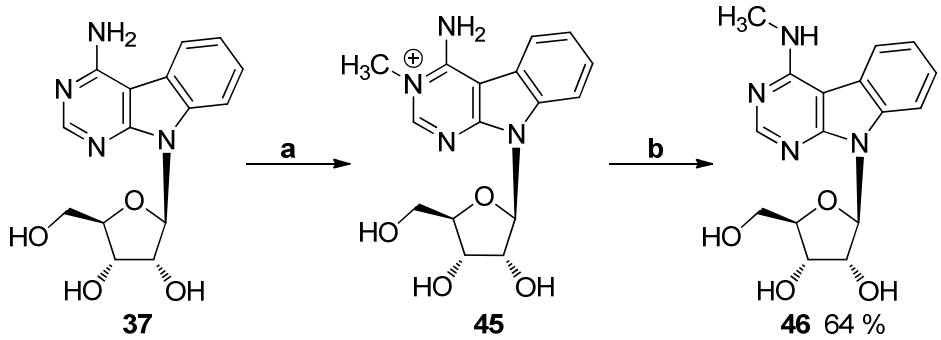
a: reagent (2 eq., see Table 12), $Pd(PPh_3)_4$ (0.05 eq), THF, r.t.; **b**: Me_2NH in THF (2 eq.), propan-2-ol, r.t., 24 h; **c**: 1M MeONa in MeOH (0.3 eq), MeOH, r.t., 24 h.

Scheme 37 Synthesis of 4-susbtituted pyrimidoindole nucleosides **44**

Table 12 Yields of 4-substituted products

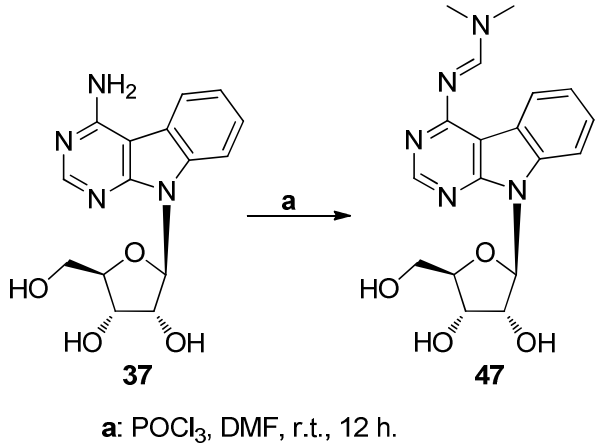
Entry	Reagent	R	Protected product	Yield [%]	Unprotected product	Yield [%]
1	Me_3Al	Me	**43a**	63	**44a**	92
2	Et_3Al	Et	**43b**	76	**44b**	85
3	cyclopropyl-ZnCl	cyclopropyl	**43c**	63	**44c**	89
4	Me_2NH	Me_2N	**43d**	74	**44d**	88

Nucleophilic substitution with methylamine surprisingly failed even though more than 10 equivalents of amine were used at 50 °C in a closed microwave vial. Also palladium catalyzed amination using X-Phos in combination with palladium acetate and sodium tert-butoxide was not successful. Desired *N*-methylamino compound **46** was finally synthesized according to a 40 years old procedure.[137] Aminopyrimidoindole nucleoside **37** was first methylated by methyl iodide in DMA in position 3 to form quarternary salt **45**, followed by sodium hydroxide induced rearrangement of quartnernary salt **45** to methylamino-pyrimidoindole nucleoside **46** in 74 % yield (Scheme 38).



Scheme 38 Synthesis of 4-*N*-methylaminopyrimidoindole nucleoside **46**

Dimethylaminomethylidene-protected nucleoside **47** (Scheme 39) was the only isolated product from all attempts on electrophilic substitution reactions mentioned in chapter 3.1.6. It was formed during the reaction of 4-aminopyrimidoindole nucleoside **37** under Vilsmeier-Haack conditions. Formation of *N*,*N*-disubstituted formamidines from primary amines and DMF in the presence of $POCl_3$ is known,[138] formamidine is formed by nucleophilic attack of Vilsmeier-Haack reagent by amine.[139] Dimethylaminomethylidene-protected nucleoside **47** did not react with excess of Vilsmeier-Haack reagent.



Scheme 39 Formation of nucleoside **47**

3.2 Biological activities of pyrimidoindole nucleosides

3.2.1 Biological activities of 4-hetaryl-6-chloro and 4,6-bishetarylnucleosides

All the title compounds **30**, **32** and **36** were tested for *in vitro* cytotoxic (human T-lymphoblastic leukemia line CCRF-CEM, promyelocytic leukemia HL-60 and cervical carcinoma HeLa S3) and antiviral activity (HCV and Dengue virus). Cytostatic activity screening was done by our collaborative laboratories at Institute of Organic Chemistry and Biochemistry AS CR. Anti-HCV activities were studied at Gilead Sciences, anti-dengue activities at Novartis Institute for Tropical Diseases, Singapore (NITD). None of these nucleosides showed any significant cytostatic/cytotoxic effect or anti-HCV activity, except for compound **32a,** which showed cytotoxicity in THP-1 and HepG2 cells with CC_{50} of 1.565 and 0.175 μM, respectively. However, three examples of 4-hetaryl-6-chloropyrimido[4,5-*b*]indole nucleosides bearing 2-furyl **32a**, 2-thienyl **32c** or 2-benzofuryl **32e** groups at position 4 showed significant (submicromolar) effects against Dengue virus (Table 13). 2-Furyl derivative **32a** was the most active compound with IC_{50} value of 10 nM in dengue virus reporter assay. Unfortunately, narrow windows of these compounds (x 10) between EC_{50} and CC_{50} in Huh-7 replicon assay were observed. This led to termination of any further studies of these compounds as nucleoside inhibitors of dengue virus in NITD. On the other hand, further structural modifications of the parent 7-deazapurine moiety in the biologically active ribonucleosides can lead to more selective and more active compounds with anti-RNA virus activities and decreased cytotoxicities.

Table 13 Anti-dengue activities of 4-hetaryl-6-chloropyrimidoindole nucleosides

comp.	Dg-reporter		Dg-replicon	
	K-562 IC_{50} (μM)	K-562 CC_{50} (μM)	Huh7 IC_{50} (μM)	Huh7 CC_{50} (μM)
32a	0.01	4.12	0.49	3.60
32b	69.51	>100	>100	>100
32c	0.51	15.75	1.71	10.71
32d	41.77	>100	46.99	>100
32e	0.13	16.50	10.04	26.26
32f	27.56	51.34	15.63	20.10
30	>100	>100	61.77	>100
36a	3.51	18.43	18.69	31.09
36b	10.91	29.91	30.00	52.98
36f	3.46	7.36	7.53	11.1

3.2.2 Biological activities of 4-aminopyrimidoindole nucleosides

All the target nucleosides **37**, **38**, **39**, **41, 42** and **45** were also studied for *in vitro* cytotoxic activity against cancer cell lines (HL60, HeLa S3, CCRF-CEM and HepG2) at IOCB. Anti-HCV activities were studied at Gilead Sciences, anti-dengue activity in group of Dr. Weber at IOCB.

The title nucleosides **37**, **38**, **39**, **41, 42** and **45** did not show any significant cytotoxicity in these assays. The only three compounds with non-negligible activities (in 11-100 μM) are shown in Table 14. The most active compound was 4-amino-5-chloropyrimidoindole nucleoside **38**. All 5- or 6-hetaryl derivatives were inactive except for benzofuryl derivative **41f**. The 4-methylpyrimidoindole nucleoside **44a** was the only cytostatic compound in the series of 4-substituted derivatives.

Table 14. Cytotoxic activities of nucleosides

	IC_{50} (μM)			
Compound	HL-60	HeLa S3	CCRF-CEM	HepG2
38	17	21	12	21
41f	87	93	32	60
44a	54	11	82	15

Results of testing against HCV genotype 1A, 1B and 2A replicons are summarized in Table 15. Several nucleosides displayed micromolar activity against HCV, but the activity was usually accompanied by cytotoxicity to MT-4 cells. The most active, but also the most cytotoxic compound was the 4-amino-5-chloro derivative **38.** However, 4-methyl derivative **44a** showed submicromolar activities in the HCV 1A and 1B replicon assays and cytotoxicity higher than 44 μM.

Table 15. Anti-HCV activities of nucleosides.

	HCV replicon 1A		HCV replicon 1B		HCV replicon 2A	
Compound	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
38	0.53	1.16	0.32	1.71	1.28	1.66
39	30.55	>44	11.05	>44	>44	>44
41d	10.03	18.10	8.75	>44	24.89	>44
41e	10.09	17.34	7.38	25.60	13.77	30.19
41f	6.13	14.47	4.83	28.0	8.77	18.00
42g	>44	>44	8.81	>44	>44	>44
44d	21.27	>44	7.07	>44	>44	>44
44a	0.56	>44	0.34	>44	>44	>44

All the nucleosides were also tested for anti-dengue activities in Vero cells. This screening was done at IOCB in group of Dr. Weber. 2-Furyl **31a** and 2-benzofuryl **31e** derivatives, which were previously shown to be active against dengue virus, were used as standards during establishment of this screening. Several derivatives showed activity in micromolar concentrations, but only benzofuryl derivative **41e** was not cytotoxic to these cells. 4-Amino-5-chloropyrimidoindole nucleoside **38** was the most active compound, but with low selectivity index (SI).

Table 16. Anti-dengue activities of nucleosides

	Vero cells, DENV-2 (μM)		
Compound	EC_{50}	CC_{50}	SI
37	43.5	39.6	0.9
38	0.85	1.14	1.3
43d	18.8	46.3	2.5
44g	14.8	62.9	4.3
43e	15.4	>100	>6.5
40	39.6	43.5	1.1

Based on these results, it can be noted that all pyrimido[4,5-*b*]indole ribonucleosides are less cytotoxic than the corresponding 7-deazapurine nucleosides and are not very promising cytostatics. Micromolar activity of 4-amino-5-chloro- **38** and 4-methylpyrimidoindole nucleoside **44a** against HCV virus is also not sufficient for further studies. On the other hand, submicromolar anti-dengue activity of three 4-hetaryl-6-chloropyrimidoindole nucleosides **31a**, **31c**, **31e** is far more interesting, especially because of the lack of any antiviral therapy or vaccine against dengue fever. 2-Furylpyrimidoindole nucleoside **31a** displayed even better activity (10 nM) than NITD-008 (0.64 μM), compound subjected to *in vivo* screening in mice.[23] The problem of pyrimidoindole derivatives is in their low selectivity index caused by quite high toxicity. Further SAR studies are needed to find less toxic compounds or improve selectivity index of active compounds. It seems that introduction of hetaryl groups into benzene ring leads to inactive compounds, but the presence of a heteroatom at the benzene ring is advantageous for antiviral activity (see Table 15 and 16, 4-aminopyrimidoindole derivative **37** vs. 4-amino-5-chloropyrimidoindole derivative **38** and 4-(2-furyl)pyrimidoindole derivative **30** vs. 4-(2-furyl)-6-chloropyrimidoindole derivative **32a**, Table 13). Based on these results, we designed new target molecules, isosteric thieno-fused 7-deazapurines substituted by hetaryl, alkyl, amino, methoxy and sulfanyl groups in position 4.

3.3 Synthesis of thienopyrrolopyrimidine nucleosides

Another class of target coumpounds were thieno-fused 7-deazapurine ribonucleosides. There are three possible isomeric bases, we were most interested in 4-chloro-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (**48**), isomer with sulphur in position 5 and 4-chloro-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (**49**) with sulphur in position 7 of thienopyrrolo-pyrimidine system (Figure 26).

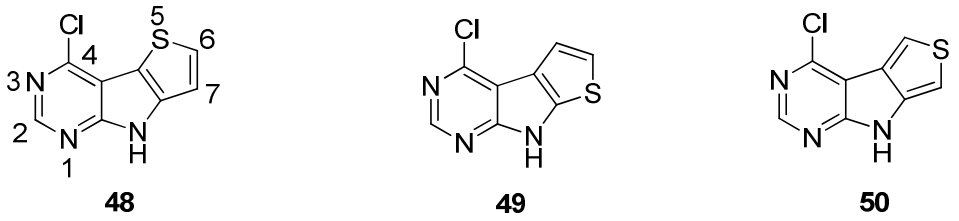


Figure 26 Stuctures of all possible thienopyrrolopyrimidines

Suggested synthesis of these tricyclic bases was based on published procedure for the only one known thienopyrrolopyrimidine derivative **LXII**.[94] In the first step, thiophene was needed to be attached to position 5 of 4,6-dichloropyrimidine, this can be in principle done by any cross-coupling reaction. As the key and also the last step of this synthesis is thermal cyclization or photocyclization of nitrene generated from azide precursor, azido group was supposed to be introduced into position 4 by nucleophilic substitution with sodium azide (Fig. 27).

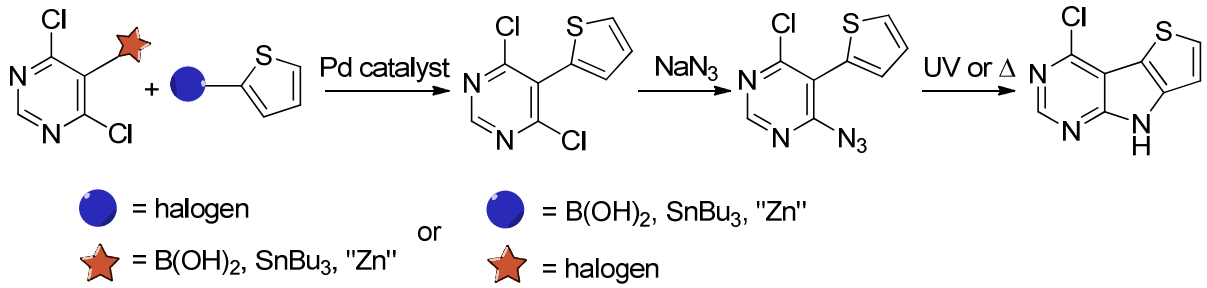
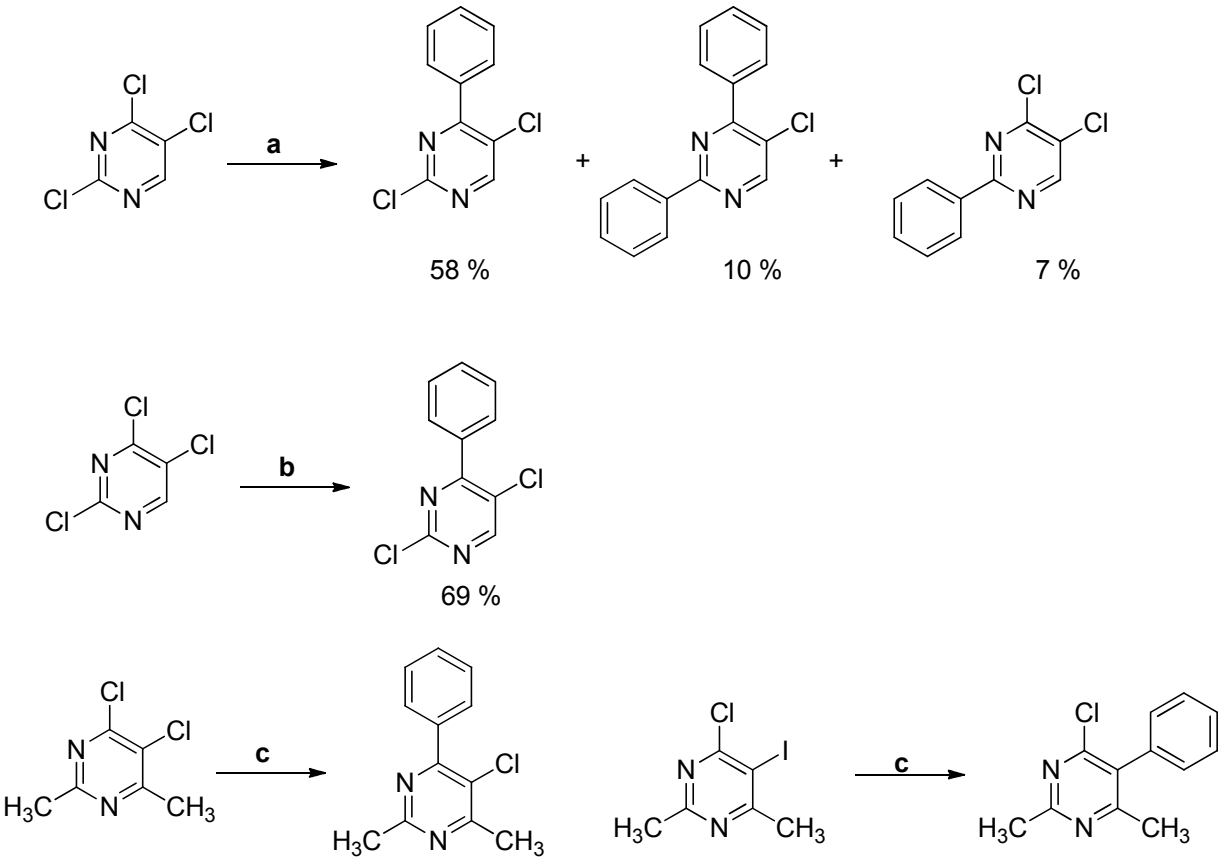


Figure 27 Suggested synthesis of thienopyrrolopyrimidines

It is known, that the halogen atom in position 4 on pyrimidine is the most reactive one in palladium catalyzed cross-coupling reactions, this was proved on 2,4,5-trichloropyrimidine. During the Suzuki coupling with phenylboronic acid, mainly 4-substituted pyrimidine (67 %) was formed, accompanied by small amount of 2-substituted (7 %) and 2,4-disubstituted product (10 %).[140] Similar results were obtained from Stille coupling of 2,4,5-trichloropyrimidine with phenyltributylstannane.[141] Substitution of chlorine atom in position 5 by iodine changed the regioselectivity of cross-coupling reaction on 2,6-dimethyl-4,5-

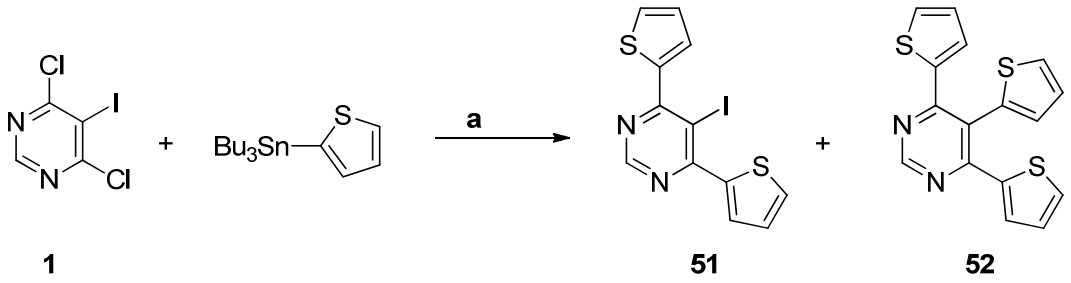
dihalopyrimidines, 4-chloro-5-iodo-2,6-dimethylpyrimidine gave only 4-chloro-5-substituted product.[142]



a: $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene–DMF (9:1), microwave irradiation, 10 min, 185 °C; **b**: $PdCl_2(PPh_3)_2$, DMF, 10 h, 80 °C;
c: $PdCl_2(PPh_3)_2$, Et_4NCl, K_2CO_3, DMF, 4 h, 110 °C

Scheme 40 Published[140,141,142] cross-couplings on halopyrimidines

Inspired by the literature,[94] Stille and Suzuki cross-coupling reactions were performed on 4,6-dichloro-5-iodopyrimidine **1**. Stille reaction of 4,6-dichloro-5-iodopyrimidine (**1**) with 2-tributylstannylthiophene was tested with $PdCl_2(PPh_3)_2$ in DMF. Although iodine is supposed to be more reactive, only 4,6-disubstituted **51** or even trisubstituted product **52** was identified in the reaction mixture by LC-MS after 8 hours at 0 °C, no desired 5-substituted compound was found (Scheme 41). At lower temperature (-15 °C), reaction did not proceed. The reaction proceeded faster at r.t., but regioselectivity was the same. As this system is different from above mentioned published examples and chlorine at position 4 seemed to be more reactive than iodine at position 5, any further optimization of Stille coupling has not been done.

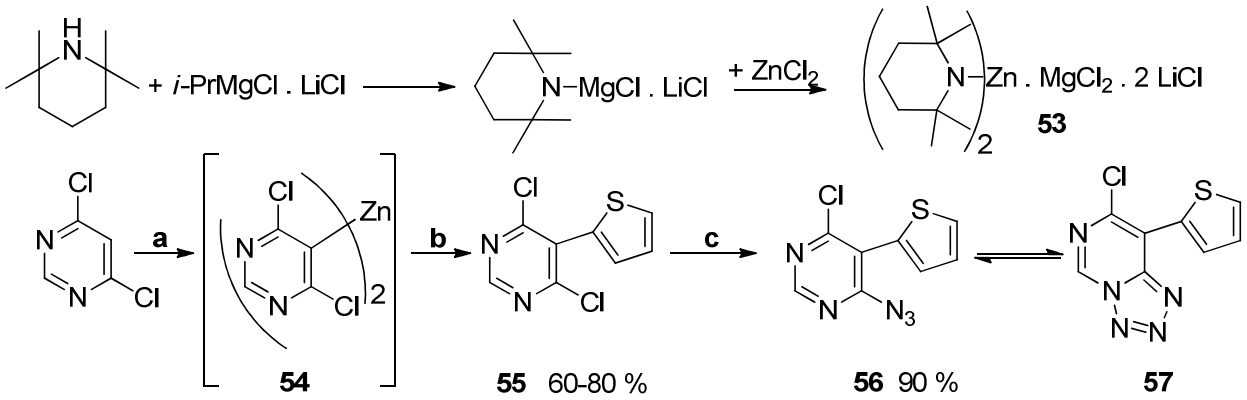


a: $PdCl_2(PPh_3)_2$, DMF, 0 °C, 8 h

Scheme 41 Stille reaction of 4,6-dichloro-5-iodopyrimidine (**1**)

Another way how to connect pyrimidine and thiophene can be Negishi coupling. Organozinc species needed for this reaction was prepared by Knochel´s procedure from 4,6-dichloropyrimidine and tetramethylpiperidinylzinc complex with magnesium chloride and lithium chloride.[108] The reaction was optimized with zinc complex **53** generated *in situ* from tetramethylpiperidine and *i*-propylmagnesium chloride complex with lithium chloride, which was then added to zinc chloride. Later on, Sigma-Aldrich started to sell final zinc complex **53**, which speeded up the whole process. Generated pyrimidinylzinc species **54** was then subjected to $Pd(PPh_3)_4$ catalyzed cross-coupling reaction with 2-iodothiophene to furnish desired product **55** (Scheme 42). Yield of this reaction usually varies from 40 to 80 % and it depends only on the yield of the zincation step, the coupling reaction is then quantitative. This was proved by NMR determined conversion of zincation step; part of the reaction mixture was quenched by deuterium oxide and ratio of 4,6-dichloropyrimidine and 5-deuterated analogue was calculated. It was found, that conversion of zincation corresponds to final isolated yield. As it is really difficult to separate product **55** from unreacted starting 4,6-dichloropyrimidine on column and because yields were lower in bigger (1 g) scale, optimization and scale up of this process was necessary. According to published procedure, THF solution of 4,6-dichloropyrimidine is added to a solution of tetramethylpiperidinylzinc complex **53** (0.55 eq.) at r.t. and then stirred at r.t. for 45 minutes.[108] The amount of tetramethylpiperidinylzinc complex **53** does not have any effect on conversion, which was the same even with 1.1 eq. of the zinc complex. Temperature is more important for this zincation, during optimization it was found out, that the best procedure is to add solution of 4,6-dichloropyrimidine in several parts to an ice-cooled solution of tetramethylpiperidinylzinc complex **53**, stir the mixture for 1 hour at 0 °C and leave it to warm to r.t. for another hour. This allows to set up the reaction from 8 grams of starting material with isolated yields 60-80 %.

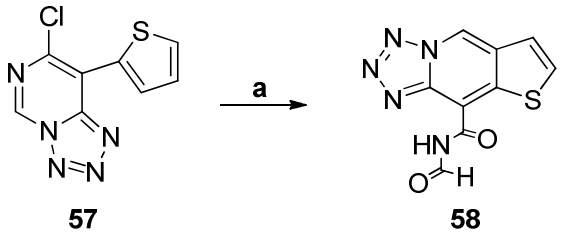
The next step was a simple nucleophilic substitution with sodium azide in DMF, which worked without any problem and furnished desired azidopyrimidine **56** in more than 90 % yield. This azidopyrimidine **56**, which is in solution present in form of tetrazole **57** can be cyclized photochemically or thermally to desired tricyclic base **48** (Scheme 44).



a: $(TMP)_2Zn.MgCl_2.2LiCl$ (0.55 eq.), THF, 0 °C, 1h, then r.t., 1h; **b**: 2-iodothiophene (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq.), THF, 65 °C, 16h; **c**: NaN_3 (1 eq.), LiCl (1 eq.), DMF, r.t.,

Scheme 42 Preparation of organozinc complex **53** and synthesis of tetrazole **57**

When the reaction mixture containing only tetrazole **57** and inorganic chlorides (LiCl, NaCl) in DMF was irradiated by UV lamp (254 nm), an unwanted fluorescent product **58** was isolated (Scheme 43). Structure of this compound was suggested from NMR spectra and confirmed by HR-MS and X-Ray crystallography (Fig. 28).



a: DMF, UV 254 nm, r.t., 12 h

Scheme 43 Formation of unwanted product **58**

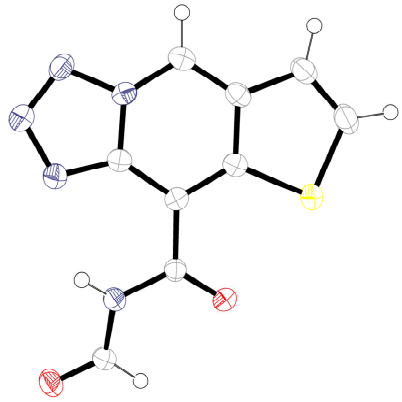


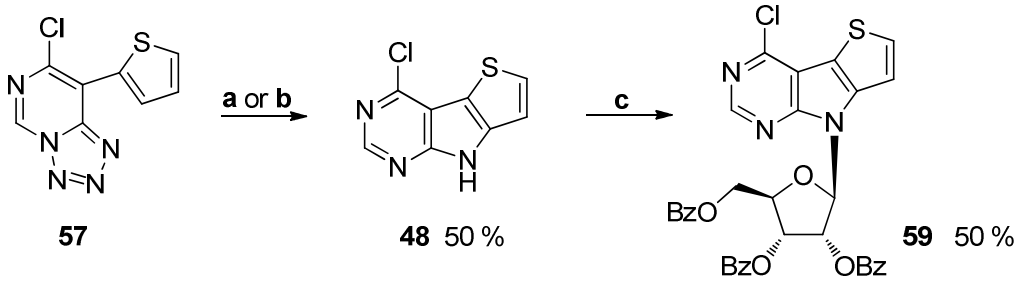
Figure 28 An ORTEP[4] view of **58**, displacement ellipsoids shown with 50 % probability

Later on it was found out that this compound is always formed if there is even a trace amount of DMF present in the reaction mixture, because it was observed also from photocyclization reaction of DMF-contaminated tetrazole **57** performed in TFA. To avoid formation of unwanted product **58**, it is important to have DMF free tetrazole **57**. To get it, it is necessary to evaporate the crude product several times with toluene, then extract it with water and ethyl-acetate and again co-evaporate several times with toluene or heptane. All published procedures for nucleophilic substitution with sodium azide on pyrimidine use DMF as a solvent, but much better way is to perform this nucleophilic substitution step in THF, the reaction is slower, but full conversion is reached in two days.

Tetrazole **57** was cyclized to desired product **48** (Scheme 44) by irradiation with 4W UV lamp in TFA at r.t. The photocyclization was successful but too slow, it took 7 days to reach full conversion. This is why the photoreactor with 400W mercury lamp was used, but only decomposed starting material was obtained. Therefore thermal cyclization of starting material **57** was accomplished by heating in 1,4-dibromobenzene to 180 °C for 30 minutes to give the desired tricyclic product **48** in 25 % yield, the rest was decomposed starting material. There are only 2 factors that can be optimized, temperature and amount of solvent. Temperature control is crucial for this reaction, at lower temperature, tetrazole **57** decomposes to unidentified black mass, at temperature higher than 180 °C, decomposition of starting material is faster and become predominant over formation of product. Then I optimized amount of the solvent and found that the best result is obtained if the ratio of tetrazole and 1,4-dibromobenzene is 1:10. However, isolated yield of compound **48** was only 50 %, the rest was decomposed starting material.

Thienopyrrolopyrimidine base **48** was then subjected to Vorbrűggen glycosylation under the same condions as in case of pyrimidoindoles. The stirring of the base **48** with BSA

for 10 minutes at room temperature was followed by addition of 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose and trimethylsilyltriflate, but no nucleoside was obtained. So I tried to heat the tricyclic base **48** with BSA for 30 minutes at 60 °C and then add trimethylsilyltriflate and 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose and heat the reaction mixture overnight. This procedure afforded the nucleoside **59** in ca. 50 % yield (Scheme 44).



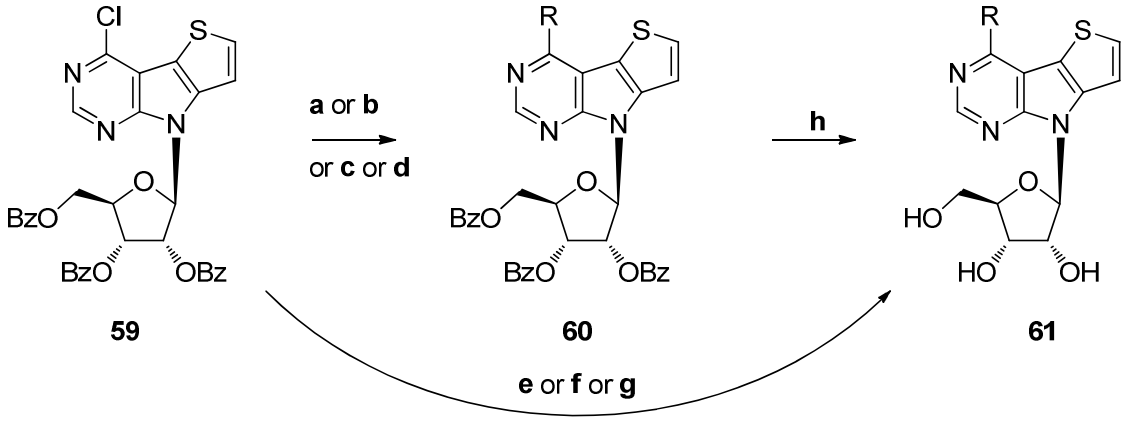
a: 1,4-dibromobenzene, 180 °C, 30 min; **b**: TFA, UV, r.t., days;
c: BSA (1 eq.), MeCN, 60 °C, 30 min; then 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose (2 eq.), TMSOTf (2 eq.), 60 °C, 8 h.

Scheme 44 Synthesis of key-intermediate nucleoside **51**

Thienopyrrolopyrimidine nucleosides have never been reported, so we decided to synthesize the first series of 8 nucleosides substituted in position 4 for initial biological activity studies. Substituents selection was based on previous results obtained in our group.[143,144] Furyl and benzofuryl as well as amino and methyl groups were selected because pyrimidoindoles bearing those groups displayed some biological activity; methoxy, methylsulfanyl and dimethylamino groups were chosen as analogues of cytostatic 7-hetaryl-7-deazapurine ribonucleosides bearing those groups in position 6.[46]

Synthesis of final nucleosides was done according to the same conditions used for synthesis of pyrimidoindole nucleosides **31** and **43**. 3-Furyl and 2-benzofuryl substituted nucleosides **60b**, **60e** were prepared from 4-chlorothienopyrrolopyrimidine nucleoside **59** by Suzuki coupling using $Pd(PPh_3)_4$ as a catalyst with potassium carbonate in toluene, 2-furyl derivative **60a** was synthesized by Stille coupling catalyzed by $PdCl_2(PPh_3)_2$ in DMF and 4-methyl derivative **60h** was obtained from palladium catalyzed alkylation by trimethylaluminium. Dimethylamino derivative **60i** was prepared by nucleophilic substitution with dimethylamine (Scheme 45). Yields of protected nucleosides **60** are summarized in Table 17. Zemplén deprotection furnished desired unprotected nucleosides in good yields. Amino, methoxy and methylsulfanyl groups were introduced into position 4 of chloroderivative **59** by nucleophilic substitution with aqueous ammonia in dioxane, sodium

methoxide or sodium thiomethoxide in methanole. Ribose moiety was simultaneously deprotected under reaction conditions and target unprotected nucleosides **61** were obtained in good yields (Table 17).



a: 2-tributylstannylfuran (1.2 eq.), $PdCl_2(PPh_3)_2$ (0.1 eq.), DMF, 100 °C, 8 h; **b**: R-boronic acid (1.5 eq.), $Pd(PPh_3)_4$ (0.05 eq), K_2CO_3 (2 eq.), toluene, 100 °C, 8 h; **c**: Me_3Al (2 eq.), $Pd(PPh_3)_4$ (0.05 eq), THF, r.t., 12 h; **d**: Me_2NH in THF (2 eq.), propan-2-ol/EtOH 1:1, r.t., 24 h; **e**: NH_3 (aq.), dioxane, 120 °C, 12 h; **f**: MeONa (1.3 eq.), MeOH, r.t., 12 h; **g**: MeSNa (1.3 eq.), MeOH, r.t., 12 h; **h**: 1M MeONa in MeOH (0.3 eq), MeOH, r.t., 24 h.

Scheme 45 Synthesis of 4-susbtituted thienopyrrolopyrimidine nucleosides **60, 61**

Table 17 Synthesis of 4-susbtituted thienopyrrolopyrimidine nucleosides

Entry	Conditions	R	Protected product	Yield [%]	Unprotected product	Yield [%]
1	a	2-furyl	**60a**	84	**61a**	62
2	b	3-furyl	**60b**	82	**61b**	70
3	b	2-benzofuryl	**60e**	87	**61e**	68
4	c	Me	**60h**	87	**61h**	82
5	d	Me_2N	**60i**	78	**61i**	49
6	e	NH_2	-	-	**61j**	75
7	f	MeO	-	-	**61k**	78
8	g	MeS	-	-	**61l**	64

The next goal was to synthesize a series of nucleosides with isomeric (8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine) base **49**. The trickiest part was as usually synthesis of the heterocyclic base. I wanted to apply the same synthetic strategy as for base **48** and just change the starting material from 2-iodothiophene to 3-iodothiophene (Scheme 46). Because 3-iodothiophene is far more expensive, I tried Negishi cross-coupling with a bromoanalogue but, unfortunately, it did not react. However, 3-bromothiophene, which is much cheaper, can be quite easily converted to an iododerivative. It was prepared by modified

published procedure[145] using sodium iodide with 5 % of copper (I) iodide, *N*,*N*-dimethylethylenediamine at 2 days reflux in a mixture of toluene and 1,2-dimethoxyethane. It is important to reach full conversion, because both thiophenes are unseparable. Negishi coupling with 3-iodothiophene worked well and product **62** was isolated in 70-80 % yield and its structure was confirmed by X-ray (Fig. 29).

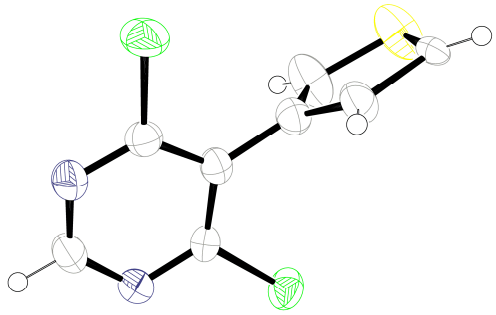
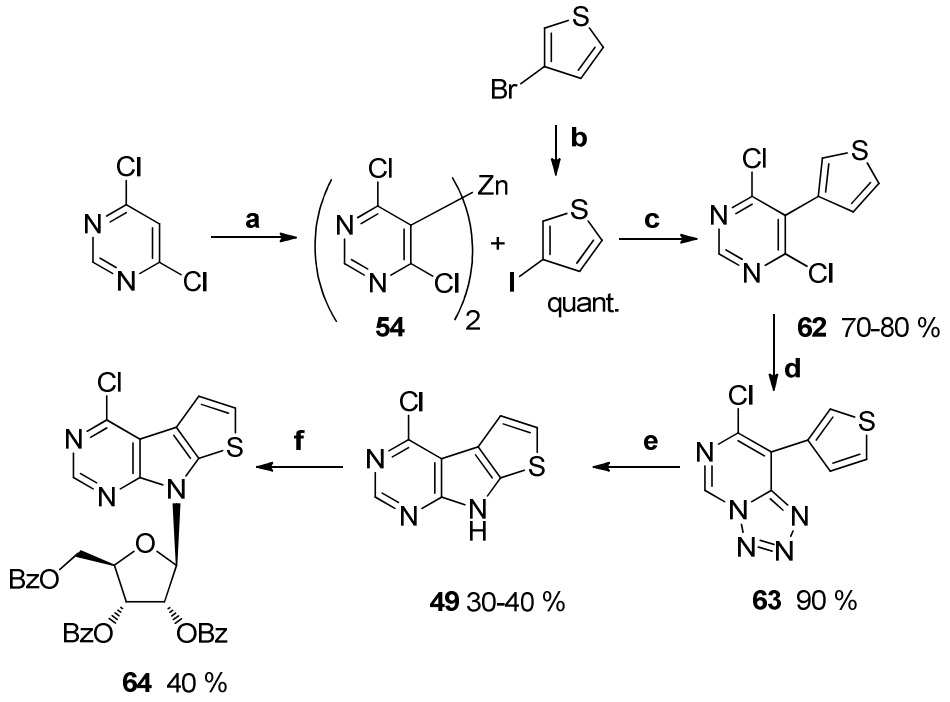


Figure 29 An ORTEP[4] view of **62**, displacement ellipsoids shown with 50 % probability.

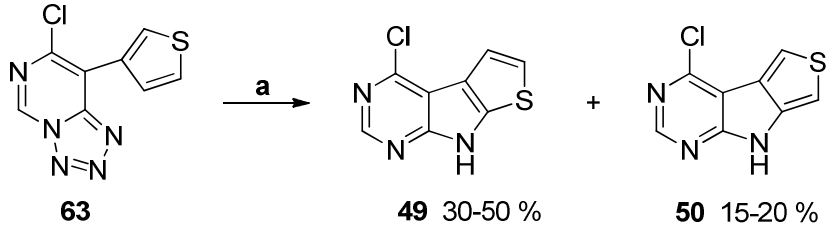
Azido group was then introduced into position 4 of pyrimidine ring of compound **62** by nucleophilic substitution with sodium azide in THF and obtained tetrazole **63** was cyclized in 1,4-dibromobenzene at 180 °C in the same manner as **57** (Scheme 45). Although there are two possible products of cyclization, only isomer **49** with sulphur in position 7 was isolated, but the yield was only 15 % and the rest was decomposed starting material. Changing the amount of dibromobenzene did not help like in case of base **48** and the yield of thermal cycliation was always around 15 %, so other solvents were tried. 1,4-dichlorobenzene and naphtalene were used as solvents for cyclization of tetrazole **63**, but yield was 3 % and 15 %, respectively. This led to conclusion that thermal cyclization is not the best approach to desired 4-chloro-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (**49**).



a: $(TMP)_2Zn.MgCl_2.LiCl$, 0 °C, 60 min, then r.t. for 45 min; **b**: NaI (2 eq.), CuI (0.05 eq.), *N*,*N*-dimethylethylenediamine (0.1 eq.), toluene/1,2-dimethoxyethane, refl., 2 days; **c**: $Pd(PPh_3)_4$ (0.1 eq.), THF, 65 °C, 12 h, **d**: NaN_3 (1 eq.), LiCl (1 eq.), THF, r.t., 2 days; **e**: TFA, UV, r.t., 4 days; **f**: BSA (1 eq.), MeCN, 60 °C, 30 min; then 1-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-ribofuranose (2 eq.), TMSOTf (2 eq.), 60 °C, 8 h.

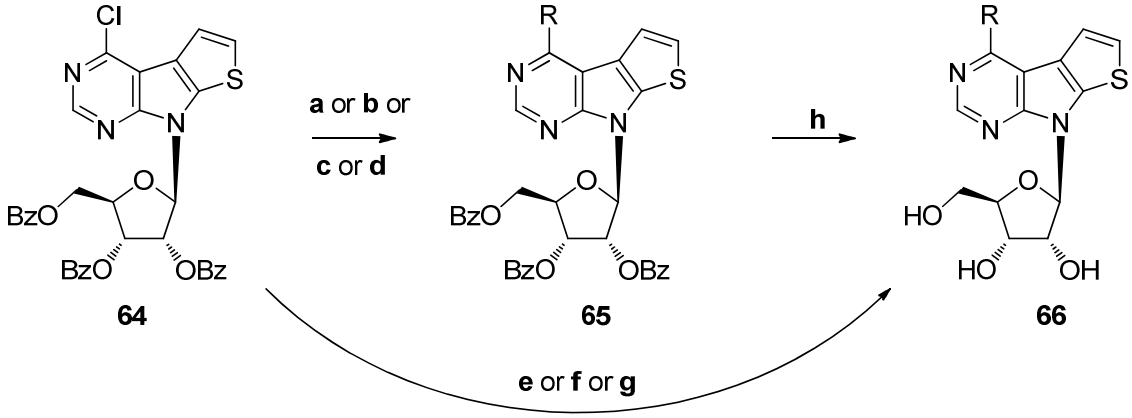
Scheme 45 Synthesis of nucleoside **64**

Photocyclization of tetrazole **63** in TFA under irradiation by 4W 254 nm UV lamp was very slow, after 4 days, there was only 50 % conversion of the starting material. However, reaction mixture contained both possible isomers **49** and **50** in ratio 2:1 (Scheme 46). Unfortunately, it is not possible to separate those isomers by crystalization or by column chromatography. Separation is possible only on HPFC system and only 150 mg of the mixture can be separated on column designed for 4 g separations. Even with such complicated separation, it was possible to get enough base **49** for synthesis of series of nucleosides. Subsequent Vorbrűggen glycosylation was performed in the same manner as in case of base **48** and gave desired key-intermediate nucleoside **64** in yields around 40 %.



Scheme 46 Photocyclization of tetrazole **63**

Target nucleosides **66** were synthesized using analogous procedures as for nucleosides **61** (Table 18, Scheme 47). Both 2- and 3-furyl, 2-benzofuryl and methyl groups were introduced into position 4 by palladium catalyzed reactions, amino, dimethylamino, methoxy and methylsulfanyl groups by nucleophilic substitution. Palladium catalyzed reactions led to protected nucleosides **65**, which were then treated by sodium methoxide in methanol to give desired unprotected nucleosides **66** in good yields (Scheme 47, Table 18). Protected methyl nucleoside **65h** was not isolated as pure compound and was directly deprotected. Yields are summarized in Table 18.



a: 2-tributylstannylfuran (1.2 eq.), $PdCl_2(PPh_3)_2$ (0.1 eq.), DMF, 100 °C, 8 h; **b**:R- boronic acid (1.5 eq.), $Pd(PPh_3)_4$ (0.05 eq), K_2CO_3 (2 eq.), toluene, 100 °C, 8 h; **c**: Me_3Al (2 eq.), $Pd(PPh_3)_4$ (0.05 eq), THF, r.t., 12 h; **d**: Me_2NH in THF (2 eq.), propan-2-ol/EtOH 1:1, r.t., 24 h; **e**: NH_3 (aq.), dioxane, 120 °C, 12 h; **f**: MeONa (1.3 eq.), MeOH, r.t., 12 h; **g**: MeSNa (1.3 eq.), MeOH, r.t., 12 h; **h**: 1M MeONa in MeOH (0.3 eq), MeOH, r.t., 24 h.

Scheme 47 Synthesis of 4-substituted thienopyrrolopyrimidine nucleosides **65, 66**

Table 18 Synthesis of 4-susbtituted thienopyrrolopyrimidine nucleosides **65, 66**

Entry	Conditions	R	Protected product	Yield [%]	Unprotected product	Yield [%]
1	**a**	2-furyl	**65a**	67	**66a**	68
2	**b**	3-furyl	**65b**	82	**66b**	83
3	**b**	2-benzofuryl	**65e**	83	**66e**	86
4	**c**	Me	**65h**	-	**66h**	70
5	**d**	Me_2N	**65i**	85	**66i**	88
6	**e**	NH_2	-	-	**66j**	78
7	**f**	MeO	-	-	**66k**	65
8	**g**	MeS	-	-	**66l**	90

In conclusion, both desired thienopyrrolopyrimidine bases were synthesized from simple 4,6-dichloropyrimidine and corresponding iodothiophene by three-step synthesis

involving Negishi coupling, nucleophilic aromatic substitution and cyclization of tetrazole. Cyclization of tetrazoles can be induced thermally or photochemically, thermal variant is better in case of base **48**, it gives 50 % yield in 30 minutes and product can be isolated by simple flash chromatography on silica. 1,4-Dibromobenzene, which is used as a solvent, is eluted by pure hexane followed by elution of product in hexane/ ethyl-acetate mixture. On the other hand, thermall cyclization of tetrazole **63** gave only 15 % of base **49**. This is a reason for using the photocyclization in this case, because even if it gives mixture of bases **49** and **50** in ratio 2:1, it can be done with full conversion. Separation of those isomers is extremely difficult, but is still better than thermal cyclization. The synthesis of all desired thienopyrrolopyrimidine nucleosides were done by the same procedures, which were successfully applied for synthesis of pyrimidoindole nucleosides, and all nucleosides were obtained in good yields and sufficient amounts for biological activity screening.

3.4 Biological activities of thienopyrrolopyrimidine nucleosides

All the title nucleosides **61, 66** are currently tested for cytostatic, antiviral and antimicrobial activities. Antiviral screening against hepatitis C virus (HCV) and respiratory syncitial virus (RSV) was done at Gilead Sciences. Compounds were also submitted to antiviral screening against dengue, influenza, coxsackie and herpes simplex virus to Dr. Weber at IOCB, for screening of cytostatic and antimicrobial activities to Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital in Olomouc.

Only results of screenings on HCV and RSV viruses are available at this moment, other studies are still in progress. All tested thieno-fused nucleosides are inactive against RSV. 2-Furyl, 3-furyl and dimethylamino derivatives **61a, 66a, 61b, 66b, 61i, 66i** were also completely inactive against HCV. Whilst benzofuryl and amino derivatives **61e, 66e, 61j, 66j** displayed only low activity agains HCV, methyl, methoxy and methylsulfanyl derivatives **61h, 66h, 61k, 66k, 66l, 61l** from both series showed sub-micromolar activity against HCV, unfortunately accompanied by micromolar cytotoxicity. Isomeric analogues **66** are more active, but also more toxic. The most active compounds are both methyl derivatives **61h, 66h**, derivative **61h** is four times less toxic than **66h** (Table 19).

Table 19. Anti-HCV activities of nucleosides.

	HCV replicon 1B		HCV replicon 2A	
Compound	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
61e	2.1	19.59	13.92	26.55
61h	0.11	44.44	0.06	44.44
61j	5.02	17.17	13.48	16.39
61k	0.95	29.24	15.00	13.91
61l	0.47	44.44	0.34	44.44
66e	2.05	44.44	14.09	44.44
66h	0.06	44.44	0.24	44.44
66j	17.52	44.44	44.44	44.44
66k	0.23	44.44	0.40	44.44
66l	0.13	44.44	0.80	44.44

3.5 Fluorescence properties of target nucleosides

Annulated 7-deazapurine nucleosides can be interesting not just for their biological activities, but also from biochemical point of view. All these tricyclic bases are supposed to be fluorescent and their corresponding 2´-deoxynucleosides can be used for preparation of modified DNA and study of its fluorescence and thermal stability, which was inspired by the Saito's applications of the related nucleosides as fluorescent probes.[91,92,93]

First of all, we studied electronic spectra and photophysical properties of the title nucleosides. UV spectra and fluorescence spectral data of pyrimidoindole nucleosides are summarized in Tables 20-22. All the title nucleosides exhibited fluorescence with emission maxima from 427 to 485 nm and low qauntum yields (0.02-0.13) in case of pyrimidoindole nucleosides bearing hetaryl group in position 4. 4-Aminopyrimidoindole nucleosides and nucleosides bearing alkyl groups in position 4 have emission maxima in broader range from 341 to 467 nm and moderate quantum yields 0.07-0.36. 4-Amino-6-(2-benzofuryl)nucleoside **43e** is the most fluorescent compound with quantum yield 0.36. Its deoxy analogue has a great potential to be used for enzymatic synthesis of modified DNA and study of fluorescence properties and stability of such modified DNA.

Table 20 UV-Vis and fluorescence spectral data of 4-hetaryl-6-chloro- and 4,6-bis(hetaryl) derivatives

	Absorption				Emission	
Comp.	λ_{max} (nm)	ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	λ_{max} (nm)	ε ($L \cdot mol^{-1} \cdot cm^{-1}$)	λ_{max} (nm)	φ
32a	264	18391	326	14702	449	0.05
32b	264	20110	303	11090	427	0.03
32c	265	16526	323	10675	452	0.04
32d	257	15295	303	7940	435	0.04
32e	272	19799	342	22087	467	0.08
32f	250	18140	299	8751	442	0.05
30a	264	23284	326	18106	447	0.13
36a	250	29235	326	25098	435	0.02
36b	249	25048	325	17721	454	0.03
36f	254	39270	286	24148	485	0.08

UV spectra were measured in methanol at 25 °C. All 5×10^{-5} M solutions. The emission spectra were measured by excitation at 320 nm.

Table 21 UV and fluorescence spectral data of 4-amino-6-hetarylpyrimidoindole nucleosides

	Absorption		Emission		
Comp.	λ_{max} (nm)	ε (L·mol^{-1}·cm^{-1})	λ_{exc} (nm)	φ	λ_{max} (nm)
38	321 292 247	13769 15071 71635	300	0.10	447
39	320 273	12786 13564	310	0.12	395
37	315 282 242	10212 16538 88024	310	0.19	341
41f	325 263	2876 33463	330	0.22	361
41a	280 229	19049 13557	320	0.24	442
41b	324 257	1147 18736	330	0.30	384
41c	282 236	30297 23989	320	0.07	435
41d	269	36191	320	0.08	467
41e	314	35291	340	0.36	427

UV spectra were measured in methanol at 25 °C. All 5×10^{-5} M solutions.

Table 22 UV and fluorescence spectral data of 4-amino-5-hetaryl and 4-substituted derivatives

	Absorption		Emission		
Comp.	λ_{max} (nm)	ε (L·mol^{-1}·cm^{-1})	λ_{exc} (nm)	φ	λ_{max} (nm)
42f	290	8993	320	0.20	452
	248	38259			
42a	323	10281	320	0.07	440
	248	51832			
42d	320	9163	320	0.07	356
	294	12085			
	248	86990			
42g	289	23021	300	0.09	454
	245	80745			
44a	284	16395	310	0.13	386
	255	45473			
	235	44282			
44c	288	26360	300	0.13	485
	255	49620			
	236	57993			
44d	326	10102	300	0.03	449
	298	13086			
	250	31625			
46	318	16054	310	0.17	341
	290	15592			
	245	63821			

UV spectra were measured in methanol at 25 °C. All 5×10^{-5} M solutions.

Table 23 UV and fluorescence spectra of thieno-fused deazapurine nucleosides

Comp.	Absorption				Emission		
	λ_{max} [nm]	ε [$L \cdot mol^{-1} \cdot cm^{-1}$]	λ_{max} [nm]	ε [$L \cdot mol^{-1} \cdot cm^{-1}$]	λ_{max} [nm]	φ	$\lambda_{exc.}$ [nm]
61a	253	4562	-	-	445	0.19	340
61b	322	11531	-	-	433	0.13	340
61e	271	26674	309 368	13549 17148	465	0.28	360
61h	250	32251	301	12250	396	0.12	320
61i	232	31141	314 327	13797 11563	358	0.07	300
61j	220	27496	300	10475	-	-	-
61k	229	21298	291	14370	368	0.15	300
61l	241	30761	313	11646	505	0.03	340
66a	319	15558	-	-	484	0.30	320
66b	253	27407	-	-	469	0.07	300
66e	260	18776	333	16844	437	0.79	320
66h	246	36961	-	-	434	0.10	290
66i	220	32814	285	11461	420	0.03	300
66j	242	20896	277	6448	382	0.03	300
66k	237	58328	-	-	408	0.14	300
66l	232	39276	262 292	25421 14256	467	0.02	290

UV spectra were measured in methanol at 25 °C. All 5×10^{-5} M solutions.

I also measured UV and fluorescence spectra of all target thienopyrrolopyrimidine nucleosides **61, 66** (Table 23). Most of those compounds exhibited at least some fluorescence except 4-amino derivative **61j**, which is not fluorescent at all. Nucleosides substituted in position 4 by amino **66j**, methylthio **61l**, **66l**, dimethylamino **61i**, **66i** groups are weakly fluorescent with quantum yields lower than 0.1, methyl and methoxy derivatives showed slightly higher fluorescence with quantum yields 0.1-0.15. The most fluorescent compounds are nucleosides bearing furyl and benzofuryl groups. Whilst quantum yields of 3-furyl derivatives **61b, 66b** are still moderate (~ 0.1), 2-furyl **61a** and **66a** derivatives exhibited stronger fluorescence with quantum yields 0.19 and 0.30, respectively. Benzofuryl derivative **66e** was the most brightly fluorescent coumpound with excellent quantum yield 0.79.

4 Conclusion

Syntheses of ribonucleosides with tricyclic bases (fused 7-deazapurines: pyrimido[4,5-*b*]indoles and thienopyrrolopyrimidines) were developed and compounds were tested for cytostatic and antiviral activities.

Although C-H arylation approach to pyrimido[4,5-*b*]indoles was not successful, synthesis of these heterocycles was developed. Pyrimidoindoles were built-up part by part from simple commercially available chloronitrobenzenes, first step was nucleophilic substitution with ethyl-cyanoacetate followed by reduction of nitro group and spontaneous cyclization to indole derivatives, pyrimidoindole motif was then formed by cyclization with formamide. 4-Chloropyrimidoindoles were obtained in the last step by reaction with phosphorus oxychloride. The whole synthesis was optimized for 30 g scale. Key-intermediate ribonucleosides bearing additional chlorine atoms in positions 4 and 5 or 6 were prepared by Vorbrűggen glycosylation method. Hetaryl groups were introduced into position 4 of 4,6-dichloropyrimido[4,5-*b*]indole nucleoside by Stille or Suzuki coupling reactions using standard conditions. Final free nucleosides were obtained by Zemplén deprotection. Biological activity screening showed interesting submicromolar anti-dengue activities of 4-hetaryl-6-chloro nucleosides bearing 2-furyl, 2-thienyl and 2-benzofuryl groups in position 4. On the other hand, there was no cytostatic effect or anti-HCV activity of these nucleosides with one exception; 2-furyl derivative displayed micromolar activity against THP-1 and HepG2 cell lines.

Since chlorine atom in position 6 is far less reactive than chlorine in position 4 and stayed untouched under standard cross-coupling conditions, I found effective catalyst-ligand system (palladium acetate and X-Phos ligand) for coupling in position 6 and prepared small series of 4,6-bis(hetaryl)nucleosides, which were completely inactive in all biological assays.

A series of pyrimidoindole nucleosides substituted only in position 4 was synthesized by nucleophilic substitution with ammonia or amines, alkyl groups were introduced into position 4 by palladium catalyzed alkylation using trialkylaluminiums or by Negishi coupling and then deprotected by Zemplén procedure. There was no significant cytostatic and anti-dengue activity in this series, but 4-methylpyrimidoindole nucleoside **44a** showed submicromolar anti-HCV activity and no toxicity.

In next part of the project, benzo-fused analogues of tubercidin were synthesized by Suzuki or Stille cross-coupling reactions of 4-amino-6-chloro- or 4-amino-5-

chloropyrimidoindole nucleosides employing previously optimized conditions (X-Phos ligand). The target 4-amino-5(6)hetarylpyrimidoindole nucleosides did not show any significant cytotoxic or antiviral activity. The most active compound was the starting 4-amino-5-chloropyrimidoindole nucleoside with sub-micromolar activities against dengue and HCV, unfortunately these activities were accompanied by cytotoxicity.

In the last part of my project, synthesis of thieno-fused 7-deazapurine nucleosides was developed. Tricyclic bases were synthesized by 3 step procedure from 4,6-dichloropyrimidine and iodothiophene, which were connected by Negishi coupling and after introduction of azido group into position 4 on pyrimidine ring, tricyclic motif was formed by thermal cyclization or photocyclization of tetrazole. Tricyclic bases were successfully glycosylated to key-intermediate 4-chlorothienopyrrolopyrimidine ribonucleosides. Two series of nucleosides with isomeric bases were synthesized by Suzuki or Stille coupling in case of hetaryl derivatives, methyl group was introduced into position 4 by palladium catalyzed alkylation. Amino, dimethylamino, methoxy and methylsulfanyl derivatives of unprotected nucleosides were obtained from the reaction of the chlorothieno-pyrrolopyrimidine nucleosides with corresponding nucleophiles. Biological acitivity screening of thieno-fused nucleosides is still in progress, only results of anti-HCV screening are available. 2-Furyl, 3-furyl and dimethylamino derivatives are completely inactive against HCV. Whilst benzofuryl and amino derivatives showed only low activity agains HCV, methyl, methoxy and methylsulfanyl derivatives from both series showed sub-micromolar activity against HCV, unfortunately accompanied by micromolar cytotoxicity. The most active compounds are both methyl derivatives.

Anti-dengue activities of some pyrimidoindole nucleosides and anti-HCV activities of thienopyrrolopyrimidine nucleosides confirm the potential of nucleosides with fused 7-deazapurine bases to have an interesting biological activities and study of such types of nucleosides will continue in the Hocek group.

5 Experimental part

5.1 General remarks

All reactions with organometalic reagents as well as all palladium catalyzed reactions were done in flame-dried glassware under argon atmosphere. THF was always freshly distilled from sodium/benzophenone. 4,6-Dichloropyrimidine, 4-chloroaniline, 2-bromo-4-chloroaniline, 2-chloronitrobenzene, 2,3-dichloronitrobenzene, 2,4-dichloronitrobenzene, 2-iodothiophene and 3-bromothiophene were purchased from Sigma-Aldrich. All other solvents and reagents were purchased from commercial suppliers and used as received. Reactions in microwave reactor were performed in The Biotage® Initiator Microwave Reactor. Photocyclizations were performed using 4W Spectroline® E-Series UV lamp (Sigma-Aldrich). Reactions were monitored by thin layer chromatography (TLC) on TLC Silica gel 60 F_{254} (Merck) and detected by UV (254 nm) or by solution of 4-anisaldehyde in ethanol and 10 % of sulphuric acid. In reasonable cases, reactions were monitored by Advion expression Compact Mass Spectrometer using electrospray ionization (ESI). Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in DMSO on Autopol IV polarimeter (Rudolph Research Analytical), $[\alpha]_D^{20}$ values are given in 10^{-1} deg.cm^2.g^{-1}. IR spectra were recorded on Bruker Alpha FT-IR spectrometer using attenuated total reflection (ATR). NMR spectra were measured on Bruker Avance 400 MHz spectrometer (400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C) or Bruker Avance 500 MHz spectrometer (499.8 MHz for ^{1}H, 125.7 MHz for ^{13}C) or Bruker Avance 600 MHz spectrometer (600.1 MHz for ^{1}H and 150.9 MHz for ^{13}C) in DMSO-*d6* (referenced to the residual solvent signal) or in $CDCl_3$ (TMS was used as internal standard). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,H-ROESY, H,C-HSQC and H,C-HMBC experiments. Low resolution mass spectra were measured on LCQ Fleet (Thermo Fisher Scientific) using electrospray ionization (ESI). High resolution mass spectra were measured on LTQ Orbitrap XL (Thermo Fisher Scientific). Elemental analyses were measured on PE 2400 Series II CHNS/O Analyzer (Perkin Elmer, USA) – C,H,N; other elements were determined on SPECTRO iQ II (Spectro Analytical Instruments, Germany). UV spectra were measured on CARY 100 BIO UV-Visible spectrophotometer (Agilent Technologies) in Microcell 80 µl, 4 mm×10 mm (Agilent Technologies) at room temperature. The fluorescence measurements of all pyrimidoindole nucleosides were performed on

Aminco Bowman Series 2 spectrofluorometer with 220−850 nm range, xenon source, excitation and emission wavelength scans, spectral bandwidth 1−16 nm, PMT detector, scan rate 3−6000 nm/min, and Seya-Namioka grating monochromator. The fluorescence measurements of all thienopyrrolopyrimidine nucleosides were performed on JASCO FP-6600 spectrofluorometer. High performance flash chromatography (HPFC) were performed with Biotage SP1 apparatus on Biotage SiO_2 columns or KP-C18 columns (reversed phase) or with ISCO Combiflash Rf system on RediSep Rf Gold Silica Gel Disposable columns or Reverse Phase (C18) RediSep Rf column. X-ray diffraction experiment of single crystals was carried out on an Xcalibur PX X-ray diffractometer (Oxford Diffraction) using $Cu_{K\alpha}$ radiation (λ=1.54180 Å). Purity of all final compounds (unprotected nucleosides) were determined by analytical HPLC or by elemental analysis and by clean NMR spectra.

5.2 General procedures

Suzuki cross-coupling in position 4 (General procedure A):

Protected nucleoside (0.5 mmol), boronic acid (1.5 equiv), K_2CO_3 (2 equiv) and $Pd(PPh_3)_4$ (0.1 eq.) were dissolved in toluene and heated to 100 °C for 6 hours. Then, the reaction mixture was diluted with water and extracted with chloroform. Organic layer was washed with saturated NH_4Cl, then with water and was dried over $MgSO_4$. After evaporation of solvent, the crude product was purified by column chromatography (hexane/EtOAc, 0 – 20 % EtOAc). Products were obtained as white solids or crystals.

Stille cross-coupling in position 4 (General procedure B):

Protected nucleoside (0.5 mmol), tributylstannane (1.2 eq) and $PdCl_2(PPh_3)_2$ (0.1 eq.) were dissolved in anhydrous DMF and heated to 100 °C for 6 – 8 hours. The volatiles were removed in vacuo and the residue was loaded on silica column containing 15 % of KF. Column was washed with 3 litres of hexane, than with gradient of ethyl-acetate in hexane (0-20 % EtOAc). Products were obtained as white solids or crystals.

Suzuki cross-coupling in position 5 or 6 (General procedure C):

Free aminochloronucleoside (0.5 mmol), boronic acid (1.5 eq.), K_2CO_3 (3 eq.), $Pd(OAc)_2$ (0.05 eq.) and X-Phos (0.1 eq.) were dissolved in DMF and heated to 100 °C for 12 hours. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Products were obtained as white powders or crystals after recrystallization from MeOH/H_2O mixtures.

Stille cross-coupling in position 5 or 6 (General procedure D):

Free aminochloronucleoside (0.5 mmol), tributylstannane (1.5 eq.), K_2CO_3 (3 eq.), $Pd(OAc)_2$ (0.05 eq.) and X-Phos (0.1 eq.) were dissolved in DMF and heated to 100 °C for 12 hours. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 column (0→100% MeOH in water). Products were obtained as white powders or crystals after recrystallization from MeOH/H_2O mixtures.

Zemplén deprotection of benzoylated nucleosides (General procedure E):

Protected nucleoside (0.2 mmol) was disolved in methanol (10 ml) and 1M solution of MeONa in MeOH (0.3 equiv.) was added. Reaction mixture was stirred at r.t. overnight. Solvent was evaporated under reduced pressure and crude products were purified using RP-HPFC (H_2O/MeOH, 0 % to 100 %, 2 L).

5.3 Synthesis of pyrimidoindoles via C-H activation

4,6-Dichloro-5-iodopyrimidine (1)

Compound **1** was prepared from 4,6-dichloropyrimidine (2.89 g, 19.5 mmol) according to literature conditions.[108] Compound **1** (3.5 g, 65 %) was obtained as yellowish crystals. ^{1}H NMR and MS are in agreement with literature.[108]

6-Chloro-*N*-(4-chlorophenyl)-5-iodopyrimidin-4-amine (3)

Compound **3** was prepared in analogy to modified literature procedure.[80] 4,6-dichloro-5-iodopyrimidine (**1**) (1.3 g, 0.47 mmol) was dissolved in isopropyl alcohol (40 ml), 4-chloroaniline (**2**) (660 mg, 5.19 mmol) was added and the reaction mixture was refluxed for 30 hours. Solvent was evaporated and solids were dissolved in ethyl-acetate, extracted with 1 M HCl, then with saturated $NaHCO_3$. Organic layers were evaporated and the crude material was purified by column chromatography on silica (hexane/EtOAc 1 to 5 %) to obtain product **3** (1.3 g, 73 %) as white crystals after recrystalliztaion from ethyl-acetate. m.p. 180-183 °C. IR (ATR): υ = 3372, 1600, 1558, 1541, 1496, 1417, 1392, 1013, 961, 825, 805, 761, 565, 502, 488. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.40 (m, 2H, H-*m*-NHC_6H_4Cl); 7.55 (m, 2H, H-*o*-NHC_6H_4Cl); 8.27 (s, 1H, H-2); 8.83 (s, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 81.42 (C-5); 125.81 (CH-*o*-NHC_6H_4Cl); 128.44 (CH-*m*-NHC_6H_4Cl); 128.74 (C-*p*-

NHC_6H_4Cl); 137.79 (C-*i*-NHC_6H_4Cl); 156.75 (CH-2); 162.03, 163.14 (C-4,6). ESI MS m/z (rel. %): 365.9 (100) [M+H], 367.9 (67) [M+H+2], 369.9 (11) [M+H+4]. HR MS (ESI) for $C_{10}H_7N_3ICl_2$ [M+H]: calcd 365.90562; found 365.90567.

6-Chloro-*N*-(4-chlorophenyl)pyrimidin-4-amine (5)

Compound **5** was obtained from several C-H arylation experiments, which were done according to general procedure for C-H arylation and are summarized in Tables 1-3, pages 43-45. ^{1}H NMR is in agreement with literature.[146]

***N*-(2-Bromo-4-chlorophenyl)-6-chloropyrimidin-4-amine (7)**

4,6-Dichloropyrimidine (1.6 g, 10.7 mmol) and 2-bromo-4-chloroaniline (2.0 g, 9.8 mmol) were dissolved in isopropyl alcohol and refluxed for 36 hours. Solvent was evaporated and the residue was dissolved in EtOAc and extracted with saturated $NaHCO_3$ and then with water. The organic layer was evaporated and crude material was purified by column chromatography on silica (hexane/EtOAc 5:1) to give pure product **7** (2.8 g, 82 %) as white crystals after recrystallization from ethyl-acetate. m.p. 195-198 °C. IR (ATR): υ = 3376, 3096, 1589, 1568, 1498, 1488, 1448, 1383, 1103, 985, 859, 812, 467, 448. ^{1}H NMR (500.0 MHz, DMSO-d_6): 6.80 (d, 1H, $J_{5,2}$ = 1.0, H-5); 7.50 (dd, 1H, $J_{5',6'}$ = 8.6, $J_{5',3'}$ = 2.4, H-5′); 7.64 (d, 1H, $J_{6',5'}$ = 8.6, H-6′); 7.85 (d, 1H, $J_{3',5'}$ = 2.4, H-3′); 8.39 (d, 1H, $J_{2,5}$ = 1.0, H-2); 9.57 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 104.63 (CH-5); 120.36 (C-2′); 128.57 (CH-5′); 129.19 (CH-6′); 130.81 (C-4′); 132.41 (CH-3′); 135.81 (C-1′); 158.64 (CH-2); 158.70, 162.18 (C-4,6). ESI MS m/z (rel. %): 318 (62) [M+H], 320 (100) [M+H+2], 322 (45) [M+H+4]. HR MS (ESI) for $C_{10}H_7N_3BrCl_2$ [M+H]: calcd 317.91949; found 317.91965.

***N*-4-(4-Chlorophenyl)-5-iodopyrimidine-4,6-diamine (8)**

Compound **3** (1.2 g, 3.3 mmol) was put into autoclave and liquid ammonia (30 ml) was added. Reaction was heated to 100 °C for 16 hours. Ammonia was then let to evaporate and crude material was purified by column chromatography on silica (hexane/EtOAc 3:1) to obtain product **8** (885 mg, 78 %) as white crystals. m.p. 235 °C. IR (ATR): υ = 3450, 3331, 3080, 3045, 2975, 1691, 1574, 1466, 1459, 1386, 1356, 1297, 1298, 1085, 1049, 1005, 921, 798, 635, 588, 567, 415. ^{1}H NMR (500.0 MHz, DMSO-d_6): 6.67 (bs, 2H, NH_2); 7.32 (m, 2H, H-*m*-NHC_6H_4Cl); 7.56 (m, 2H, H-*o*-NHC_6H_4Cl); 7.89 (s, 1H, H-2); 7.98 (s, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 59.06 (C-5); 123.76 (CH-*o*-NHC_6H_4Cl); 126.56 (C-*p*-NHC_6H_4Cl); 128.20 (CH-*m*-NHC_6H_4Cl); 139.22 (C-*i*-NHC_6H_4Cl); 156.69 (CH-2); 159.47 (C-

6); 163.74 (C-4). ESI MS m/z (rel. %): 347 (100) [M+H]. HR MS (ESI) for $C_{10}H_9N_4ICl$ [M+H]: calcd 346.95549; found 346.9551.

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

Compound **9** was isolated from two C-H arylation experiments done according to the general procedure for C-H arylation: a) from reaction of **8**, just with palladium acetate (0.1 eq.) without any ligand and with KOAc (2 eq.) and TBAB (1 eq.) at 150 °C for 48 h. b) reaction of **8** with palladium acetate (0.1 eq.), $(4\text{-FPh})_3P$ (0.2 eq.), K_2CO_3 (3 eq.), PivOH (0.3 eq.) in DMF at 130 °C for 48 h (page 49, Table 4, Entries 4,5). m.p. 215-220 °C. IR (ATR): υ = 3467, 3404, 3293, 3063, 1641, 1601, 1568, 1488, 1474, 1415, 1390, 1354, 1312, 1293, 1276, 983, 815, 763, 518, 495, 422. ^{1}H NMR (499.8 MHz, DMSO-d_6): 7.31 (bs, 2H, NH_2); 7.35 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 2.0, H-7); 7.43 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,NH}$ = 0.5, H-8); 8.25 (s, 1H, H-2); 8.45 (d, 1H, $J_{5,7}$ = 2.0, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 94.91 (C-4a); 112.45 (CH-8); 120.81 (CH-5); 121.39 (C-4b); 124.48 (CH-7); 124.90 (C-6); 135.00 (C-8a); 155.69 (CH-2); 156.47 (C-9a); 157.87 (C-4). ESI MS m/z (rel. %): 219 (100) [M+H]. HR MS (ESI) for $C_{10}H_8N_4Cl$ [M+H]: calcd 219.04320; found 219.04319.

***N*-4-(4-Chlorophenyl)pyrimidine-4,6-diamine (10)**

Compound **10** was obtained from several C-H arylation experiments, which were done according to the general procedure for C-H arylation and are summarized in Table 4, page 46. ^{1}H NMR is in agreement with literature. [147]

General procedure for Pd-catalyzed C-H arylation

All the reactions were carried out in flame-dried Biotage microwave vial under argon atmosphere. Starting halogenated *N*-phenylpyrimidin-4-amine (**3**,**7** or **8**) (100 mg) was placed into vial and palladium catalyst (0.1 eq.), ligand (0.2 eq), base and additive were added. Vial was evacuated and flushed with argon (3 times). Solvent (6 ml) was added and the reaction mixture was placed into pre-heated oil bath or into microwave reactor. Detailed conditions and results are summarized in Tables 1-4, pages 46-50.

5.4 Synthesis of 4-hetaryl- and 4,6-bis(hetaryl)pyrimidoindole nucleosides

Ethyl 2-(2-nitrophenyl)-2-cyanoacetate (**14**)

Compound **14** was prepared from 2-chloronitrobenzene (**11**) (20 g, 127 mmol) and ethyl cyanoacetate (27.1 ml, 2 eq, 254 mmol) according to literature conditions.[85] Compound **14** was obtained as dark oil. For analysis, the oil was purified by column chromatography

(hexane/EtOAc 0 – 10 % EtOAc). ^{1}H NMR is in agreement with literature.[148] The crude material was used directly for the next step.

Ethyl 2-(6-chloro-2-nitrophenyl)-2-cyanoacetate (15)

Compound **15** was prepared from 2,3-dichloronitrobenzene (**12**) (20 g; 104 mmol) and ethyl cyanoacetate (26.12 ml; 208.4 mmol) according to literature conditions.[85] Compound **15** (21 g; 86 %) was obtained as a dark oil. The crude material was used directly for the next step. For analysis, the oil was purified by column chromatography (hexane/EtOAc 0 – 10 % EtOAc). ^{1}H NMR is in agreement with literature.[85]

Ethyl 2-(5-chloro-2-nitrophenyl)-2-cyanoacetate (16)

Compound **16** was prepared from 2,4-dichloronitrobenzene (**13**) (6.0 g, 31.3 mmol) and ethyl cyanoacetate (6.66 ml, 2 eq., 62.6 mmol) according to literature conditions.[85] Compound **16** was obtained as a dark oil. For analysis, the oil was purified by column chromatography (hexane/EtOAc 0 – 10 % EtOAc). ^{1}H NMR is in agreement with literature.[149] The crude material was used directly for the next step.

Ethyl 2-amino-1*H*-indole-3-carboxylate (17)

Compound **17** was prepared as described for **19** from crude **14** (44.2 g), product **17** (33.1g, 86 %) was obtained as brown powder. ^{1}H NMR is in agreement with literature.[150] Crude material was used directly for the next step.

Ethyl 2-amino-4-chloro-1*H*-indole-3-carboxylate (18)

Compound **18** was prepared as described for **19** from crude **15** (27 g; 115 mmol). Compound **18** (21.0 g; 66 %) was obtained as brown solid. For analysis, the crude compound was purified by column chromatography (hexane/chloroform, 0-60 % chloroform). m.p. 147–149 °C. IR (ATR): ν = 3 459, 3 347, 1 633, 1 596, 1 492, 1 426, 1 378, 1 330, 1 318, 1 232, 1 177, 1 143, 731 cm^{-1}. ^{1}H NMR is in agreement with literature.[85] Crude material was used directly for the next step.

Ethyl 2-amino-5-chloro-1H-indole-3-carboxylate (19)

Compound **19** was prepared according to modified literature conditions.[85] Crude **16** (60 g, 221 mmol) was dissolved in glacial acetic acid (600 ml) and zinc dust (40 g) was added in 5 parts during 45 minutes. The mixture was stirred for 2 hours without external heating (reaction is exothermic, temperature in the flask reached 55-60 °C during the reaction) and filtered through a pad of cellite. The pad was washed with acetic acid (400 ml) and solution

was evaporated. The residue was then washed well with water and dried under reduced pressure. Compound **19** (46.1 g, 88 %) was obtained as brown powder. For analysis, the crude compound was further purified by column chromatography (hexane/chloroform, 0-60 % chloroform). m.p. 190 – 193 °C. ^{1}H NMR is in agreement with literature.[150] Crude material was used directly for the next step.

3*H*-Pyrimido[4,5-*b*]indol-4(9*H*)-one (20)

Compound **20** was prepared as described for **22** from crude **17** (20.0 g, 108 mmol) to give 15.8 g, (87 %) of **20** as dark powder. The crude compound was used directly in the next step. For analysis, the crude compound was further purified by column chromatography (chloroform/MeOH, 3 % MeOH); mp: > 300 °C. IR (ATR): ν = 3031, 2359, 2342, 1640, 1587, 1541, 1377, 1248, 750 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.23 (ddd, 1H, $J_{6,5}$ = 7.9, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.0, H-6); 7.32 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.48 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.8, H-8); 7.99 (ddt, 1H, $J_{5,6}$ = 7.9, $J_{5,7}$ = 1.2, $J_{5,8}$ = $J_{5,NH}$ = 0.8, H-5); 8.12 (s, 1H, H-2), 12.17, 12.20 (2 × bs, 2 × 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 100.4 (C-4a); 111.9 (CH-8); 120.9 (CH-5); 121.4 (CH-6); 122.3 (C-4b); 124.4 (CH-7); 135.7 (C-8a); 147.7 (CH-2); 153.9 (C-9a); 158.6 (C-4). ESI MS m/z (rel. %): 186 (12) [M+H], 208 (100) [M+Na]. HR MS (ESI) for $C_{10}H_7N_3ONa$ [M+Na]: calcd 208.04813; found 208.04815.

5-Chloro-3*H*-pyrimido[4,5-*b*]indol-4(9*H*)-one (21)

Compound **21** was prepared as described for **22** from crude **18** (20.5 g; 73.8 mmol). Desired product **21** (15.0 g; 93 %) was obtained as brown powder. For analysis, it was purified by column chromatography (chloroform/MeOH, 3 % MeOH); m.p. > 300 °C. IR (ATR): ν = 3 176, 3 062, 2 959, 2 920, 1 669, 1 635, 1 623, 1 587, 1 551, 1 422, 1 354, 1 343, 1 313, 1 084, 992, 766. ^{1}H NMR (499.8 MHz, DMSO-d_6): 7.23 (dd, 1H, $J_{6,5}$ = 7.8, $J_{6,8}$ = 1.1, H-6); 7.28 (t, 1H, $J_{7,6}$ = $J_{7,8}$ = 7.8, H-7); 7.40 (dd, 1H, $J_{8,7}$ = 7,8, $J_{8,6}$ = 1.1, H-8); 8,13 (d, 1H, J = 3,5, H-2); 12.19 (bs, 1H, NH-3); 12.51 (bs, 1H, NH-9). ^{13}C NMR (125.7 MHz, DMSO-d_6): 99.64 (C-4a); 1110.68 (CH-8); 120.93 (C-4b); 122.57 (CH-6); 125.26 (CH-7); 125.88 (C-5); 137.16 (C-8a); 148.68 (CH-2); 154.51 (C-9a); 157.16 (C-4). ESI MS m/z (rel. %): 242 (100) [M+Na], 244 (33) [M+2+Na]. HR MS (ESI) for $C_{10}H_6ClN_3ONa$ [M+Na]: calc. 242.00916; found 242.00915.

6-Chloro-3*H*-pyrimido[4,5-*b*]indol-4(9*H*)-one (22)

Indole derivative **19** (30 g, 126 mmol) was dissolved in formamide (100 ml, 2.5 mol) and after heating to 190 °C for 18 hours, the reaction mixture was cooled down, precipitate was filtered out, washed well with water and dried under reduced pressure. Compound **22** (23.6 g, 85 %) was obtained as dark powder. For analysis, the crude compound **22** was purified by column chromatography (chloroform/MeOH, 3 % MeOH); mp: 188 – 193 °C. IR (ATR): ν = 2924, 1661, 1588, 1447, 1359, 1250, 794 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 7.34 (ddd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 2.2, $J_{7,NH}$ = 0.4, H-7); 7.49 (ddd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.6, $J_{8,NH}$ = 0.4, H-8); 7.91 (ddt, 1H, $J_{5,7}$ = 2.2, $J_{5,8}$ = 0.6, $J_{5,NH}$ = 0.4, H-5); 8.16 (bd, 1H, $J_{2,NH}$ = 3.5, H-2), 12.31 (bs, 1H, NH-3); 12.36 (bs, 1H, NH-9). ^{13}C NMR (150.9 MHz, DMSO-d_6): 99.9 (C-4a); 113.5 (CH-8); 119.7 (CH-5); 123.4 (C-4b); 124.2 (CH-7); 125.6 (C-6); 134.1 (C-8a); 148.4 (CH-2); 154.6 (C-9a); 158.3 (C-4). ESI MS m/z (rel. %): 242 (100) [M+Na], 244 (33) [M+2+Na]. HR MS (ESI) for $C_{10}H_6ClN_3ONa$ [M+Na]: calcd 242.00916; found 242.00915.

4-Chloro-9*H*-pyrimido[4,5-*b*]indole (23)

Compound **23** was prepared as described for **4** from crude **20** (10.0 g, 54.0 mmol) to give **23** (9.9 g, 90 %) as brown crystals; m.p. 188 - 190 °C, IR (ATR): ν = 2361, 2340, 1719, 1558, 1265, 1225, 704 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.43 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 6.0, $J_{6,8}$ = 2.2, H-6); 7.64 (m, 2H, H-7,8); 8.29 (bd, 1H, $J_{5,6}$ = 8.0, H-5); 8.78 (s, 1H, H-2), 12.79 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 111.3 (C-4a); 112.5 (CH-8); 117.9 (C-4b); 121.9 (CH-6); 122.6 (CH-5); 128.6 (CH-7); 138.7 (C-8a); 151.4 (C-4); 154.1 (CH-2); 156.1 (C-9a). ESI MS m/z (rel. %): 204 (100) [M+H], 206 (33) [M+2+H]. HR MS (ESI) for $C_{10}H_6ClN_3$ [M+H]: calcd 204.03230; found 204.03229.

4,5-Dichloro-9*H*-pyrimido[4,5-*b*]indole (24)

Compound **24** was prepared as described for **4** from crude **21** (15.0 g; 68.3 mmol). Desired product **24** (12.0 g; 74 %) was obtained as brown powder. For analysis, it was purified by column chromatography (chloroform/MeOH, 3 % MeOH); m.p. 252 °C. IR (ATR): ν = 3 048, 2 967, 2 804, 1 590, 1 559, 1 541, 1 441, 1 397, 1 306, 1 159, 988, 775.^{1}H NMR (500.0 MHz, DMSO-d_6): 7.44 (m, 1H, H-6); 7.59 (m, 2H, H-7, H-8); 8.80 (s, 1H, H-2); 13.17 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 110.45 (C-4a); 111.50 (CH-8); 115.73 (C-4b); 123.91 (CH-6); 127.23 (C-5); 129.47 (CH-7); 140.42 (C-8a); 151.52 (C-4); 154.16 (CH-2); 156.45 (C-9a).ESI MS m/z (rel. %): 238 (100) [M+H], 240 (66) [M+2+H], 242 (16) [M+4+H]. HR MS (ESI) for $C_{10}H_5Cl_2N_3$ [M+H]: calc. 236.9861; found 236.9860.

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

Compound **22** (24.0 g, 0.11 mol) was dissolved in $POCl_3$ (330 ml) and heated to 120 °C for two days. Then, solvent was evaporated under reduced pressure, residue was diluted with water and slowly neutralized with aqueous ammonia to pH 7. Crude product was filtered out, washed well with cold water, then with hydrochloric acid and again with cold water. After drying under reduced pressure, the desired product **4** (21.5 g, 83 %) was obtained as dark powder. For analysis, the crude compound was purified by column chromatography (chloroform/MeOH, 3 % MeOH), recrystallization (propan-2-ol/ethanol) furnished compound **4** as white crystals; mp: 258 °C (sublimation). IR (ATR): ν = 2953, 2362, 2342, 1604, 1560, 1436, 1269, 1230, 1195 cm^{-1}. ^1H NMR (499.8 MHz, DMSO-d_6): 7.65 (d, 2H, $J_{7,5\&8,5}$ = 1.4, H-7,8); 8.21 (td, 1H, $J_{5,7}$ = $J_{5,8}$ = 1.4, $J_{5,NH}$ = 0.6, H-5); 8.81 (s, 1H, H-2), 12.94 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 110.63 (C-4a); 114.20 (CH-8); 119.11 (C-4b); 121.67 (CH-5); 126.14 (C-6); 128.55 (CH-7); 137.19 (C-8a); 152.02 (C-4); 154.75 (CH-2); 156.46 (C-9a). ESI MS m/z (rel. %): 238 (100) [M+H], 240 (66) [M+2+H], 242 (16) [M+4+H]. HR MS (ESI) for $C_{10}H_5Cl_2N_3$ [M+H]: calcd 236.9861; found 236.9860.

4-Chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (25)

Compound **25** was prepared as described for **27** from pyrimidoindole **23** (300 mg, 1.5 mmol) to give nucleoside **25** (440 mg, 46 %) as white crystals; m.p. 178 – 182 °C, IR (ATR): ν = 2360, 2336, 1718, 1546, 1442, 1261, 1090, 1068, 704 cm^{-1}. ^1H NMR (600.1 MHz, DMSO-d_6): 4.69 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.2, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.2, 3.2, H-4'); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.61 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.6, H-2'); 7.03 (d, 1H, $J_{1',2'}$ = 4.6, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 4H, H-*m*-Bz-3',5'); 7.51 (ddd, 1H, $J_{6,5}$ = 7.8, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.4, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.62 (m, 1H, H-*p*-Bz-2'); 7.68 (m, 2H, H-*p*-Bz-3',5'); 7.84 (m, 2H, H-*o*-Bz-2'); 7.93 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.10 (ddd, 1H, $J_{8,7}$ = 8.4, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.35 (ddd, 1H, $J_{5,6}$ = 7.8, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.7, H-5); 8.78 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.1 (CH_2-5'); 70.3 (CH-3'); 72.2 (CH-2'); 78.8 (CH-4'); 86.2 (CH-1'); 112.1 (CH-8); 112.4 (C-4a); 118.0 (C-4b); 122.9 (CH-5); 123.3 (CH-6); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-*m*-Bz); 129.0 (CH-7); 129.3 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.8, 134.1 (CH-*p*-Bz); 138.4 (C-8a); 152.1 (C-4); 154.0 (C-2-furyl); 154.0 (CH-2); 155.3 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.5 (COPh-5'). ESI MS m/z (rel. %): 670 (100) [M+Na], 672 (33) [M+2+Na]. HR MS (ESI) for $C_{36}H_{27}ClN_3O_7$ [M+H]: calcd 648.15320; found 648.15318.

4,5-Dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (26)

Compound **26** was prepared as described for **27** from crude **24** (1.0 g; 4.2 mmol). Desired product **26** (1.4 g; 52 %) was obtained as white crystals after flash chromatography in hexane/EtOAc 7:1 and crystallization in chloroform/methanol mixture. m.p. 169–173 °C. IR (ATR): 3 071, 2 925, 1 732, 1 720, 1 560, 1 451, 1 279, 1 261, 1 244, 1 109, 1 088, 1 068, 1 051, 1 034, 1 023, 703. ^{1}H NMR (499.8 MHz, DMSO d_6): 4.69 (dd, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.2, H-5'b); 4.85 (dd, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5'a); 4.90 (ddd, $J_{4',3'}$ = 6.7, $J_{4',5'}$ = 4.2, 3.3, H-4'); 6.37 (t, $J_{3',2'}$ = $J_{3',4'}$ = 6.7, H-3'); 6.54 (dd, $J_{2',3'}$ = 6.7, $J_{2',1'}$ = 4.3, H-2'); 7.08 (d, $J_{1',2'}$ = 4.3, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.49, 7.50 (2 × m, 2 × 2H, H-*m*-Bz-3',5'); 7.53 (m, 2H, H-6,7); 7.62 (m, 1H, H-*p*-Bz-2'); 7.68 (m, 2H, H-*p*-Bz-3',5'); 7.83 (m, 2H, H-*o*-Bz-2'); 7.92 (m, 2H, H-*o*-Bz-5'); 7.98 (m, 2H, H-*o*-Bz-3'); 8.10 (m, 1H, H-8); 8.81 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.05 (CH_2-5'); 70.17 (CH-3'); 72.40 (CH-2'); 78.80 (CH-4'); 86.52 (CH-1'); 110.99 (CH-8); 111.70 (C-4a); 116.19 (C-4b); 125.45 (CH-6); 127.66 (C-5); 128.64, 128.79 (C-*i*-Bz); 128.90, 128.95, 128.97 (CH-*m*-Bz); 129.33 (CH-*o*-Bz-5', C-*i*-Bz); 129.51 (CH-*o*-Bz-2'); 129.61 (CH-*o*-Bz-3'); 129.84 (CH-7); 133.80, 134.10 (CH-*p*-Bz); 140.01 (C-8a); 152.14 (C-4); 153.91 (CH-2); 155.62 (C-9a); 164.81 (COPh-2'); 164.98 (COPh-3'); 165.52 (COPh-5'). ESI MS m/z (rel. %): 682 (100) [M+H], 684 (66) [M+2+H], 686 (26) [M+4+H]. HR MS (ESI) for $C_{36}H_{26}Cl_2N_3O_7$ [M+H]: calc. 682.11480; found 682.11423; for $C_{36}H_{25}Cl_2N_3O_7Na$ [M+Na]: calc. 704.09660; found 704.09618.

4,6-Dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (27)

Pyrimidoindole **4** (200 mg, 0.84 mmol) was suspended in acetonitrile (5 ml) and BSA (206 μl, 0.84 mmol) was added. The reaction mixture was stirred for 10 min at r.t., then TMSOTf (308 μl, 1.68 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (430 mg, 0.84 mmol) were added. The mixture was heated to 60 °C for 8 hours. After cooling to r.t., the mixture was extracted with EtOAc and water, organic layer was washed with $NaHCO_3$ and again with water, dried over $MgSO_4$ and evaporated under reduced pressure. Crude product was purified using column chromatography (hexane/EtOAc, 10–40 % EtOAc). After crystallization from chloroform/methanol mixture, nucleoside **27** (572 mg, 54 %) was observed as white crystals; mp: 164-165 °C. IR (ATR): ν = 1722, 1545, 1472, 1433, 1292, 1266, 1119, 1098, 714 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6):): 4.68 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.90 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.59 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.4, H-2'); 7.02 (d, 1H, $J_{1',2'}$ = 4.4, H-1'); 7.42 (m, 2H, H-*m*-Bz-2'); 7.49 (m, 4H, H-*m*-Bz-3',5'); 7.60 (dd,

1H, $J_{7,8}$ = 9.1, $J_{7,5}$ = 2.2, H-7); 7.62 (m, 1H, H-*p*-Bz-2'); 7.68 (m, 2H, H-*p*-Bz-3',5'); 7.84 (m, 2H, H-*o*-Bz-2'); 7.91 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.15 (dd, 1H, $J_{8,7}$ = 9.1, $J_{8,5}$ = 0.5, H-8); 8.28 (dd, 1H, $J_{5,7}$ = 2.2, $J_{5,8}$ = 0.5, H-5); 8.81 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.1 (CH_2-5'); 70.3 (CH-3'); 72.5 (CH-2'); 78.9 (CH-4'); 86.4 (CH-1'); 111.7 (C-4a); 114.0 (CH-8); 119.4 (C-4b); 122.0 (CH-5); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-7); 128.9, 128.9, 129.0 (CH-*m*-Bz); 129.3 (CH-*o*-Bz-5'); 129.3 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.8, 134.1 (CH-*p*-Bz); 137.1 (C-8a); 152.7 (C-4); 154.6 (CH-2); 155.5 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.5 (COPh-5'). ESI MS m/z (rel. %): 682 (100) [M+H], 684 (66) [M+2+H], 686 (26) [M+4+H]. HR MS (ESI) for $C_{36}H_{26}Cl_2N_3O_7$ [M+H]: calcd 682.11480; found 682.11423; for $C_{36}H_{25}Cl_2N_3O_7Na$ [M+Na]: calcd 704.09660; found 704.09618.

6-Chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3*H*, 9*H*-pyrimido[4,5-*b*]indol-4-one (28)

Nucleoside **28** was prepared in the same manner as nucleosides **25-27** from ketobase **22** (500 mg, 2.28 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (2.30 g, 4.56 mmol). Nucleoside **28** (340 mg, 22 %) was obtained as yellowish solid. ^{1}H NMR (499.8 MHz, DMSO-d_6): 4.67 (dd, J_{gem} = 12.1, $J_{5'b,4'}$ = 4.6, H-5'b); 4.79 (dd, J_{gem} = 12.1, $J_{5'a,4'}$ = 3.4, H-5'a); 4.82 (ddd, $J_{4',3'}$ = 7.5, $J_{4',5'}$ = 4.6, 3.4, H-4'); 6.20 (dd, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 2.9, H-2'); 6.27 (dd, $J_{3',4'}$ = 7.5, $J_{3',2'}$ = 6.5, H-3'); 6.40 (d, $J_{1',2'}$ = 2.9, H-1'); 7.39 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 2.2, H-7); 7.43-7.51 (m, 6H, H-*m*-Bz); 7.54 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.6, H-8); 7.63-7.68 (m, 3H, H-*p*-Bz); 7.90-7.93 (m, 4H, H-*o*-Bz-2',3'); 7.96 (dd, 1H, $J_{5,7}$ = 2.2, $J_{5,8}$ = 0.6, H-5); 8.08 (m, 2H, H-*o*-Bz-5'); 8.63 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.18 (CH_2-5'); 70.39 (CH-3'); 74.28 (CH-2'); 79.08 (CH-4'); 91.36 (CH-1'); 99.05 (C-4a); 114.00 (CH-8); 119.83 (CH-5); 123.40 (C-4b); 124.63 (CH-7); 126.01 (C-6); 128.75 (CH-*m*-Bz); 128.78, 128.81 (C-*i*-Bz); 128.78, 128.92 (CH-*m*-Bz); 129.51, 129.53, 129.63 (CH-*o*-Bz); 133.64, 133.99, 134.10 (CH-*p*-Bz); 134.93 (C-8a); 149.81 (CH-2); 153.93 (C-4); 156.98 (C-9a); 164.89 (COPh-2'); 164.94 (COPh-3'); 165.73 (COPh-5'). ESI MS m/z (rel. %): 664 (100) [M+H]. HR MS (ESI) for $C_{36}H_{26}ClN_3O_8$ [M+H]: calcd 663.1408; found 663.1412.

4-(Furan-2-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (29)

Nucleoside **29** was synthesized according to general procedure B. Protected nucleoside **25** (450 mg, 0.69 mmol) and 2-(tributylstannyl)furan (296 mg, 0.83 mmol) were used. Desired product **29** (390 mg, 82 %) was obtained as yellowish powder. m.p. 89 – 91 °C. IR (ATR): ν = 2932, 2363, 2331, 1742, 1578, 1547, 1452, 1258, 804 cm^{-1}. ^{1}H NMR (600.1

MHz, DMSO-d_6): 4.71 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.2, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.1, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.7, $J_{4',5'}$ = 4.2, 3.1, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.7, H-3'); 6.64 (dd, 1H, $J_{2',3'}$ = 6.7, $J_{2',1'}$ = 4.7, H-2'); 6.89 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.07 (d, 1H, $J_{1',2'}$ = 4.7, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.45 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.3, H-6); 7.48 (ddd, 1H, $J_{7,8}$ = 8.0, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.50 (m, 4H, H-*m*-Bz-3',5'); 7.60 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.61 (m, 1H, H-*p*-Bz-2'); 7.66, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.84 (m, 2H, H-*o*-Bz-2'); 7.97 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.04 (ddd, 1H, $J_{8,7}$ = 8.0, $J_{8,6}$ = 1.3, $J_{8,5}$ = 0.6, H-8); 8.29 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.892 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.6, H-5); 8.893 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.3 (CH-3'); 72.1 (CH-2'); 78.6 (CH-4'); 86.0 (CH-1'); 108.4 (C-4a); 111.4 (CH-8); 113.1 (CH-4-furyl); 115.2 (CH-3-furyl); 119.1 (C-4b); 122.7 (CH-5); 125.0 (CH-6); 128.2 (CH-7); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 138.5 (C-8a); 146.8 (CH-5-furyl); 147.8 (C-4); 152.4 (C-2-furyl); 153.8 (CH-2); 156.2 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 702 (100) [M+Na], 704 (29) [M+2+Na], 680 (15) [M+H]. HR MS (ESI) for $C_{40}H_{30}N_3O_8$ [M+H]: calcd 680.20274; found 680.20275.

4-(Furan-2-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (30)

Deprotection of **29** (250 mg, 0.37 mmol) according to the general procedure E afforded compound **30** (108 mg, 80 %) as white crystals: m.p. 106 – 110 °C, IR (ATR): ν = 3326, 1591, 1561, 1540, 1490, 1461, 1466, 1440, 1121, 1091, 1074, 741. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.69 (ddd, 1H, J_{gem}= 12.0, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 4.0, H-5'b); 3.75 (ddd, 1H, J_{gem}= 12.0, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.4, H-5'a); 4.00 (dt, 1H, $J_{4',5'}$ = 4.0, 3.4, $J_{4',3'}$ = 3.1, H-4'); 4.25 (ddd, 1H, $J_{3',2'}$ = 5.9, $J_{3',OH}$ = 4.9, $J_{3',4'}$ = 3.1, H-3'); 4.84 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.9, H-2'); 5.17 (d, 1H, $J_{OH,3'}$ = 4.9, OH-3'); 5.18 (dd, 1H, $J_{OH,5'}$ = 5.6, 5.1, OH-5'); 5.25 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.57 (d, $J_{1',2'}$ = 7.3, H-1'); 6.89 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.7, H-4-furyl); 7.44 (ddd, 1H, $J_{6,5}$ = 8.1, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.0, H-6); 7.59 (dd, 1H, $J_{3,4}$ = 3.4, $J_{3,5}$ = 0.9, H-3-furyl); 7.60 (ddd, 1H, $J_{7,8}$ = 8.4, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 8.08 (ddd, 1H, $J_{8,7}$ = 8.4, $J_{8,6}$ = 1.0, $J_{8,5}$ =0.6, H-8); 8.29 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl); 8.90 (ddd, 1H, $J_{5,6}$ = 8.1, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.94 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.2 (CH-3'); 70.4 (CH-2'); 85.6 (CH-4'); 87.1 (CH-1'); 108.1 (C-4a); 112.9 (CH-8); 113.1 (CH-4-furyl); 114.9 (CH-3-furyl); 119.2 (C-4b); 122.2 (CH-6); 124.7 (CH-5); 128.1 (CH-7); 138.3 (C-8a); 146.6 (CH-5-furyl); 147.6 (C-4); 152.5 (C-2-furyl); 153.7 (CH-2); 156.7 (C-9a). ESI

MS m/z (rel. %): 390 (100) [M+H]. HR MS (ESI) for $C_{19}H_{18}N_3O_5$ [M+H]: calcd 368.12410; found 368.12408. Anal. Calcd for $C_{19}H_{17}N_3O_5$: C, 62.12 %; H, 4.66 %; N, 11.44 %; O 21.78 %. Found C, 62.18 %; H, 4.69 %; N, 11.38 %.

6-Chloro-4-(furan-2-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*] indole (31a)

Nucleoside **31a** was synthesized according to the general procedure B. Protected nucleoside **27** (200 mg, 0.29 mmol) and 2-(tributylstannyl)furan (126 mg, 0.35 mmol) were used. Desired product **31a** (165 mg, 79 %) was obtained as yellowish powder; mp: 92 - 93 °C. IR (ATR): ν = 2360, 2342, 1720, 1262, 1091, 1068, 706 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.69 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.90 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.62 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.5, H-2'); 6.91 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.04 (d, 1H, $J_{1',2'}$ = 4.5, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.49 (m, 4H, H-*m*-Bz-3',5'); 7.53 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.2, H-7); 7.62 (m, 1H, H-*p*-Bz-2'); 7.64 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.66, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.84 (m, 2H, H-*o*-Bz-2'); 7.94 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.10 (dd, 1H, $J_{8,7}$ = 8.8, H-8); 8.38 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.82 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.91 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.3 (CH-3'); 72.3 (CH-2'); 78.7 (CH-4'); 86.2 (CH-1'); 107.5 (C-4a); 113.2 (CH-8); 113.4 (CH-4-furyl); 115.8 (CH-3-furyl); 120.5 (C-4b); 124.1 (CH-5); 127.0 (C-6); 128.1 (CH-7); 128.7, 128.8 (C-*i*-Bz); 128.9, 128.9, 129.0 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 137.2 (C-8a); 147.2 (CH-5-furyl); 148.3 (C-4); 152.2 (C-2-furyl); 154.4 (CH-2); 156.4 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 736 (100) [M+Na], 738 (42) [M+2+Na], 714 (50) [M+H]. HR MS (ESI) for $C_{40}H_{29}ClN_3O_8$ [M+H]: calcd 714.16382; found 714.16377.

6-Chloro-4-(furan-3-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (31b)

Nucleoside **31b** was synthesized according to the general procedure A. Protected nucleoside **27** (500 mg, 0.73 mmol) and furan-3-boronic acid (125 mg, 1.1 mmol) were used. Desired product **31b** (360 mg, 69 %) was obtained as white powder; mp: 120 - 124 °C. IR (ATR): ν = 2360, 2342, 1719, 1260, 1095, 1067, 705 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.70 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.90 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.65 (dd, 1H,

$J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.6, H-2'); 7.03 (d, 1H, $J_{1',2'}$ = 4.6, H-1'); 7.12 (dd, 1H, $J_{4,5}$ = 1.8, $J_{4,2}$ = 0.8, H-4-furyl); 7.42 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 5H, H-7, H-*m*-Bz-3',5'); 7.62 (m, 1H, H-*p*-Bz-2'); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.85 (m, 2H, H-*o*-Bz-2'); 7.94 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.02 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,2}$ = 1.5, H-5-furyl); 8.09 (d, 1H, $J_{5,7}$ = 2.0, H-5); 8.10 (dd, 1H, $J_{8,7}$ = 9.0, H-8); 8.56 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.8, H-2-furyl); 8.93 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.4 (CH-3'); 72.2 (CH-2'); 78.7 (CH-4'); 86.2 (CH-1'); 110.7 (C-4a); 110.7 (CH-4-furyl); 113.4 (CH-8); 120.5 (C-4b); 121.9 (CH-5); 124.2 (C-3-furyl); 126.7 (C-6); 128.0 (CH-7); 128.6, 128.8 (C-*i*-Bz); 128.9, 128.9, 129.0 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.8, 134.1 (CH-*p*-Bz); 136.9 (C-8a); 144.7 (CH-2-furyl); 144.9 (CH-5-furyl); 153.5 (C-4); 154.6 (CH-2); 155.7 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 714 (100) [M+H], 716 (39) [M+2+H], 736 (10) [M+Na]. HR MS (ESI) for $C_{40}H_{29}ClN_3O_8$ [M+H]: calcd 714.16370; found 714.16377.

6-Chloro-4-(thiophen-2-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (31c)

Nucleoside **31c** was synthesized according to the general procedure B. Protected nucleoside **27** (500 mg, 0.73 mmol) and 2-(tributylstannyl)thiophene (326 mg, 0.9 mmol) were used. Desired product **31c** (416 mg, 78 %) was obtained as yellowish powder; mp: 148 - 155 °C; IR (ATR): ν = 2359, 2341, 1720, 1560, 1261, 1106, 1068, 1025, 704 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 4.69 (dd, 1H, J_{gem}= 12.3, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.90 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.64 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.4, H-2'); 7.05 (d, 1H, $J_{1',2'}$ = 4.4, H-1'); 7.42 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.6, H-4-thienyl); 7.425 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 4H, H-*m*-Bz-3',5'); 7.53 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.62 (m, 1H, H-*p*-Bz-2'); 7.66, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.85 (m, 2H, H-*o*-Bz-2'); 7.94 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.02 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.1, H-5-thienyl); 8.09 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.1, H-3-thienyl); 8.13 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.24 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.92 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.3 (CH-3'); 72.3 (CH-2'); 78.7 (CH-4'); 86.3 (CH-1'); 109.4 (C-4a); 113.6 (CH-8); 120.4 (C-4b); 121.7 (CH-5); 126.7 (C-6); 128.2 (CH-7); 128.7 (C-*i*-Bz); 128.8 (CH-4-thienyl); 128.8 (C-*i*-Bz); 128.9, 129.0 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.5 (CH-*o*-Bz-2'); 129.7 (CH-*o*-Bz-3'); 130.3 (CH-3-thienyl); 131.9 (CH-5-thienyl); 133.8, 134.1 (CH-*p*-Bz); 137.1 (C-8a); 140.8 (C-2-thienyl); 153.8 (C-4); 154.4 (CH-2); 156.1 (C-9a); 164.9 (COPh-2'); 165.1 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %):

752.3 (50) [M+Na], 730.3 (100) [M+H. HR MS (ESI) for $C_{40}H_{29}ClN_3O_7S$ [M+H]: calcd 730.14112; found 730.14093.

6-Chloro-4-(thiophen-3-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (31d)

Nucleoside **31d** was synthesized according to the general procedure A. Protected nucleoside **27** (500 mg, 0.73 mmol) and thiophene-3-boronic acid (140 mg, 1.1 mmol) were used. Desired product **31d** (375 mg, 70 %) was obtained as yellowish powder; mp: 171 - 184 °C; IR (ATR): ν = 2360, 2342, 1721, 1561, 1264, 1106, 1068, 705 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.70 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.90 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.40 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.66 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.6, H-2'); 7.04 (d, 1H, $J_{1',2'}$ = 4.6, H-1'); 7.42 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 5H, H-7, H-*m*-Bz-3',5'); 7.62 (m, 1H, H-*p*-Bz-2'); 7.67 (m, 3H, H-4-thienyl, H-*p*-Bz-3',5'); 7.85 (m, 2H, H-*o*-Bz-2'); 7.88 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,2}$ = 2.9, H-5-thienyl); 7.95 (d, 1H, $J_{5,7}$ = 2.0, H-5); 7.96 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.11 (dd, 1H, $J_{8,7}$ = 9.0, H-8); 8.34 (dd, 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 8.96 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.4 (CH-3'); 72.3 (CH-2'); 78.7 (CH-4'); 86.2 (CH-1'); 110.6 (C-4a); 113.5 (CH-8); 120.6 (C-4b); 121.8 (CH-5); 126.6 (C-6); 127.6, 128.0, 128.2 (CH-7, CH-4,5-thienyl); 128.6, 128.8 (C-*i*-Bz); 128.9, 128.9, 129.0 (CH-2-thienyl, CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.8, 134.1 (CH-*p*-Bz); 137.0 (C-8a); 139.0 (C-3-thienyl); 154.6 (CH-2); 155.8 (C-9a); 155.9 (C-4); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 752 (100) [M+Na], 754 (48) [M+2+H], 730 (13) [M+H]. HR MS (ESI) for $C_{40}H_{29}ClN_3O_7S$[M+H]: calcd 730.14089; found 730.14093.

4-(Benzofuran-2-yl)-6-chloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido-[4,5-*b*]indole (31e)

Nucleoside **31e** was synthesized according to the general procedure A. Protected nucleoside **27** (500 mg, 0.73 mmol) and benzofuran-2-boronic acid (180 mg, 1.1 mmol) were used. Desired product **31e** (358 mg, 64 %) was obtained as yellow powder; mp: 149 - 158 °C. IR (ATR): ν = 1716, 1582, 1559, 1446, 1280, 1125, 1065, 707 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 4.71 (dd, 1H, J_{gem}= 12.3, $J_{5'b,4'}$ = 4.3, H-5'b); 4.87 (dd, 1H, J_{gem}= 12.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.40 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.64 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.6, H-2'); 7.06 (d, 1H, $J_{1',2'}$ = 4.6, H-1'); 7.42 (m, 3H, H-*m*-Bz-2', H-5-benzofuryl); 7.49 (m, 4H, H-*m*-Bz-3',5'); 7.50 (dd, 1H, $J_{7,8}$ = 9.0, $J_{7,5}$ = 2.1, H-7); 7.55

(ddd, 1H, $J_{6,7}$ = 8.3, $J_{6,5}$ = 7.1, $J_{6,4}$ = 1.2, H-6-benzofuryl); 7.62 (m, 1H, H-*p*-Bz-2'); 7.65, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.78 (ddt, 1H, $J_{7,6}$ = 8.3, $J_{7,3}$ = 1.0, $J_{7,4}$ = $J_{7,5}$ = 0.8, H-7-benzofuryl); 7.85 (m, 2H, H-*o*-Bz-2'); 7.87 (ddd, 1H, $J_{4,5}$ = 7.8, $J_{4,6}$ = 1.2, $J_{4,7}$ = 0.8, H-4-benzofuryl); 7.95 (m, 2H, H-*o*-Bz-5'); 7.99 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 8.00 (m, 2H, H-*o*-Bz-3'); 8.10 (dd, 1H, $J_{8,7}$ = 8.8, $J_{8,5}$ = 0.5, H-8); 8.92 (dd, 1H, $J_{5,7}$ = 2.2, $J_{5,8}$ = 0.5, H-5); 8.97 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.3 (CH-3'); 72.3 (CH-2'); 78.7 (CH-4'); 86.2 (CH-1'); 108.9 (C-4a); 111.1 (CH-3-benzofuryl); 111.5 (CH-7-benzofuryl); 113.2 (CH-8); 120.2 (C-4b); 123.0 (CH-4-benzofuryl); 124.4 (CH-5-benzofuryl); 124.6 (CH-5); 127.1 (C-6); 127.4 (CH-6-benzofuryl); 127.6 (C-3-benzofuryl); 128.2 (CH-7); 128.7, 128.8 (C-*i*-Bz); 128.9, 128.9, 128.9 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 137.4 (C-8a); 148.3 (C-4); 153.6 (C-2-benzofuryl); 154.3 (CH-2); 155.3 (C-7a-benzofuryl); 156.5 (C-9a) 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 786 (100) [M+Na], 788 (45) [M+2+H], 764 (10) [M+H]. HR MS (ESI) for $C_{44}H_{30}ClN_3O_8Na$[M+H]: calcd 786.16098; found 786.16136.

6-Chloro-4-phenyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (31f)

Nucleoside **31f** was synthesized according to the general procedure A. Protected nucleoside **27** (500 mg, 0.73 mmol) and phenylboronic acid (134 mg, 1.1 mmol) were used. Desired product **31f** (382 mg, 72 %) was obtained as white powder. m.p. 175 - 180 °C. IR (ATR): ν = 1721, 1560, 1265, 1108, 701 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 4.70 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.3, H-5'b); 4.85 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.3, 3.2, H-4'); 6.41 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.68 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.5, H-2'); 7.06 (d, 1H, $J_{1',2'}$ = 4.5, H-1'); 7.42 (m, 2H, H-*m*-Bz-2'); 7.48-7.52 (m, 5H, H-*m*-Bz-3',5', H-7); 7.63 (m, 1H, H-*p*-Bz-2'); 7.66-7.70 (m, 6H, H-*p*-Bz-3',5', H-*m*,*p*-Ph, H-5); 7.86 (m, 2H, H-*o*-Bz-2'); 7.89 (m, 2H, H-*o*-Ph); 7.96 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.12 (d, 1H, $J_{8,7}$ = 8.9, H-8); 9.01 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.4 (CH-3'); 72.4 (CH-2'); 78.7 (CH-4'); 86.3 (CH-1'); 110.9 (C-4a); 113.6 (CH-8); 120.5 (C-4b); 121.6 (CH-5); 126.6 (C-6); 128.2 (CH-7); 128.7, 128.8 (C-*i*-Bz); 129.0 (CH-*m*-Bz, CH-*o*-Ph); 129.1 (CH-*m*-Ph); 129.4 (CH-*o*-Bz-5'); 129.6 (CH-*o*-Bz-2'); 129.7 (CH-*o*-Bz-3'); 130.8 (CH-*p*-Ph); 133.8, 134.1, 134.2 (CH-*p*-Bz); 137.2 (C-8a); 137.5 (C-*i*-Ph); 154.8 (CH-2); 155.8 (C-9a); 160.7 (C-4); 164.9 (COPh-2'); 165.1 (COPh-3'); 165.6 (COPh-

5'). ESI MS m/z (rel. %): 746.3 (100) [M+Na], 748.3 (35) [M+2+Na], 724.3 (33) [M+H]. HR MS (ESI) for $C_{42}H_{31}ClN_3O_7$ [M+H]: calcd 724.18463; found 724.18450.

6-Chloro-4-(furan-2-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (32a)

Deprotection of **31a** (150 mg, 0.21 mmol) according to the general procedure E afforded compound **32a** (72 mg, 86 %) as white crystals. m.p. 188-192 °C; $[\alpha]_D$ -33.1 (c 0.45). IR (ATR): ν = 3320, 2922, 1592, 1565, 1538, 1460, 1435, 1398, 1308, 1291, 1221, 1130, 1075, 1037, 1012 cm^{-1}. ^1H NMR (600.1 MHz, DMSO-*d*6): 3.69 (ddd, 1H, J_{gem} = 12.0, *J*5'b,OH = 5.3, $J_{5'b,4'}$ = 3.9, H-5'b); 3.74 (ddd, 1H, J_{gem} = 12.0, $J_{5'a}$,OH = 5.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.00 (dt, 1H, $J_{4',5'}$ = 3.9, 3.2, $J_{4',3'}$ = 3.2, H-4'); 4.24 (ddd, 1H, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 3.2, H-3'); 4.77 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.7, H-2'); 5.23 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.24 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.31 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.57 (d, $J_{1',2'}$ = 7.4, H-1'); 6.91 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.62 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl); 7.63 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.2, H-7); 8.17 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.38 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl); 8.84 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.98 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.7 (CH_2-5'); 70.2 (CH-3'); 70.6 (CH-2'); 85.8 (CH-4'); 87.0 (CH-1'); 107.1 (C-4a); 113.4 (CH-4-furyl); 114.8 (CH-8); 115.6 (CH-3-furyl); 120.7 (C-4b); 123.9 (CH-5); 126.6 (C-6); 127.9 (CH-7); 136.8 (C-8a); 147.0 (CH-5-furyl); 148.0 (C-4); 152.4 (C-2-furyl); 154.5 (CH-2); 157.1 (C-9a). ESI MS m/z (rel. %): 424 (100) [M+Na], 426 (34) [M+2+Na], 402 (26) [M+H] 404 (8) [M+2+H]. HR MS (ESI) for $C_{19}H_{17}ClN_3O_5$ [M+H]: calcd 402.08520; found 402.08512. Anal. Calcd. for $C_{19}H_{16}ClN_3O_5$: C, 56.80 %; H, 4.01 %; Cl, 8.82 %; N, 10.46 %. Found C, 56.97 %; H, 4.02 %; N, 10.45 %.

6-Chloro-4-(furan-3-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (32b)

Deprotection of **31b** (400 mg, 0.56 mmol) according to the general procedure E and crystallization ($CHCl_3$/MeOH) afforded compound **32b** (205 mg, 91 %) as white crystals. m.p. 238 - 242 °C; $[\alpha]_D$ -27.0 (c 0.28). IR (ATR): ν = 3317, 3136, 2934, 1587, 1561, 1471, 1447, 1296, 1173, 1158, 1104, 1070 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-*d*6): 3.69 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 3.8, H-5'b); 3.74 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 3.4, H-5'a); 4.01 (ddd, 1H, $J_{4',5'}$ = 3.8, 3.4, $J_{4',3'}$ = 3.0, H-4'); 4.24 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.0, H-3'); 4.78 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 5.8, H-2'); 5.21 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.21 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.28 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2'); 6.54 (d, $J_{1',2'}$ = 7.4, H-1'); 7.13 (d, 1H, $J_{4,5}$ = 2.1, H-4-furyl); 7.59 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 8.03 (dd, 1H, $J_{5,4}$ = 2.1, $J_{5,2}$ = 1.3, H-5-furyl); 8.11 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.16 (d, 1H, $J_{8,7}$ = 8.9, H-8);

8.55 (dd, 1H, $J_{2,5}$ = 1.3, $J_{2,4}$ = 0.8, H-2-furyl); 9.01 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.7 (CH_2-5'); 70.2 (CH-3'); 70.7 (CH-2'); 85.8 (CH-4'); 87.0 (CH-1'); 110.3 (C-4a); 110.7 (CH-4-furyl); 115.0 (CH-8); 120.7 (C-4b); 121.7 (CH-5); 124.3 (C-3-furyl); 126.3 (C-6); 127.8 (CH-7); 136.5 (C-8a); 144.5 (CH-2-furyl); 144.9 (CH-5-furyl); 153.2 (C-4); 154.7 (CH-2); 156.3 (C-9a). ESI MS m/z (rel. %): 402 (100) [M+H], 404 (35) [M+2+H]. HR MS (ESI) for $C_{19}H_{17}ClN_3O_5$ [M+H]: calcd 402.08520; found 402.08512. Anal. Calcd for $C_{19}H_{16}ClN_3O_5$: C, 56.80 %; H, 4.01 %; Cl, 8.82 %; N, 10.46 %. Found C, 56.74 %; H, 4.06 %; Cl, 8.81 %; N, 10.42 %.

6-Chloro-4-(thiophen-2-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (32c)

Deprotection of **31c** (340 mg, 0.47 mmol) according to the general procedure E and lyophilization (*t*-BuOH) afforded compound **32c** (181 mg, 93 %) as white lyophilisate. m.p. 245-249 °C; $[\alpha]_D$ -16.7 (c 0.23). IR (ATR): ν = 3327, 3307, 3289, 2928, 1561, 1461, 1439, 1396, 1288, 1119, 1077, 1044. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.70 (ddd, 1H, J_{gem}= 12.0, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 3.8, H-5'b); 3.74 (ddd, 1H, J_{gem}= 12.0, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 3.4, H-5'a); 4.02 (ddd, 1H, $J_{4',5'}$ = 3.8, 3.4, $J_{4',3'}$ = 3.1, H-4'); 4.25 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 3.1, H-3'); 4.78 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.8, H-2'); 5.22 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.23 (d, 1H, $J_{OH,3'}$ = 4.3, OH-3'); 5.30 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.56 (d, $J_{1',2'}$ = 7.4, H-1'); 7.43 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,3}$ = 3.7, H-4-thienyl); 7.61 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 8.01 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,3}$ = 1.2, H-5-thienyl); 8.085 (dd, 1H, $J_{3,4}$ = 3.7, $J_{3,5}$ = 1.2, H-3-thienyl); 8.19 (d, 1H, $J_{8,7}$ = 8.9, H-8); 8.26 (d, 1H, $J_{5,7}$ = 2.2, H-5); 8.99 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.7 (CH_2-5'); 70.2 (CH-3'); 70.7 (CH-2'); 85.8 (CH-4'); 87.1 (CH-1'); 109.0 (C-4a); 115.1 (CH-8); 120.6 (C-4b); 121.4(CH-5); 126.3 (C-6); 128.0 (CH-7); 128.7 (CH-4-thienyl); 129.4 (CH-3-thienyl); 131.6 (CH-5-thienyl); 136.6 (C-8a); 141.0 (C-2-thienyl); 153.4 (C-4); 154.4 (CH-2); 156.7 (C-9a). ESI MS m/z (rel. %): 440 (33) [M+Na], 442 (12) [M+2+Na], 418 (100) [M+H], 420 (40) [M+2+H]. HR MS (ESI) for $C_{19}H_{17}ClN_3O_4S$[M+H]: calcd 418.06228; found 418.06228. Anal. Calcd for $C_{19}H_{16}ClN_3O_4S$: C, 54.61 %; H, 3.86 %; Cl, 8.48 %; N, 10.06 %. Found C, 54.51 %; H, 4.00 %; N, 9.95 %.

6-Chloro-4-(thiophen-3-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (32d)

Deprotection of **31d** (120 mg, 0.16 mmol) according to the general procedure E and crystallization ($CHCl_3$/MeOH) afforded compound **32d** (59 mg, 86 %) as white crystals. m.p. 257-261 °C; $[\alpha]_D$ -29.2 (c 0.31). IR (ATR): ν = 3356, 3272, 3107, 2920, 1566, 1468, 1442, 1289, 1129, 1078, 1037. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.69 (ddd, 1H, J_{gem}= 12.0, $J_{5'b,OH}$

= 5.3, $J_{5'b,4'}$ = 3.8, H-5'b); 3.74 (ddd, 1H, J_{gem}= 12.0, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 3.4, H-5'a); 4.01 (ddd, 1H, $J_{4',5'}$ = 3.8, 3.4, $J_{4',3'}$ = 2.9, H-4'); 4.24 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.9, H-3'); 4.78 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.8, H-2'); 5.24 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.25 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.32 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.55 (d, $J_{1',2'}$ = 7.4, H-1'); 7.59 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.67 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,2}$ = 1.3, H-4-thienyl); 7.89 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,2}$ = 2.9, H-5-thienyl); 7.96 (dd, 1H, $J_{5,7}$ = 2.1, $J_{5,8}$ = 0.3, H-5); 8.17 (dd, 1H, $J_{8,7}$ = 8.8, $J_{8,5}$ = 0.3, H-8); 8.33 (dd, 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 9.03 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.3 (CH-3'); 70.7 (CH-2'); 85.8 (CH-4'); 87.0 (CH-1'); 110.2 (C-4a); 115.1 (CH-8); 120.8 (C-4b); 121.5 (CH-5); 126.2 (C-6); 127.8 (CH-7); 128.0 (CH-5-thienyl); 128.2 (CH-4-thienyl); 128.7 (CH-2-thienyl); 136.6 (C-8a); 139.2 (C-3-thienyl); 154.7 (CH-2); 155.7 (C-4); 156.4 (C-9a). ESI MS m/z (rel. %): 440 (100) [M+Na], 442 (33) [M+2+Na]. HR MS (ESI) for $C_{19}H_{17}ClN_3O_4S$[M+H]: calcd 418.06228; found 418.06227. Anal. Calcd for $C_{19}H_{16}ClN_3O_4S$: C, 54.61 %; H, 3.86 %; Cl, 8.48 %; N, 10.06 %. Found C, 54.51 %; H, 4.00 %; N, 9.95 %.

4-(Benzofuran-2-yl)-6-chloro-9-β-D-ribofuranosyl)-9H-pyrimido[4,5-b]indole (32e)

Deprotection of **31e** (200 mg, 0.26 mmol) according to the general procedure E afforded compound **32e** (92 mg, 78 %) as yellow crystals. m.p. 231-238 °C; $[\alpha]_D$ -32.2 (c 0.28). IR (ATR): ν = 3294, 2922, 1585, 1564, 1532, 1467, 1437, 1289, 1102, 989 cm^{-1}. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.71, 3.75 (2 × m, 2 × 1H, H-5'); 4.02 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.4, H-4'); 4.25 (bm, 1H, H-3'); 4.78 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.6, H-2'); 5.24 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5'); 5.25 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.33 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 6.60 (d, 1H, $J_{1',2'}$ = 7.4, H-1'); 7.44 (ddd, 1H, $J_{5,4}$ = 7.7, $J_{5,6}$ = 7.2, $J_{5,7}$ = 0.9, H-5-benzofuryl); 7.57 (ddd, 1H, $J_{6,7}$ = 8.4, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.2, H-6-benzofuryl); 7.66 (dd, 1H, $J_{7,8}$ = 8.7, $J_{7,5}$ = 2.1, H-7); 7.82 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 0.9, H-7-benzofuryl); 7.90 (ddd, 1H, $J_{4,5}$ = 7.7, $J_{4,6}$ = 1.2, $J_{4,7}$ = 0.9, H-4-benzofuryl); 8.03 (d, 1H, $J_{3,7}$ = 0.9, H-3-benzofuryl); 8.21 (d, 1H, $J_{8,7}$ = 8.7, H-8); 8.99 (d, 1H, $J_{5,7}$ = 2.1, H-5); 9.08 (s, 1H, H-2).^{13}C NMR (125.7 MHz, DMSO-d_6): 61.7 (CH_2-5'); 70.2 (CH-3'); 70.6 (CH-2'); 85.8 (CH-4'); 87.1 (CH-1'); 108.6 (C-4a); 111.0 (CH-3-benzofuryl); 111.6 (CH-7-benzofuryl); 114.9 (CH-8); 120.5 (C-4b); 123.0 (CH-4-benzofuryl); 124.4 (CH-5, CH-5-benzofuryl); 126.7 (C-6); 127.3 (CH-6-benzofuryl); 127.7 (C-3-benzofuryl); 128.1 (CH-7); 137.0 (C-8a); 148.1 (C-4); 153.8 (C-2-benzofuryl); 154.5 (CH-2); 155.3 (C-7a-benzofuryl); 157.2 (C-9a). ESI MS m/z (rel. %): 452 (100) [M+H], 454 (33) [M+2+H]. HR MS (ESI) for $C_{23}H_{19}ClN_3O_5$ [M+H]: calc. 452.10075; found

452.10077. Anal. Calcd for $C_{23}H_{19}ClN_3O_5$ (451.9): C 61.14 %, H 4.02 %, Cl 7.85 %, N 9.30 %, O 17.70 %. Found C 61.19 %, H 4.05 %, N 9.25 %.

6-Chloro-4-phenyl-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (32f)

Deprotection of **31f** (180 mg, 0.25 mmol) according to the general procedure E and lyophilization (*t*-BuOH) afforded compound **32f** (91 mg, 89 %) as white lyophilisate. m.p. 254-257 °C; $[\alpha]_D$ -24.5 (c 0.31). IR (ATR): ν = 3294, 3063, 2920, 1554, 1464, 1446, 1434, 1287, 1075, 1024 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.69 (ddd, 1H, J_{gem}= 12.0, $J_{5'b,OH}$ = 5.3, $J_{5'b,4'}$ = 3.8, H-5'b); 3.74 (ddd, 1H, J_{gem}= 12.0, $J_{5'a,OH}$ = 5.3, $J_{5'a,4'}$ = 3.4, H-5'a); 4.02 (ddd, 1H, $J_{4',5'}$ = 3.8, 3.4, $J_{4',3'}$ = 2.9, H-4'); 4.25 (ddd, 1H, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 2.9, H-3'); 4.80 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.7, H-2'); 5.21 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.23 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.31 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.56 (d, $J_{1',2'}$ = 7.4, H-1'); 7.58 (dd, 1H, $J_{7,8}$ = 8.9, $J_{7,5}$ = 2.2, H-7); 7.66-7.72 (m, 4H, H-5, H-*m*,*p*-Ph); 7.90 (m, 2H, H-*o*-Ph); 8.165 (d, 1H, $J_{8,7}$ = 8.9, H-8); 9.09 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.2 (CH-3'); 70.7 (CH-2'); 85.8 (CH-4'); 87.1 (CH-1'); 110.4 (C-4a); 115.1 (CH-8); 120.7 (C-4b); 121.3 (CH-5); 126.1 (C-6); 127.8 (CH-7); 128.9 (CH-*o*-Ph); 129.1 (CH-*m*-Ph); 130.7 (CH-*p*-Ph); 136.6 (C-8a); 137.7 (C-*i*-Ph); 154.8 (CH-2); 156.3 (C-9a); 160.4 (C-4). ESI MS m/z (rel. %): 434 (25) [M+Na], 436 (6) [M+2+Na], 412 (100) [M+H], 414 (33) [M+2+H]. HR MS (ESI) for $C_{21}H_{19}ClN_3O_4$ [M+H]: calcd 412.10593; found 412.10586. Anal. Calcd for $C_{21}H_{19}ClN_3O_4$: C, 61.24 %; H, 4.41 %; Cl, 8.61 %; N, 10.20 %; O, 15.54 %. Found C, 61.14 %; H, 4.45 %; N, 10.20 %.

4,6-Diphenyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (33) and 4-phenyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (34)

Free nucleoside **32f** (50 mg, 0.12 mmol), phenylboronic acid (22 mg, 0.18 mmol), K_2CO_3 (33 mg, 0.24 mmol), $Pd(OAc)_2$ (1.3 mg, 7.5 μmol) and CataXCium F (8.3 mg, 15 μmol) were dissolved in mixture of *n*-butanol (2.5 ml) and water (1 ml) and heated to 95 °C for 12 hours. Solvents were evaporated under reduced pressure and the reaction mixture was separated by column chromatography (1-3 % of MeOH in $CHCl_3$). Unseparable mixture of compounds **33** and **34** was obtained as white powder. Products were identified and characterised by NMR and MS spectra.

For **33**: ^1H NMR (500.0 MHz, DMSO-d_6): 3.66-3.80 (m, 2H, H-5'a, H-5'b); 4.04 (q, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 3.5, H-4'); 4.28 (ddd, 1H, $J_{3',2'}$ = 3.1, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.5, H-3'); 4.89 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 3.1, H-2'); 5.25 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.26 (t, 1H, $J_{OH,5'}$

= 5.4, OH-5'); 5.33 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2'); 6.60 (d, $J_{1',2'}$ = 7.4, H-1'); 7.34 (m, 1H, H-*p*-Ph-6); 7.45 (m, 2H, H-*m*-Ph-6); 7.56 (m, 2H, H-*o*-Ph-6); 7.63-7.73 (m, 3H, H-*m,p*-Ph-4); 7.84 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.9, H-7); 7.98 (m, 2H, H-*o*-Ph-4); 8.04 (d, 1H, $J_{5,7}$ = 1.9, H-5); 8.19 (d, 1H, $J_{8,7}$ = 8.6, H-8); 9.08 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.85 (CH_2-5'); 70.31 (CH-3'); 70.70 (CH-2'); 85.72 (CH-4'); 87.13 (CH-1'); 111.26 (C-4a); 113.78 (CH-8); 119.91 (C-4b); 120.16 (CH-5); 126.80 (CH-*o*-Ph-6); 127.09 (CH-7); 127.31 (CH-*p*-Ph-6); 128.95; 129.09 (CH-*o*-Ph-4); 129.27 (CH-*m*-Ph-6); 130.56 (CH-*p*-Ph-4); 134.16 (CH-6); 137.63 (C-8a); 137.99 (C-*i*-Ph-4); 140.49 (C-*i*-Ph-6); 154.31 (CH-2); 156.42 (C-9a); 159.87 (C-4).

For **34**: ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.66-3.80 (m, 2H, H-5'a, H-5'b); 4.02 (q, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 3.5, H-4'); 4.27 (ddd, 1H, $J_{3',2'}$ = 3.1, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.5, H-3'); 4.87 (ddd, 1H, $J_{2',1'}$ = 7.4, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 3.1, H-2'); 5.22 (t, 1H, $J_{OH,5'}$ = 5.4, OH-5'); 5.23 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.30 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2'); 6.57 (d, $J_{1',2'}$ = 7.4, H-1'); 7.26 (ddd, 1H, $J_{6,5}$= 8.1, $J_{6,7}$ = 7.3, $J_{6,8}$= 0.9, H-6); 7.80 (ddd, 1H, $J_{5,6}$= 8.1, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 7.63-7.73 (m, 3H, H-*m,p*-Ph-4); 7.90 (m, 2H, H-*o*-Ph-4); 8.09 (ddd, 1H, $J_{8,7}$= 8.4, $J_{8,6}$ = 0.9, $J_{8,5}$ = 0.6, H-8); 9.05 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.85 (CH_2-5'); 70.27 (CH-3'); 70.54 (CH-2'); 85.63 (CH-4'); 87.13 (CH-1'); 111.15 (C-4a); 113.26 (CH-8); 119.20 (C-4b); 121.85 (CH-6); 122.13 (CH-5); 128.08 (CH-7); 128.91, 128.94 (CH-*o,m*-Ph-4); 130.35 (CH-*p*-Ph-4); 138.10 (C-*i*-Ph-4); 138.17 (C-8a); 154.07 (CH-2); 155.98 (C-9a); 159.67 (C-4). ESI MS m/z (rel. %): 378 (70) [**33**+H]; 400 (100) [**33**+Na]; 454 (25) [**34**+H]; 476 (32) [**34**+Na]. HR MS (ESI) for $C_{21}H_{19}N_3O_4$ [M+H]: calcd 377.1376; found 377.1375; for $C_{27}H_{23}N_3O_4$ [M+H]: calcd 453.16886; found 453.16890.

4,6-Bis(furan-2-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (35a)

Protected nucleoside **31a** (500 mg, 0.7 mmol), 2-furyltributylstannane (300 mg, 0.84 mmol), $Pd(OAc)_2$ (7.9 mg, 0.035mmol) and X-Phos (33.4 mg, 0.07 mmol) were dissolved in anhydrous DMF and heated to 95 °C for 3 hours. Then, solvent was evaporated under reduced pressure. Crude product was purified using column chromatography (hexane/EtOAc, 10-20 % EtOAc). Nucleoside **35a** (416 mg, 79 %) was obtained as white powder. m.p. 104 – 108 °C. IR (ATR): ν = 2360, 2349, 1724, 1560, 1533, 1468, 1449, 1266, 1094, 1027, 711 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.70 (dd, 1H, J_{gem}= 12.4, $J_{5'b,4'}$ = 4.1, H-5'b); 4.86 (dd, 1H, J_{gem}= 12.4, $J_{5'a,4'}$ = 2.9, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.7, $J_{4',5'}$ = 4.1, 2.9, H-4'); 6.40 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.7, H-3'); 6.649 (dd, 1H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.8, H-4-furyl-6); 6.652 (dd, $J_{2',3'}$ = 6.7, $J_{2',1'}$

= 4.5, H-2'); 6.94 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl-4); 7.00 (dd, 1H, $J_{3,4}$ = 3.3, $J_{3,5}$ = 0.7, H-3-furyl-6); 7.06 (d, $J_{1',2'}$ = 4.5, H-1'); 7.42 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 4H, H-*m*-Bz-3',5'); 7.62 (m, 1H, H-*p*-Bz-2'); 7.63 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl-4); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.79 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.7, H-5-furyl-6); 7.81 (dd, 1H, $J_{7,8}$ = 8.7, $J_{7,5}$ = 1.7, H-7); 7.85 (m, 2H, H-*o*-Bz-2'); 7.96 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.09 (dd, 1H, $J_{8,7}$ = 8.7, $J_{8,5}$ = 0.3, H-8); 8.39 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl-4); 8.90 (s, 1H, H-2); 9.14 (dd, 1H, $J_{5,7}$ = 1.7, $J_{5,8}$ = 0.3, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.3 (CH-3'); 72.2 (CH-2'); 78.6 (CH-4'); 86.1 (CH-1'); 105.3 (CH-3-furyl-6); 108.4 (C-4a); 112.0 (CH-8); 112.4 (CH-4-furyl-6); 113.3 (CH-4-furyl-4); 115.4 (CH-3-furyl-4); 119.5 (CH-5); 119.5 (C-4b); 124.1 (CH-7); 125.4 (C-6); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 137.8 (C-8a); 143.0 (CH-5-furyl-6); 146.9 (CH-5-furyl-4); 148.1 (C-4); 152.3 (C-2-furyl-4); 153.3 (C-2-furyl-6); 154.1 (CH-2); 156.6 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). ESI MS m/z (rel. %): 746 (100) [M+H], 768 (95) [M+Na]. HR MS (ESI) for $C_{44}H_{32}N_3O_9$ [M+H]: calcd 746.21331; found 746.21328.

4-(Furan-2-yl)-6-(furan-3-yl)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (35b)

Method A: Protected nucleoside **31a** (400mg, 0.56 mmol), 3-furanboronic acid (94 mg, 0.84 mmol), K_2CO_3 (232 mg, 1.68 mmol), $Pd(OAc)_2$ (6.3 mg, 0.028mmol) and X-Phos (26.7 mg, 0.056 mmol) were dissolved in anhydrous DMF and heated to 95 °C for 3 hours. Solvent was evaporated under reduced pressure. Crude product was purified using column chromatography (hexane/EtOAc, 10 – 20 % EtOAc). Nucleoside **35b** (192 mg, 46 %) was obtained as white crystals. m.p. 87 – 91 °C. IR (ATR): ν = 2356, 2346, 1720, 1563, 1538, 1471, 1452, 1263, 1092, 1024, 706 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.71 (dd, 1H, J_{gem}= 12.3, $J_{5'b,4'}$ = 4.2, H-5'b); 4.87 (dd, 1H, J_{gem}= 12.3, $J_{5'a,4'}$ = 3.2, H-5'a); 4.91 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.2, 3.2, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.64 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.6, H-2'); 6.92 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl-4); 7.04 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl-6); 7.06 (d, $J_{1',2'}$ = 4.6, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.50 (m, 4H, H-*m*-Bz-3',5'); 7.61 (m, 1H, H-*p*-Bz-2'); 7.62 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl-4); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.71 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.8, H-7); 7.82 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.6, H-5-furyl-6); 7.84 (m, 2H, H-*o*-Bz-2'); 7.97 (m, 2H, H-*o*-Bz-5'); 8.00 (m, 2H, H-*o*-Bz-3'); 8.06 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.5, H-8); 8.27 (dd, 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl-6); 8.48 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl-4); 8.89 (s, 1H, H-2); 8.82 (dd, 1H, $J_{5,7}$ = 1.8, $J_{5,8}$ =

0.5, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.4 (CH-3'); 72.2 (CH-2'); 78.6 (CH-4'); 86.1 (CH-1'); 108.3 (C-4a); 109.1 (CH-4-furyl-6); 111.9 (CH-8); 113.2 (CH-4-furyl-4); 115.4 (CH-3-furyl-4); 119.6 (C-4b); 121.3 (CH-5); 126.1 (C-3-furyl-6); 126.2 (CH-7); 126.6 (C-6); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-*m*-Bz); 129.4 (CH-*o*-Bz-5'); 129.4 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 137.6 (C-8a); 139.1 (CH-2-furyl-6); 144.7 (CH-5-furyl-6); 147.2 (CH-5-furyl-4); 147.9 (C-4); 152.3 (C-2-furyl-4); 153.9 (CH-2); 156.5 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). MS ESI MS m/z (rel. %): 746 (100) [M+H], 768 (95) [M+Na]. HR MS (ESI) for $C_{44}H_{32}N_3O_9$ [M+H]: calcd 746.21346; found 746.21331.

Method B: Protected nucleoside **31a** (400mg, 0.56 mmol), K_2CO_3 (232 mg, 1.68 mmol), $Pd(OAc)_2$ (6.3 mg, 0.028mmol), X-Phos (26.7 mg, 0.056 mmol) and one third of all amount of 3-furanboronic acid (94 mg, 0.84 mmol) were dissolved in anhydrous DMF (20 ml) and heated to 95 °C for 1 hour. Second third of boronic acid was added and the reaction was stirred at 95 °C for one hour. Then, last third of boronic acid was added and the reaction was heated for another 6 hours at 95 °C. Solvent was evaporated under reduced pressure and crude product was purified by flash chromatography (hexane/EtOAc, 10 – 20 % EtOAc). Nucleoside **35b** was obtained (88 mg, 75 %) as white crystals.

4-(Furan-2-yl)-6-phenyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (35f)

Compound **35f** was prepared as described for **35a** from **31a** (Method A and Method B) (300 mg, 0.42 mmol). Nucleoside **35f** was obtained (196 mg, 62 %, Method A), (202 mg, 64 %, Method B) as white powder. m.p. 92 - 96 °C. IR (ATR): ν = 2359, 2342, 1723, 1470, 1264, 1093, 1069, 708 cm^{-1}.^{1}H NMR (500.0 MHz, DMSO-d_6): 4.72 (dd, 1H, J_{gem}= 12.3, $J_{5'b,4'}$ = 4.1, H-5'b); 4.88 (dd, 1H, J_{gem}= 12.3, $J_{5'a,4'}$ = 3.1, H-5'a); 4.93 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.1, 3.1, H-4'); 6.39 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.65 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.7, H-2'); 6.91 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.09 (d, $J_{1',2'}$ = 4.7, H-1'); 7.40 (m, 1H, H-*p*-Ph); 7.41 (m, 2H, H-*m*-Bz-2'); 7.51 (m, 4H, H-*m*-Bz-3',5'); 7.54 (m, 2H, H-*m*-Ph); 7.62 (m, 1H, H-*p*-Bz-2'); 7.63 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.67, 7.69 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.73 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.9, H-7); 7.75 (m, 2H, H-*o*-Ph); 7.84 (m, 2H, H-*o*-Bz-2'); 7.99 (m, 2H, H-*o*-Bz-5'); 8.01 (m, 2H, H-*o*-Bz-3'); 8.12 (d, 1H, $J_{8,7}$ = 8.6, H-8); 8.34 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.91 (s, 1H, H-2); 9.08 (dd, 1H, $J_{5,7}$ = 1.9, $J_{5,8}$ = 0.5, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.2 (CH_2-5'); 70.4 (CH-3'); 72.1 (CH-2'); 78.7 (CH-4'); 86.1 (CH-1'); 108.5 (C-4a); 112.0 (CH-8); 113.3 (CH-4-furyl); 115.3 (CH-3-furyl); 119.7 (C-4b);

122.7 (CH-5); 127.0 (CH-*o*-Ph); 127.2 (CH-7); 127.3 (CH-*p*-Ph); 128.6, 128.8 (C-*i*-Bz); 128.9 (CH-*m*-Bz); 129.4 (CH-*m*-Ph, CH-*o*-Bz-5'); 129.5 (C-*i*-Bz); 129.5 (CH-*o*-Bz-2'); 129.6 (CH-*o*-Bz-3'); 133.7, 134.1 (CH-*p*-Bz); 134.9 (C-6); 137.9 (C-8a); 140.6 (C-*i*-Ph); 146.8 (CH-5-furyl); 148.0 (C-4); 152.4 (C-2-furyl); 154.0 (CH-2); 156.6 (C-9a); 164.8 (COPh-2'); 165.0 (COPh-3'); 165.6 (COPh-5'). MS: ESI MS m/z (rel. %): 756 (62) [M+H], 778 (100) [M+Na]. HR MS (ESI) for $C_{46}H_{34}N_3O_8$ [M+H]: calcd 756.23404; found 756.23371.

4,6-Bis(furan-2-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (36a)

Deprotection of **35a** (300 mg, 0.40 mmol) according to the general procedure E afforded compound **36a** (72 mg, 86 %) as white crystals. m.p. 92 – 95 °C, $[\alpha]_D$ -32.3 (c 0.29). IR (ATR): ν = 3297, 2363, 2339, 1590, 1563, 1539, 1466, 1094, 1075, 1042, 743 cm^{-1}. ^1H NMR (600.1 MHz, DMSO-d_6): 3.71, 3.76 (2 × bdt, 2 × H, J_{gem}= 11.7, $J_{5',4'}$ = $J_{5',OH}$ = 3.5, H-5'); 4.01 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 3.5, H-4'); 4.27 (bm, 1H, H-3'); 4.84 (bddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.1, $J_{2',3'}$ = 4.7, H-2'); 5.20 (bm, 2H, OH-3',5'); 5.29 (d, 1H, $J_{OH,2'}$ = 6.1, OH-2'); 6.57 (d, $J_{1',2'}$ = 7.3, H-1'); 6.64 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.8, H-4-furyl-6); 6.93 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl-4); 7.03 (dd, 1H, $J_{3,4}$ = 3.3, $J_{3,5}$ = 0.8, H-3-furyl-6); 7.62 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl-4); 7.79 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H-5-furyl-6); 7.95 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.8, H-7); 8.15 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.4, H-8); 8.38 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl-4); 8.96 (s, 1H, H-2); 9.17 (dd, 1H, $J_{5,7}$ = 1.8, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.2 (CH-3'); 70.6 (CH-2'); 85.7 (CH-4'); 87.1 (CH-1'); 105.2 (CH-3-furyl-6); 108.1 (C-4a); 112.4 (CH-4-furyl-6); 113.3 (CH-4-furyl-4); 113.4 (CH-8); 115.2 (CH-3-furyl-4); 119.3 (CH-5); 119.6 (C-4b); 124.0 (CH-7); 124.9 (C-6); 137.6 (C-8a); 142.8 (CH-5-furyl-6); 146.7 (CH-5-furyl-4); 147.8 (C-4); 152.5 (C-2-furyl-4); 153.6 (C-2-furyl-6); 154.0 (CH-2); 157.1 (C-9a). ESI MS m/z (rel. %): 434 (100) [M+H]. HR MS (ESI) for $C_{23}H_{20}N_3O_6$ [M+H]: calcd 434.13466; found 434.13460. $C_{23}H_{19}N_3O_6$ (433.4): calc. C 63.74, H 4.42, N 9.70, O 22.15; found C, 63.68 %, H 4.38 %, N 9.75 %.

4-(Furan-2-yl)-6-(furan-3-yl)-9-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (36b)

Deprotection of **35b** (200 mg, 0.27 mmol) according to the general procedure E afforded compound **36b** (68 mg, 90 %) as white crystals. m.p. 110 – 115 °C, $[\alpha]_D$ -33.0 (c 0.25). IR (ATR): ν = 3359, 2365, 2342, 1748, 1561, 1540, 1472, 1094, 1042, 748 cm^{-1}.^1H NMR (499.8 MHz, DMSO-d_6): 3.70 (ddd, 1H, J_{gem}= 11.9, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 4.0, H-5'b); 3.76 (ddd, 1H, J_{gem}= 11.9, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.3, H-5'a); 4.01 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.3, $J_{4',3'}$ = 2.9, H-4'); 4.26 (ddd, 1H, $J_{3',2'}$ = 6.0, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 2.9, H-3'); 4.83 (ddd, 1H, $J_{2',1'}$ = 7.3,

$J_{2',OH}$ = 6.4, $J_{2',3'}$ = 6.0, H-2'); 5.20 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.22 (d, 1H, $J_{OH,5'}$ = 5.6, 5.0, OH-5'); 5.29 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.57 (d, $J_{1',2'}$ = 7.3, H-1'); 6.92 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl-4); 7.09 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl-6); 7.61 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl-4); 7.82 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl-6); 7.87 (dd, 1H, $J_{7,8}$ = 8.5, $J_{7,5}$ = 1.7, H-7); 8.12 (d, 1H, $J_{8,7}$ = 8.5, H-8); 8.31 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl-6); 8.48 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl-4); 8.95 (s, 1H, H-2); 9.03 (d, 1H, $J_{5,7}$ = 1.7, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.2 (CH-3'); 70.6 (CH-2'); 85.6 (CH-4'); 87.1 (CH-1'); 108.0 (C-4a); 109.2 (CH-4-furyl-6); 113.2 (CH-4-furyl-4); 113.4 (CH-8); 115.1 (CH-3-furyl-4); 119.7 (C-4b); 121.1 (CH-5); 126.1 (CH-7); 126.1 (C-6); 126.3 (C-3-furyl-6); 137.3 (C-8a); 139.1 (CH-2-furyl-6); 144.7 (CH-5-furyl-6); 147.0 (CH-5-furyl-4); 147.7 (C-4); 152.5 (C-2-furyl-4); 153.9 (CH-2); 157.0 (C-9a). ESI MS m/z (rel. %): 434 (100) [M+H]. HR MS (ESI) for $C_{23}H_{20}N_3O_6$ [M+H]: calcd 434.13466; found 434.13452. Anal. Calcd for $C_{23}H_{20}N_3O_6$: C, 63.74 %; H, 4.42 %; N, 9.70 %; O 22.15 %. Found C, 63.62 %; H, 4.39 %; N, 9.63 %.

4-(Furan-2-yl)-6-phenyl-9-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (36f)

Deprotection of **35f** (130 mg, 0.17 mmol) according to the general procedure E afforded compound **36f** (62 mg, 81 %) as white crystals. m.p. 174 -177 °C, $[\alpha]_D$ -30.3 (c 0.26). IR (ATR): ν = 3296, 2359, 2342, 1734, 1561, 1541, 1470, 1094, 1042, 758 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.72 (ddd, 1H, J_{gem}= 12.0, $J_{5'b,OH}$ = 5.6, $J_{5'b,4'}$ = 3.9, H-5'b); 3.77 (ddd, 1H, J_{gem}= 12.0, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 3.3, H-5'a); 4.02 (ddd, 1H, $J_{4',5'}$ = 3.9, 3.3, $J_{4',3'}$ = 3.0, H-4'); 4.27 (ddd, 1H, $J_{3',2'}$ = 5.9, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.0, H-3'); 4.85 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.9, H-2'); 5.19 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.22 (d, 1H, $J_{OH,5'}$ = 5.6, 5.2, OH-5'); 5.28 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.59 (d, $J_{1',2'}$ = 7.3, H-1'); 6.92 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.40 (m, 1H, H-*p*-Ph); 7.54 (m, 2H, H-*m*-Ph); 7.62 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.9, H-3-furyl); 7.80 (m, 2H, H-*o*-Ph); 7.90 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.8, H-7); 8.18 (d, 1H, $J_{8,7}$ = 8.6, H-8); 8.34 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.9, H-5-furyl); 8.97 (s, 1H, H-2); 9.11 (d, 1H, $J_{5,7}$ = 1.8, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.8 (CH_2-5'); 70.2 (CH-3'); 70.5 (CH-2'); 85.6 (CH-4'); 87.1 (CH-1'); 108.2 (C-4a); 113.2 (CH-4-furyl); 113.4 (CH-8); 115.1 (CH-3-furyl); 119.9 (C-4b); 122.5 (CH-5); 127.0 (CH-*o*-Ph); 127.0 (CH-7); 127.2 (CH-*p*-Ph); 129.4 (CH-*m*-Ph); 134.4 (C-6); 137.7 (C-8a); 140.8 (C-*i*-Ph); 146.6 (CH-5-furyl); 147.8 (C-4); 152.5 (C-2-furyl); 153.9 (CH-2); 157.1 (C-9a). ESI MS m/z (rel. %): 444 (100) [M+H]. HR MS (ESI) for $C_{25}H_{21}N_3O_5$ [M+H]: calcd 444.15540; found 444.15513. Anal. Calcd for

$C_{25}H_{21}N_3O_5$: C, 67.71 %; H, 4.77 %; N, 9.48 %; O 18.04 %. Found C, 67.93 %; H, 4.83 %; N, 9.39 %.

5.5 Synthesis of 4-amino-(5)6-hetarylpyrimidoindole nucleosides

4-Amino-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (37)

Compound **37** was prepared as described for **39** from protected nucleoside **25** (0.8 g; 1.24 mmol). Pure nucleoside **37** (282 mg, 72 %) was obtained as white crystals. m. p. 237-240 °C; $[\alpha]_D$ -47.0 (0.30); IR (ATR): ν = 3467, 3444, 3341, 2373, 2169, 2039, 1656, 1596, 1583, 1567, 1449, 1316, 1198, 1089, 1075, 1024, 740. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.63 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.9, $J_{5'b,4'}$ = 3.7, H-5'b); 3.71 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.2, H-5'a); 3.96 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 3.0, H-4'); 4.19 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 3.0, H-3'); 4.83 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.8, H-2'); 5.15 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.20 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2'); 5.48 (dd, 1H, $J_{OH,5'}$ = 6.9, 4.5, OH-5'); 6.33 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 7.29 (ddd, 1H, $J_{6,5}$ = 7.8, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.32 (bs, 2H, NH_2); 7.39 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 7.82 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.34 (dd, 1H, $J_{5,6}$ = 7.8, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.35 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.13 (CH_2-5'); 70.56 (CH-3'); 70.76 (CH-2'); 85.60 (CH-4'); 87.28 (CH-1'); 95.95 (C-4a); 111.67 (CH-8); 120.29 (C-4b); 121.22 (CH-6); 121.47 (CH-5); 124.90 (CH-7); 136.29 (C-8a); 154.53 (CH-2); 155.25 (C-9a); 157.93 (C-4). ESI MS m/z (rel. %): 317 (11) [M+H] 339 (100) [M+Na]. HR MS (ESI) for $C_{15}H_{17}N_4O_4$ [M+H]: calcd 317.12443; found 317.12448; for $C_{15}H1_6N_4O_4Na$ [M+Na]: calcd 339.10638; found 339.10638. Anal. Calcd. for $C_{15}H_{16}N_4O_4$ 1 H_2O: C, 53.89; H, 5.43; N, 16.76. Found C, 54.05; H, 5.28; N, 16.62.

4-Amino-5-chloro-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (38)

Compound **38** was prepared as described for **39** from 4,5-dichloro-9-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-pyrimido[4,5-*b*]indole (**26**) (0.5 g; 1.4 mmol) to give a pure nucleoside **38** (256 mg, 76 %) as white crystals. m.p. 117–122 °C; $[\alpha]_D$ -32.4 (0.26). IR (ATR): ν = 3 471, 3 321, 3 188, 2 925, 2 859, 1 627, 1 567, 1 556, 1 449, 1 319, 1 188, 1 120, 1 079, 1 041, 986, 770. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.7, H-5'b); 3.71 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.9, $J_{5'a,4'}$ = 3.1, H-5'a); 3.96 (dt, $J_{4',5'}$ = 3.7, 3.1, $J_{4',3'}$ = 3.1, H-4'); 4.19 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.1, H-3'); 4.75 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.7, H-2'); 5.13 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.19 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 5.33 (dd, 1H, $J_{OH,5'}$ = 6.1, 4.9, OH-5'); 6.43 (d, $J_{1',2'}$ = 7.3, H-1'); 7.26 (bs, 1H, NH_a**H_b**); 7.36

(dd, 1H, $J_{6,7}$ = 7.8, $J_{6,8}$ = 1.2, H-6); 7.39 (dd, 1H, $J_{7,8}$ = 8.0, $J_{7,6}$ = 7.8, H-7); 7.65 (bs, 1H, N**H**$_a$H$_b$); 7.94 (dd, 1H, $J_{8,7}$ = 8.0, $J_{8,6}$ = 1.2, H-8); 8.32 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.86 (CH_2-5'); 70.25 (CH-3'); 70.55 (CH-2'); 85.66 (CH-4'); 87.23 (CH-1'); 94.50 (C-4a); 111.66 (CH-8); 118.92 (C-4b); 122.78 (CH-6); 124.47 (C-5); 126.30 (CH-7); 137.98 (C-8a); 155.18 (CH-2); 155.80 (C-9a); 157.77 (C-4). ESI MS m/z (rel. %): 351 (100) [M+H], 373 (40) [M+Na]. HR MS (ESI) for $C_{15}H_{16}Cl_1N_4O_4$ [M+H]: calc. 351.08546; found 351.08552. Anal. Calcd for $C_{15}H_{15}ClN_4O_4 \cdot 0.55\ CH_3OH$: C, 50.70 %; H, 4.71 %; N, 15.21 %. Found C, 50.85 %; H, 4.76 %; N, 15.09 %.

4-Amino-6-chloro-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (39)

4,6-dichloro-9-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (**27**) (2.8 g; 4.1 mmol) was dissolved in dioxane (10 ml) and 30 % aqueous ammonia (30 ml) was added. Reaction mixture was stirred in screw-cap pressure glass tube at 100 °C for 24 hours, then cooled to r.t. and filtered. After drying under reduced pressure, desired product **39** (1.38 g, 96 %) was observed as white crystals. m.p. 174 °C, $[\alpha]_D$ -35.8 (0.29). IR (ATR): ν = 3426, 3325, 3159, 2956, 2931, 1721, 1703, 1663, 1644, 1597, 1569, 1451, 1433, 1288, 1269, 1177, 1131, 1096, 1062, 1024, 1008, 905, 848, 829, 794, 708. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.63 (ddd, J_{gem} = 12.1, $J_{5'b,OH}$ = 6.4, $J_{5'b,4'}$ = 3.7, H-5'b); 3.70 (ddd, J_{gem} = 12.1, $J_{5'a,OH}$ = 4.6, $J_{5'a,4'}$ = 3.3, H-5'a); 3.96 (ddd, $J_{4',5'}$ = 3.7, 3.3, $J_{4',3'}$ = 2.8, H-4'); 4.18 (ddd, $J_{3',2'}$ = 5.9, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.8, H-3'); 4.77 (ddd, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.9, H-2'); 5.14 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.20 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2'); 5.42 (dd, 1H, $J_{OH,5'}$ = 6.4, 4.6, OH-5'); 6.33 (d, $J_{1',2'}$ = 7.5, H-1'); 7.39 (dd, 1H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 7.46 (bs, 2H, NH_2); 7.88 (d, 1H, $J_{8,7}$ = 8.8, H-8); 8.30 (s, 1H, H-2); 8.51 (d, 1H, $J_{5,7}$ = 2.1, H-5).^{13}C NMR (125.7 MHz, DMSO-d_6): 62.01 (CH_2-5'); 70.47 (CH-3'); 70.82 (CH-2'); 85.68 (CH-4'); 87.23 (CH-1'); 95.29 (C-4a); 113.32 (CH-8); 120.80 (CH-5); 121.76 (C-4b); 124.63 (CH-7); 126.06 (C-6); 134.72 (C-8a); 155.23 (CH-2); 155.88 (C-9a); 157.97 (C-4). ESI MS m/z (rel. %): 373 (100) [M+Na], 351 (70) [M+H]. HR MS (ESI) for $C_{15}H_{16}O_4N_4Cl$ [M+H]: calc. 351.08546; found 351.08549. Anal. Calcd for $C_{15}H_{15}ClN_4O_4 \cdot 0.2\ CH_3OH$: C, 51.11 %; H, 4.46 %; N, 15.69 %. Found C, 51.62 %; H, 4.56 %; N, 15.89 %.

6-Chloro-4-(1,4-dioxan-2-yl)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (40)

Nucleoside **40** was isolated from reaction of nucleoside **27** (300 mg, 0.43 mmol) with aqueous ammonia (2 ml) in dioxane (2 ml). Reaction mixture was heated to 100 °C for 16 h, solvents were evaporated and crude material was purified by column chromatography on silica (chloroform/methanol from 1 to 5 %). Unexpected nucleoside **40** (30 mg, 16 %) was

isolated as white solid. The rest of the material was 5-O´-benzoylated derivative of amino nucleoside **39**. Spectra for **40** (1:1 mixture of diastereoisomers): ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.65 (m, 2H, H-5b-dioxane); 3.67-3.74 (m, 4H, H-5'); 3.76 (m, 2H, H-5a-dioxane); 3.78-3.86 (m, 4H, H-3b,6b-dioxane); 3.94 (m, 2H, H-6a-dioxane); 4.00 (m, 2H, H-4'); 4.05, 4.08 (2 × dd, 2 × 1H, $J_{3a,3b}$ = 11.3, $J_{3a,2}$ = 2.9, H-3a-dioxane); 4.25 (m, 2H, H-3'); 4.77-4.84 (m, 4H, H-2', H-2-dioxane); 5.13 (bt, 2H, $J_{OH,5'}$ = 5.5, OH-5'); 5.258, 5.261 (2 × d, 2 × 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.30, 5.31 (2 × d, 2 × 1H, $J_{OH,2'}$ = 6.4, OH-2'); 6.447, 6.450 (2 × d, 2 × 1H, $J_{1',2'}$ = 7.4, H-1'); 7.58 (dd, 2H, $J_{7,8}$ = 8.8, $J_{7,5}$ = 2.1, H-7); 8.09, 8.10 (2 × d, 2 × 1H, $J_{8,7}$ = 8.8, H-8); 8.46 (d, 2H, $J_{5,7}$ = 2.1, H-5); 9.536, 9.538 (2 × s, 2 × 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.83 (CH_2-5'); 65.93 (CH_2-5-dioxane); 66.20, 66.22 (CH_2-6-dioxane); 69.51, 69.54 (CH_2-3-dioxane); 70.33 (CH-3'); 70.61, 70.62 (CH-2-dioxane); 70.33 (CH-2'); 85.76, 85.78 (CH-4'); 86.75, 86.78 (CH-1'); 112.77, 112.78 (C-4a); 114.90, 114.92 (CH-8); 120.77 (C-4b); 121.77 (CH-5); 126.64 (C-6); 127.85 (CH-7); 136.83, 136.84 (C-8a); 150.05, 150.06 (CH-2); 157.79, 155.80 (C-9a); 162.49 (C-4). ESI MS m/z (rel. %): 444 (100) [M+Na], 422 (78) [M+H]. HR MS (ESI) for $C_{19}H_{20}O_6N_3Cl$ [M+H]: calc. 421.1041; found 421.1046.

4-Amino-6-furan-2-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (41a)

Free aminonucleoside **39** (150 mg, 0.43 mmol) and 2-(tributylstannyl)furan (230 mg, 0.64 mmol) were used. Desired product **41a** (111 mg, 68 %) was obtained as white powder. m.p. 219-222 °C; $[\alpha]_D$ -34.8 (0.28). IR (ATR): ν = 3343, 3215, 3128, 1633, 1597, 1570, 1463, 1311, 1084, 1043, 1012, 800, 736. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.64 (dd, J_{gem} = 11.9, $J_{5'b,4'}$ = 3.7, H-5'b); 3.72 (dd, J_{gem} = 11.9, $J_{5'a,4'}$ = 3.2, H-5'a); 3.97 (ddd, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 2.9, H-4'); 4.20 (dd, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 2.9, H-3'); 4.82 (dd, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.8, H-2'); 5.45 (bs, 3H,OH-2',3',5'); 6.33 (d, $J_{1',2'}$ = 7.2, H-1'); 6.62 (dd, 1H, $J_{4,3}$ = 3.3, $J_{4,5}$ = 1.8, H-4-furyl); 7.04 (dd, 1H, $J_{3,4}$ = 3.3, $J_{3,5}$ = 0.8, H-3-furyl); 7.46 (bs, 2H, NH_2); 7.737 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.743 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H-5-furyl); 7.87 (d, 1H, $J_{8,7}$ = 8.6, H-8); 8.29 (s, 1H, H-2); 8.64 (d, 1H, $J_{5,7}$ = 1.7, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.11 (CH_2-5'); 70.58 (CH-3'); 70.94 (CH-2'); 85.67 (CH-4'); 87.38 (CH-1'); 95.93 (C-4a); 104.96 (CH-3-furyl); 112.01 (CH-8); 112.19 (CH-4-furyl); 116.58 (CH-5); 120.58 (CH-7); 120.80 (C-4b); 124.36 (C-6); 135.67 (C-8a); 142.33 (CH-5-furyl); 154.14 (C-2-furyl); 154.83 (CH-2); 155.79 (C-9a); 157.98 (C-4). ESI MS m/z (rel. %): 383 (100) [M+H]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13516. Anal. Calcd for $C_{19}H_{18}N_4O_5 \cdot 1\ CH_3OH$: C, 57.97 %; H, 5.35 %; N, 13.52 %. Found C, 58.06 %; H, 5.32 %; N, 13.60 %.

4-Amino-6-furan-3-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (41b)

Free aminonucleoside **39** (100 mg, 0.29 mmol) and furan-3-ylboronic acid (48 mg, 0.43 mmol) were used. Desired product **41b** (111 mg, 72 %) was obtained as white powder. m.p. 121-122 °C; $[\alpha]_D$ -41.6 (0.33). IR (ATR): ν = 3330, 3164, 1652, 1593, 1571, 1464, 1307, 1077, 1046, 1029, 794, 596. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.64 (bddd, J_{gem} = 11.8, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.3, H-5'b); 3.72 (bdt, J_{gem} = 11.8, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 3.3, H-5'a); 3.97 (td, $J_{4',5'}$ = 3.3, $J_{4',3'}$ = 2.7, H-4'); 4.20 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.7, H-3'); 4.82 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.7, H-2'); 5.13 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.19 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2'); 5.47 (bdd, 1H, $J_{OH,5'}$ = 6.1, 3.3, OH-5'); 6.33 (d, $J_{1',2'}$ = 7.3, H-1'); 7.19 (dd, 1H, $J_{4,5}$ = 1.8, $J_{4,2}$ = 0.8, H-4-furyl); 7.43 (bs, 2H, NH_2); 7.67 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.76 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,2}$ = 1.5, H-5-furyl); 7.83 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.4, H-8); 8.25 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.8, H-2-furyl); 8.28 (s, 1H, H-2); 8.51 (dd, 1H, $J_{5,7}$ = 1.7, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.10 (CH_2-5'); 70.56 (CH-3'); 70.89 (CH-2'); 85.64 (CH-4'); 87.33 (CH-1'); 95.93 (C-4a); 109.30 (CH-4-furyl); 112.01 (CH-8); 117.99 (CH-5); 120.83 (C-4b); 122.64 (CH-7); 125.52 (C-6); 126.55 (C-3-furyl); 135.35 (C-8a); 138.94 (CH-2-furyl); 144.16 (CH-5-furyl); 154.68 (CH-2); 155.59 (C-9a); 157.90 (C-4). ESI MS m/z (rel. %): 383 (30) [M+H]; 405 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13495; for $C_{19}H_{18}N_4O_5Na$ [M+Na]: calcd 405.11694; found 405.11683. Anal. Calcd for $C_{19}H_{18}N_4O_5 \cdot 1\ H_2O$: C, 57.00 %; H, 5.03 %; N, 13.99 %. Found C, 56.85 %; H, 4.89 %; N, 13.96 %.

4-Amino-6-thiophen-2-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (41c)

Free aminonucleoside **39** (100 mg, 0.29 mmol) and 2-(tributylstannyl)thiophene (160 mg, 0.43 mmol) were used. Desired product **41c** (83 mg, 73 %) was obtained as white powder. m.p. 228-230 °C; $[\alpha]_D$-50.0 (0.18). IR (ATR): ν = 3396, 3301, 1688, 1674, 1659, 1545, 1511, 1502, 1401, 1381, 1243, 1160, 965, 762. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.64 (bd, J_{gem} = 12.4, H-5'b); 3.72 (bdd, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.1, H-5'a); 3.97 (q, $J_{4',5'}$ = $J_{4',3'}$ = 3.1, H-4'); 4.20 (dd, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 3.1, H-3'); 4.82 (dd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.7, H-2'); 5.20 (bs, 2H, OH-2',3'); 5.47 (bs, 1H, OH-5'); 6.33 (d, $J_{1',2'}$ = 7.3, H-1'); 7.17 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,3}$ = 3.6, H-4-thienyl); 7.51 (bs, 2H, NH_2); 7.51 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,3}$ = 1.2, H-5-thienyl); 7.63 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.8, H-7); 7.69 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.2, H-3-thienyl); 7.89 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.4, H-8); 8.29 (s, 1H, H-2); 8.63 (dd, 1H, $J_{5,7}$ = 1.8, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.11 (CH_2-5'); 70.57 (CH-3'); 70.89 (CH-2'); 85.68 (CH-4'); 87.28 (CH-1'); 95.91 (C-4a); 112.26 (CH-8); 117.93 (CH-5); 121.05 (C-4b); 122.92 (CH-7);

123.54 (CH-3-thienyl); 124.98 (CH-5-thienyl); 127.67 (C-6); 128.50 (CH-4-thienyl); 135.73 (C-8a); 144.41 (C-2-thienyl); 154.90 (CH-2); 155.85 (C-9a); 158.01 (C-4). ESI MS m/z (rel. %): 399 (65) [M+H]; 421 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11219. Anal. Calcd for $C_{19}H_{18}N_4O_4S \cdot 1.1\ H_2O$: C, 54.56 %; H, 4.87 %; N, 13.40 %; S, 7.67 %. Found C, 54.31 %; H, 4.78 %; N, 13.28 %; S, 7.58 %.

4-Amino-6-thiophen-3-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-b]indole (41d)

Nucleoside **41d** was prepared according to the general procedure C. Free aminonucleoside **39** (50 mg, 0.14 mmol) and thiophene-3-boronic acid (27.0 mg, 0.21 mmol) were used. Desired product **41d** (43 mg, 77 %) was obtained as white powder. m.p. 167-169 °C; $[\alpha]_D$ -38.9 (0.30). IR (ATR): ν = 3373, 3364, 1640, 1630, 1469, 1080, 782. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.7, $J_{5'b,4'}$ = 3.7, H-5'b); 3.72 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.4, H-5'a); 3.99 (ddd, $J_{4',5'}$ = 3.7, 3.4, $J_{4',3'}$ = 2.8, H-4'); 4.21 (bdd, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.8, H-3'); 4.83 (bdd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.5, H-2'); 5.14 (bs, 1H, OH-3'); 5.20 (bs, 1H, OH-2'); 5.47 (dd, 1H, $J_{OH,5'}$ = 6.7, 4.5, OH-5'); 6.34 (d, $J_{1',2'}$ = 7.3, H-1'); 7.46 (bs, 2H, NH_2); 7.66 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,2}$ = 3.0, H-5-thienyl); 7.79 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.7, H-7); 7.82 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,2}$ = 1.4, H-4-thienyl); 7.85 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.5, H-8); 7.97 (dd, 1H, $J_{2,5}$ = 3.0, $J_{2,4}$ = 1.4, H-2-thienyl); 8.29 (s, 1H, H-2); 8.63 (dd, 1H, $J_{5,7}$ = 1.7, $J_{5,8}$ = 0.5, H-5). ^{13}C NMR (150.9 MHz, DMSO-d_6): 62.09 (CH_2-5'); 70.54 (CH-3'); 70.87 (CH-2'); 85.62 (CH-4'); 87.31 (CH-1'); 96.03 (C-4a); 111.99 (CH-8); 118.55 (CH-5); 119.96 (CH-2-thienyl); 120.90 (C-4b); 123.22 (CH-7); 126.74 (CH-5-thienyl); 126.93 (CH-4-thienyl); 128.93 (C-6); 135.46 (C-8a); 142.14 (C-3-thienyl); 154.69 (CH-2); 155.70 (C-9a); 157.93 (C-4). ESI MS m/z (rel. %): 399 (100) [M+H]; 421 (95) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11211. Anal. Calcd for $C_{19}H_{18}N_4O_4S \cdot 1.15\ H_2O$: C, 54.45 %; H, 4.88 %; N, 13.37 %. Found C, 54.39 %; H, 4.92 %; N, 13.47 %.

4-Amino-6-benzofuran-2-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (41e)

Nucleoside **41e** was prepared according to the general procedure C. Free aminonucleoside **39** (150 mg, 0.43 mmol) and 2-benzofurylboronic acid (140.0 mg, 0.86 mmol) were used. Desired product **41e** (138 mg, 75 %) was obtained as yellowish crystals. m.p. 265-268 °C; $[\alpha]_D$ -53.3 (0.30). IR (ATR): ν = 3342, 3202, 2940, 2372, 1632, 1594, 1569, 1452, 1084, 1042, 799, 750. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.66 (bddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 3.6, H-5'b); 3.74 (bdt, J_{gem} = 12.0, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 3.6, H-5'a); 4.00 (td, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.8, H-4'); 4.22 (bdd, $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 2.8, H-3'); 4.86 (bdd, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.6, H-2'); 5.16 (bs, 1H,OH-3'); 5.24 (bs, 1H,OH-2'); 5.47 (bdd, 1H, $J_{OH,5'}$ = 6.1, 3.6, OH-5'); 6.37 (d, $J_{1',2'}$

= 7.2, H-1'); 7.27 (ddd, 1H, $J_{5,4}$ = 7.5, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.31 (ddd, 1H, $J_{6,7}$ = 8.1, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.5, H-6-benzofuryl); 7.54 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.55 (bs, 2H, NH_2); 7.64 (dtd, 1H, $J_{7,6}$ = 8.1, $J_{7,3}$ = $J_{7,5}$ = 1.0, $J_{7,4}$ = 0.7, H-7-benzofuryl); 7.68 (ddd, 1H, $J_{4,5}$ = 7.5, $J_{4,6}$ = 1.5, $J_{4,7}$ = 0.7, H-4-benzofuryl); 7.96 (dd, 1H, $J_{7,8}$ = 8.6, $J_{7,5}$ = 1.5, H-7); 7.98 (dd, 1H, $J_{8,7}$ = 8.6, $J_{8,5}$ = 0.6, H-8); 8.33 (s, 1H, H-2); 8.89 (dd, 1H, $J_{5,7}$ = 1.5, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.09 (CH_2-5'); 70.57 (CH-3'); 70.91 (CH-2'); 85.73 (CH-4'); 87.36 (CH-1'); 95.90 (C-4a); 101.03 (CH-3-benzofuryl); 111.14 (CH-7-benzofuryl); 112.17 (CH-8); 117.97 (CH-5); 120.94 (C-4b); 120.97 (CH-3-benzofuryl); 121.60 (CH-7); 123.32 (CH-5-benzofuryl); 123.44 (C-6); 124.42 (CH-6-benzofuryl); 129.37 (C-3a-benzofuryl); 136.60 (C-8a); 154.33 (C-7a-benzofuryl); 155.02 (CH-2); 155.97 (C-9a); 156.49 (C-2-benzofuryl); 158.03 (C-4). ESI MS m/z (rel. %): 433 (92) [M+H]; 455 (100) [M+Na]. HR MS (ESI) for $C_{23}H_{21}N_4O_5$ [M+H]: calcd 433.15065; found 433.15073; calcd 455.13259; for $C_{23}H_{20}N_4O_5Na$ [M+H]: found 455.13266. Anal. Calcd. for $C_{23}H_{20}N_4O_5 \cdot 1.3\ H_2O$: C, 60.60; H, 5.00; N, 12.29. Found C, 60.85; H, 4.89; N, 12.04.

4-Amino-6-phenyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (41f)

Nucleoside **39** (50 mg, 0.14 mmol), phenylboronic acid (25 mg, 0.21 mmol)), K_2CO_3 (58 mg, 0.42 mmol), $Pd(OAc)_2$ (1.6 mg, 0.007 mmol) and X-Phos (6.7 mg, 0.014 mmol) were dissolved in acetonitrile/water mixture (3:2, 5 ml) and heated to 100 °C for 16 hours. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Product **41f** (52 mg, 93 %) was obtained as white powder after recrystallization from MeOH/H_2O mixture. m.p. 216-218 °C; $[\alpha]_D$ -49.8 (0.20). IR (ATR): ν =3437, 3329, 3156, 1651, 1629, 1594, 1570, 1468, 1405, 1316, 114, 1045, 1022, 988, 717.^{1}H NMR (499.8 MHz, DMSO-d_6): 3.65 (ddd, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.8, $J_{5'b,4'}$ = 3.7, H-5'b); 3.73 (ddd, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.5, $J_{5'a,4'}$ = 3.2, H-5'a); 3.98 (ddd, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 2.9, H-4'); 4.20 (ddd, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.9, H-3'); 4.84 (ddd, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 5.7, H-2'); 5.15 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3'); 5.22 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2'); 5.50 (dd, 1H, $J_{OH,5'}$ = 6.8, 4.5, OH-5'); 6.36 (d, $J_{1',2'}$ = 7.3, H-1'); 7.35 (m, 1H, H-*p*-Ph); 7.48 (m, 4H, NH_2, H-*m*-Ph); 7.71 (dd, 1H, $J_{7,8}$ = 8.7, $J_{7,5}$ = 1.9, H-7); 7.86 (m, 2H, H-*o*-Ph); 7.91 (dd, 1H, $J_{8,7}$ = 8.9, $J_{8,5}$ = 0.4, H-8); 8.29 (s, 1H, H-2); 8.62 (dd, 1H, $J_{5,7}$ = 1.9, $J_{5,8}$ = 0.4, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.15 (CH_2-5'); 70.61 (CH-3'); 70.89 (CH-2'); 85.69 (CH-4'); 87.32 (CH-1'); 96.13 (C-4a); 112.13 (CH-8); 119.36 (CH-5); 121.08 (C-4b); 123.81 (CH-7); 127.00 (CH-*p*-Ph); 127.25 (CH-*o*-Ph); 128.96 (CH-*m*-Ph); 133.82 (C-6); 135.83 (C-8a); 140.78 (C-*i*-Ph); 154.74 (CH-2); 155.77 (C-9a); 158.03 (C-4). ESI MS m/z (rel. %): 393 (100) [M+H]. HR MS

(ESI) for $C_{21}H_{21}N_4O_4$ [M+H]: calcd 393.15573; found 393.15563. Anal. Calcd for $C_{21}H_{20}N_4O_4$: C, 61.05 %; H, 5.44 %; N, 13.56 %. Found C, 60.92 %; H, 5.35 %; N, 13.44 %.

4-Amino-5-furan-2-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (42a)

Compound **42a** was prepared according to the general procedure D. Free aminonucleoside **38** (150 mg, 0.43 mmol), $Pd(OAc)_2$ (12 mg, 0.05 mmol), X-Phos (50 mg, 0.1 mmol) and 2-(tributylstannyl)furan (230 mg, 0.64 mmol) were used. RP-HPFC purification furnished nucleoside **42a** (54 mg, 33 %) as white solid; m.p. 112-113 °C; $[\alpha]_D$ -37.8 (0.21). IR (ATR): ν = 3329, 3285, 2373, 2351, 2170, 1557, 1451, 1073, 1040, 788, 739. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.66 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.4, $J_{5'b,4'}$ = 3.7, H-5'b); 3.73 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 4.7, $J_{5'a,4'}$ = 3.2, H-5'a); 3.98 (ddd, 1H, $J_{4',5'}$ = 3.7, 3.2, $J_{4',3'}$ = 3.0, H-4'); 4.21 (ddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.0, H-3'); 4.82 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.9, H-2'); 5.15 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.22 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 5.41 (dd, 1H, $J_{OH,5'}$ = 6.4, 4.7, OH-5'); 6.44 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 6.74 (dd, 1H, $J_{3,4}$ = 3.2, $J_{3,5}$ = 0.8, H-3-furyl); 6.77 (dd, 1H, $J_{4,3}$ = 3.2, $J_{4,5}$ = 1.9, H-4-furyl); 7.31 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.46 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.94 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,3}$ = 0.8, H-5-furyl); 8.01 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.27 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.00 (CH_2-5'); 70.41 (CH-3'); 70.65 (CH-2'); 85.66 (CH-4'); 87.22 (CH-1'); 95.56 (C-4a); 110.77 (CH-3-furyl); 111.92 (CH-4-furyl); 112.93 (CH-8); 119.61 (C-4b); 124.05 (CH-6); 125.22 (C-5); 124.78 (CH-7); 136.86 (C-8a); 144.01 (CH-5-furyl); 152.53 (C-2-furyl); 154.68 (CH-2); 155.93 (C-9a); 157.79 (C-4). ESI MS m/z (rel. %): 383 (43) [M+H]; 405 (100) [M+Na]. HR MS (ESI) for $C_{19}H_{19}N_4O_5$ [M+H]: calcd 383.13500; found 383.13505. Anal. Calcd for $C_{19}H_{18}N_4O_5$: C, 59.68 %; H, 4.74 %; N, 14.65 %. Found C, 59.96 %; H, 4.86 %; N, 14.89 %.

4-Amino-5-thiophen-3-yl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (42d)

Free nucleoside **38** (200 mg, 0.57 mmol), K_2CO_3 (236 mg, 1.71 mmol), $Pd(OAc)_2$ (12.8 mg, 0.053 mmol), X-Phos (54.0 mg, 0.110 mmol) and one third of all amount of 3-furanboronic acid (146 mg, 1.14 mmol) were dissolved in anhydrous DMF (20 ml) and heated to 120 °C for 3 hours. Second third of boronic acid was added and reaction was stirred at 120 °C for 3 hours. Then, last third of boronic acid was added and reaction was heated for another 3 hours at 120 °C. Solvent was evaporated under reduced pressure and crude product was purified by RP-HPFC ($MeOH/H_2O$, 0 –> 100 % MeOH). Nucleoside **42d** was obtained (54 mg, 24 %) as white crystals; m.p. 141 °C; $[\alpha]_D$-39.4 (0.32). IR (ATR): ν = 3464, 3313, 3200, 2919, 2870, 2372, 2346, 1629, 1577, 1559, 1454, 1320, 1077, 1042, 738. ^{1}H NMR

(500.0 MHz, DMSO-d_6): 3.65 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 3.5, H-5'b); 3.73 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.2, H-5'a); 3.98 (dt, 1H, $J_{4',5'}$ = 3.5, 3.2, $J_{4',3'}$ = 3.2, H-4'); 4.21 (dd, 1H, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 3.2, H-3'); 4.84 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.8, H-2'); 5.20-5.50 (bm, 3H, OH-2',3',5'); 6.44 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 7.14 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.36 (dd, 1H, $J_{4,5}$ = 4.8, $J_{4,2}$ = 1.3, H-4-thienyl); 7.42 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.74 (dd, 1H, $J_{2,5}$ = 2.9, $J_{2,4}$ = 1.3, H-2-thienyl); 7.80 (dd, 1H, $J_{5,4}$ = 4.8, $J_{5,2}$ = 2.9, H-5-thienyl); 7.91 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.23 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.04 (CH_2-5'); 70.43 (CH-3'); 70.60 (CH-2'); 85.60 (CH-4'); 87.27 (CH-1'); 95.71 (C-4a); 111.33 (CH-8); 119.75 (C-4b); 123.65 (CH-6); 124.71 (CH-7); 125.65 (CH-2-thienyl); 127.10 (CH-5-thienyl); 130.34 (C-5); 130.58 (CH-4-thienyl); 136.75 (C-8a); 142.07 (C-3-thienyl); 154.38 (CH-2); 155.76 (C-9a); 157.56 (C-4). ESI MS m/z (rel. %): 399 (100) [M+H]. HR MS (ESI) for $C_{19}H_{19}N_4O_4S$ [M+H]: calcd 399.11215; found 399.11218. Anal. Calcd for $C_{19}H_{18}N_4O_4S \cdot 1.0\ H_2O$: C, 54.80 %; H, 4.84 %; N, 13.45 %; S, 7.70 %. Found C, 54.68 %; H, 4.86 %; N, 13.55 %; S, 7.60 %.

4-Amino-5-phenyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (42f)

Nucleoside **38** (200 mg, 0.57 mmol), phenylboronic acid (139 mg, 1.14 mmol)), K_2CO_3 (236 mg, 1.14 mmol), $Pd(OAc)_2$ (12.0 mg, 0.05 mmol) and X-Phos (54.0 mg, 0.11 mmol) were dissolved in DMF (5 ml) and heated to 120 °C for 16 hours. The volatiles were removed in vacuo and the residue was purified by RP-HPFC on C-18 (0→100% MeOH in water). Product **42f** (62 mg, 28 %) was obtained as white powder after recrystallization from MeOH/H_2O mixture; m.p. 185-157 °C; $[\alpha]_D$ -28.2 (0.26). IR (ATR): ν = 3469, 3378, 2934, 1578, 1561, 1456, 1321, 1092, 1029, 794, 765, 705. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.66 (dd, J_{gem} = 12.0, $J_{5'b,4'}$ = 3.8, H-5'b); 3.73 (dd, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.1, H-5'a); 3.98 (dt, $J_{4',5'}$ = 3.8, 3.1, $J_{4',3'}$ = 3.1, H-4'); 4.22 (dd, 1H, $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 3.1, H-3'); 4.86 (dd, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.8, H-2'); 5.28, 5.43 (2 × bs, 3H, OH-2',3',5'); 6.46 (d, $J_{1',2'}$ = 7.3, H-1'); 7.13 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 1.0, H-6); 7.45 (dd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.4, H-7); 7.51 (bm, 2H, H-*o*-Ph); 7.56 (m, 3H, H-*m*,*p*-Ph); 7.92 (dd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, H-8); 8.22 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.07 (CH_2-5'); 70.45 (CH-3'); 70.62 (CH-2'); 85.62 (CH-4'); 87.31 (CH-1'); 95.58 (C-4a); 111.20 (CH-8); 119.21 (C-4b); 123.39 (CH-6); 124.86 (CH-7); 128.61 (CH-*p*-Ph); 128.67 (CH-*m*-Ph); 130.04 (CH-*o*-Ph); 135.39 (C-5); 136.72 (C-8a); 141.73 (C-*i*-Ph); 154.37 (CH-2); 155.86 (C-9a); 157.37 (C-4). ESI MS m/z (rel. %): 393 (100) [M+H]; 415 (89) [M+Na]. HR MS (ESI) for $C_{21}H_{21}N_4O_4$ [M+H]: calcd 393.15573; found 393.15573.

Anal. Calcd for $C_{21}H_{20}N_4O_4 \cdot 1\ H_2O$: C, 61.46 %; H, 5.40 %; N, 13.65 %. Found C, 61.32 %; H, 5.52 %; N, 13.48 %.

4-Amino-5-butyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (42g)

Compound **42g** was obtained as unexpected product according to the general procedure D. Free aminonucleoside **38** (150 mg, 0.43 mmol) and 2-(tributylstannyl)thiophene (230 mg, 0.64 mmol) were used. Unexpected product **42g** (66 mg, 42 %) was obtained as white powder (as the only product); m.p. 125-127 °C; $[\alpha]_D$ -31.2 (0.24). IR (ATR): ν = 3542, 3384, 3363, 2965, 2169, 1637, 1591, 1559, 1456, 1327, 1056, 1023, 991, 863. ^{1}H NMR (500.0 MHz, DMSO-d_6): 0.88 (t, 3H, J_{vic} = 7.4, C**H$_3$**CH$_2$CH$_2$CH$_2$); 1.33 (m, 2H, CH$_3$C**H$_2$**CH$_2$CH$_2$); 1.63 (m, 2H, CH$_3$CH$_2$C**H$_2$**CH$_2$); 3.22 (dd, 2H, J_{vic} = 8.4, 7.1, CH$_3$CH$_2$CH$_2$C**H$_2$**); 3.63 (ddd, J_{gem} = 11.9, $J_{5'b,OH}$ = 6.5, $J_{5'b,4'}$ = 4.0, H-5'b); 3.71 (ddd, J_{gem} = 11.9, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.2, H-5'a); 3.95 (dt, $J_{4',5'}$ = 4.0, 3.3, $J_{4',3'}$ = 3.3, H-4'); 4.20 (bm, 1H, H-3'); 4.83 (bddd, $J_{2',1'}$ = 7.1, $J_{2',OH}$ = 6.3, $J_{2',3'}$ = 4.3, H-2'); 5.12 (bs, 1H, OH-3'); 5.17 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2'); 5.39 (dd, 1H, $J_{OH,5'}$ = 6.5, 4.8, OH-5'); 6.39 (d, $J_{1',2'}$ = 7.1, H-1'); 6.76 (bs, 2H, NH$_2$); 7.10 (dd, 1H, $J_{6,7}$ = 7.4, $J_{6,8}$ = 0.8, H-6); 7.31 (dd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.4, H-7); 7.68 (dd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 0.8, H-8); 8.26 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 13.99 (**C**H$_3$CH$_2$CH$_2$CH$_2$); 21.82 (CH$_3$**C**H$_2$CH$_2$CH$_2$); 33.27 (CH$_3$CH$_2$**C**H$_2$CH$_2$); 35.64 (CH$_3$CH$_2$CH$_2$**C**H$_2$); 62.04 (CH$_2$-5'); 70.37 (CH-3'); 70.44 (CH-2'); 85.46 (CH-4'); 87.34 (CH-1'); 96.82 (C-4a); 109.68 (CH-8); 119.33 (C-4b); 123.00 (CH-6); 125.27 (CH-7); 135.86 (C-5); 137.13 (C-8a); 153.68 (CH-2); 155.63 (C-9a); 158.24 (C-4). ESI MS m/z (rel. %): 373 (100) [M+H]. HR MS (ESI) for $C_{19}H_{25}N_4O_4$ [M+H]: calcd 373.18703; found 373.18707. Anal. Calcd. for $C_{19}H_{24}N_4O_4 \cdot 0.85\ CH_3OH$: C, 59.66; H, 6.91; N, 14.02. Found C, 59.86; H, 6.52; N, 13.65.

5.6 Synthesis of pyrimidoindole nucleosides substituted in position 4

4-Methyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (43a)

(Me)$_3$Al (310 μl, 2M in toluene) was added to solution of nucleoside **25** (200 mg, 0.31 mmol) and Pd(PPh$_3$)$_4$ (17.9 mg, 0.015 mmol) in THF (8 ml) and the reaction mixture was stirred at 70 °C for 12 hours. Volatiles were removed under reduced pressure and crude product was purified by HPFC (10 – 50 % EtOAc in hexane) to give **43a** (120 mg, 63 %) as white solid; m.p. 155-158 °C; IR (ATR): ν = 1733, 1497, 1457, 1420, 1278, 1263, 1136, 1113, 1091, 1072, 727, 707. ^{1}H NMR (499.8 MHz, DMSO-d_6): 2.94 (s, 3H, CH$_3$); 4.68 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 4.1, H-5'b); 4.82 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 3.2, H-5'a); 4.89 (ddd,

1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.1, 3.2, H-4'); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.65 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.8, H-2'); 6.99 (d, 1H, $J_{1',2'}$ = 4.8, H-1'); 7.40 (m, 2H, H-*m*-Bz-2'); 7.44 (m, 1H, H-6); 7.45 (m, 1H, H-7); 7.49, 7.51 (2 × m, 2 × 2H, H-*m*-Bz-3',5'); 7.61 (m, 1H, H-*p*-Bz-2'); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.82 (m, 2H, H-*o*-Bz-2'); 7.95 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.01 (m, 1H, H-8); 8.20 (m, 1H, H-5); 8.78 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 23.00 (CH_3); 63.24 (CH_2-5'); 70.39 (CH-3'); 72.08 (CH-2'); 78.61 (CH-4'); 85.96 (CH-1'); 111.65 (CH-8); 112.77 (C-4a); 119.84 (C-4b); 122.69 (CH-6); 123.40 (CH-5); 127.71 (CH-7); 128.63, 128.87 (C-*i*-Bz); 128.97, 129.01, 129.03 (CH-*m*-Bz); 129.45 (CH-*o*-Bz-5', C-*i*-Bz); 129.53 (CH-*o*-Bz-2'); 129.67 (CH-*o*-Bz-3'); 133.84, 134.17 (CH-*p*-Bz); 137.98 (C-8a); 154.01 (CH-2); 154.45 (C-9a); 160.64 (C-4); 164.84 (COPh-2'); 165.10 (COPh-3'); 165.66 (COPh-5'). ESI MS m/z (rel. %): 628 (39) [M+H] 650 (100) [M+Na]. HR MS (ESI) for $C_{37}H_{30}N_3O_7$ [M+H]: calcd 628.20783; found 628.20790; for $C_{37}H_{29}N_3O_7Na$ [M+Na]: calcd 650.18977; found 650.18975.

4-Ethyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (43b)

$(Et)_3Al$ (760 μl, 1M in hexane) was added to solution of nucleoside **25** (250 mg, 0.38 mmol) and $Pd(PPh_3)_4$ (22.0 mg, 0.019 mmol) in THF (12 ml) and the reaction mixture was stirred at 70 °C for 12 hours. Volatiles were removed under reduced pressure and crude product was purified by HPFC (10 – 50 % EtOAc in hexane) to give **43b** (188 mg, 75 %) as white solid; m.p. 145-146 °C; IR (ATR): ν = 2982, 2934, 1728, 1587, 1572, 1451, 1256, 1135, 1108, 1090, 1070, 707. ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.38 (t, 3H, J_{vic} = 7.5, C**H**$_3$CH_2); 3.30 (q, 2H, J_{vic} = 7.5, CH_3C**H**$_2$); 4.68 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.2, H-5'b); 4.82 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.1, H-5'a); 4.89 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.2, 3.1, H-4'); 6.37 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.67 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.7, H-2'); 7.01 (d, 1H, $J_{1',2'}$ = 4.7, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.42 (m, 1H, H-6); 7.47 (m, 1H, H-7); 7.49, 7.51 (2 × m, 2 × 2H, H-*m*-Bz-3',5'); 7.61 (m, 1H, H-*p*-Bz-2'); 7.68 (m, 2H, H-*p*-Bz-3',5'); 7.83 (m, 2H, H-*o*-Bz-2'); 7.96 (m, 2H, H-*o*-Bz-5'); 7.99 (m, 2H, H-*o*-Bz-3'); 8.03 (m, 1H, H-8); 8.19 (m, 1H, H-5); 8.84 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 11.77 (**C**H_3CH_2); 28.88 (CH_3**C**H_2); 63.24 (CH_2-5'); 70.36 (CH-3'); 72.08 (CH-2'); 78.54 (CH-4'); 85.92 (CH-1'); 111.65 (CH-8); 111.95 (C-4a); 119.40 (C-4b); 122.70 (CH-6); 123.35 (CH-5); 127.63 (CH-7); 128.62, 128.84 (C-*i*-Bz); 128.91, 128.95, 128.97 (CH-*m*-Bz); 129.40 (CH-*o*-Bz-5'); 129.42 (C-*i*-Bz); 129.49 (CH-*o*-Bz-2'); 129.62 (CH-*o*-Bz-3'); 133.77, 134.10 (CH-*p*-Bz); 137.99 (C-8a); 154.15 (CH-2); 154.56 (C-9a); 164.79 (COPh-2'); 165.03 (COPh-3'); 165.09 (C-4); 165.59 (COPh-5'). ESI

MS m/z (rel. %): 642 (85) [M+H] 664 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{32}N_3O_7$ [M+H]: calcd 642.22334; found 642.22348.

4-Cyclopropyl-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (43c)

Nucleoside **43c** was prepared in analogy to literature conditions.[136] THF (2 ml) was added to dried zinc chloride (84.5 mg, 0.62 mmol) under argon atmosphere. Mixture was cooled to -10 °C and cyclopropylmagnesium chloride (1.24 ml, 0.5 M in THF) was added dropwise. After 40 minutes of stirring the solution of nucleoside **25** (200 mg, 0.31 mmol) and $Pd(PPh_3)_4$ (35.8 mg, 0.03 mmol) in THF (5 ml) was added. The mixture was stirred at 40 °C for 2 hours. The reaction mixture was dilluted with water (20 ml) and extracted with ethyl-acetate (3 × 50 ml). The collected organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (20 – 40 % EtOAc in hexane) furnished **43c** (128 mg, 63 %) as white solid; m.p. 202-204 °C. ^{1}H NMR (600.1 MHz, DMSO-d_6): 1.24-1.33 (m, 4H, CH_2-cyclopropyl); 2.91 (ttd, 1H, J_{vic} = 7.7, 4.7, ^5J = 0.5, CH-cyclopropyl); 4.68 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.1, H-5'b); 4.82 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.2, H-5'a); 4.89 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.1, 3.2, H-4'); 6.36 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.66 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.7, H-2'); 6.99 (d, 1H, $J_{1',2'}$ = 4.7, H-1'); 7.41 (m, 2H, H-*m*-Bz-2'); 7.42 (m, 1H, H-6); 7.45 (m, 1H, H-7); 7.49, 7.50 (2 × m, 2 × 2H, H-*m*-Bz-3',5'); 7.61 (m, 1H, H-*p*-Bz-2'); 7.67, 7.68 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.83 (m, 2H, H-*o*-Bz-2'); 7.95 (m, 2H, H-*o*-Bz-5'); 7.98 (m, 2H, H-*o*-Bz-3'); 8.02 (m, 1H, H-8); 8.41 (m, 1H, H-5); 8.72 (d, 1H, ^5J = 0.5, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 11.22 (CH_2-cyclopropyl); 15.04 (CH-cyclopropyl); 63.24 (CH_2-5'); 70.38 (CH-3'); 72.05 (CH-2'); 78.55 (CH-4'); 85.94 (CH-1'); 111.64 (CH-8); 112.20 (C-4a); 119.75 (C-4b); 122.59 (CH-6); 123.01 (CH-5); 127.46 (CH-7); 128.62, 128.84 (C-*i*-Bz); 128.91, 128.95, 128.97 (CH-*m*-Bz); 129.40 (CH-*o*-Bz-5'); 129.42 (C-*i*-Bz); 129.49 (CH-*o*-Bz-2'); 129.62 (CH-*o*-Bz-3'); 133.77, 134.09 (CH-*p*-Bz); 137.92 (C-8a); 154.21 (CH-2); 154.23 (C-9a); 164.80 (COPh-2'); 165.03 (COPh-3'); 165.31 (C-4); 165.60 (COPh-5'). ESI MS m/z (rel. %): 654 (62) [M+H] 676 (100) [M+Na]. HR MS (ESI) for $C_{39}H_{32}N_3O_7$ [M+H]: calcd 654.22348; found 654.22349.

4-(*N*,*N*-Dimethylamino)-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-pyrimido[4,5-*b*]indole (43d)

Dimethylamine (230 μl, 2M in THF) was added to solution of nucleoside **25** (200 mg, 0.31 mmol) in propan-2-ol (10 ml) and the reaction mixture was stirred at r.t. for 24 hours. Volatiles were removed under reduced pressure and crude product was purified by HPFC (15 % EtOAc in hexane) to give **43d** (150 mg, 74 %) as white solid; m.p. 64-67 °C; IR

(ATR): ν = 1724, 1576, 1556, 1512, 1269, 1251, 1099, 1070, 710. ^{1}H NMR (499.8 MHz, DMSO-d_6): 3.24 (s, 6H, $(CH_3)_2N$); 4.67 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.2, H-5'b); 4.81 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5'a); 4.86 (ddd, 1H, $J_{4',3'}$ = 6.6, $J_{4',5'}$ = 4.2, 3.3, H-4'); 6.35 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 6.6, H-3'); 6.64 (dd, 1H, $J_{2',3'}$ = 6.6, $J_{2',1'}$ = 4.8, H-2'); 6.97 (d, 1H, $J_{1',2'}$ = 4.8, H-1'); 7.29 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.34 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.1, H-6); 7.42 (m, 2H, H-*m*-Bz-2'); 7.48, 7.51 (2 × m, 2 × 2H, H-*m*-Bz-3',5'); 7.61 (m, 1H, H-*p*-Bz-2'); 7.67, 7.69 (2 × m, 2 × 1H, H-*p*-Bz-3',5'); 7.84 (m, 2H, H-*o*-Bz-2'); 7.93 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.7, H-8); 7.95 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.7, H-8); 7.97 (m, 2H, H-*o*-Bz-3'); 7.98 (m, 2H, H-*o*-Bz-5'); 8.40 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.10 ($(CH_3)_2N$); 63.36 (CH_2-5'); 70.41 (CH-3'); 72.11 (CH-2'); 78.44 (CH-4'); 85.92 (CH-1'); 98.31 (C-4a); 111.10 (CH-8); 120.13 (C-4b); 121.99 (CH-6); 123.19 (CH-5); 125.23 (CH-7); 128.66, 128.85 (C-*i*-Bz); 128.92, 128.97, 128.98 (CH-*m*-Bz); 129.44 (CH-*o*-Bz-5', C-*i*-Bz); 129.50 (CH-*o*-Bz-2'); 129.60 (CH-*o*-Bz-3'); 133.79, 134.08, 134.10 (CH-*p*-Bz); 136.48 (C-8a); 153.51 (CH-2); 156.13 (C-9a); 160.12 (C-4); 164.81 (COPh-2'); 165.03 (COPh-3'); 165.63 (COPh-5'). ESI MS m/z (rel. %): 657 (79) [M+H] 679 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{33}N_4O_7$ [M+H]: calcd 657.23438; found 657.23432; for $C_{38}H_{32}N_4O_7Na$ [M+Na]: calcd 679.21632; found 679.21619.

4-Methyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (44a)

Deprotection of **43a** (80 mg, 0.12 mmol) according to the general procedure E afforded compound **44a** (36 mg, 92 %) as white solid. m.p. 212-215 °C; $[\alpha]_D$ -25.6 (0.21). IR (ATR): ν = 3341, 3253, 1598, 1457, 1433, 1111, 1056, 1039, 1012, 994, 749, 739. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.95 (s, 3H, CH_3); 3.66 (bdt, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = $J_{5'b,OH}$ = 4.0, H-5'b); 3.73 (bdt, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = $J_{5'a,OH}$ = 3.3, H-5'a); 3.98 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.3, $J_{4',3'}$ = 3.1, H-4'); 4.23 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.1, H-3'); 4.83 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.9, H-2'); 5.22 (bm, 1H, OH-5'); 5.27, 5.31 (2 × bs, 2 × 1H, OH-2',3'); 6.47 (dd, 1H, $J_{1',2'}$ = 7.2, $J_{1',3'}$ = 0.4, H-1'); 7.43 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.04 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.21 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.84 (d, 1H, ^{5}J = 0.3, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 22.93 (CH_3); 61.88 (CH_2-5'); 70.30 (CH-3'); 70.57 (CH-2'); 85.56 (CH-4'); 87.08 (CH-1'); 112.32 (C-4a); 112.95 (CH-8); 119.93 (C-4b); 122.09 (CH-6); 123.07 (CH-5); 127.43 (CH-7); 137.77 (C-8a); 153.82 (CH-2); 154.83 (C-9a); 160.12 (C-4). ESI MS m/z (rel. %): 316 (15) [M+H] 338 (100) [M+Na]. HR MS (ESI) for $C_{16}H_{18}N_3O_4$ [M+H]: calcd 316.12918; found

316.12924; for $C_{16}H_{17}N_3O_4Na$ [M+Na]: calcd 338.11113; found 338.11105. Anal. Calcd. for $C_{16}H_{17}N_3O_4 \cdot 0.6\ H_2O$: C, 58.92; H, 5.62; N, 12.88. Found C, 58.67; H, 5.28; N, 12.58.

4-Ethyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (44b)

Deprotection of **43b** (100 mg, 0.16 mmol) according to the general procedure E afforded compound **44b** (39 mg, 76 %) as white solid. m.p 181-183 °C, $[\alpha]_D$ -30.2 (0.25). IR (ATR): ν = 3402, 3296, 2986, 1688, 1675, 1659, 1545, 1510, 1402, 1243, 1161, 965, 916, 761. ^{1}H NMR (499.8 MHz, DMSO-d_6): 1.40 (t, 3H, J_{vic} = 7.5, C**H$_3$**CH$_2$); 3.31 (m, 2H, CH$_3$C**H$_2$**); 3.66 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.8, $J_{5'b,4'}$ = 4.0, H-5'b); 3.73 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.2, $J_{5'a,4'}$ = 3.5, H-5'a); 3.99 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.5, $J_{4',3'}$ = 3.1, H-4'); 4.23 (dddd, 1H, $J_{3',2'}$ = 5.8, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.1, $J_{3',1'}$ = 0.4, H-3'); 4.84 (ddd, 1H, $J_{2',1'}$ = 7.2, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 5.8, H-2'); 5.18 (d, 1H, $J_{OH,3'}$ = 4.8, OH-3'); 5.21 (dd, 1H, $J_{OH,5'}$ = 5.8, 5.2, OH-5'); 5.24 (d, 1H, $J_{OH,2'}$ = 6.5, OH-2'); 6.48 (dd, 1H, $J_{1',2'}$ = 7.2, $J_{1',3'}$ = 0.4, H-1'); 7.43 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.05 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.20 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.89 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 11.90 (**C**H$_3$CH$_2$); 28.89 (CH$_3$**C**H$_2$); 61.89 (CH$_2$-5'); 70.31 (CH-3'); 70.52 (CH-2'); 85.57 (CH-4'); 87.03 (CH-1'); 111.53 (C-4a); 113.00 (CH-8); 119.51 (C-4b); 122.18 (CH-6); 123.07 (CH-5); 127.42 (CH-7); 137.79 (C-8a); 154.00 (CH-2); 155.01 (C-9a); 164.73 (C-4). ESI MS m/z (rel. %): 330 (15) [M+H] 352 (100) [M+Na]. HR MS (ESI) for $C_{17}H_{20}N_3O_4$ [M+H]: calcd 330.14483; found 330.14484; for $C_{17}H_{20}N_3O_4Na$ [M+Na]: calcd 352.12678; found 352.12670. Anal. Calcd. for $C_{19}H_{24}N_4O_4 \cdot 0.85\ CH_3OH$: C, 59.66; H, 6.91; N, 14.02. Found C, 59.86; H, 6.52; N, 13.65.

4-Cyclopropyl-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (44c)

Deprotection of **43c** (80 mg, 0.12 mmol) according to the general procedure E afforded compound **44c** (37 mg, 89 %) as white solid. m.p. 230-231 °C, $[\alpha]_D$ -31.3 (0.26). IR (ATR): ν = 3389, 3228, 2185, 2000, 1597, 1085, 1053, 1020, 752 cm^{-1}. ^{1}H NMR (499.8 MHz, DMSO-d_6): 1.22-1.36 (m, 4H, CH$_2$-cyclopropyl); 2.92 (tt, 1H, J_{vic} = 8.0, 4.7, CH-cyclopropyl); 3.66, 3.73 (2 × bd, 2 × 2H, J_{gem} = 11.7, H-5'); 3.98 (td, 1H, $J_{4',5'}$ = 3.8, $J_{4',3'}$ = 3.0, H-4'); 4.23 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.0, H-3'); 4.83 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.9, H-2'); 5.22 (bs, 3H, OH-2',3',5'); 6.46 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 7.42 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.56 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.03 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.7, H-8); 8.42 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.7, H-5); 8.77 (d, 1H, ^5J = 0.2, H-2). ^{13}C NMR (12.57 MHz, DMSO-d_6): 10.98, 11.03 (CH$_2$-cyclopropyl); 14.97 (CH-cyclopropyl); 61.89 (CH$_2$-5'); 70.30 (CH-3'); 70.51 (CH-2'); 85.55 (CH-4'); 87.07 (CH-1');

111.86 (C-4a); 112.95 (CH-8); 119.86 (C-4b); 122.05 (CH-6); 122.77 (CH-5); 127.25 (CH-7); 137.76 (C-8a); 154.04 (CH-2); 154.63 (C-9a); 164.87 (C-4). ESI MS m/z (rel. %): 342 (15) [M+H] 364 (100) [M+Na]. HR MS (ESI) for $C_{18}H_{20}N_3O_4$ [M+H]: calcd 342.14483; found 342.14486; for $C_{18}H_{19}N_3O_4Na$ [M+Na]: calcd 364.12678; found 364.12668. Anal. Calcd for $C_{18}H_{19}N_3O_4 \cdot 1.8\ H_2O$: C, 57.84 %; H, 6.09 %; N, 11.24 % Found.C, 57.94 %; H, 6.12 %; N, 11.20 %.

4-(*N,N*-Dimethylamino)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (44d)

Deprotection of **43c** (80 mg, 0.12 mmol) according to the general procedure E afforded compound **44d** (37 mg, 88 %) as white solid. m.p. 98-101 °C; $[\alpha]_D$ -28.5 (c 0.29). IR (ATR): ν = 3279, 3243, 1580, 1556, 1113, 1070, 1043, 749.^{1}H NMR (499.8 MHz, DMSO-d_6): 3.24 (s, 6H, $(CH_3)_2N$); 3.64 (bdd, 1H, J_{gem} = 11.6, $J_{5'b,4'}$ = 4.0, H-5'b); 3.72 (bdd, 1H, J_{gem} = 11.6, $J_{5'a,4'}$ = 3.2, H-5'a); 3.96 (ddd, 1H, $J_{4',5'}$ = 4.0, 3.2, $J_{4',3'}$ = 3.1, H-4'); 4.21 (dd, 1H, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 3.1, H-3'); 4.84 (dd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.9, H-2'); 5.21 (bs, 2H, OH-2',3'); 5.35 (bs, 1H, OH-5'); 6.42 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 7.34 (ddd, 1H, $J_{6,5}$ = 8.0, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.1, H-6); 7.43 (ddd, 1H, $J_{7,8}$ = 8.1, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.3, H-7); 7.91 (ddd, 1H, $J_{8,7}$ = 8.1, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.5, H-8); 7.95 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.5, H-5); 8.43 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 40.12 ($(CH_3)_2N$); 62.04 (CH_2-5'); 70.41 (CH-3'); 70.66 (CH-2'); 85.53 (CH-4'); 87.28 (CH-1'); 98.19 (C-4a); 112.20 (CH-8); 120.03 (C-4b); 121.45 (CH-6); 122.94 (CH-5); 125.12 (CH-7); 136.49 (C-8a); 153.22 (CH-2); 156.33 (C-9a); 160.25 (C-4).ESI MS m/z (rel. %): 345 (100) [M+H] 367 (75) [M+Na]. HR MS (ESI) for $C_{17}H_{21}N_4O_4$ [M+H]: calcd 345.15573; found 345.15577. Anal. Calcd. for $C_{17}H_{20}N_4O_4$: C, 56.35 %; H, 6.12 %; N, 15.46 %. Found C, 56.22 %; H, 6.01 %; N, 15.33 %.

4-(*N*-Methylamino)-9-β-D-ribofuranosyl-9*H*-pyrimido[4,5-*b*]indole (46)

Nucleoside **46** was prepared in analogy to literature procedure.[137] Aminonucleoside **37** (100 mg, 0.32 mmol) was dissolved in DMA (5 ml) and MeI (0.2 ml, 0.62 mmol) was added. The reaction mixture was stirred overnight at r.t, poured into diethylether and precipitated hydroiodide **45** was filtered out and dried under vacuum. IR (ATR): ν = 3397, 3302, 2985, 1667, 1502, 1462, 1411, 1243, 1119, 1034, 966, 786, 760. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.70 (ddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 5.1, $J_{5'b,4'}$ = 3.9, H-5'b); 3.73 (ddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.2, H-5'a); 3.89 (s, 3H, CH_3); 4.00 (ddd, 1H, $J_{4',5'}$ = 3.9, 3.2, $J_{4',3'}$ = 2.8, H-4'); 4.21 (ddd, 1H, $J_{3',2'}$ = 5.7, $J_{3',OH}$ = 4.9, $J_{3',4'}$ = 2.8, H-3'); 4.69 (ddd, 1H, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.7, H-2'); 5.19 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5'); 5.28 (d, 1H, $J_{OH,3'}$ = 4.9, OH-3'); 5.29 (d, 1H, $J_{OH,2'}$

= 6.6, OH-2'); 6.44 (d, 1H, $J_{1',2'}$ = 7.5, H-1'); 7.51 (ddd, 1H, $J_{6,5}$ = 7.7, $J_{6,7}$ = 7.3, $J_{6,8}$ = 1.0, H-6); 7.57 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.3, $J_{7,5}$ = 1.2, H-7); 8.22 (d, 1H, $J_{8,7}$ = 8.3, H-8); 8.62 (d, 1H, $J_{5,6}$ = 7.7, H-5); 8.88 (s, 1H, H-2); 8.94 (bs, 2H, NH_2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 38.21 (CH_3); 61.62 (CH_2-5'); 70.08 (CH-3'); 71.03 (CH-2'); 86.07 (CH-4'); 87.08 (CH-1'); 95.58 (C-4a); 114.21 (CH-8); 119.26 (C-4b); 121.81 (CH-5); 123.21 (CH-6); 126.88 (CH-7); 136.37 (C-8a); 149.87 (CH-2); 151.11 (C-4); 152.32 (C-9a). ESI MS m/z (rel. %): 331 (100) [M+H]. HR MS (ESI) for $C_{16}H_{19}O_4N_4$[M+H]: calcd 331.14008; found 331.14006.

Crude hydroiodide salt **45** was dissolved in 1M NaOH (5ml) and heated to 100 °C for 1.5 hr. Reaction mixture was cooled to r.t., filtered and the filtrate was neutralized with 2M HCl. The product crystallized from solution after standing at 4 °C for 48 hours. Filtration furnished desired compound **46** (66 mg, 64 %) as white crystals. m.p. 242-244 °C; $[\alpha]_D$ -61.8 (0.14). IR (ATR): ν = 3395, 3119, 2261, 1626, 1608, 1576, 1027, 1011, 999, 740 cm^{-1}. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.10 (d, 3H, J_{vic} = 4.6, CH_3N); 3.63 (bddd, 1H, J_{gem} = 11.8, $J_{5'b,OH}$ = 6.6, $J_{5'b,4'}$ = 3.8, H-5'b); 3.71 (bddd, 1H, J_{gem} = 11.8, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.8, H-5'a); 3.96 (td, 1H, $J_{4',5'}$ = 3.8, $J_{4',3'}$ = 2.9, H-4'); 4.19 (dd, 1H, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 2.9, H-3'); 4.83 (bdd, 1H, $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 5.7, H-2'); 5.15, 5.20 (2 × bs, 2 × 1H, OH-2',3'); 5.47 (bdd, 1H, $J_{OH,5'b}$ = 6.6, 4.8, OH-5'); 6.35 (d, 1H, $J_{1',2'}$ = 7.3, H-1'); 7.29 (bq, 1H, J_{vic} = 4.6, NH); 7.32 (ddd, 1H, $J_{6,5}$ = 7.9, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.0, H-6); 7.40 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.84 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.6, H-8); 8.35 (ddd, 1H, $J_{5,6}$ = 7.9, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.39 (d, 1H, ^5J = 0.4, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 28.05 (CH_3N); 62.14 (CH_2-5'); 70.56 (CH-3'); 70.83 (CH-2'); 85.60 (CH-4'); 87.31 (CH-1'); 96.38 (C-4a); 111.79 (CH-8); 119.96 (C-4b); 121.18 (CH-5,6); 124.84 (CH-7); 136.09 (C-8a); 154.51 (C-9a); 154.53 (CH-2); 157.14 (C-4). ESI MS m/z (rel. %): 331 (100) [M+H]. HR MS (ESI) for $C_{16}H_{19}O_4N_4$[M+H]: calcd 331.14008; found 331.14005. Anal. Calcd for $C_{16}H_{18}N_4O_4 \cdot 1\ H_2O$: C, 55.17 %; H, 5.79 %; N, 16.08 %. Found C, 55.23 %; H, 5.71 %; N, 16.02 %.

_N,N_-dimethyl-_N´_-(9-β-D-ribofuranosyl-9_H_-pyrimido[4,5-_b_]indol-4-yl)methanamidine (47)

$POCl_3$ (32 µl, 0.32 mmol) was mixed with an ice-cooled DMF (2 ml) and stirred for 30 min. at r.t., then, solution of nucleoside **37** (100 mg, 0.32 mmol) in DMF (2 ml) was added and the reaction mixture was stirred overnight at r.t. The reaction mixture was neutralized with saturated $NaHCO_3$, solvents were evaporated and crude material was purified by column chromatography on silica (DCM/MeOH 10:1) to obtain nucleoside **47** (40 mg, 34 %) as white solid. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.24 (d, 3H, ^4J = 0.4, CH_3N); 3.26 (d, 3H, ^4J = 0.6,

CH_3N); 3.66 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 6.3, $J_{5'b,4'}$ = 3.9, H-5'b); 3.73 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 4.8, $J_{5'a,4'}$ = 3.5, H-5'a); 3.99 (ddd, 1H, $J_{4',5'}$ = 3.9, 3.5, $J_{4',3'}$ = 2.6, H-4'); 4.22 (bdd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 2.6, H-3'); 4.87 (bm, 1H, H-2'); 5.20 (bs, 1H, OH-3'); 5.27 (bd, 1H, $J_{OH,2'}$ = 4.9, OH-2'); 5.43 (bdd, 1H, $J_{OH,5'}$ = 6.3, 4.8, OH-5'); 6.37 (d, 1H, $J_{1',2'}$ = 7.2, H-1'); 7.33 (ddd, 1H, $J_{6,5}$ = 7.7, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.0, H-6); 7.43 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.3, H-7); 7.85 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.0, $J_{8,5}$ = 0.6, H-8); 8.38 (ddd, 1H, $J_{5,6}$ = 7.7, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.6, H-5); 8.52 (s, 1H, H-2); 9.05 (m, 1H, ^4J = 0.6, 0.4, HC=N). ^{13}C NMR (125.7 MHz, DMSO-d_6): 34.99, 40.89 ($(CH_3)_2N$); 62.13 (CH_2-5'); 70.57 (CH-3'); 70.80 (CH-2'); 85.59 (CH-4'); 87.36 (CH-1'); 104.56 (C-4a); 111.78 (CH-8); 121.18 (C-4b); 121.45 (CH-6); 123.16 (CH-5); 125.74 (CH-7); 137.23 (C-8a); 153.93 (CH-2); 156.33 (C-9a); 157.29 (CH=N); 161.56 (C-4). ESI MS m/z (rel. %): 372 (100) [M+H]. HR MS (ESI) for $C_{18}H_{22}O_4N_5$ [M+H]: calcd 372.16720; found 372.16726.

5.7 Synthesis of thienopyrrolopyrimidine nucleosides

4-Chloro-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (48)

Compound **48** was prepared in analogy to literature conditions.[94] Tetrazole **57** (750 mg, 3.2 mmol) and 1,4-dibromobenzene (4 g) were heated to 180 °C for 35 min. Crude mixture was purified by HPFC (500 ml hexane, then 10-30 % ethyl-acetate in hexane). Compound **48** (345 mg, 52 %) was obtained as white powder. m.p. 238-240 °C, IR (ATR): ν = 3028, 2948, 2865, 1615, 1596, 1501, 1468, 1430, 1315, 1265, 1230, 1107, 1068, 917, 840, 162. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.39 (d, 1H, $J_{7,6}$ = 5.2, H-7); 7.96 (d, 1H, $J_{6,7}$ = 5.2, H-6); 8.67 (s, 1H, H-2); 13.02 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 111.89 (C-4a); 112.12 (C-4b); 112.92 (CH-7); 132.43 (CH-6); 143.65 (C-7a); 147.89 (C-4); 151.08 (CH-2); 156.20 (C-8a). APCI MS m/z (rel%): 209 (100) [M+H]. HR MS (APCI) for $C_8H_5N_3ClS$ [M+H]: calcd 209.98872; found 209.98875.

4-Chloro-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (49) and **4-chloro-8*H*-thieno [3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (50)**

Compound **49** was prepared in analogy to literature conditions.[94] Tetrazole **63** (200 mg, 0.84 mmol)) was dissolved in TFA (20 ml) and stirred at r.t under irradiation by 4W UV bulb for 24 h. UV bulb was placed inside the flask with the reaction mixture. Solvent was evaporated and crude material containing compounds **49** and **50** in ratio 2:1 (determined by NMR) was purified by HPFC (40 g silica cartridge, gradient hexane/EtOAc, 20-30 % of EtOAc) to obtain tricyclic bases **49** (98 mg, 56 %) and **50** (32 mg, 18 %) as white solids.

Characterization of **49**: m.p. 258-261 °C. IR (ATR): ν = 3047, 2931, 2861, 2804, 2663, 1607, 1568, 1499, 1470, 1425, 1313, 1267, 1229, 1107, 1071, 917, 835, 783, 635. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.41 (d, 1H, $J_{6,5}$ = 5.3, H-6); 7.50 (d, 1H, $J_{5,6}$ = 5.3, H-5); 8.65 (s, 1H, H-2); 13.23 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 111.11 (C-4a); 118.01 (CH-5); 119.59 (C-4b); 121.46 (CH-6); 142.54 (C-7a); 148.48 (C-4); 150.69 (CH-2); 156.69 (C-8a). APCI MS m/z (rel%): 209 (100) [M+H]. HR MS (APCI) for $C_8H_5N_3ClS$ [M+H]: calcd 209.98872; found 209.98874.

Characterization of **50**: m.p. 215-217 °C. IR (ATR): ν = 2931, 2861, 1602, 1560, 1503, 1440, 1368, 1238, 1197, 1158, 793, 749, 709. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.16 (d, 1H, $J_{5,7}$ = 2.4, H-5); 8.04 (d, 1H, $J_{7,5}$ = 2.4, H-7); 8.62 (s, 1H, H-2); 12.08 (bs, 1H, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 97.45 (CH-5); 109.38 (C-4a); 116.34 (CH-7); 126.32 (C-4b); 141.00 (C-7a); 149.69 (C-4); 154.79 (CH-2); 164.02 (C-8a). APCI MS m/z (rel%): 209 (100) [M+H]. HR MS (APCI) for $C_8H_5N_3ClS$ [M+H]: calcd 209.98872; found 209.98871.

5-Iodo-4,6-bis(thiophen-2-yl)pyrimidine (51) and 4,5,6-tri(thiophen-2-yl)pyrimidine (52)

4,6-dichloro-5-iodopyrimidine (100 mg, 0.36 mmol) and $PdCl_2(PPh_3)_2$ (25 mg, 0.036 mmol) were dissolved in DMF (5 ml) and 2-(tributylstannyl)thiophene (202 mg, 0.54 mmol) was added and the reaction mixture was stirred at 0 °C for 1 hour. Solvent was evaporated under reduced pressure and crude reaction mixture was analyzed by HPLC/ESI. Only 4,6-disubstituted **51** and trisubstituted **52** products were observed. ESI MS **for 51** m/z (rel%): 371 (12) [M+H]; 393 (100) [M+Na]. ESI MS **for 52** m/z (rel%): 327 (100) [M+H].

Tetramethylpiperidinylzinc complex with magnesium chloride and lithium chloride (53)

Zinc complex **53** was prepared according to literature procedure.[151] Dry and argon-flushed sealed-flask was filled with *i*-PrMgCl·LiCl (1.3 M in THF) (5.0 ml, 7.5 mmol) and 2,2,6,6-dimethylpiperidine (1.48 ml, 7.88 mmol) was added dropwise and the reaction was stirred overnight at r.t. for 32 hours until gas evolution stopped. Formed solution of TMP·MgCl·LiCl was added dropwise to zinc chloride (500 mg, 3.75 mmol) and the reaction was stirred at r.t. for 12 hours to give zinc complex **53**, which was used directly to the next step.

Bis(4,6-dichloropyrimidin-5-yl)zinc (54)

Organozinc species **54** was prepared according to modified literature procedure.[108] 4,6-dichloropyrimidine (8 g, 53.4 mmol) was dissolved in THF (20 ml) and added dropwise into an ice-cooled solution of $(TMP)_2Zn·MgCl_2·LiCl$ **53** in THF (0.35 M, 85 ml, 29.8 mmol)

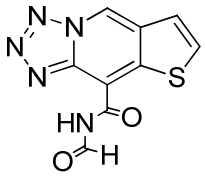
and the reaction mixture was stirred at 0 °C for 1 hour, then let to warm to r.t. for one hour and used directly in the next step. Conversion of this reaction was determined by NMR from small sample of the reaction mixture, which was quenched by deuterium oxide.

4,6-Dichloro-5-(thiophen-2-yl)pyrimidine (55)

Zincated pyrimidine **54** was prepared as described above from 4,6-dichloropyrimidine (1.0 g, 6.7 mmol). Solution of 2-iodothiophene (0.74 ml, 6.7 mmol) and $Pd(PPh_3)_4$ (775 mg, 0.67 mmol) in THF (3 ml), which was pre-stirred at r.t. for 20 min., was added to a solution of zincated pyrimidine **54** and stirred at 65 ° for 16 hrs. After that, solvent was evaporated under reduced pressure and crude mixture was purified by HPFC (0-1 % ethyl-acetate in hexane) to obtain **55** (950 mg, 62 %) as white solid. m.p. 174-175 °C. ^{1}H NMR (499.8 MHz, $CDCl_3$): 7.16 (dd, 1H, $J_{3,4}$ = 3.6, $J_{3,5}$ = 1.3, H-3-thienyl); 7.18 (dd, 1H, $J_{4,5}$ = 5.0, $J_{4,3}$ = 3.6, H-4-thienyl); 7.58 (dd, 1H, $J_{5,4}$ = 5.0, $J_{5,3}$ = 1.3, H-5-thienyl); 8.77 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 127.32 (CH-4-thienyl); 128.32 (C-5); 128.54 (CH-5-thienyl); 129.97 (CH-3-thienyl); 131.86 (C-2-thienyl); 156.90 (CH-2); 162.54 (C-4,6).MS

4-azido-6-chloro-5-(thiophen-2-yl)pyrimidine (56)

Compound **56** was prepared in analogy to modified literature conditions[94] from **55** (900 mg, 3.9 mmol), which was dissolved in DMF (10 ml), NaN_3 (254 mg, 3.9 mmol) and LiCl (163 mg, 3.9 mmol) were added and the reaction mixture was stirred overnight. Solvent was co-evaporated several times with toluene, dissolved in ethyl-acetate and extracted with water. Organic layer was evaporated several times with toluene and crude material was purified by column chromatography on silica (hexane/EtOAc 6:1). Desired product **56** (845 mg, 92 %) was obtained as yellow solid and according to NMR and IR spectra is present in form of tetrazole **57**; m.p. 78-79 °C. IR (ATR): ν = 3388, 3074, 2145 (weak), 1577, 1505, 1399, 1324, 1182, 1086, 967, 898, 794, 763, 633, 504. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.39 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,3}$ = 3.8, H-4-thienyl); 8.04 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,3}$ = 1.2, H-5-thienyl); 8.48 (dd, 1H, $J_{3,4}$ = 3.8, $J_{3,5}$ = 1.2, H-3-thienyl); 10.14 (s, 1H, H-2).



***N*-formyltetrazolo[1,5-*a*]thieno[2,3-*d*]pyridine-9-carboxamide (58)**

The crude reaction mixture containing tetrazole **57** in DMF was irradiated by UV lamp for 24 h at r.t. Solvent was evaporated and crude material was purified by HPFC on silica (gradient 20-40 % EtOAc in hexane) to give **58** (14 mg) as the only isolated compound. ^{1}H

NMR (500.0 MHz, DMSO-d_6): 7.69 (dd, 1H, $J_{6,7}$ = 5.8, $J_{6,5}$ = 0.5, H-6); 8.12 (dd, 1H, $J_{7,6}$ = 5.8, $J_{7,5}$ = 0.5, H-6); 9.40 (d, 1H, J = 9.5, CHO); 10.35 (t, 1H, $J_{5,6}$ = $J_{5,7}$ = 0.5, H-5); 11.46 (bd, 1H, J =9.5, NH). ^{13}C NMR (125.7 MHz, DMSO-d_6): 107.45 (C-5a); 121.05 (CH-6); 126.20 (CH-5); 133.53 (C-9); 136.70 (CH-7); 145.06 (C-9a); 150.55 (C-8a); 162.50 (CO); 162.92 (CHO). ESI MS m/z (rel%): 270 (100) [M+Na]. HR MS (ESI) for $C_9H_5N_5O_2SNa$ [M+Na]: calcd 270.00620; found 270.00580.

4-chloro-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (59)

Tricyclic base **48** (250 mg; 1.2 mmol) was dissolved in MeCN (30 ml) and BSA (295 μl, 1.2 mmol) was added. Reaction mixture was heated at 60 °C for 30 minutes, then, TMSOTf (538 μl, 2.98 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.2 g, 2.4 mmol) were added. Mixture was heated to 60 °C for 12 hours. After cooling to r.t., the mixture was extracted with EtOAc and water, organic layer was washed with $NaHCO_3$ and again with water, dried over $MgSO_4$ and evaporated under reduced pressure. Crude product was purified using column chromatography (hexane/EtOAc, 15–35 % EtOAc). Nucleoside **59** (486 mg, 62 %) was obtained as white crystals. m.p. 165-168 °C, IR (ATR): ν = 1728, 1604, 1542, 1495, 1455, 1432, 1271, 1239, 1185, 1126, 1114, 1090, 1071, 1028, 1003, 973, 824, 719, 708. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.72 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.7, H-5′b); 4.83 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.92 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.7, 3.3, H-4'); 6.25 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 5.9, H-3'); 6.47 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 5.1, H-2'); 6.95 (d, 1H, $J_{1',2'}$ = 5.1, H-1'); 7.41, 7.49, 7.50 (3 × m, 3 × 2H, H-*m*-Bz); 7.62, 7.67, 7.68 (3 × m, 3 × 1H, H-*p*-Bz); 7.77 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.82, 7.93, 7.99 (3 × m, 3 × 2H, H-*o*-Bz); 8.00 (d, 1H, $J_{6,7}$ = 5.3, H-6); 8.67 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.44 (CH_2-5'); 70.48 (CH-3'); 72.57 (CH-2'); 79.16 (CH-4'); 86.84 (CH-1'); 112.92 (C-4a); 113.22 (C-4b); 113.23 (CH-7); 128.48, 128.79 (C-*i*-Bz); 128.94, 129.00 (CH-*m*-Bz); 129.35 (C-*i*-Bz); 129.37, 129.50, 129.63 (CH-*o*-Bz); 133.12 (CH-6); 133.79, 134.11, 134.17 (CH-*p*-Bz); 143.46 (C-7a); 148.57 (C-4); 151.25 (CH-2); 155.11 (C-8a); 164.68, 164.99, 165.57 (CO-Bz). ESI MS m/z (rel%): 654 (24) [M+H]; 676 (100) [M+Na]. HR MS (ESI) for $C_{34}H_{25}N_3O_7S$ [M+H]: calcd 654.10962; found 654.10989.

4-(Furan-2-yl)-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[2‘,3‘:4,5]pyrrolo[2,3-*d*]pyrimidine (60a)

Nucleoside **60a** was prepared according to the general procedure B. Protected nucleoside **59** (200 mg, 0.31 mmol) and 2-(tributylstannyl)furan (131 mg, 0.37 mmol) were used. Desired product **60a** (175 mg, 84 %) was obtained as yellowish powder. m.p. 110-113 °C. ^{1}H NMR (600.1 MHz, DMSO-d_6): 4.72 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.7, H-5′b); 4.82 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.91 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.7, 3.3, H-4'); 6.28 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 5.9, H-3'); 6.51 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 5.2, H-2'); 6.87 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 6.96 (d, 1H, $J_{1',2'}$ = 5.2, H-1'); 7.41 (m, 2H, H-*m*-Bz); 7.47-7.53 (m, 5H, H-3-furyl, H-*m*-Bz); 7.61, 7.65, 7.68 (3 ×m, 3 × 1H, H-*p*-Bz); 7.71 (d, 1H, $J_{7,6}$ = 5.4, H-7); 7.82 (m, 2H, H-*o*-Bz); 7.88 (d, 1H, $J_{6,7}$ = 5.4, H-6); 7.95, 7.99 (2 × m, 2 × 2H, H-*o*-Bz); 8.23 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.78 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 63.54 (CH_2-5'); 70.54 (CH-3'); 72.41 (CH-2'); 78.93 (CH-4'); 86.44 (CH-1'); 107.95 (C-4a); 112.98 (CH-7); 113.14 (CH-3-furyl); 113.30 (CH-4-furyl); 115.16 (C-4b); 128.50, 128.82 (C-*i*-Bz); 128.92, 128.98 (CH-*m*-Bz); 129.40 (CH-*o*-Bz, C-*i*-Bz); 129.48, 129.62 (CH-*o*-Bz); 132.01 (CH-6); 133.75, 134.11, 134.14 (CH-*p*-Bz); 143.20 (C-7a); 144.31 (C-4); 146.59 (CH-5-furyl); 151.58 (CH-2); 152.21 (C-2-furyl); 156.20 (C-8a); 164.70, 165.01, 165.62 (CO-Bz). ESI MS m/z (rel%): 686 (100) [M+H]; 708 (78) [M+Na]. HR MS (ESI) for $C_{38}H_{28}N_3O_8S$ [M+H]: calcd 686.15916; found 686.15943; for $C_{38}H_{27}N_3O_8SNa$ [M+Na]: calcd 708.14111; found 708.14121.

4-(Furan-3-yl)-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (60b)

Nucleoside **60b** was prepared accroding to the general procedure A. Protected nucleoside **59** (350 mg, 0.53 mmol) and furan-3-boronic acid (90 mg, 1.1 mmol) were used. Desired product **60b** (301 mg, 82 %) was obtained as white powder. m.p. 147-149 °C. IR (ATR): ν = 2934, 2862, 1724, 1605, 1563, 1551, 1454, 1262, 1093, 1070, 1027, 707. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.73 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.8, H-5′b); 4.83 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.91 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.7, 3.3, H-4'); 6.28 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 5.9, H-3'); 6.52 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 5.2, H-2'); 6.98 (d, 1H, $J_{1',2'}$ = 5.2, H-1'); 7.24 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.41, 7.50, 7.51 (3 × m, 3 × 2H, H-*m*-Bz); 7.61, 7.66, 7.68 (3 ×m, 3 × 1H, H-*p*-Bz); 7.77 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.83 (m, 2H, H-*o*-Bz); 7.90 (d, 1H, $J_{6,7}$ = 5.3, H-6); 7.96, 8.00 (2 × m, 2 × 2H, H-*o*-Bz); 8.02 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl);

8.59 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 8.82 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.50 (CH_2-5'); 70.53 (CH-3'); 72.38 (CH-2'); 78.93 (CH-4'); 86.50 (CH-1'); 109.40 (CH-4-furyl); 110.46 (C-4a); 113.14 (CH-7); 113.95 (C-4b); 125.03 (C-3-furyl); 128.46, 128.79 (C-*i*-Bz); 128.87, 128.88, 128.93 (CH-*m*-Bz); 129.35 (CH-*o*-Bz); 129.38 (C-*i*-Bz); 129.44, 129.57 (CH-*o*-Bz); 130.80 (CH-6); 133.70, 134.05, 134.08 (CH-*p*-Bz); 143.08 (C-7a); 143.98 (CH-2-furyl); 145.34 (CH-5-furyl); 148.61 (C-4); 151.63 (CH-2); 155.73 (C-8a); 164.65, 164.97, 165.57 (CO-Bz). ESI MS m/z (rel%): 686 (13) [M+H]; 708 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{28}N_3O_8S$ [M+H]: calcd 686.15916; found 686.15937.

4-(Benzofuran-2-yl)-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8H-thieno[2',3':4,5] pyrrolo[2,3-d]pyrimidine (60e)

Nucleoside **60e** was prepared accroding to general procedure A. Protected nucleoside **59** (350 mg, 0.53 mmol) and benzofurane-3-boronic acid (173 mg, 1.1 mmol) were used. Desired product **60e** (341 mg, 87 %) was obtained as white powder. m.p. 124-125 °C. IR (ATR): ν = 2933, 2862, 1724, 1605, 1563, 1552, 1495, 1454, 1424, 1264, 1093, 1070, 1028, 707. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.74 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.8, H-5′b); 4.84 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.3, H-5′a); 4.93 (ddd, 1H, $J_{4',3'}$ = 5.9, $J_{4',5'}$ = 4.7, 3.3, H-4'); 6.29 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 5.9, H-3'); 6.53 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 5.2, H-2'); 6.99 (d, 1H, $J_{1',2'}$ = 5.2, H-1'); 7.38-7.44 (m, 3H, H-5-benzofuryl, H-*m*-Bz); 7.47-7.56 (m, 5H, H-6-benzofuryl, H-*m*-Bz); 7.61, 7.65, 7.68 (3 ×m, 3 × 1H, H-*p*-Bz); 7.75 (d, 1H, $J_{7,6}$ = 5.4, H-7); 7.80-7.89 (m, 4H, H-4,7-benzofuryl, H-*o*-Bz); 7.92 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.94-7.98 (m, 3H, H-6, H-*o*-Bz); 8.00 (m, 2H, H-*o*-Bz); 8.87 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.53 (CH_2-5'); 70.55 (CH-3'); 72.43 (CH-2'); 78.99 (CH-4'); 86.51 (CH-1'); 108.53 (CH-3-benzofuryl); 109.31 (C-4a); 111.64 (CH-7-benzofuryl); 113.09 (CH-7); 115.30 (C-4b); 122.89 (CH-4-benzofuryl); 124.30 (CH-5-benzofuryl); 127.03 (CH-6-benzofuryl); 127.87 (C-3a-benzofuryl); 128.50, 128.81 (C-*i*-Bz); 128.90, 128.96 (CH-*m*-Bz); 129.38, 129.47, 129.60 (C-*i*-Bz, CH-*o*-Bz); 132.83 (CH-6); 133.72, 134.08, 134.11 (CH-*p*-Bz); 143.70 (C-7a); 144.12 (C-4); 151.55 (CH-2); 153.67 (C-2-benzofuryl); 155.33 (C-7a-benzofuryl); 156.46 (C-8a); 164.70, 165.01, 165.60 (CO-Bz). ESI MS m/z (rel%): 736 (14) [M+H]; 758 (100) [M+Na]. HR MS (ESI) for $C_{42}H_{30}N_3O_8S$ [M+H]: calcd 736.17481; found 736.17504.

4-Methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (60h)

Compound **60h** was prepared as described for **43a** from **59** (300 mg, 0.46 mmol). Nucleoside **60h** (252 mg, 87 %) was obtained as white solid. m.p. 169-171 °C. IR (ATR): ν = 2927, 1729, 1456, 1435, 1261, 1134, 1121, 1090, 1042, 706. ^{1}H NMR (500.0 MHz, DMSO-d_6): 2.74 (s, 3H, CH_3); 4.71 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 4.7, H-5′b); 4.81 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.3, H-5′a); 4.90 (ddd, 1H, $J_{4',3'}$ = 5.8, $J_{4',5'}$ = 4.7, 3.3, H-4'); 6.25 (dd, 1H, $J_{3',2'}$ = 6.5, $J_{3',4'}$ = 5.8, H-3'); 6.50 (dd, 1H, $J_{2',3'}$ = 6.5, $J_{2',1'}$ = 5.3, H-2'); 6.92 (d, 1H, $J_{1',2'}$ = 5.3, H-1'); 7.41, 7.50 (2 × m, 6H, H-*m*-Bz); 7.61, 7.67, 7.68 (3 × m, 3 × 1H, H-*p*-Bz); 7.71 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.82 (m, 2H, H-*o*-Bz); 7.88 (d, 1H, $J_{6,7}$ = 5.3, H-6); 7.95, 7.99 (2 × m, 2 × 2H, H-*o*-Bz); 8.69 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 22.22 (CH_3**)**; 63.51 (CH_2-5'); 70.55 (CH-3'); 72.33 (CH-2'); 78.90 (CH-4'); 86.40 (CH-1'); 113.00 (CH-7); 113.11 (C-4a); 114.40 (C-4b); 128.45, 128.80 (C-*i*-Bz); 128.90, 128.95 (CH-*m*-Bz); 129.38 (CH-*o*-Bz); 129.39 (C-*i*-Bz); 129.44, 129.58 (CH-*o*-Bz); 131.15 (CH-6); 133.72, 134.07, 134.11 (CH-*p*-Bz); 142.03 (C-7a); 151.65 (CH-2); 154.60 (C-8a); 156.89 (C-4); 164.64, 164.99, 165.58 (CO-Bz). ESI MS m/z (rel%): 634 (71) [M+H]; 656 (100) [M+Na]. HR MS (ESI) for $C_{35}H_{28}N_3O_7S$ [M+H]: calcd 634.16425; found 634.16453; for $C_{35}H_{28}N_3O_7SNa$ [M+Na]: calcd 656.14619; found: 656.14633.

4-*N*,*N*-Dimethylamino-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (60i)

Compound **60i** was prepared in the same manner as **43d** from **59** (300 mg, 0.46 mmol). Nucleoside **60i** (235 mg, 78 %) was obtained as white solid. m.p. 145-148 °C. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.42 (s, 6H, $(CH_3)_2$N); 4.70 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 4.8, H-5′b); 4.78 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.3, H-5′a); 4.85 (ddd, 1H, $J_{4',3'}$ = 5.5, $J_{4',5'}$ = 4.8, 3.3, H-4'); 6.22 (dd, 1H, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 5.5, H-3'); 6.43 (dd, 1H, $J_{2',3'}$ = 6.3, $J_{2',1'}$ = 5.4, H-2'); 6.93 (d, 1H, $J_{1',2'}$ = 5.4, H-1'); 7.41, 7.49, 7.51 (3 × m, 3 × 2H, H-*m*-Bz); 7.52 (d, 1H, $J_{6,7}$ = 5.4, H-6); 7.56 (d, 1H, $J_{7,6}$ = 5.4, H-7); 7.61, 7.67, 7.68 (3 × m, 3 × 1H, H-*p*-Bz); 7.82, 7.97, 7.98 (3 × m, 3 × 2H, H-*o*-Bz); 8.22 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 38.94 ((CH_3**)**$_2$N); 63.67 (CH_2-5'); 70.58 (CH-3'); 72.29 (CH-2'); 78.72 (CH-4'); 86.27 (CH-1'); 98.60 (C-4a); 113.01 (CH-7); 116.08 (C-4b); 127.03 (CH-6); 128.50, 128.82 (C-*i*-Bz); 128.93, 128.95, 128.97 (CH-*m*-Bz); 129.43 (C-*i*-Bz); 129.44, 129.47, 129.58 (CH-*o*-Bz); 133.77, 134.08, 134.13 (CH-*p*-Bz); 138.76 (C-7a); 151.52 (CH-2); 155.17 (C-8a); 156.30 (C-4); 164.67,

165.00, 165.64 (CO-Bz). ESI MS m/z (rel%): 663 (25) [M+H]; 685 (100) [M+Na]. HR MS (ESI) for $C_{36}H_{31}N_4O_7S$ [M+H]: calcd 663.19080; found 663.19101.

4-(Furan-2-yl)-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61a)

Deprotection of **60a** (150 mg, 0.22 mmol) according to the general procedure E afforded compound **61a** (51 mg, 62 %) as white crystals: m.p. 205-208 °C, $[\alpha]_D$ -7.0 (c 0.17), IR (ATR): ν = 3294, 1604, 1433, 1126, 623. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.68 (m, 2H, H-5'); 3.99 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 2.6, H-4'); 4.19 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.6, H-3'); 4.62 (ddd, 1H, $J_{2',1'}$ = 7.6, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.5, H-2'); 5.17 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5'); 5.21 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.31 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2'); 6.46 (d, 1H, $J_{1',2'}$ = 7.6, H-1'); 6.87 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.50 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.76 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.90 (d, 1H, $J_{6,7}$ = 5.3, H-6); 8.23 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.84 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 61.86 (CH_2-5'); 70.49 (CH-3'); 71.88 (CH-2'); 85.70 (CH-4'); 86.50 (CH-1'); 107.46 (C-4a); 112.82 (CH-3-furyl); 113.22 (CH-4-furyl); 114.31 (CH-7); 115.10 (C-4b); 131.37 (CH-6); 142.82 (C-7a); 143.99 (C-4); 146.37 (CH-5-furyl); 151.48 (CH-2); 152.43 (C-2-furyl); 156.76 (C-8a). ESI MS m/z (rel%): 396 (100) [M+Na]. HR MS (ESI) for $C_{17}H_{16}N_3O_5S$ [M+H]: calcd 374.08052; found 374.08064; for $C_{17}H_{15}N_3O_5SNa$ [M+Na]: calcd 396.06246; found 396.06255.

4-(Furan-3-yl)-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61b)

Deprotection of **60b** (280 mg, 0.41 mmol) according to the general procedure E afforded compound **61b** (107 mg, 70 %) as white crystals: m.p. 190 °C decomp.; $[\alpha]_D$ -15.6 (c 0.25). IR (ATR): ν = 3068, 1590, 1574, 1564, 1496, 1435, 1345, 1330, 1264, 1159, 1129, 1076, 1055, 877, 786, 731, 590. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.68 (dd, 2H, $J_{5',OH}$ = 5.3, $J_{5',4'}$ = 3.7, H-5'); 4.00 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 2.4, H-4'); 4.19 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.4, H-3'); 4.60 (ddd, 1H, $J_{2',1'}$ = 7.6, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.5, H-2'); 5.20 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.23 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.32 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 6.48 (d, 1H, $J_{1',2'}$ = 7.6, H-1'); 7.26 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.83 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.92 (d, 1H, $J_{6,7}$ = 5.3, H-6); 8.03 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl); 8.60 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 8.88 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.82 (CH_2-5'); 70.46 (CH-3'); 71.97 (CH-2'); 85.71 (CH-4'); 86.58 (CH-1'); 109.46 (CH-4-furyl); 109.94 (C-4a); 113.87 (C-4b); 114.50 (CH-7); 125.22 (C-3-furyl); 130.17 (CH-6); 142.68 (C-7a); 143.79 (CH-2-furyl); 145.28 (CH-5-furyl); 148.19 (C-4); 151.55 (CH-2); 156.31 (C-8a). ESI MS m/z

(rel%): 396 (100) [M+Na]. HR MS (ESI) for $C_{17}H_{16}N_3O_5S$ [M+H]: calcd 374.08052; found 374.08063.

4-(Benzofuran-2-yl)-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61e)

Nucleoside **60e** (260 mg, 0.35 mmol) was deprotected according to the general procedure E to obtain **61e** (101 mg, 68 %) as yellowish crystals: m.p. 82-86 °C, $[\alpha]_D$ -35.8 (c 0.26), IR (ATR): ν = 3324, 3087, 2947, 1562, 1492, 1454, 1430, 1341, 1123, 1080, 1052, 1034, 976, 831, 792, 739, 715, 658, 573. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.68, 3.71 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.3, $J_{5',4'}$ = 3.6, H-5'); 4.01 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.4, H-4'); 4.20 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.4, H-3'); 4.64 (ddd, 1H, $J_{2',1'}$ = 7.6, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.5, H-2'); 5.20 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.24 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.36 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 6.50 (d, 1H, $J_{1',2'}$ = 7.6, H-1'); 7.41 (ddd, 1H, $J_{5,4}$ = 7.9, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.54 (ddd, 1H, $J_{6,7}$ = 8.4, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.3, H-6-benzofuryl); 7.82 (d, 1H, $J_{7,6}$ = 5.4, H-7); 7.87 (ddd, 1H, $J_{4,5}$ = 7.9, $J_{4,6}$ = 1.3, $J_{4,7}$ = 1.0, H-4-benzofuryl); 7.89 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 1.0, H-7-benzofuryl); 7.92 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.99 (d, 1H, $J_{6,7}$ = 5.4, H-6); 8.94 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.83 (CH_2-5'); 70.47 (CH-3'); 71.91 (CH-2'); 85.74 (CH-4'); 86.58 (CH-1'); 108.24 (CH-3-benzofuryl); 108.81 (C-4a); 111.63 (CH-7-benzofuryl); 114.42 (CH-7); 115.23 (C-4b); 122.82 (CH-4-benzofuryl); 124.26 (CH-5-benzofuryl); 126.89 (CH-6-benzofuryl); 127.91 (C-3a-benzofuryl); 132.22 (CH-6); 143.33 (C-7a); 143.74 (C-4); 151.49 (CH-2); 153.92 (C-2-benzofuryl); 155.28 (C-7a-benzofuryl); 157.02 (C-8a). ESI MS m/z (rel%): 424 (15) [M+H]; 446 (100) [M+Na]. HR MS (ESI) for $C_{21}H_{18}N_3O_5S$ [M+H]: calcd 424.09617; found 424.09618.

4-Methyl-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61h)

Deprotection of **60h** (220 mg, 0.35 mmol) according to the general procedure E afforded compound **61h** (91 mg, 82 %) as white crystals: m.p. 150-153 °C, $[\alpha]_D$ -40.5 (c 0.20). IR (ATR): ν = 3287, 3088, 2933, 1602, 1560, 1488, 1437, 1339, 1312, 1204, 1127, 1111, 1080, 1050, 981, 887, 723, 668, 593, 533. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.76 (s, 3H, CH_3); 3.64, 3.67 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.2, $J_{5',4'}$ = 3.6, H-5'); 3.97 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.4, H-4'); 4.16 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.4, H-3'); 4.59 (ddd, 1H, $J_{2',1'}$ = 7.6, $J_{2',OH}$ = 6.7, $J_{2',3'}$ = 5.5, H-2'); 5.18 (t, 1H, $J_{OH,5'}$ = 5.2, OH-5'); 5.21 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.29 (d, 1H, $J_{OH,2'}$ = 6.7, OH-2'); 6.39 (d, 1H, $J_{1',2'}$ = 7.6, H-1'); 7.75 (d, 1H, $J_{7,6}$ = 5.2,

H-7); 7.89 (d, 1H, $J_{6,7}$ = 5.2, H-6); 8.75 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 22.23 (CH_3); 61.89 (CH_2-5'); 70.54 (CH-3'); 72.00 (CH-2'); 85.67 (CH-4'); 86.55 (CH-1'); 112.60 (C-4a); 114.29 (C-4b); 114.10 (CH-7); 130.54 (CH-6); 141.69 (C-7a); 151.59 (CH-2); 155.15 (C-8a); 156.38 (C-4). ESI MS m/z (rel%): 322 (17) [M+H]; 344 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{14}N_3O_4S$ [M+H]: calcd 322.08560; found 322.08576.

4-(Dimethylamino)-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61i)

Deprotection of **60i** (220 mg, 0.33 mmol) according to the general procedure E afforded compound **61i** (57 mg, 49 %) as white crystals: m.p. 200-204 °C, $[\alpha]_D$ -47.9 (c 0.28). IR (ATR): ν = 3257, 2932, 1580, 1548, 1505, 1420, 1402, 1332, 1127, 1100, 1075, 1041, 719, 587. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.43 (s, 6H, $(CH_3)_2$N); 3.64 (bm, 2H, H-5'); 3.93 (td, 1H, $J_{4',5'}$ = 3.7, $J_{4',3'}$ = 2.5, H-4'); 4.13 (dd, 1H, $J_{3',2'}$ = 5.7, $J_{3',4'}$ = 2.5, H-3'); 4.56 (dd, 1H, $J_{2',1'}$ = 7.4, $J_{2',3'}$ = 5.7, H-2'); 5.16, 5.21 (2 × bs, 2 × 1H, OH-2',3'); 5.25 (bt, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 6.33 (d, 1H, $J_{1',2'}$ = 7.4, H-1'); 7.55 (d, 1H, $J_{6,7}$ = 5.4, H-6); 7.58 (d, 1H, $J_{7,6}$ = 5.4, H-7); 8.23 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 39.23 ($(CH_3)_2$N); 62.24 (CH_2-5'); 70.79 (CH-3'); 72.10 (CH-2'); 85.74 (CH-4'); 87.13 (CH-1'); 98.51 (C-4a); 114.51 (CH-7); 116.01 (C-4b); 126.72 (CH-6); 138.94 (C-7a); 151.48 (CH-2); 155.81 (C-8a); 156.58 (C-4). ESI MS m/z (rel%): 351 (47) [M+H]; 373 (100) [M+Na]. HR MS (ESI) for $C_{15}H_{19}N_4O_4S$ [M+H]: calcd 351.11215; found 351.11228.

4-Amino-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61j)

Compound **61j** was prepared as described for **39** from **59** (200 mg, 0.31 mmol). The crude product was purified by RP-HPFC (gradient water/methanol 10-100 %). Nucleoside **61j** (74 mg, 75 %) was obtained as white powder. m.p. 210 °C decomp., $[\alpha]_D$ -28.2 (c 0.22). IR (ATR): ν = 3349, 3250, 3120, 2929, 2875, 1581, 1549, 1400, 1380, 1129, 1090, 1053, 1027, 716, 659. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.61 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 5.9, $J_{5'b,4'}$ = 3.6, H-5′b); 3.65 (ddd, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.6, H-5′a); 3.93 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.5, H-4′); 4.13 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.5, H-3′); 4.60 (ddd, 1H, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.8, $J_{2',3'}$ = 5.5, H-2′); 5.13 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3′); 5.21 (d, 1H, $J_{OH,2'}$ = 6.8, OH-2′); 5.28 (dd, 1H, $J_{OH,5'}$ = 5.9, 5.0, OH-5′); 6.26 (d, 1H, $J_{1',2'}$ = 7.5, H-1′); 7.11 (bs, 2H, NH_2); 7.54 (d, 1H, $J_{7,6}$ = 5.2, H-7); 7.62 (d, 1H, $J_{6,7}$ = 5.2, H-6); 8.16 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 62.04 (CH_2-5′); 70.63 (CH-3′); 71.88 (CH-2′); 85.47 (CH-4′); 87.02 (CH-1′); 97.37 (C-4a); 113.24 (CH-7); 114.17 (C-4b); 127.19 (CH-6); 139.08 (C-7a);

152.25 (CH-2); 155.14 (C-8a); 155.89 (C-4). ESI MS m/z (rel%): 323 (23) [M+H]; 345 (100) [M+Na]. HR MS (ESI) for $C_{13}H_{14}N_4O_4SNa$ [M+Na]: calcd 345.06280; found 345.06304.

4-Methoxy-8-(β-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (61k)

Nucleoside **39** (280 mg, 0.43 mmol) was suspended in methanol (30 ml) and sodium methoxide (46 mg, 0.85 mmol) was added. The reaction mixture was stirred overnight at r.t., then, solvent was evaporated and crude material was purified by RP-HPFC chromatography (gradient water/methanol 10-100 %). Nucleoside **61k** (95 mg, 66 %) was obtained as white powder. m.p. 139-141 °C, $[\alpha]_D$ -54.3 (c 0.14), IR (ATR): ν = 3246, 2932, 1582, 1548, 1439, 1423, 1402, 1343, 1124, 1080, 1054, 1033, 720. ^{1}H NMR (600.1 MHz, DMSO-d_6): 3.64, 3.67 (2 × ddd, 2 × 1H, J_{gem} = 11.8, $J_{5',OH}$ = 5.3, $J_{5',4'}$ = 3.6, H-5'); 3.97 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.5, H-4'); 4.14 (s, 3H, CH_3O); 4.16 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.5, $J_{3',4'}$ = 2.5, H-3'); 4.59 (ddd, 1H, $J_{2',1'}$ = 7.5, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 5.5, H-2'); 5.15 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5'); 5.18 (d, 1H, $J_{OH,3'}$ = 4.5, OH-3'); 5.27 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2'); 6.36 (d, 1H, $J_{1',2'}$ = 7.5, H-1'); 7.69 (d, 1H, $J_{7,6}$ = 5.2, H-7); 7.77 (d, 1H, $J_{6,7}$ = 5.2, H-6); 8.53 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, DMSO-d_6): 54.16 (CH_3O); 61.88 (CH_2-5'); 70.51 (CH-3'); 72.06 (CH-2'); 85.63 (CH-4'); 86.92 (CH-1'); 99.77 (C-4a); 113.24 (C-4b); 114.10 (CH-7); 129.19 (CH-6); 140.60 (C-7a); 151.36 (CH-2); 156.48 (C-8a); 161.04 (C-4). ESI MS m/z (rel%): 360 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{15}N_3O_5SNa$ [M+Na]: calcd 360.06246; found 360.06256.

4-(Methylsulfanyl)-8-(β-D-ribofuranosyl)-thieno[2',3':4,5]pyrrolo[2,3-d]pyrimidine (61l)

Nucleoside **39** (150 mg, 0.23 mmol) was suspended in methanol (10 ml) and sodium thiomethoxide (32 mg, 0.46 mmol) was added. The reaction mixture was stirred overnight at r.t., then, solvent was evaporated and crude material was purified by RP-HPFC chromatography (gradient water/methanol 10-100 %). Nucleoside **61l** (52 mg, 64 %) was obtained as white powder. m.p. 150-155 °C, $[\alpha]_D$ -35.3 (c 0.22). IR (ATR): ν = 3076, 2935, 1575, 1554, 1493, 1432, 1124, 1054, 978, 965, 728, 536. ^{1}H NMR (500.0 MHz, DMSO-d_6): 2.76 (s, 3H, CH_3S); 3.66 (bd, 2H, $J_{5',4'}$ = 3.6, H-5'); 3.97 (td, 1H, $J_{4',5'}$ = 3.6, $J_{4',3'}$ = 2.4, H-4'); 4.17 (dd, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 2.4, H-3'); 4.58 (dd, 1H, $J_{2',1'}$ = 7.5, $J_{2',3'}$ = 5.5, H-2'); 5.10-5.40 (bm, 3H, OH-2',3',5'); 6.37 (d, 1H, $J_{1',2'}$ = 7.5, H-1'); 7.76 (d, 1H, $J_{7,6}$ = 5.2, H-7); 7.91 (d, 1H, $J_{6,7}$ = 5.2, H-6); 8.74 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 11.69 (CH_3S); 61.86 (CH_2-5'); 70.52 (CH-3'); 72.11 (CH-2'); 85.74 (CH-4'); 86.68 (CH-1'); 110.57 (C-4a); 113.67 (C-4b); 114.35 (CH-7); 130.93 (CH-6); 141.06 (C-7a); 151.29 (CH-2); 153.21 (C-8a); 158.07

(C-4). ESI MS m/z (rel%): 376 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{16}N_3O_4S_2$ [M+H]: calcd 354.05767; found 354.05778.

4,6-Dichloro-5-(thiophen-3-yl)pyrimidine (62)

Compound **62** was prepared in the same manner as **55**. Desired product **62** (890 mg, 58 %) was obtained as white solid. m.p. 178-180 °C. IR (ATR): ν = 2932, 2862, 1510, 1404, 1326, 813, 774. ^{1}H NMR (600.1 MHz, $CDCl_3$): 7.15 (dd, 1H, $J_{4,5}$ = 4.9, $J_{4,2}$ = 1.4, H-4-thienyl); 7.47 (dd, 1H, $J_{2,5}$ = 3.0, $J_{2,4}$ = 1.4, H-2-thienyl); 7.48 (dd, 1H, $J_{5,4}$ = 4.9, $J_{5,2}$ = 3.0, H-5-thienyl); 8.75 (s, 1H, H-2). ^{13}C NMR (150.9 MHz, $CDCl_3$): 126.12 (CH-5-thienyl); 126.91 (CH-2-thienyl); 128.08 (CH-4-thienyl); 129.83 (C-5); 131.56 (C-3-thienyl); 156.44 (CH-2); 161.55 (C-4,6). APCI MS m/z (rel%): 231 (100) [M+H]. HR MS (APCI) for $C_8H_5N_2Cl_2S$ [M+H]: calcd 230.95450; found 230.95456.

4-azido-6-chloro-5-(thiophen-2-yl)pyrimidine (**63**)

Compound **63** was prepared according to modified literature conditions[94] from **55** (1.1 g, 4.9 mmol), which was dissolved in THF (10 ml), NaN_3 (320 mg, 4.9 mmol) and LiCl (204 mg, 4.9 mmol) were added and the reaction mixture was stirred for 2 days at r.t. Solvent was evaporated and crude material was purified by column chromatography on silica (hexane/EtOAc 6:1). Desired product **63** (1.0 g, 90 %) was obtained as yellow solid and according to NMR and IR spectra is present mainly in tetrazole form. m.p. 85 °C. IR (ATR): ν = 3390, 3086, 2148 (weak), 1587, 1514, 1406, 1382, 1324, 1182, 1086, 978, 898, 816, 794, 763, 633, 504. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.75 (dd, 1H, $J_{4,5}$ = 5.1, $J_{4,2}$ = 1.3, H-4-thienyl); 7.82 (dd, 1H, $J_{5,4}$ = 5.1, $J_{5,2}$ = 3.0, H-5-thienyl); 8.34 (dd, 1H, $J_{2,5}$ = 3.0, $J_{2,4}$ = 1.3, H-2-thienyl); 10.17 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 117.66 (C-5); 126.71 (CH-5-thienyl); 128.78 (CH-4-thienyl); 129.39 (C-3-thienyl); 130.22 (CH-2-thienyl); 137.98 (CH-2); 144.00, 150.95 (C-4,6).

4-Chloro-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (64)

Compound **64** was prepared as desribed for compound **59** from base **49** (150 mg, 0.7 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (724 mg, 1.4 mmol). Protected nucleoside **64** (187 mg, 40 %) was obtained as white solid. m.p. 166-169 °C. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.72 (dd, 1H, J_{gem} = 12.3 $J_{5'b,4'}$ = 4.6, H-5′b); 4.84 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.1, H-5′a); 5.03 (td, 1H, $J_{4',5'}$ = 4.6, 3.1, $J_{4',3'}$ = 4.6, H-4′); 6.11 (dd, 1H, $J_{3',2'}$ = 6.1, $J_{3',4'}$

= 4.6, H-3′); 6.32 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 5.7, H-2′); 6.96 (d, 1H, $J_{1',2'}$ = 5.7, H-1′); 7.43 (m, 2H, H-*m*-Bz); 7.47 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.48, 7.52 (2 × m, 2 × 2H, H-*m*-Bz); 7.53 (d, 1H, $J_{5,6}$ = 5.4, H-5); 7.62, 7.66, 7.69 (3 × m, 3 × 1H, H-*p*-Bz); 7.82, 7.95, 7.99 (3 × m, 3 × 2H, H-*o*-Bz); 8.66 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.76 (CH_2-5′); 70.94 (CH-3′); 72.53 (CH-2′); 79.85 (CH-4′); 86.54 (CH-1′); 112.08 (C-4a); 117.97 (CH-5); 120.41 (C-4b); 123.39 (CH-6); 128.40, 128.76 (C-*i*-Bz); 128.85, 128.90, 128.99 (CH-*m*-Bz); 129.32 (C-*i*-Bz); 129.38, 129.46, 129.58 (CH-*o*-Bz); 133.73, 134.12 (CH-*p*-Bz); 140.99 (C-7a); 149.15 (C-4); 150.91 (CH-2); 155.64 (C-8a); 164.58, 164.90, 165.59 (CO-Bz). ESI MS m/z (rel%): 676 (100) [M+Na]. HR MS (ESI) for $C_{34}H_{24}N_3O_7ClSNa$ [M+Na]: calcd 676.09157; found 676.09181.

4-(2-Furyl) -8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (65a)

Compound **65a** was prepared according to general procedure B from protected nucleoside **64** (200 mg, 0.31 mmol) and 2-(tributylstannyl)furan (131 mg, 0.38 mmol). Protected nucleoside **65a** (140 mg, 67 %) was obtained as white solid. m.p. 114-118 °C. IR (ATR): ν = 2933, 2862, 1722, 1605, 1562, 1446, 1435, 1289, 1264, 1134, 1110, 1091, 1055, 1029, 708, 687. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.73 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.7, H-5′b); 4.84 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.1, H-5′a); 5.02 (td, 1H, $J_{4',5'}$ = 4.7, 3.1, $J_{4',3'}$ = 4.7, H-4′); 6.13 (dd, 1H, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 4.7, H-3′); 6.33 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 5.9, H-2′); 6.84 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.00 (d, 1H, $J_{1',2'}$ = 5.9, H-1′); 7.40 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.42 (m, 2H, H-*m*-Bz); 7.47 – 7.56 (m, 5H, H-3-furyl, H-*m*-Bz); 7.62, 7.65, 7.70 (3 × m, 3 × 1H, H-*p*-Bz); 7.81 (m, 2H, H-*o*-Bz); 7.93 (d, 1H, $J_{5,6}$ = 5.4, H-5); 7.99-8.02 (m, 4H, H-*o*-Bz); 8.24 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.76 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.84 (CH_2-5′); 70.92 (CH-3′); 72.15 (CH-2′); 79.51 (CH-4′); 85.94 (CH-1′); 107.30 (C-4a); 112.99 (CH-4-furyl); 113.63 (CH-3-furyl); 121.21 (CH-6); 121.61 (CH-5); 121.71 (C-4b); 128.39, 128. 80 (C-*i*-Bz); 128.91, 128.94, 129.04 (CH-*m*-Bz); 129.41 (C-*i*-Bz); 129.48, 129.62 (CH-*o*-Bz); 133.76, 134.16 (CH-*p*-Bz); 140.83 (C-7a); 145.22 (C-4); 146.86 (CH-5-furyl); 151.21 (CH-2); 152.36 (C-2-furyl); 156.98 (C-8a); 164.60, 164.96, 165.67 (CO-Bz). ESI MS m/z (rel%): 686 (45) [M+H]; 708 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{28}N_3O_8S$ [M+H]: calcd 686.15916; found 686.15935.

4-(3-Furyl) -8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (65b)

Compound **65b** was prepared according to the general procedure A from protected nucleoside **64** (250 mg, 0.38 mmol) and furan-3-boronic acid (85 mg, 0.76 mmol). Nucleoside **65b** containing 30 % of inpurities (312 mg, 83 %) was obtained as yellow solid and was directly deprotected. ESI MS m/z (rel%): 686 (19) [M+H]; 708 (100) [M+Na]. HR MS (ESI) for $C_{38}H_{27}N_3O_8SNa$ [M+Na]: calcd 708.14111; found 708.14120.

4-(2-Benzofuryl)-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5] pyrrolo[2,3-*d*]pyrimidine (65e)

Compound **65e** was prepared according to the general procedure A from protected nucleoside **64** (250 mg, 0.38 mmol) and benzofuran-2-boronic acid (124 mg, 0.76 mmol). Nucleoside **65e** (228 mg, 82 %) was obtained as yellow solid. m.p. 110-113 °C. IR (ATR): ν = 2965, 2938, 1728, 1603, 1454, 1433, 1290, 1267, 1112, 1070, 1030, 709, 688. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.75 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.6, H-5′b); 4.86 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.1, H-5′a); 5.04 (td, 1H, $J_{4',5'}$ = 4.6, 3.1, $J_{4',3'}$ = 4.6, H-4′); 6.15 (dd, 1H, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 4.6, H-3′); 6.35 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 5.8, H-2′); 7.03 (d, 1H, $J_{1',2'}$ = 5.8, H-1′); 7.39 (ddd, 1H, $J_{5,4}$ = 8.0, $J_{5,6}$ = 7.3, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.42 (m, 2H, H-*m*-Bz); 7.45 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.48-7.55 (m, 5H, H-6-benzofuryl, H-*m*-Bz); 7.62, 7.64, 7.70 (3 × m, 3 × 1H, H-*p*-Bz); 7.82 (m, 2H, H-*o*-Bz); 7.85 (ddd, 1H, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.3, $J_{4,7}$ = 1.0, H-4-benzofuryl); 7.93 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.98 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 1.0, H-7-benzofuryl); 8.00, 8.01 (2 × m, 2 × 2H, H-*o*-Bz); 8.08 (d, 1H, $J_{5,6}$ = 5.4, H-5); 8.86 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.88 (CH_2-5′); 70.97 (CH-3′); 72.26 (CH-2′); 79.61 (CH-4′); 86.08 (CH-1′); 108.58 (C-4a); 109.31 (CH-3-benzofuryl); 112.06 (CH-7-benzofuryl); 121.55 (C-4b); 121.68 (CH-6); 121.83 (CH-5); 122.76 (CH-4-benzofuryl); 124.21 (CH-5-benzofuryl); 126.82 (CH-6-benzofuryl); 127.83 (C-3a-benzofuryl); 128.42, 128.82 (C-*i*-Bz); 128.94, 128.97, 129.07 (CH-*m*-Bz); 129.42 (C-*i*-Bz); 129.50, 129.65 (CH-*o*-Bz); 133.79, 134.19 (CH-*p*-Bz); 141.60 (C-7a); 145.00 (C-4); 151.19 (CH-2); 153.99 (C-2-benzofuryl); 155.62 (C-7a-benzofuryl); 157.19 (C-8a); 164.65, 164.98, 165.70 (CO-Bz). ESI MS m/z (rel%): 758 (100) [M+Na]. HR MS (ESI) for $C_{42}H_{29}N_3O_8S$ [M+H]: calcd 736.17481; found 736.17495.

4-Methyl-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5] pyrrolo[2,3-*d*]pyrimidine (65h)

Compound **65h** was prepared as described for **60h** from protected nucleoside **64** (150 mg, 0.23 mmol) and 2M solution of trimethylaluminium in toluene (345 µl, 0.69 mmol). Nucleoside **65h** (137 mg) was obtained in 90 % purity and was directly deprotected. ESI MS m/z (rel%): 634 (18) [M+H]; 656 (100) [M+Na]. HR MS (ESI) for $C_{35}H_{28}N_3O_7S$ [M+H]: calcd 634.16425; found 634.16474.

4-*N*,*N*-dimethylamino-8-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5] pyrrolo[2,3-*d*]pyrimidine (65i)

Compound **65i** was prepared as described for **60i** from protected nucleoside **64** (220 mg, 0.34 mmol). Nucleoside **65i** (190 mg, 85 %) was obtained as white solid. m.p. 148-151 °C. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.35 (s, 6H, $(CH_3)_2N$); 4.70 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.6, H-5′b); 4.81 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.1, H-5′a); 4.96 (td, 1H, $J_{4',5'}$ = 4.6, 3.1, $J_{4',3'}$ = 4.6, H-4′); 6.09 (dd, 1H, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 4.6, H-3′); 6.26 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.1, H-2′); 6.90 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 7.23 (d, 1H, $J_{6,5}$ = 5.5, H-6); 7.39-7.44 (m, 3H, H-5, H-*m*-Bz); 7.51, 7.52 (2 × m, 2 × 2H, H-*m*-Bz); 7.62, 7.678, 7.684 (3 × m, 3 × 1H, H-*p*-Bz); 7.81, 7.97, 8.03 (3 × m, 3 × 2H, H-*o*-Bz); 8.20 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 39.36 ($(CH_3)_2N$); 63.91 (CH_2-5′); 70.88 (CH-3′); 71.79 (CH-2′); 79.17 (CH-4′); 85.59 (CH-1′); 97.93 (C-4a); 119.86 (CH-6); 121.21 (CH-5); 122.04 (C-4b); 128.37, 128.79 (C-*i*-Bz); 128.96, 129.04 (CH-*m*-Bz); 129.46 (C-*i*-Bz); 129.49, 129.54, 129.60 (CH-*o*-Bz); 133.80, 134.15, 134.19 (CH-*p*-Bz); 135.17 (C-7a); 151.12 (CH-2); 156.29 (C-8a); 157.01 (C-4); 164.59, 164.97, 165.71 (CO-Bz). ESI MS m/z (rel%): 663 (15) [M+H]; 685 (100) [M+Na]. HR MS (ESI) for $C_{36}H_{31}N_4O_7S$ [M+H]: calcd 663.19080; found 663.19088.

4-(2-Furyl)-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66a)

Compound **65a** (140 mg, 0.20 mmol) was deprotected according to the general procedure E. Nucleoside **66a** (52 mg, 68 %) was obtained as white solid. m.p. 189-192 °C, $[\alpha]_D$ -65.1 (c 0.19). IR (ATR): ν = 3242, 2930, 1579, 1548, 1504, 1420, 1401, 1331, 1127, 1099, 1041, 719. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.63, 3.67 (2 × bdd, 2 × 1H, J_{gem} = 11.5, $J_{5',4'}$ = 5.2, H-5′); 3.98 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 2.8, H-4′); 4.13 (dd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 2.8, H-3′); 4.60 (dd, 1H, $J_{2',1'}$ = 7.1, $J_{2',3'}$ = 5.4, H-2′); 5.01 (bs, 1H, OH-5′); 5.35 (bs, 1H, OH-3′); 5.49 (bs, 1H, OH-2′); 6.48 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 6.85 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.43 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.50 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H-3-furyl); 7.96 (d, 1H, $J_{5,6}$ = 5.4, H-

5); 8.25 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.83 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.19 (CH_2-5′); 70.76 (CH-3′); 71.11 (CH-2′); 85.47 (CH-4′); 86.45 (CH-1′); 106.96 (C-4a); 112.97 (CH-4-furyl); 113.41 (CH-3-furyl); 121.09 (CH-5); 121.38 (CH-6); 121.41 (C-4b); 140.88 (C-7a); 145.00 (C-4); 146.68 (CH-5-furyl); 151.14 (C-2-furyl); 152.55 (CH-2); 157.45 (C-8a). ESI MS m/z (rel%): 396 (100) [M+Na]. HR MS (ESI) for $C_{17}H_{15}N_3O_5SNa$ [M+Na]: calcd 396.06246; found 396.06251.

4-(3-Furyl)- 8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66b)

Crude compound **65b** (300 mg, 70 % purity, 0.31 mmol) was deprotected according to the general procedure E. Nucleoside **66b** (95 mg, 83 %) was obtained as white crystals. m.p. 192-195 °C, $[\alpha]_D$ -1.6 (c 0.19). IR (ATR): ν = 3424, 3225, 3161, 1565, 1496, 1453, 1441, 1301, 1261, 1132, 1051, 1016, 878, 817, 795, 652, 643, 596. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.62, 3.67 (2 × ddd, 2 × 1H, J_{gem} = 11.5, $J_{5',OH}$ = 5.5, $J_{5',4'}$ = 5.2, H-5′); 3.98 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 2.8, H-4′); 4.12 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.6, $J_{3',4'}$ = 2.8, H-3′); 4.59 (ddd, 1H, $J_{2',1'}$ = 7.3, $J_{2',OH}$ = 6.3, $J_{2',3'}$ = 5.5, H-2′); 5.04 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5′); 5.35 (d, 1H, $J_{OH,3'}$ = 4.6, OH-3′); 5.50 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2′); 6.48 (d, 1H, $J_{1',2'}$ = 7.3, H-1′); 7.19 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.43 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.62 (d, 1H, $J_{5,6}$ = 5.4, H-5); 7.96 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.6, H-5-furyl); 8.57 (dd, 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl); 8.86 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.24 (CH_2-5′); 70.82 (CH-3′); 71.22 (CH-2′); 85.54 (CH-4′); 86.54 (CH-1′); 109.79 (C-4a); 110.32 (CH-4-furyl); 119.50 (CH-5); 120.69 (C-4b); 121.74 (CH-6); 125.17 (C-3-furyl); 140.65 (C-7a); 144.42 (CH-2-furyl); 144.90 (CH-5-furyl); 149.34 (C-4); 151.29 (CH-2); 156.92 (C-8a). ESI MS m/z (rel%): 396 (100) [M+Na]. HR MS (ESI) for $C_{17}H_{15}N_3O_5SNa$ [M+Na]: calcd 396.06246; found 396.06237.

4-(2-Benzofuryl)-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66e)

Compound **65e** was deprotected according to the general procedure E. Nucleoside **66e** (100 mg, 86 %) was obtained as yellowish crystals. m.p. 118-119 °C, $[\alpha]_D$ -29.6 (c 0.13). IR (ATR): ν = 3259, 1563, 1494, 1435, 1300, 1276, 1129, 1054, 1044, 1025, 794, 744, 644, 599. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.65, 3.69 (2 × ddd, 2 × 1H, J_{gem} = 11.5, $J_{5',OH}$ = 5.5, $J_{5',4'}$ = 5.2, H-5′); 3.99 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 2.8, H-4′); 4.14 (ddd, 1H, $J_{3',2'}$ = 5.3, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.8, H-3′); 4.62 (ddd, 1H, $J_{2',1'}$ = 7.1, $J_{2',OH}$ = 6.3, $J_{2',3'}$ = 5.3, H-2′); 5.03 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5′); 5.36 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.52 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2′); 6.52 (d, 1H,

$J_{1',2'}$ = 7.1, H-1′); 7.40 (ddd, 1H, $J_{5,4}$ = 7.9, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.50 (d, 1H, $J_{6,5}$ = 5.4, H-6); 7.52 (ddd, 1H, $J_{6,7}$ = 8.4, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.3, H-6-benzofuryl); 7.86 (ddd, 1H, $J_{4,5}$ = 7.9, $J_{4,6}$ = 1.3, $J_{4,7}$ = 1.0, H-4-benzofuryl); 7.95 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 8.00 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 1.0, H-7-benzofuryl); 8.12 (d, 1H, $J_{5,6}$ = 5.4, H-5); 8.94 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.18 (CH_2-5′); 70.77 (CH-3′); 71.16 (CH-2′); 85.55 (CH-4′); 86.52 (CH-1′); 108.22 (C-4a); 109.12 (CH-3-benzofuryl); 112.07 (CH-7-benzofuryl); 121.27 (C-4b); 121.60 (CH-5,6); 122.72 (CH-4-benzofuryl); 124.20 (CH-5-benzofuryl); 126.73 (CH-6-benzofuryl); 127.88 (C-3a-benzofuryl); 141.64 (C-7a); 144.75 (C-4); 151.15 (CH-2); 154.19 (C-2-benzofuryl); 155.58 (C-7a-benzofuryl); 157.68 (C-8a). ESI MS m/z (rel%): 424 (31) [M+H]; 446 (100) [M+Na]. HR MS (ESI) for $C_{21}H_{17}N_3O_5S$ [M+H]: calcd 423.0889; found 423.0894.

4-Methyl-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66h)

Crude compound **65h** (125 mg, 0.23 mmol) was deprotected according to the general procedure E. Nucleoside **66h** (43 mg, 70 %) was obtained as white lyophilizate. m.p. 126-128 °C, $[\alpha]_D$ -62.3 (c 0.20). IR (ATR): ν = 3524, 3131, 2848, 1608, 1504, 1450, 1402, 1323, 1257, 1113, 1051, 654. ^{1}H NMR (500.0 MHz, DMSO-d_6): 2.84 (s, 3H, CH_3); 3.58-3.67 (bm, 2H, H-5′); 3.95 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 2.8, H-4′); 4.11 (dd, 1H, $J_{3',2'}$ = 5.3, $J_{3',4'}$ = 2.8, H-3′); 4.56 (dd, 1H, $J_{2',1'}$ = 7.2, $J_{2',3'}$ = 5.3, H-2′); 5.01 (bs, 1H, OH-5′); 5.34 (bs, 1H, OH-3′); 5.47 (bs, 1H, OH-2′); 6.41 (d, 1H, $J_{1',2'}$ = 7.2, H-1′); 7.42 (d, 1H, $J_{6,5}$ = 5.3, H-6); 7.63 (d, 1H, $J_{5,6}$ = 5.3, H-5); 8.73 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 22.28 (CH_3); 62.22 (CH_2-5′); 70.80 (CH-3′); 71.24 (CH-2′); 85.42 (CH-4′); 86.42 (CH-1′); 112.20 (C-4a); 118.75 (CH-5); 121.52 (C-4b); 121.83 (CH-6); 139.32 (C-7a); 151.25 (CH-2); 155.73 (C-8a); 157.35 (C-4). ESI MS m/z (rel%): 322 (25) [M+H]; 344 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{15}N_3O_4S$ [M+H]: calcd 321.0783; found 321.0789.

4-*N*,*N*-dimethylamino-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66i)

Compound **65i** (90 mg, 0.14 mmol) was deprotected according to the general procedure E. Nucleoside **66i** (42 mg, 88 %) was obtained as white crystals. m.p. 117 °C, $[\alpha]_D$ -8.5 (c 0.16). IR (ATR): ν = 3258, 1583, 1460, 1442, 1420, 1314, 1112, 1053, 787, 646. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.34 (s, 6H, $(CH_3)_2N$); 3.59, 3.64 (2 × dd, 2 × 1H, J_{gem} = 11.6, $J_{5',4'}$ = 5.2, H-5′); 3.92 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 3.0, H-4′); 4.08 (dd, 1H, $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 3.0, H-3′); 4.55 (dd, 1H, $J_{2',1'}$ = 7.1, $J_{2',3'}$ = 5.5, H-2′); 5.07, 5.37 (2 × bs, 3H, OH-2′,3′,5′); 6.35

(d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.27 (d, 1H, $J_{6,5}$ = 5.5, H-6); 7.44 (d, 1H, $J_{5,6}$ = 5.5, H-5); 8.23 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 39.39 ($(CH_3)_2N$); 62.29 (CH_2-5′); 70.82 (CH-3′); 71.00 (CH-2′); 85.20 (CH-4′); 86.80 (CH-1′); 97.76 (C-4a); 119.65 (CH-6); 120.88 (CH-5); 121.53 (C-4b); 135.58 (C-7a); 150.85 (CH-2); 156.55 (C-8a); 157.10 (C-4). ESI MS m/z (rel%): 351 (39) [M+H]; 373 (100) [M+Na]. HR MS (ESI) for $C_{15}H_{19}N_4O_4S$ [M+H]: calcd 351.11215; found 351.11223.

4-Amino-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66j)

Compound **66j** was prepared as described for **39** from protected nucleoside **64** (280 mg, 0.42 mmol). Nucleoside **66j** (107 mg, 78 %) was obtained as white crystals. m.p. 98 °C, $[\alpha]_D$ -24.7 (c 0.15). IR (ATR): ν = 3452, 3347, 3073, 2933, 2862, 1723, 1605, 1551, 1453, 1263, 1093, 1068, 1028, 707. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.58, 3.63 (2 × bdd, 2 × 1H, J_{gem} = 11.7, $J_{5',4'}$ = 5.0, H-5′); 3.92 (td, 1H, $J_{4',5'}$ = 5.0, $J_{4',3'}$ = 2.8, H-4′); 4.08 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.3, $J_{3',4'}$ = 2.8, H-3′); 4.55 (ddd, 1H, $J_{2',1'}$ = 7.1, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 5.5, H-2′); 5.08 (bs, 1H, OH-5′); 5.25 (bd, 1H, $J_{OH,3'}$ = 4.3, OH-3′); 5.40 (bd, 1H, $J_{OH,2'}$ = 6.5, OH-2′); 6.26 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.18 (bs, 2H, NH_2); 7.26 (d, 1H, $J_{6,5}$ = 5.3, H-6); 7.82 (d, 1H, $J_{5,6}$ = 5.3, H-5); 8.16 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.33 (CH_2-5′); 70.89 (CH-3′); 71.22 (CH-2′); 85.22 (CH-4′); 86.77 (CH-1′); 96.80 (C-4a); 118.91 (CH-6); 119.96 (CH-5); 121.48 (C-4b); 135.57 (C-7a); 151.95 (CH-2); 155.89 (C-8a); 156.19 (C-4). ESI MS m/z (rel%): 323 (15) [M+H]; 345 (100) [M+Na]. HR MS (ESI) for $C_{13}H_{15}N_4O_7S$ [M+H]: calcd 323.08085; found 323.08091.

4-Methoxy-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (66k)

Compound **66k** was prepared as described for **61k** from protected nucleoside **64** (130 mg, 0.20 mmol). Nucleoside **66k** (43 mg, 65 %) was obtained as white crystals. m.p. 159-160 °C, $[\alpha]_D$ -47.3 (c 0.15, DMSO). IR (ATR): ν = 3617, 3480, 2951, 1610, 1564, 1443, 1335, 1308, 1205, 1127, 1052, 1023, 975, 635, 602. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.60, 3.64 (2 × ddd, 2 × 1H, J_{gem} = 11.6, $J_{5',OH}$ = 5.6, $J_{5',4'}$ = 5.1, H-5′); 3.95 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 2.8, H-4′); 4.10 (dd, 1H, $J_{3',2'}$ = 5.3, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.8, H-3′); 4.13 (s, 3H, CH_3O); 4.55 (dd, 1H, $J_{2',1'}$ = 7.1, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.3, H-2′); 5.01 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.32 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.47 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2′); 6.38 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.38 (d, 1H, $J_{5,6}$ = 5.3, H-5); 7.39 (d, 1H, $J_{6,5}$ = 5.3, H-6); 8.53 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 54.11 (CH_30); 62.22 (CH_2-5′); 70.80 (CH-3′); 71.29 (CH-2′); 85.42 (CH-4′); 86.72 (CH-1′); 99.35 (C-4a); 118.11 (CH-5); 120.51 (C-4b); 122.00 (CH-6); 137.53 (C-7a); 151.17 (CH-2);

157.17 (C-8a); 161.53 (C-4). ESI MS m/z (rel%): 360 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{15}N_3O_5SNa$ [M+Na]: calcd 360.06246; found 360.06254.

4-Methylsulfanyl-8-(β-D-ribofuranosyl)-8*H*-thieno[3',2':4,5]pyrrolo[2,3-d]pyrimidine (66l)

Compound **66l** was prepared as described for **61l** from protected nucleoside **64** (80 mg, 0.12 mmol). Nucleoside **66l** (33 mg, 90 %) was obtained as white crystals. m.p. 148-152 °C, $[\alpha]_D$ -50.9 (c 0.16). IR (ATR): ν = 3305, 1576, 1556, 1497, 1471, 1431, 1321, 1265, 1246, 1136, 1094, 1055, 912, 821, 719, 645. ^{1}H NMR (500.0 MHz, DMSO-d_6): 2.74 (s, 3H, CH_3S); 3.61, 3.64 (2 × dd, 2 × 1H, J_{gem} = 11.6, $J_{5',4'}$ = 5.2, H-5′); 3.96 (td, 1H, $J_{4',5'}$ = 5.2, $J_{4',3'}$ = 2.8, H-4′); 4.11 (dd, 1H, $J_{3',2'}$ = 5.3, $J_{3',4'}$ = 2.8, H-3′); 4.55 (dd, 1H, $J_{2',1'}$ = 7.1, $J_{2',3'}$ = 5.3, H-2′); 4.90-5.70 (bm, 3H, OH-2′,3′,5′); 6.38 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.43 (d, 1H, $J_{5,6}$ = 5.3, H-5); 7.45 (d, 1H, $J_{6,5}$ = 5.3, H-6); 8.73 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 11.57 (CH_3S); 62.19 (CH_2-5′); 70.77 (CH-3′); 71.33 (CH-2′); 85.49 (CH-4′); 86.54 (CH-1′); 110.05 (C-4a); 118.07 (CH-5); 120.83 (C-4b); 122.46 (CH-6); 138.67 (C-7a); 151.00 (CH-2); 153.70 (C-8a); 159.00 (C-4). ESI MS m/z (rel%): 376 (100) [M+Na]. HR MS (ESI) for $C_{14}H_{16}N_3O_4S_2$ [M+H]: calcd 354.05767; found 354.05772.

3-Iodothiophene

3-Iodothiophene was prepared according to modified literature conditions.[145] CuI (1.75 g, 9.0 mmol) and NaI (55.0 g, 367 mmol) were suspended in toluene (100 ml) and dimethoxyethane (30 ml), *N*,*N*-dimethylethylenediamine (2 ml, 20 mmol) and 3-bromothiophene (17.3 ml, 184 mmol) were added and reaction mixture was heated to 110 °C for 40 hrs. Volatiles were removed under reduced pressure and crude mixture was washed with hexane (3 × 100 ml). Hexane was evaporated to give 3-iodothiophene (27 g, 70 %) of NMR pure liquid. NMR spectra are in agreement with literature.[145]

5.8 X-ray crystallography

The single crystal data were collected at 170K (**4** and **26**) and 180K (**58** and **62**) on Xcalibur PX diffractometer with the graphite monochromatized $Cu_{K\alpha}$ radiation (λ=1.54180 Å). CrysAlisProCCD[152] was used for data collection, cell refinement and data reduction. The structures were solved by direct methods (SIR92)[153] (**4** and **62**) and by charge flipping (SUPERFLIP)[154]. They were all refined by full-matrix least-squares based on F with CRYSTALS.[155] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms

were found on difference Fourier map, but those attached to carbon atoms were recalculated into idealized positions and refined with riding constraints.

Crystal data for 4 (light brown, 0.18 x 0.22 x 0.58 mm)**:**

$C_{10}H_5Cl_2N_3$, monoclinic, space group $P2_1/c$, a = 7.6521(4) Å, b = 6.9509(3) Å, c = 17.7525(8) Å, β = 91.496(4)°, V = 943.91(8) Å^3, Z = 4, M = 238.08, 7781 reflections measured, 1967 independent reflections. Final R = 0.038, wR = 0.050, GoF = 0.991 for 1773 reflections with $I > 2\sigma(I)$ and 137 parameters.

Crystal data for 26 (colourless, 0.13 x 0.28 x 0.64 mm)**:**

$C_{36}H_{25}Cl_2N_3O_7$, monoclinic, space group $P2_1$, a = 6.4327(4) Å, b = 17.1451(17) Å, c = 14.5954(15) Å, β = 99.062(9)°, V = 1589.6(3) Å^3, Z = 2, M = 682.52, 12428 reflections measured, 6315 independent reflections. Final R = 0.065, wR = 0.078, GoF = 1.080 for 4230 reflections with $I > 2\sigma(I)$ and 434 parameters, Flack parameter x = -0.03(3).

Crystal data for 58 (yellow, 0.07 x 0.18 x 0.21 mm)**:**

$C_9H_5N_5O_2S_1$, orthorhombic, space group $P2_12_12_1$, a = 6.1658(3) Å, b = 9.2302(4) Å, c = 17.2207(7) Å, V = 980.05(7) Å^3, Z = 4, M = 247.24, 3601 reflections measured, 1971 independent reflections. Final R = 0.032, wR = 0.034, GoF = 1.028 for 1893 reflections with $I > 2\sigma(I)$ and 156 parameters, Flack parameter x = 0.013(17).

Crystal data for 62 (colourless, 0.12 x 0.33 x 0.73 mm)**:**

$C_8H_4Cl_2N_2S_1$, orthorhombic, space group $P2_12_12_1$, a = 6.7583(3) Å, b = 7.3412(4) Å, c = 18.8485(8) Å, V = 935.15(8) Å^3, Z = 4, M = 231.10, 8246 reflections measured, 1923 independent reflections. Final R = 0.058, wR = 0.068, GoF = 0.969 for 1866 reflections with $I > 2\sigma(I)$ and 120 parameters, Flack parameter x = 0.0(3).

6 References

[1] Legraverend, M. *Tetrahedron* **2008**, *64*, 8585-8603.

[2] Legraverend, M.; Grierson, D. *Bioorg. Med. Chem.* **2006**, *14*, 3987-4006.

[3] Rosemeyer, H. *Chem. Biodivers.* **2004**, *1*, 361–401.

[4] Müller, Ch. E.; Deters, D.; Dominik, A.; Pawlowski, M. *Synthesis* **1998**, 1428-1436.

[5] Letham, D. S. *Life Sci.* **1963**, *8*, 569-573.

[6] Searle, P. A.; Molinsk, T. F. *J. Nat. Prod.* **1994**, *57*, 1452-1454.

[7] Evans, B.; Wolfenden, R. *J. Am. Chem. Soc.* **1970**, *92*, 4751-4752.

[8] http://www.who.int/mediacentre/factsheets/fs360/en/# Section accessed [07/10/2014]

[9] Darbyshire, J. H. *The Lancet* **1996**, *348*, 283-291.

[10] Justesen, U. *Basic Clin. Pharmacol. Toxicol.* **2006**, *98*, 20–31.

[11] Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Section accessed [07/10/2014]

[12] De Clercq, E.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holý, A. *Antiviral Res.* **1987**, *8*, 261-272.

[13] Wiktor, S.; Ford, N.; Ball, A.; Hirnschall, G. *J. Int. AIDS Soc.* **2014**, *17:*19323.

[14] http://www.who.int/mediacentre/factsheets/fs164/en/ Accessed [07/10/2014]

[15] Houghton, M. *J. Hepatol.* **2009**, *51*, 939–48.

[16] Kronenberger, B.; Zeuzem, S. *Ann. Hepatol.* **2009**, *8*, 103–112.

[17] Eldrup, A.; Allerson, C.; Bennett, C.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M.; Brooks, J.; Burlein, C.; Carroll, S.; Cook, P.; Getty, K.; MacCoss, M.; McMasters, D.; Olsen, D.; Prakash, T.; Prhavc, M.; Song, Q.; Tomassini, J.; Xia, J. *J. Med. Chem.* **2004**, *47*, 2283-2295.

[18] Carroll, S.; Tomassini, J.; Bosserman, M.; Getty, K.; Stahlhut, M.; Eldrup, A.; Bhat, B.; Hall, D.; Simcoe, A.; LaFemina, R.; Rutkowski, C.; Wolanski, B.; Yang, Z.; Migliaccio, G.; Francesco, R.; Kuo, L.; MacCoss, M.; Olsen, D. *J. Biol. Chem.* **2003**, *278*, 11979–11984.

[19] Kwong, A. *ACS Med. Chem. Lett.* **2014**, *5*, 214–220.

[20] Oishi, K.; Saito, M.; Mapua, C.; Natividad, F. *J. Infect. Chemother.* **2007**, *13*, 125-133.

[21] http://www.who.int/mediacentre/factsheets/fs117/en/ Accessed [07/10/2014]

[22] Lim, S.; Wang, Q.-Y.; Noble, C.; Chen, Y.-L.; Dong, H.; Zou, B.; Yokokawa, F.; Nilar, S.; Smith, P.; Beer, D.; Lescar, J.; Shi, P.-Y. *Antiviral Res.* **2013**, *100*, 500–519.

[23] Yin, Z.; Chen, Y.-L.; Schul, W.; Wang, Q.-Y.; Gu, F.; Duraiswamy, J.; Kondreddi, R.; Niyomrattanakit, P.; Lakshminarayana, S.; Goh, A.; Xu, H.; Liu, W.; Liu, B.; Lim, J.; Ng, C.; Qing, M.; Lim, C.; Yip, A.; Wang, G.; Chan, W.; Tan, H.; Lin, K.; Zhang, B.; Zou, G.; Bernard, K.; Garrett,

C.; Beltz, K.; Dong, M.; Weaver, M.; He, H.; Pichota, A.; Dartois, V.; Keller, T.; Shi, P.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20435–20439.

[24] Wu, R.; Smidansky, E.; Oh, H.; Takhampunya, R.; Padmanabhan, R.; Cameron, C.; Peterson, B. *J. Med. Chem.* **2010**, *53*, 7958–7966.

[25] http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx Accessed [07/10/2014]

[26] Jordheim, L.; Durantel, D.; Zoulim, F.; Dumontet, C. *Nat. Rev. Drug Discov.* **2013**, *12*, 447–464.

[27] Cano-Soldado, P.; Pastor-Anglada, M. *Med. Res. Rev.* **2012**, *32*, 428–457.

[28] Minuesa, G.; Huber-Ruano, I.; Pastor-Anglada, M.; Koepsell, H.; Clotet, B.; Martinez-Picado, J. *Pharmacol. Ther*. **2011**, *132*, 268–279.

[29] Galmarini, C.; Mackey, J.; Dumontet, C. *Leukemia* **2001**, *15*, 875-890.

[30] Galmarini, C.; Mackey, J.; Dumontet, C. *Lancet Oncol.* **2002**, *3*, 415-424.

[31] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817-1825.

[32] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2000**, *65*, 1683-1697.

[33] Hocek, M.; Šilhár, P.; Pohl, R. *Collect. Czech. Chem. Commun.* **2006**,*71*, 1484-1496.

[34] Hocek, M.; Šilhár, P.; Shih, I.; Mabery, E.; Mackman, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5290-5293.

[35] Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2001**, *66*, 483-499.

[36] Hocek, M.; Nauš, P.; Pohl, R.; Votruba, I.; Furman, P.; Tharnish, P.; Otto, M. *J. Med. Chem.* **2005**, *48*, 5869-5873.

[37] Hocek, M.; Holý, A.; Dvořáková, H. *Collect. Czech. Chem. Commun.* **2002**, *67*, 325-335.

[38] Hocek, M.; Hocková, D.; Štambaský, J. *Collect. Czech. Chem. Commun.* **2003**, *68*, 837-848.

[39] Kimoto, M.; Moriyama, K.; Yokoyama, S.; Hirao, I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5582-5585.

[40] Nauš, P.; Kuchař, M.; Hocek, M. *Collect. Czech. Chem. Commun.* **2008**, *73*, 665-678.

[41] Nauš, P.; Pohl, R.; Votruba, I.; Džubák, P.; Hajdúch, M.; Ameral, R.; Birkuš, G.; Wang, T.; Ray, A.; Mackman, R.; Cihlar, T.; Hocek, M. *J. Med. Chem.* **2010**, *53*, 460–470.

[42] Perlíková, P.; Konečný, P.; Nauš, P.; Snášel, J.; Votruba, I.; Džubák, P.; Pichová, I.; Hajdúch, M.; Hocek, M. *Med. Chem. Comm.* **2013**, *4*, 1497–1500.

[43] Acs, G.; Reich, E.; Mori, M. *P. Natl. Acad. Sci. USA* **1964**, *52*, 493-501.

[44] Bourderioux, A.; Nauš, P.; Perlíková, P.; Pohl, R.; Pichová, I.; Votruba, I.; Džubák, P.; Konečný, P.; Hajdúch, M.; Stray, K.; Wang, T.; Ray, A.; Feng, J.; Birkuš, G.; Cihlar, T.; Hocek, M. *J. Med. Chem.* **2011**, *54*, 5498–507.

[45] Perlíková, P. Unpublished results

[46] Nauš, P.; Caletková, O.; Konečný, P.; Džubák, P.; Bogdanová, K.; Kolář, M.; Vrbková, J.; Slavětínská, L.; Tloušťová, E.; Perlíková, P.; Hajdúch, M.; Hocek, M. *J. Med. Chem.* **2014**, *57*, 1097-1110.

[47] Spáčilová, P.; Nauš, P.; Pohl, R.; Votruba, I.; Snášel, J.; Zábranská, H.; Pichová, I.; Ameral, R.; Birkuš, G.; Cihlář, T.; Hocek, M. *Chem. Med. Chem.* **2010**, *5*, 1386-1396.

[48] Perlíková, P.; Pohl, R.; Votruba, I.; Shih, R.; Birkuš, G.; Cihlář, T.; Hocek, M. *Bioorg. Med. Chem.* **2011**, *19*, 229–242.

[49] Nauš, P.; Perlíková, P.; Pohl, R.; Hocek, M. *Collect. Czech. Chem. Commun.* **2011**, *76*, 957-988.

[50] Perlíková, P.; Jornet Martínez, N.; Slavětínská, L.; Hocek, M. *Tetrahedron* **2012**, *68*, 8300-8310.

[51] Perlíková, P.; Eberlin, L.; Ménová, P.; Raindlová, V.; Slavětínská, L.; Tloušťová, E.; Bahador, G.; Lee, Y.; Hocek, M. *Chem. Med. Chem.* **2013**, *8*, 832–846.

[52] Snášel, J.; Nauš, P.; Dostál, J.; Hnízda, A.; Fanfrlík, J.; Brynda, J.; Bourderioux, A.; Dušek, M.; Dvořáková, H.; Stolaříková, J.; Zábranská, H.; Pohl, R.; Konečný, P.; Džubák, P.; Votruba, I.; Hajdúch, M.; Řezáčová, P.; Veverka, V.; Hocek, M.; Pichová, I. *J. Med. Chem.* **2014**, *57*, 8268 – 8279.

[53] Hyatt, J.; Swenton, J. *J. Heterocycl. Chem.* **1972**, *9*, 409–410.

[54] Temple, C.; McKee, R. L.; Montgomery, J. A. *J. Org. Chem.* **1965**, *30*, 829–834.

[55] P. G. Gassman, *Acc. Chem. Res.* **1970**, *9*, 26-33.

[56] Hyatt, J.; Swenton, J. *J. Org. Chem.* **1972**, *37*, 3216–3220.

[57] Eger, K.; Lanzner, W.; Rothenhäusler, K. *Liebigs Ann. Chem.* **1993**, 465–470.

[58] Xu, G.; Zheng, L.; Wang, S.; Dang, Q.; Bai, X. *Synlett* **2009**, 3206-3210.

[59] Xu, G.; Zheng, L.; Dang, Q.; Bai, X. *Synthesis* **2013**, *45*, 743-752.

[60] Majumder, S.; Bhuyan, P. *J. Iran. Chem. Soc.* **2013**, *11*, 993-996.

[61] Mizar, P.; Myrboh, B. *Tetrahedron Lett.* **2008**, *49*, 5283-5285.

[62] Hubschwerlen, C.; Pflieger, P.; Specklin, J.-L.; Gubernator, K.; Gmiinder, H.; Angehrn, P.; Kompi I. *J. Med. Chem.* **199**, *35*, 1387-1392.

[63] a) Bridges, A.; Zhou, H. *J. Heterocycl. Chem.* **1997**, *34*, 1163-1172. b) Schneller, S.; Clough, F. *J. Heterocycl. Chem.* **1974**, *11*, 975-977. c) Venugopalan, B.; Desai, P.; Souza, N. *J. Heterocycl. Chem.* **1988**, *25*, 1633-1639. d) Showalter, H.; Bridges, A.; Zhou, H.; Sercel, A.; McMichael, A.; Fry, D. *J. Med. Chem.* **1999**, *42*, 5464-5474.

[64] a) Lim, M.I.; Ren, W.Y.; Otter, B.A.; Klein, R.S. *J. Org. Chem.* **1983,** *48*, 780-788. b) Anderson, R.; Hsiao, Y. *J. Heterocycl. Chem.* **1975**, *12*, 883-887. c) Semeraro, T.; Mugnaini, C.; Corelli, F. *Tetrahedron Lett.* **2008**, *49*, 5965-5967. d) Kamath, V.; Ananth, S.; Bantia, S.; Morris, P. *J. Med. Chem.* **2004**, *47*, 1322-1324. e) Dishington, A.; Johnson, P.; Kettle, J. *Tetrahedron Lett.* **2004**, *45*, 3733-3735.

[65] Allen, G.; Pidacks, C.; Weiss, M. *J. Am. Chem. Soc.* **1966**, *88*, 2536–2544.

[66] Dotzauer, B.; Troschütz, R. *Synlett* **2004**, 1039–1043.

[67] a) Bellina, F.; Rossi, R. *Tetrahedron* **2009**, *65*, 10269-10310. b) Rossi, R.; Bellina, F.; Lessi, M.; Manzini, C. *Adv. Synth. Catal.* **2014**, *356*, 17–117. c) Alberico, D.; Scott, M.; Lautens, M. *Chem. Rev.* **2007**, *107*, 174-238. d) Seregin, I. V; Gevorgyan, V. *Chem. Soc. Rev.* **2007**, *36*, 1173–1193.

[68] a) Majumdar, K.; Sinha, B.; Maji, P.; Chattopadhyay, S. *Tetrahedron* **2009**, *65*, 2751-2756. b) Yoon, W.; Lee, S.; Kang, S.; Ha, D.-C.; Ha, J. *Tetrahedron Lett.* **2009**, *50*, 4492-4494.

[69] a) Campeau, L.-C.; Parisien, M.; Jean, A.; Fagnou, K. *J. Am. Chem. Soc*. **2006**, *128*, 581-590. b) René, O.; Fagnou, K. *Adv. Synth. Catal*. **2010**, *352*, 2116-2120.

[70] Campeau, L.-C.; Parisien, M.; Jean, A.; Fagnou, K. *J. Am. Chem. Soc.* **2006**, *128*, 581-590.

[71] Ma, Z.; Xiang, Z.; Luo, T.; Lu, K.; Xu, Z.; Chen, J.; Yang, Z. *J. Comb. Chem.* **2006**, *8*, 696-704.

[72] Campo, M.; Huang, Q.; Yao, T.; Tian, Q.; Larock, R. *J. Am. Chem. Soc.* **2003**, *125*, 11506-11507.

[73] Caron, L.; Campeau, L.-C.; Fagnou, K. *Org. Lett.* **2008**, *10*, 4533–4536.

[74] Lafrance, M.; Lapointe, D.; Fagnou, K. *Tetrahedron* **2008**, *64*, 6015-6020.

[75] a) Martín-Matute, B.; Mateo, C.; Cárdenas, D.; Echavarren, A. *Chem. Eur. J.* **2001**, *7*, 2341–2348. b) Livendahl, M.; Echavarren , A. *Isr. J. Chem.* **2010**, *50*, 630–651.

[76] a) Davies, D.; Donald, S.; Macgregor, S. *J. Am. Chem. Soc*. **2005**, *127*, 1375413755. b) Gorelsky, S.; Lapointe, D.; Fagnou, K. *J. Am. Chem. Soc.* **2008**,*130*, 10848–10849.

[77] Lafrance, M.; Fagnou, K. *J. Am. Chem. Soc.* **2006**, *128*, 16496-16497.

[78] Liégault, B.; Lapointe, D.; Caron, L.; Vlassova, A.; Fagnou, K. *J. Org. Chem.* **2009**, *74*, 1826–1834.

[79] Ackermann, L.; Vicente, R.; Kapdi, A. *Angew. Chem. Int. Ed.* **2009**, *48*, 9792–9826.

[80] Zhang, Y.-M.; Razler, T.; Jackson, P. *Tetrahedron Lett.* **2002**, *43*, 8235-8239.

[81] Zaware, N.; Sharma, H.; Yang, J.; Devambatla, R.; Queener, S.; Anderson, K.; Gangjee, A. *ACS Med. Chem. Lett.* **2013**, *4*, 1148-1151.

[82] Tari, L.; Li, X.; Trzoss, M.; Bensen, D.; Chen, Z.; Lam, T.; Zhang, J.; Lee, S.; Hough, G.; Phillipson, D.; Akers-Rodriguez, S.; Cunningham, M.; Kwan, B.; Nelson, K.; Castellano, A.; Locke, J.; Brown-Driver, V.; Murphy, T.; Ong, V.; Pillar, C.; Shinabarger, D.; Nix, J.; Lightstone, F.; Wong, S.; Nguyen, T.; Shaw, K.; Finn, J. *PLoS ONE* **2013**, *8*.

[83] Tessier, P.; Nicolau, D. *Antimicrob. Agents Chemother.* **2013**, *57*, 2887–2889.

[84] Andrus, P.; Fleck, T.; Oostveen, J.; Hall, E. *J. Neurosci. Res.* **1997**, *47*, 650–654.

[85] Gangjee, A.; Zaware, N.; Raghavan, S.; Ihnat, M.; Shenoy, S.; Kisliuk, R. *J. Med. Chem.* **2010**, *53*, 1563–1578.

[86] Gangjee, A.; Zaware, N.; Devambatla, R.; Raghavan, S.; Westbrook, C.; Dybdal-Hargreaves, N.; Hamel, E.; Mooberry, S. *Bioorg. Med. Chem.* **2013**, *21*, 891-902.

[87] Gangjee, A.; Zaware, N.; Raghavan, S.; Disch, B.; Thorpe, J.; Bastian, A.; Ihnat, M. *Bioorg. Med. Chem.* **2013**, *21*, 1857-1864.

[88] Traxler, P.; Furet, P.; Mett, H.; Buchdunger, E.; Meyer, T.; Lydon, N. *J. Med. Chem.* **1996**, *39*, 2285-2292.

[89] Showalter, H.; Bridges, A.; Zhou, H.; Sercel, A.; McMichael, A.; Fry, D. *J. Med. Chem.* **1999**, *42*, 5464-5474.

[90] Műller, Ch. E.; Geis, U.; Grahner, B.; Lanzner, W.; Eger, K. *J. Med. Chem.* **1996**, *39*, 2482-2491.

[91] a) Okamoto, A.; Tanaka, K.; Nishiza, K.; Saito, I. *Bioorg. Med. Chem.* **2004**, *12*, 5875-5880. b) Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 9458-9463.

[92] Okamoto, A.; Tanaka, K.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 5066-5071.

[93] a) Okamoto, A.; Kamei, T.; Saito, I. *J. Am. Chem. Soc.* **2006**, *128*, 658-662. b) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003**, *125*, 9296-9297.

[94] Kondo, Y.; Watanabe, R.; Sakamoto, T.; Yamanaka, H. *Chem. Pharm. Bull.* **1989**, *37*, 2933-2936.

[95] Reader, J.; Matthews, T.; Klair, S.; Cheung, K.-M.; Scanlon, J.; Proisy, N.; Addison, G.; Ellard, J.; Piton, N.; Taylor, S.; Cherry, M.; Fisher, M.; Boxall, K.; Burns, S.; Walton, M.; Westwood, I.; Hayes, A.; Eve, P.; Valenti, M.; Brandon, A.; Box, G.; Montfort, R.; Williams, D.; Aherne, G.; Raynaud, F.; Eccles, S.; Garrett, M.; Collins, I. *J. Med. Chem.* **2011**, *54*, 8328–8342.

[96] Li, Z.; Wang, X.; Eksterowicz, J.; Gribble, M.; Alba, G.; Ayres, M.; Carlson, T.; Chen, A.; Chen, X.; Cho, R.; Connors, R.; DeGraffenreid, M.; Deignan, J.; Duquette, J.; Fan, P.; Fisher, B.; Fu, J.; Huard, J.; Kaizerman, J.; Keegan, K.; Li, C.; Li, K.; Li, Y.; Liang, L.; Liu, W.; Lively, S.; Lo, M.-C.; Ma, J.; McMinn, D.; Mihalic, J.; Modi, K.; Ngo, R.; Pattabiraman, K.; Piper, D.; Queva, C.; Ragains, M.; Suchomel, J.; Thibault, S.; Walker, N.; Wang, X.; Wang, Z.; Wanska, M.; Wehn, P.; Weidner, M.; Zhang, A.; Zhao, X.; Kamb, A.; Wickramasinghe, D.; Dai, K.; McGee, L.; Medina, J. *J. Med. Chem.* **2014**, *57*, 3430-3449.

[97] Chen, X.; Dai, K.; Duquette, J.; Gribble, M. W. Jr.; Huard, J. N.; Keegan, K. S.; Li, Z.; Lively, S. E.; McGee, L. R.; Ragains, M. L.; Wang, X.; Weidner, M. F.; Zhang, J. WO 2012/129344 A1, September 27, 2012.

[98] Connors, R. V.; Dai, K.; Eksterowicz, J.; Fan, P.; Fisher, B.; Fu, J.; Li, K.; Li, Z.; McGee, L. R.; Sharma, R.; Wang, X.; McMinn, D.; Mihalic, J.; Deignan, J. WO 2009/085185 A1, July 9, 2009.

[99] Keegan, K.; Li, C.; Li, Z.; Ma, J.; Ragains, M.; Coberly, S.; Hollenback, D.; Eksterowicz, J.; Liang, L.; Weidner, M.; Huard, J.; Wang, X.; Alba, G.; Orf, J.; Lo, M.-C.; Zhao, S.; Ngo, R.; Chen, A.; Liu, L.; Carlson, T.; Quéva, C.; McGee, L.; Medina, J.; Kamb, A.; Wickramasinghe, D.; Dai, K. *Mol. Cancer Ther.* **2014**,*13*, 880–889.

[100] Im, H.; Im, W.; Carter, D.; Schwartz, T.; Bundy, G.; Voigtlander, P. *Br. J. Pharmacol.* **1997**, *122*, 821–824.

[101] Hammond, M.; Zhao, Z. WO2011/056739 A1, May 12, 2011.

[102] Asadi, A.; Patrick, B.; Perrin, D. *J. Am. Chem. Soc.* **2008**, *130*, 12860–12861.

[103] a) Bennsen, D.; Borchardt, A.; Chen, Z.; Finn, J. M.; Lam, T. T.; Lee, S. J.; Li, X.; Tari, L. W.; Teng, M.; Trzoss, M.; Zhang, J.; Jung, M. E.; Lightstone, F. C.; Wong, S. E.; Nguyen, T. B. WO2014/043272 A1, March 20, 2014. b) Bennsen, D.; Finn, J. M.; Lee, S. J.; Chen, Z.; Lam, T. T.; Li, X.; Trzoss, M.; Jung, M. E.; Nguyen, T. B.; Lightstone, F. C.; Tari, L. W.; Zhang, J.; Arristoff, P.; Phillipson, D. W.; Wong, S. E. WO2012/125746 A1, September 20, 2012.

[104] Hernandez, M.; Chung, F.-L.; Earl, R.; Townsend, L. *J. Org. Chem.* **1981**, *46*, 3941-3945.

[105] Chung, F.-L.; Schram, K. H.; Panzica, R. P.; Earl, R.A.; Wotring, L.L.; Townsend, L. B. *J. Med. Chem.* **1980**, *23*, 1158.

[106] Zhou, L.; Amblard, F.; Zhang, H.; McBrayer, T.; Detorio, M.; Whitaker, T.; Coats, S.; Schinazi, R. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3385-3388.

[107] Tolman, R. L.; Townsend, L. B. *Tetrahedron Lett.* **1968**, *46*, 4815-4818.

[108] Mosrin, M.; Knochel, P. *Chem. Eur. J.* **2009**, *15*, 1468–1477.

[109] Koubachi, J.; Berteina-Raboin, S.; Mouaddib, A.; Guillaumet, G. *Tetrahedron* **2010**, *66*, 1937-1946.

[110] Bedford, R.; Betham, M. *J. Org. Chem.* **2006**, *71*, 9403-9410.

[111] Ackermann, L.; Althammer, A.; Mayer, P. *Synthesis* **2009**, 3493-3503.

[112] Gangjee, A.; Zaware, N.; Raghavan, S.; Ihnat, M.; Shenoy, S.; Kisliuk, R. L. *J. Med. Chem.* **2010**, *53*, 1563–1578.

[113] Kang, F.-A.; Sui, Z.; Murray, W. *J. Am. Chem. Soc.* **2008**, *130*, 11300–11302.

[114] Hall, D. G.: *Boronic acids*. WILEY-VCH Weinheim, Germany, 2005.

[115] Harrowven, D. C.; Guy, L. I. *Chem. Comm.* **2004**, 1968-1969.

[116] a) Zhou, J.; Guo, X.; Tu, C.; Li, X.; Sun, H. *J. Organomet. Chem.* **2009**, *694*, 697-702. b) Kantam, M.; Roy, M.; Roy, S.; Sreedhar, B.; Madhavendra, S.; Choudary, B.; De, R. *Tetrahedron* **2007**, *63*, 8002-8009. c) Lee, D.-H.; Lee, Y.; Kim, D.; Kim, Y.; Lim, W.; Harrowfield, J.; Thuéry, P.; Jin, M.-J.; Park, Y.; Lee, I.-M. *Tetrahedron* **2008**, *64*, 7178-7182. d) Yuan, B.; Pan, Y.; Li, Y.; Yin, B.; Jiang, H. *Angew. Chem. Int. Ed.* **2010**, *49*, 4054–8. e) Nájera, C.; Gil-Moltó, J.; Karlström, S. *Adv. Synth. Catal.* **2004**, *346*, 1798–1811. f) Kudo, N.; Perseghini, M.; Fu, G. *Angew. Chem. Int. Ed.* **2006**, *45*, 1282–1284.

[117] a) Hocek, M.; Fojta, M. *Org. Biomol. Chem.* **2008**, *6*, 2233–41. b) Hocek, M. *J. Org. Chem.* **2014**, *79*, 9914-9921.

[118] Fleckenstein, C.; Plenio, H. *J. Org. Chem.* **2008**, *73*, 3236–44.

[119] a) Martin, R.; Buchwald, S. *Acc. Chem. Res.* **2008**, *41*, 1461–73. b) Billingsley, K.; Anderson, K.; Buchwald, S. *Angew. Chem. Int. Ed.* **2006**, *45*, 3484–3488. c) Walker, S.; Barder, T.; Martinelli, J.; Buchwald, S. *Angew. Chem. Int. Ed.* **2004**, *43*, 1871–1876.

[120] Kinzel, T.; Zhang, Y.; Buchwald, S. *J. Am. Chem. Soc.* **2010**, *132*, 14073–14075.

[121] a) Shekhar, S.; Hartwig, J. *Organometallics* **2007**, *26*, 340-351. b) Stambuli, J.; Kuwano, R.; Hartwig, J. *Angew. Chem. Int. Ed.* **2002**, *41*, 4746–4748.

[122] Surry, D.; Buchwald, S. *Chem. Sci.* **2011**, *2*, 27-50.

[123] a) Old, D.; Harris, M.; Buchwald, S. *Org. Lett.* **2000**, *2*, 1403-1406. b) Harris, M.; Huang, X.; Buchwald, S. *Org. Lett.* **2002**, *4*, 2885-2888. c) Charles, M.; Schultz, P.; Buchwald, S. *Org. Lett.* **2005**, *7*, 3965-3968.

[124] a) Macé, Y.; Kapdi, A.; Fairlamb, I.; Jutand, A. *Organometallics* **2006**, *25*, 1795-1800. b) Amatore, Ch.; Jutand, A. *Coord. Chem. Rev.* **1998**, 511-528.

[125] Bedford, R.; Cazin, C.; Coles, S.; Gelbrich, T.; Horton, P.; Hursthouse, M.; Light, M. *Organometallics* **2003**, *22*, 987-999.

[126] Wolfe, J.; Tomori, H.; Sadighi, J.; Yin, J.; Buchwald, S. *J. Org. Chem.* **2000**, *65*, 1158-1174.

[127] Lee, B.; Biscoe, M.; Buchwald, S. *Tetrahedron Lett.* **2009**, *50*, 3672-3674.

[128] a) Henderson, J.; Buchwald, S. *Org. Lett.* **2010**, *12*, 4442–4445. b) Henderson, J.; McDermott, S.; Buchwald, S. *Org. Lett.* **2010**, *12*, 4438–4441.

[129] Maiti, D.; Fors, B.; Henderson, J.; Nakamura, Y.; Buchwald, S. *Chem. Sci.* **2011**, *2*, 57-68.

[130] a) Kawate, T.; Allerson, C.; Wolfe, J. *Org. Lett.* **2005**, *7*, 3865-3868. b) Kawate, T.; Wang, B.; Allerson, C.; Wolfe, J. *Synthesis* **2006**, 3280-3290.

[131] a) Olah, G.; Narang, S.; Olah, J.; Lammertsma, K. *Proc. Natl. Acad. Sci.* **1982**, *79*, 4487–4494. b) Olah, G. A.; Kuhn, S. J. *J. Am. Chem. Soc.* **1962,** *84*, 3684-3687. C) Kuhn, S. J.; Olah, G. A. *J. Am. Chem. Soc.* **1961**, 83, 4564-4571.

[132] Abbey, E.; Zakharov, L.; Liu, S.-Y. *J. Am. Chem. Soc.* **2010**, *132*, 16340–16342.

[133] Ramzaeva, N.; Rosemeyer, H. *Molbank* **2007**, *2007*, M522.

[134] Kukhareva, T.; Krasnova, V.; Koroteev, M.; Kaziev, G.; Kuleshova, L.; Korlyukov, A.; Antipin, M.; Nifanťev, E. *Russ. J. Org. Chem.* **2004**, *40*, 1190-1193.

[135] Williams, J.; Chen, J.; Drach, J.; Townsend, L. *J. Med. Chem.* **2004**, *47*, 5753-5765.

[136] Kuchař, M.; Pohl, R.; Klepetářová, B.; Votruba, I.; Hocek, M. *Org. Biomol. Chem.* **2008**, *6*, 2377-2387.

[137] Gerster, J. F.; Carpenter, B.; Robins, R. K.; Townsend, L. B. *J. Med. Chem.* **1967**, *10*, 326-331.

[138] Mandel, G.; Hill, A. J. *J. Am. Chem. Soc.* **1954**, *76*, 3978-3982.

[139] Mekhalfia, A.; Mutter, R.; Heal, W.; Chen, B. *Tetrahedron*, **2006**, *62*, 5617-5625.

[140] Ceide, S. C.; Montalban, A. G. *Tetrahedron Lett.* **2006**, *47*, 4415-4418.

[141] Solberg, J.; Undheim, K. *Acta Chem. Scand.* **1989**, *43*, 62–68.

[142] Kondo, Y.; Watanabe, R.; Sakamoto, T.; Yamanaka, H. *Chem. Pharm. Bull.* **2008**, *37*, 2814-2816.

[143] Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y. L.; Yokokawa, F.; Shi, P.-Y.; Hocek, M. *Bioorg. Med. Chem.* **2012**, *20*, 6123-6133.

[144] Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Lee, Y.-J.; Hocek, M. *Bioorg. Med. Chem.* **2013**, *21*, 5362-5372.

[145] Nagarjuna, G.; Yurt, S.; Jadhav, K.; Venkataraman, D. *Macromolecules* **2010**, *43*, 8045-8050.

[146] Mihovilovic, M.; Schnuerch, M.; Koley, M.; Hilber, K.; Koenig, X.: US 2012/0294835 A1, November 22, 2012.

[147] Whitehead, C. W.; Traverso, J. J. *J. Am. Chem. Soc.* **1958**, *80*, 2185-2189.

[148] Stazi, F.; Maton, W.; Castoldi, D.; Westerduin, P.; Curcuruto, O.; Bacchi, S. *Synthesis* **2010**, 3332-3338.

[149] Makosza, M.; Jagusztyn-Grochowska, J. M. *Roczniki Chem.* **1976**, *50*, 1841-1857.

[150] Kobayashi, K.; Komatsu, T.; Yokoi, Y.; Konitshi, H. *Synthesis* **2011**, 764-768.

[151] Wunderlich, S. H.; Knochel, P. Angew. Chem. Int. Ed. 2007, 46, 7685–7688.

[152] CrysAlisPro, Oxford Diffraction, 2002.

[153] Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K. ; Watkin, D. J. *J. Appl. Cryst.* **2003**, *36*, 1487.

[154] Palatinus, L.; Chapuis, G. *J. Appl. Cryst.* **2007**, *40*, 786-790.

[155] Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994**, *27*, 435-436.