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Synthesis, Biological Profiling and Photophysical Properties of Polycyclic Hetero-Fused 7-Deazapurine Nucleosides

by

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Disertační práce

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

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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, 30.10.2022

Podpis

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Abstract

This thesis describes the synthesis, photophysical properties and biological profiling of several series of polycyclic hetero-fused 7-deazapurine nucleosides.

Modified 7-deazapurine ribonucleosides display a variety of biological effects. Previously, small (hetero)aromatic rings-fused 7-deazapurine nucleosides show submicromolar cytostatic effects or antiviral activities. Thus, the two isomeric series of new benzothieno-fused deazapurine nucleosides were designed as the extended analogues to the cytotoxic thieno-fused nucleosides and hetero-analogues of antiviral naphtho-fused nucleosides. The goal of the first part of my work was to synthesize these target compounds. Key steps include Negishi coupling of zincated pyrimidine with iodobenzothiophene, thermal or photochemical cyclization, glycosylation and final diversification. The furyl and benzofuryl derivatives exerted moderate anticancer and anti-HCV activities. Most of the free nucleosides showed moderate to strong fluorescence, and the corresponding 2′-deoxyribonucleoside triphosphate was incorporated into modified DNA and their fluorescence properties were studied

The tri- and tetracyclic fused nucleobases can be synthesized either by multistep heterocyclization approach or through cross-coupling of zincated pyrimidine with hetaryl halides, but for some heterocycles, the corresponding halides are inaccessible, expensive or unreactive. The second part of my work aimed to overcome this synthetic problem. A new approach for synthesizing polycyclic hetero-fused 7-deazapurine heterocycles and the corresponding nucleosides was developed based on C-H functionalization of diverse (hetero)aromatics with dibenzothiophene-*S*-oxide followed by the Negishi cross-cooupling with bis(4,6-dichloropyrimidin-5-yl)zinc. This cross-coupling afforded a series of (het)aryl-pyrimidines that were used to obtain the corresponding 2'-deoxy- and ribonucleosides through the classic approach as in the first project . Most of the deoxyribonucleosides showed good cytotoxic activity, especially for CCRF-CEM cell line. Phenyl- and thienyl-thieno-fused 7-deazapurine nucleosides were fluorescent and the former one was converted to 2'-deoxyribo-nucleoside triphosphate for enzymatic synthesis of labeled oligonucleotides.

Abstrakt

Tato práce popisuje syntézu, fotofyzikální vlastnosti a biologické testování několika sérií polycyklických hetero-fúzovaných 7-deazapurinových nukleosidů.

Modifikované 7-deazapurinové ribonukleosidy vykazují různé biologické účinky. Např. nukleosidy nesoucí 7-deazapurin anelovaný s malými (hetero)aromatickými kruhy vykázaly submikromolární cytostatické účinky nebo antivirové aktivity. Proto byly navrženy a připraveny dvě izomerní řady nových benzothieno-fúzovaných deazapurinových nukleosidů jako objemnější analogy cytotoxických thieno-fúzovaných nukleosidů a heteroanalogy antivirových nafto-fúzovaných nukleosidů. Cílem první části mé práce bylo syntetizovat tyto cílové sloučeniny. Klíčové kroky zahrnují Negishiho kapling pyrimidinylzinku s jodobenzothiofenem, tepelnou nebo fotochemickou cyklizaci, glykosylaci a konečnou derivatizaci. Furylové a benzofurylové deriváty vykazovaly mírné protirakovinné a anti-HCV aktivity. Většina volných nukleosidů vykazovala střední až silnou fluorescenci a odpovídající 2'-deoxyribonukleosidtrifosfát byl inkorporován do modifikované DNA a byly studovány její fluorescenční vlastnosti.

Tri- a tetracyklické kondenzované nukleobáze mohou být syntetizovány buď vícestupňovým heterocyklizačním přístupem nebo cross-couplingem pyrimidinylzinku s hetarylhalogenidy, ale pro některé heterocykly jsou odpovídající halogenidy nedostupné, drahé nebo nereaktivní. Druhá část mé práce měla za cíl tento syntetický problém překonat. Byl vyvinut nový přístup k syntéze polycyklických hetero-kondenzovaných 7-deazapurinových heterocyklů a odpovídajících nukleosidů založený na C-H funkcionalizaci různých (hetero)aromátů s dibenzothiofen-*S*-oxidem a následně Negishiho cross-couplingu s bis(4,6-dichlorpyrimidin-5-yl)zinkem. Tato nová reakce poskytla řadu (het)arylpyrimidinů, které byly použity k získání odpovídajících 2'-deoxyribo- a ribonukleosidů klasickým přístupem jako v prvním projektu. Většina deoxyribonukleosidů vykazovala dobrou cytotoxickou aktivitu, zejména proti buněčné linii CCRF-CEM. Fenyl- a thienyl-thieno-fúzované 7-deazapurinové nukleosidy byly fluorescenční a první z nich byl převeden na 2'-deoxyribonukleosidtrifosfát pro enzymatickou syntézu značených oligonukleotidů.

List of Publications Relevant to this Thesis

1. **Yang, C.**; Pohl, R.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis, Photophysical Properties, and Biological Profiling of Benzothieno-Fused 7-Deazapurine Ribonucleosides. *J. Org. Chem.* **2020**, *85*, 8085–8101.

2. **Yang, C.**; Slavětínská, L. P.; Fleuti, M.; Klepetářová, B.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis of Polycyclic Hetero-Fused 7-Deazapurine Heterocycles and Nucleosides through C–H Dibenzothiophenation and Negishi Coupling. *J. Am. Chem. Soc.* **2022,** *144*, 19437-19446.

List of Abbreviations

Ac	acetyl
Ad	Adamantly
AIDS	acquired immunodeficiency syndrome
BCNAs	bicyclic pyrimidine nucleoside analogues
bipy	2,2′-bipyridine
Bn	benzyl
BSA	*N,O*-bis(trimethylsilyl)acetamide
Bz	Benzoyl
calcd	calculated
cAMP	cyclic adenosine monophosphate
Ch	chalcogen atom (e.g., S, Se, Te)
ChB	chalcogen bonding
CMV	Cytomegalovirus
COD	1,5-cyclooctadiene
COSY	correlation spectroscopy (NMR)
Cy	Cyclohexyl
dATP	2′-deoxyadenosine triphosphate
dba	dibenzylideneacetone
DBT	Dibenzothiophene
DENV	dengue virus
DMAc	dimethylacetamide
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EBOV	Ebola virus
EDTA	ethylenediaminetetraacetic acid

ESI	electrospray ionization
Et	ethyl
FAD	flavin adenine dinucleotide
HMBC	heteronuclear multiple bond correlation (NMR)
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPFC	high performance flash chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence (NMR)
HSV	herpes simplex virus
IR	infrared
J	coupling constant (NMR)
MERS-CoV	Middle East respiratory syndrome coronavirus
m.p.	melting point
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
NTP	nucleoside triphosphate
ON	oligonucleotides
PC	photoredox catalyst
Ph	phenyl
pppGpp	guanosine pentaphosphate
RNA	ribonucleic acid
ROESY	rotating frame Overhauser effect spectroscopy (NMR)
RSV	respiratory syncytial virus
r.t.	room temperature
SAR	structure–activity relationship
TBE	tris/borate/EDTA
t-Bu	*tert*-butyl

TDA-1	tris[2-(2-methoxyethoxy)ethyl]amine
TEAB	triethylammonium bicarbonate
Tf	triflate
TFA	trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TFT	tetrafluorothianthene
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidine
TMS	trimethylsilyl
TT	Thianthrene
TTO	thianthrene-*S*-oxide
VZV	varicella zoster virus

Contents

1 Introduction

1.1 Modified Nucleosides in Medicinal Chemistry

Nucleosides are essential endogenous biomolecules that are structural subunits of nucleic acids and thereby play central roles in both the storage and expression of genetic information. Their derivatives also participate in various cellular processes such as cell signaling, enzyme regulation and metabolism.[1, 2] Nucleosides are glycosylamines obtained by chemical or enzymatic decomposition of nucleic acids and consist of a molecule of five-carbon sugar linked to a nitrogenous heterocycle (Figure 1). Nucleosides can be phosphorylated on 5′-OH to form nucleotides, which are the monomeric units of nucleic acids.

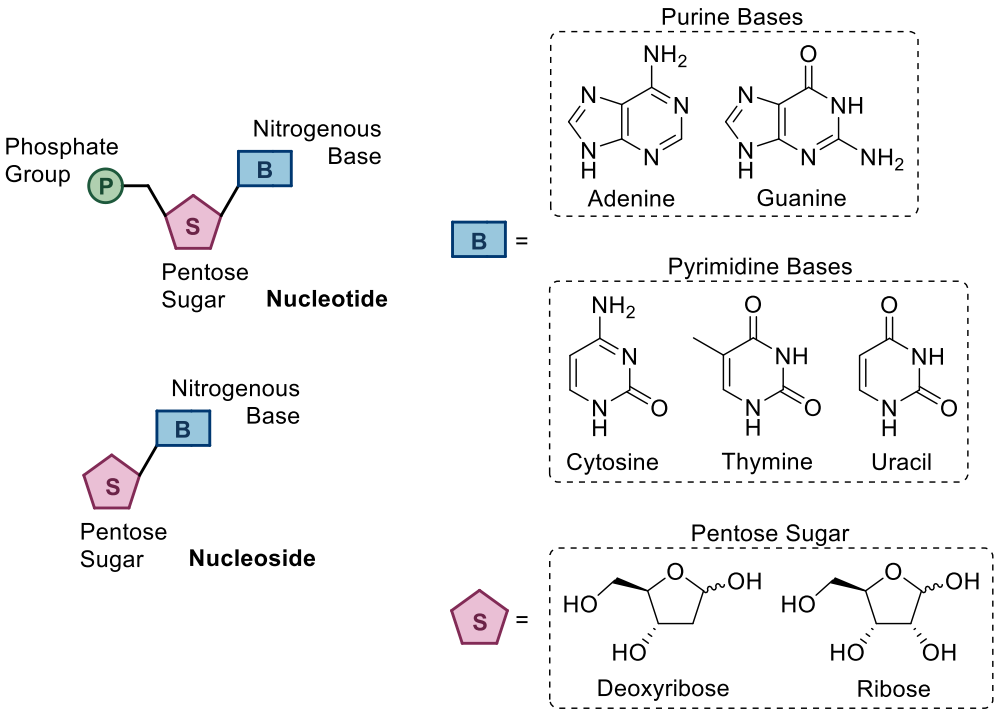


Figure 1. The structures of naturally occurring canonical nucleosides and nucleotides

DNA is constructed from four types of canonical deoxyribonucleosides, including deoxyadenosine, thymidine, deoxyguanosine and deoxycytidine, while RNA contains four ribonucleosides, namely adenosine, uridine, guanosine and

cytidine. The nucleobases of nucleosides or nucleotides include purine bases and pyrimidine bases, the former have adenine and guanine, and the latter have cytosine, thymine and uracil. DNA is replicated from nucleoside triphosphates in presence of DNA polymerases, and RNA polymerases carry out transcription that is the process of copying a segment of DNA into RNA. Nucleosides and their derivatives are not only the basic units of genetic material in all living things, they also play important roles in many biological processes (Figure 2). Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is a cofactor used in anabolic reactions, such as the Calvin cycle and lipid and nucleic acid syntheses, which require NADPH as a reducing agent.[3] Guanosine pentaphosphate (pppGpp) is a nucleotide signaling molecule involved in the stringent response in bacteria that leads to the inhibition of RNA synthesis when there is a shortage of amino acids.[4] Flavin adenine dinucleotide (FAD), a redox-active coenzyme, is a key factor for several enzymatic reactions in metabolism.[5] Another important nucleoside derivative is cyclic adenosine monophosphate (cAMP), which is a second messenger involved in intracellular signal transduction in many different organisms.[6]

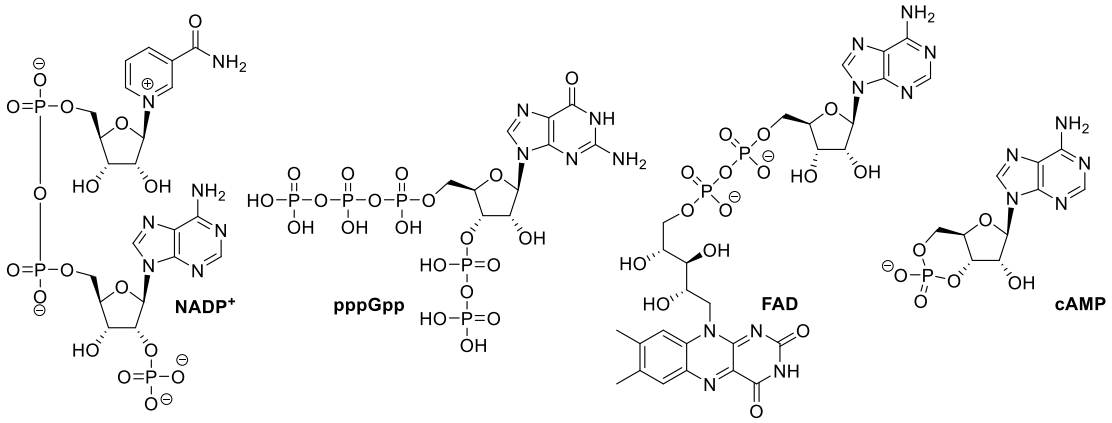


Figure 2. Examples of non-nucleic acid nucleotides

Nucleosides have attracted the attentions of medicinal chemists for decades since they function not only as building blocks in genetic materials but also as key factors in many biological processes of metabolism. The modification of natural nucleosides is always a hot research area, and chemists continually found new types of modified

nucleosides. In the last few decades, numerous modified nucleosides were synthesized and used as antiviral or anticancer agents.[7-10] There are four main types of nucleoside/tide modification: 1) base-modified nucleosides, 2) sugar-modified nucleosides, 3) phosphate-modified nucleotides, 4) combination of different types of modifications.

1.1.1 Base-modified nucleosides

Many different types of modifications of the nucleobase moieties were accomplished to achieve good biological activities, especially antiviral and antitumor activities (Figure 3).

The first class of the base modifications is introducing small substituents onto the pyrimidine ring. Examples of this kind of modification include idoxuridine, trifluridine, brivudine, and floxuridine. Idoxuridine is an analog of deoxyuridine with iodine substituted on position 5. It inhibits viral DNA synthesis and is approved as an antiviral agent in 1962.[11] The similar strategy was used for the design and synthesis of trifluridine, of which a trifluoromethyl group was introduced onto the uracil moiety. And this drug is a nucleoside metabolic inhibitor used mainly for eye infections caused by herpes simplex virus.[12] Brivudine and floxuridine have same type of chemical structures with vinyl and fluorine instead. Brivudine is utilized for treating VZV[13] and floxuridine is most often used in the treatment of colorectal cancer[14]. Brivudine firstly undergo phosphorylations by viral thymidine kinase and nucleoside-diphosphate kinase to form brivudine 5'-triphosphate, which is the active form. After that brivudine 5'-triphosphate is incorporated into the viral DNA, and then blocks the action of DNA polymerases, thus inhibiting viral replication.[13] Floxuridine rapidly undergoes catabolism to form 5-fluorouracil, which is the active form of the drug and primarily works by interfering with DNA synthesis. 5-Fluorouracil is used for treatment of colorectal cancer, oesophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer.[15]

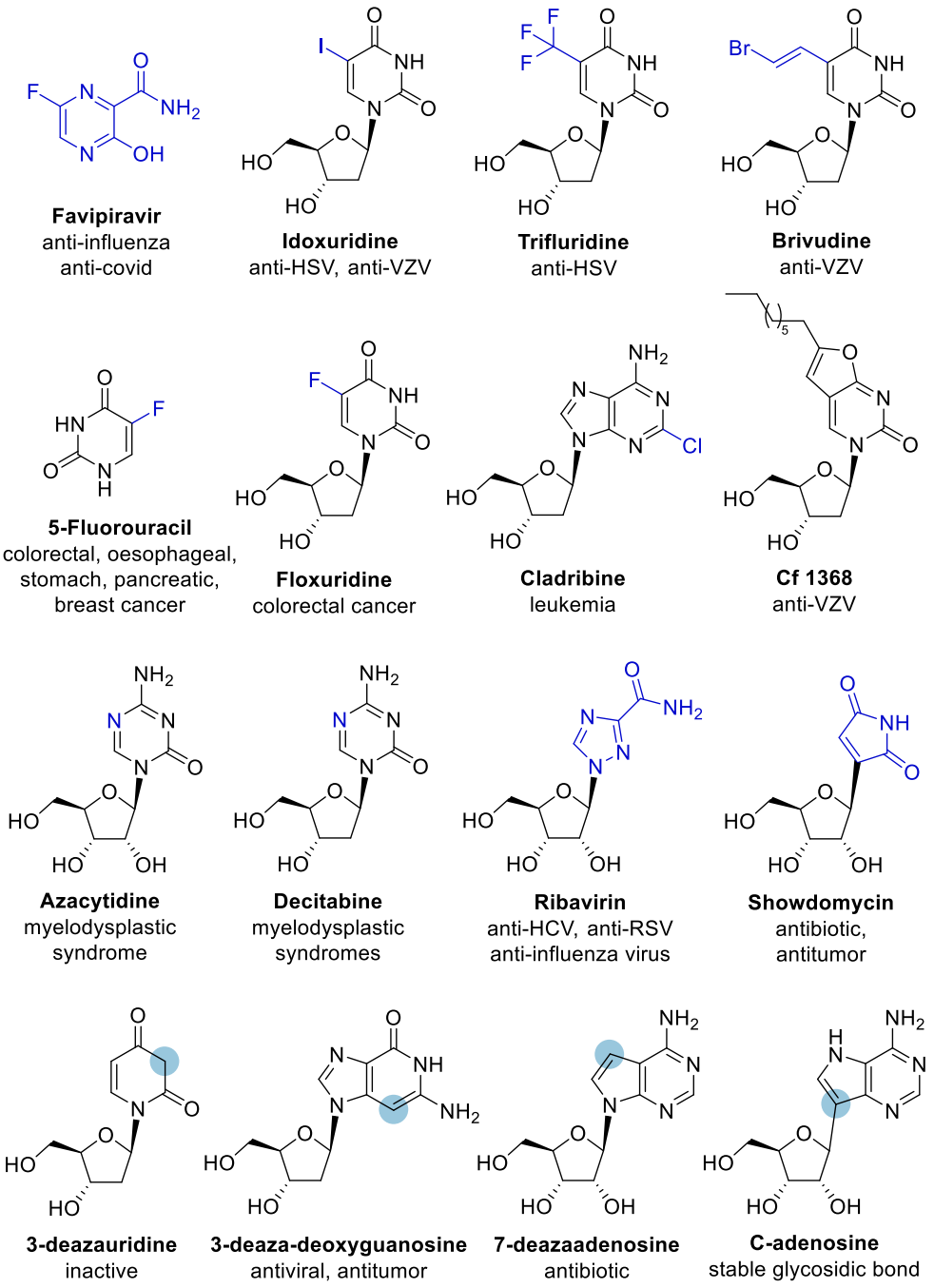


Figure 3. Examples of base-modified nucleosides with their medical uses

The strategy of introducing a substituent onto the pyrimidine bases was quite successful for achieving a series of drugs for the treatment of cancers and virus infections. This also proved true for the purine nucleobases. For example, cladribine, a medication used to treat leukemia, has a chlorine atom at C2 position, which renders it partially resistant to breakdown by adenosine deaminase.[16]

Except for substitution of the nucleobases, some heterocycles were utilized to

obtain hetero-fused pyrimidine bases. Cf 1368 is one of the successful examples, which has good anti-VZV activity. The pyrimidine base is fused with a furan, bearing a long aliphatic chain.

Replacing or relocating various atoms from the nucleobase is another common modification that was routinely tried in an effort to find better activity or temper side effects. Changing a nitrogen on the nucleobase heterocycle by a carbon and vice versa could change dramatically the hydrogen bonding interactions in enzyme binding sites that can have a profound affect.[8] There are many examples using this strategy, such as azacytidine, decitabine, 3-deazauridine, 3-deaza-deoxyguanosine, 7-deazaadenosine and C-adenosine. Azacytidine, an antibiotic analogue originally isolated from *streptoverticillium ladakanus*, has a nitrogen on position 5 of pyrimidine ring instead of a carbon, which led to profound anticancer properties. And it is used for the treatment of myelodysplastic syndrome, myeloid leukemia, and juvenile myelomonocytic leukemia.[17] Azacitidine and its deoxy derivative (decitabine) were first synthesized in Czechoslovakia as potential chemotherapeutic agents for cancer.[18] Replacing the nitrogen on pyrimidine or purine bases by a carbon give 3-deazauridine, 3-deaza-deoxyguanosine, 7-deazaadenosine. Among them, 3-deazauridine, an analogue of nucleoside uridine lacking a ring nitrogen in the 3-position, did not show any useful bioactivity. However, 3-deaza-deoxyguanosine, of which the N3 is replaced by a CH, displayed not only a broad spectrum of antiviral activity, but also potent antitumor properties against leukemia L1210 and P388 cell lines.[19] 7-Deazaadenosine is also a successful example of deaza analogue. The N7 of adenosine was replaced by a CH group, and this modification endowed 7-deazaadenosine with potent antibiotic activity against *streptococcus faecalis*.[20] Since purine and pyrimidine nucleoside phosphorylases can cleave the glycosidic bond of nucleosides, some chemists tried to replace the N9 nitrogen in the purine ring with carbon to create C-adenosine to increase the stability.[21]

The nitrogen atoms can also be relocated on the pyrimidine ring to obtain a diverse variety of nucleobase analog. This modification may introduce alternative

hydrogen bond donors or acceptors to that can therefore interact with new areas of the enzyme binding site. For example, favipiravir, a pyrazine carboxamide derivative, was approved for the treatment of influenza and SARS-CoV-2.[22]

Other more complicated modifications use some other types of heterocycles to replace the pyrimidine or purine base. For example, ribavirin contains a ribose linked with a triazole carboxamide and is an antiviral drug used for the treatment of HCV, RSV and influenza.[23] Another example is showdomycin, a C-glycosyl nucleoside analog.[24] It showed not only effective antibacterial activity against several Gram-positive and Gram-negative bacteria, but also antitumor activity against Ehrlich mouse ascites as well as HeLa cells in vitro.[25]

1.1.2 Sugar-modified nucleosides

As well as the diverse modifications made on the nucleobase moiety, medicinal chemists did plenty of significant works to modify the sugar part of nucleosides. The modifications of the sugar moiety not only lead to many clinical medications, but also reveal the mechanism of how these modified nucleosides works as antitumor or antiviral reagents. The modifications of the sugar part include: simply introducing or removing substituents, replacing the oxygen atom on the furanose ring, changing the strereo configuration of sugar, decreasing the size of sugar ring and acyclic nucleosides (Figure 4).

Firstly, different substituents to 2′-position of sugar were investigated. One example is Vidarabine, of which the 2′-OH group was inverted to β configuration. The inversion of the configuration resulted in strong biological activities against herpes simplex virus (HSV) and varicella zoster virus (VZV).[26] Another important example is gemcitabine, which is a chemotherapy medication used to treat a broad spectrum of cancers, including testicular cancer, breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer, and bladder cancer.[27] Gemcitabine is a 2′,2′-difluoro deoxycytidine analogue and its mechanism of action involves influx through the cell

membrane, intracellular conversion to gemcitabine diphosphate and triphosphate, incorporation into DNA, resistance to DNA repair, and thus leading to DNA strand termination.[28] Besides, 3′-modified nucleoside analogues were also synthesized and tested for biological activities. For example, azidothymidine, a thymidine analogue with 3′-N_3 group, was approved in the United States in 1987 and was the first medication for HIV/AIDS.[29]

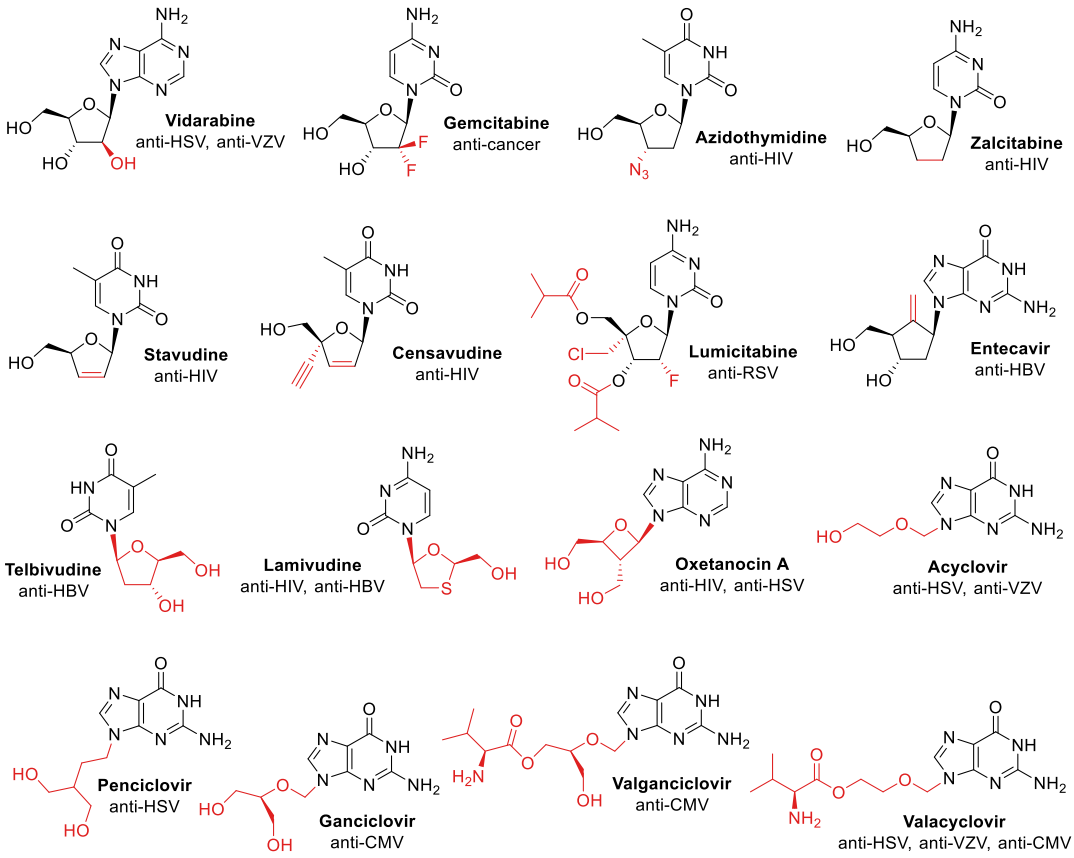


Figure 4. Examples of sugar-modified nucleosides with their medical uses

Another type of sugar-modified nucleoside analogue is 2′,3′-dideoxy nucleoside. Since the incoming nucleotide is linked to the 3′-OH of the template chain during DNA replication, the 3′-deoxy nucleoside was designed and assumed to be the "chain terminators".[30] Zalcitabine was found under this design. It is 2′,3′-dideoxycytidine and used for the treatment of HIV/AIDS.[31] One other medication for HIV infection is stavudine,[32] which has a double bond between 2′ and 3′ carbons.

Medicinal chemists also studied the biological activities and mechanism of action of 4′-Modified Nucleosides[33], which represent an important subclass of

modified nucleosides for antiviral therapies. Censavudine, also called 4′-ethynyl stavudine, has unsaturated sugar moiety like stavudine, and a unique 4′-α-ethynyl group, which is crucial for the improvement of anti-HIV-1 III_B activity.[34] Moreover, carbocyclic nucleosides were also designed by removing the oxygen atom from the furanose ring. These carbocyclic nucleosides were more stable due to the lack of hemiaminal ether, but they were less active comparing with the corresponding ribonucleosides.[35] However, after introducing an exocyclic double bond, entecavir became a very efficient anti-HBV drug.[36]

Stereo-configuration is always an important issue in medicinal chemistry. Although most of the natural nucleosides and modified nucleosides contain a D-(*β*)-ribose, new type of nucleosides with a L-(*β*)-ribose were also prepared to test their biological activities. Telbivudine is the L-isomer of thymidine, which is used for the treatment of hepatitis B infection and less likely to cause resistance.[37] An other example of L-(*β*)-nucleoside is Lamivudine, which is an antiretroviral medication used to treat HIV/AIDS and chronic hepatitis B.[38] The structural features of Lamivudine include a L-(*β*)-deoxyribose and the replacement of 3′-carbon with a sulfur atom. Furthermore, the four-membered ring, instead of pentose sugar, was linked with nucleobases to form novel modified nucleosides, such as oxetanocin A,[39] which showed potent anti-HIV and anti-HSV activities.

Last but not least, the acyclic nucleosides represent a large variety of chemical modifications featured with a flexible aliphatic chain. Removing 2′-carbon and 3′-carbon from guanosine ended up with acyclovir, which was approved in 1981 for the treatment of herpes simplex virus and varicella zoster virus infections.[40] And valacyclovir is the prodrug of acyclovir in the form of valine ester for the sake of better oral bioavailability.[41] Similarly, removing 2′-carbon from guanosine resulted in ganciclovir and valganciclovir, and valganciclovir is the valine esterified prodrug of ganciclovir. Both of them are used for treatment of cytomegalovirus retinitis in people who have AIDS.[42, 43] One example of more complicated modification of the sugar moiety are penciclovir, of which the aliphatic diol is quite different from the ribose.

Topical penciclovir is used to treat the symptoms of herpes simplex virus infections around the mouth.[44]

1.1.3 Nucleosides with combined modifications

There is a great need for novel types of nucleoside and nucleotide derivatives in development of antiviral drugs against emerging viruses or anticancer agents against drug resistant tumors or leukemias. For this purpose, many patterns of modifications used on the nucleobase moiety and on the sugar moiety are combined in different ways to create more structurally diverse nucleoside analogues (Figure 5).

Some nucleoside analogues, designed with more than one modification, are anti-HIV medications. Emtricitabine, a synthetic nucleoside analogue with activity against HIV-1, is the (-) enantiomer of a thio analogue of cytidine, which differs from other cytidine analogs in that it has a fluorine in the 5-position.[45] It also shows potent anti-HBV activity.[46] Due to the both modifications to base and sugar moieties, emtricitabine works as an inhibitor of HIV-1 reverse transcriptase, competing with the natural deoxycytidine 5'-triphosphate. By inhibiting this key enzyme, emtricitabine can lower down the amount of HIV in a patient's body.[45] Another example of combined modofications is islatravir, which is an investigational drug for the treatment of HIV infection.[47] It bears 2-F group and 4′-ethynyl. Didanosine contains a 2′,3′-dideoxyribose and a guanine without 2-amino group, approved for the treatment of HIV/AIDS.[48] Similarly, abacavir contains a 2′,3′-unsaturated carbocyclic sugar and a 2,6-diaminopurine, of which 6-amino is substituted by a cyclopropyl group. And it is a medication used to prevent and treat HIV/AIDS.[49]

Another two nucleoside analogues with modifications to both base and sugar moieties are NITD449 and Famciclovir. NITD449 has a carbamoyl 7-deazaadenine linked with a 2′-ethynylribose, and it demonstrates moderate anti-DENV activity.[50] Famciclovir is constructed from a guanine analogue with oxygen removed and an acyclic sugar moiety protected by acetyl groups, and it was approved for the treatment

of several infection symptoms caused by herpes viruses, including genital herpes, cold sores (herpes labialis), and shingles (herpes zoster).[51]

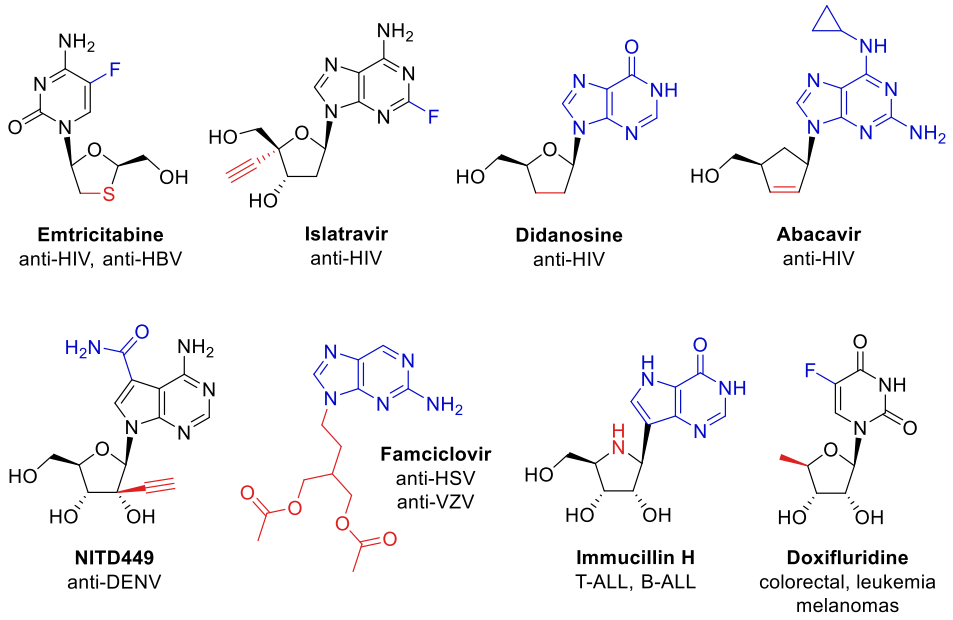


Figure 5. Examples of complex modifications to the nucleoside scaffold

This type of nucleoside analogues with combined modification also sees several successful developments of anti-cancer drugs, such as immucillin H and doxifluridine. Immucillin H is used for the treatment of T-cell acute lymphoblastic leukemia (T-ALL) and B-cell acute lymphocytic leukemia (B-ALL).[52] Doxifluridine, featuring 5′-methyl, is a prodrug developed by Roche and used as a cytostatic agent in chemotherapy.[53]

1.1.4 Phosphate-modified nucleotides

Modified nucleosides play an essential role in the treatment of cancer and viruses. However, nucleoside analogues are polar molecules and have limited membrane permeability, which leads to poor oral bioavailability. In addition, intracellular phosphorylation of many therapeutic nucleoside analogues into their active triphosphate metabolites is a prerequisite for their pharmacological activity, during which the rate-limiting step is conversion of nucleoside analogues to their monophosphates. Over the past decade, several creative prodrug strategies have been utilized to overcome the rate-limiting phosphorylation step, and to overcome issues

with delivery.[54, 55]

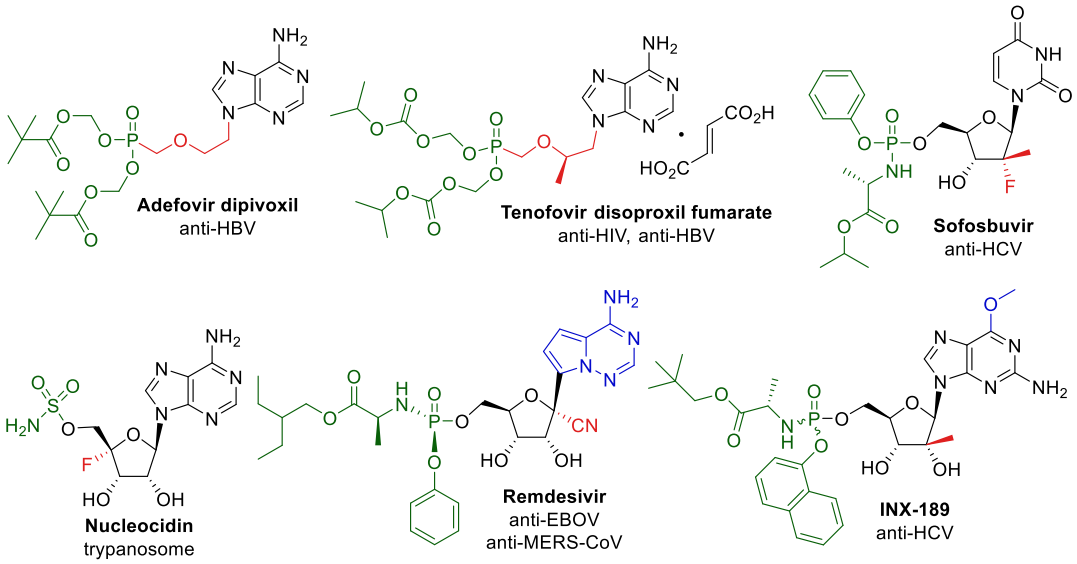


Figure 6. Phosphate-modified nucleotides as prodrugs

One of these prodrug strategies features an artificial sugar together with a modified phosphate (Figure 6). Among them are adefovir and tenofovir. Both of these two drugs are acyclic nucleoside phosphornates, and were first synthesized by Antonín Holý at IOCB. Adefovir is an adenosine analogue used to treat of chronic hepatitis B by blocking the reverse transcriptase of the hepatitis B virus.[56] The sugar moiety of adefovir was changed to an ether chain and the phosphonate was protected with dipivoxil to form its prodrug. Tenofovir has similar modifications, namely an ether chain and a protected phosphonate. Tenofovir is often used in the disoproxil prodrug form for treatment of chronic hepatitis B and HIV/AIDS.[57, 58] Tenofovir disoproxil is firstly decomposed to tenofovir, which is then phosphorylated to form tenofovir diphosphate that is the active form working as an inhibitor of reverse transcriptase by chain termination.[59] Another two examples are sofosbuvir and nucleocidin. Their structures include 2′-fluoro or 4′-fluoro ribose, respectively. Sofosbuvir, a medication for the treatment of hepatitis C,[60] is a prodrug of the ProTide (PROdrug + nucleoTIDE) type bearing a 5′-phosphoramide group. Nucleocidin, isolated from *Streptomyces calvus*, has a unique 5′-*O*-sulphamoyl group. It shows strong activity against trypanosome.[61]

What's more, there is a type of more complicated nucleoside analogues that combine three modifications, such as remdesivir and INX-189. The base, sugar and phosphate moieties of remdesivir are all modified with rational design. Its molecular contains amino pyrrolotriazine as nucleobase, 1′-cyano ribose, and 5′-*O*-phosphoramidate. Remdesivir was initially developed for the treatment of Ebola virus (EBOV), and then authorized for emergency use to treat COVID-19 in many countries.[62, 63] Since many modified nucleosides are firstly phosphorylated to from their active form during which the rate-limiting step is the synthesis of monophosphate, remdesivir was designed to bear a 5′-phosphoramide group so that it can be converted to its monophosphate by esterases and a phosphoamidase, to avoid the slow process of monophosphorylation.[64] INX-189 was designed with similar strategy with three modifications on each moiety of its molecule, and it demonstrated potent anti-HCV activity.[65] The methoxy group at C6 position of the guanine base was proved to be essential for improving the activity against the HCV.

Thanks to the continual development of nucleoside and nucleotide analogues and chemists' creative design, nowadays we have a large number of modified nucleoside drugs for treatment and prevention of various infections caused by different viruses and many kinds of tumors or leukemias. However, the newly emerging viruses and drug resistance provide new challenges for medicinal chemists. The legend of modified nucleosides should be continued.

1.2 Modified Nucleobases and Nucleosides Developed in Our Group

As described in section 1.1, modified nucleosides and their derivatives are of great importance in the design and development of anticancer and antiviral drugs, and there is still a great need for new types of nucleoside analogues due to the newly emerging viruses and drug resistance. Based on these facts, our group has been working on the medicinal projects of novel modified nucleosides for the last two decades. Most of our work is focus on the modifications of nucleobase moiety, especially the adenine base. Till now, we have designed and synthesized many series of substituted and fused purine or 7-deazapurine nucleosides, and a few series among them demonstrated nanomolar cytostatic or anti-HCV activities, which could be promising anti-cancer drug candidates (Figure 7).

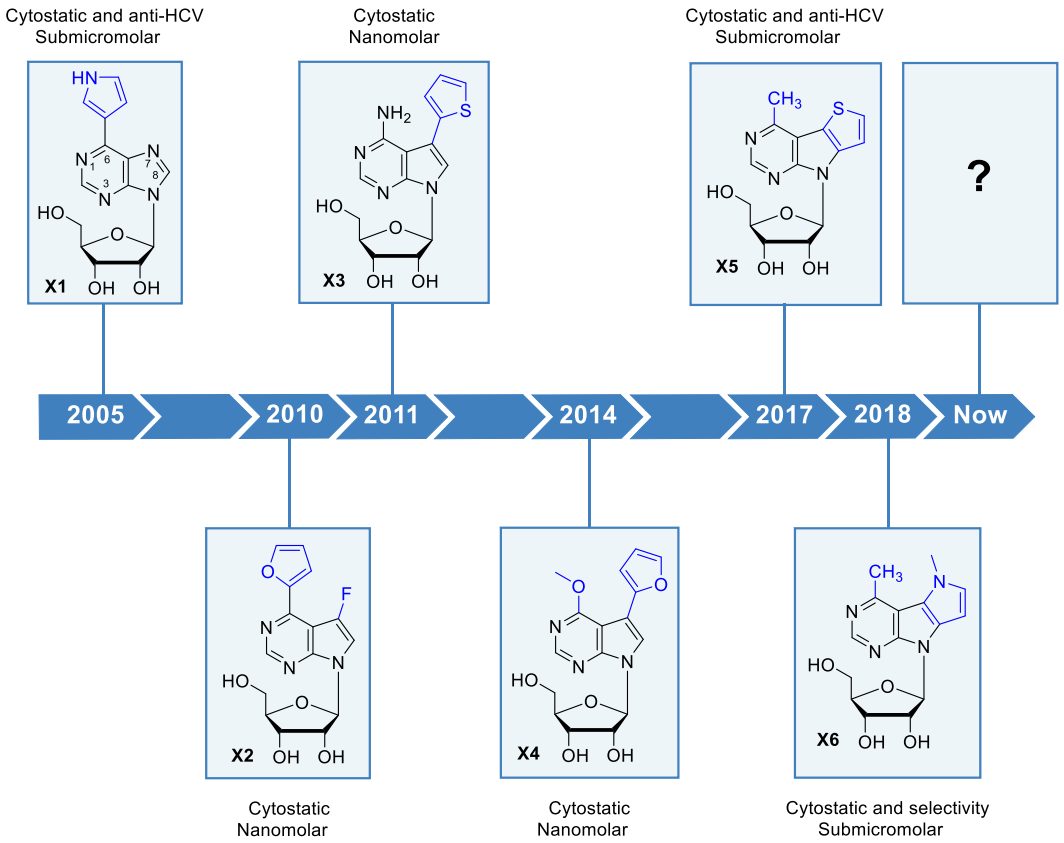


Figure 7. Systematic study of adenosine analogues in Hocek group

1.2.1 Substituted 7-deazapurine ribonucleosides

Early in 2005, Hocek et al. reported the synthesis and biological profiling of an extended series of 6-hetarylpurine nucleosides.[66] These compounds were obtained by heterocyclizations or by cross-couplings of 6-chloropurine nucleosides with hetarylboronic acids, -stannanes, or -zinc halides, and among them, the pyrrol-3-yl and 2-furyl derivatives showed the most significant anti-HCV activity with EC_{90} in the submicromolar range.

Since 6-Arylpurine ribonucleosides and 6-hetarylpurine nucleosides displayed potent cytostatic or anti-HCV activities, it encouraged us to do deep investigation of more novel types of base-modified nucleosides. In order to achieve more diverse modification on the base moiety, the N atom in position 7 of purine was changed by CH group, which resulted in 7-deazapurine. The replacement by CH group allowed the introduction of the many different types of substituents to position 7, including halogens, small alkyl groups and (het)aryl groups.

In 2010, a large series of 7-deazapurine ribonucleosides bearing a diverse substituent in position 6 and H, or halogen atom in position 7 were synthesized and their biological activities were studied.[67] The substituents in position 6 were introduced by several transition metal-catalyzed cross-coupling reactions from the key intermediate protected 6-chloro-7-deazapurine ribonucleosides. Many derivatives among them demonstrated potent cytostatic activity against a few cancer cell lines with nanomolar IC_{50}. The most active ones possessed the structural features bearing 7-H or 7-F and 6-furyl- or 6-thienyl-groups. The study of mechanism of action involved the phosphorylation and inhibition of total RNA synthesis.

One year later, a similar work was reported by Aurelie Bourderioux et al. in our group. A range of aryl and hetaryl groups were introduced to positon 7 on 7-deazaadenosine by the cross-coupling reactions.[68] The derivatives bearing 5-membered heterocycles at position 7 showed promising anti-cancer activity both in vitro and in vitro; however, the compounds substituted by aryl group at positon 7

displayed much weaker activities. Similarly, these modified nucleosides also inhibited the RNA synthesis and then led to the apoptosis.

Based on the SAR study from the above two projects, a type of more complicated double-modification was applied to build a diverse library of nucleoside analogues. A series of 7-deazapurine derivatives with modifications at both potion 6 and position 7 on the purine moiety were designed and synthesized.[69] Different types of small substituents, like methoxy, methylsulfanyl, methylamino, dimethylamino, methyl group, were introduced at position 6; several heterocycles, meanwhile, were linked with C-7 of the deazapurine. A few derivatives in this series substituted with a furyl or ethynyl group at position 7 displayed potent cytotoxic activity at nanomolar concentrations. And some compounds in this series also showed promising anti-HCV activity. On the other hand, those compounds with substituents at position 2 of the deazapurine were inactive.

1.2.2 Fused 7-deazapurine ribonucleosides

The modifications at positon 6 and position 7 on 7-deazapurine were quite successful, and we even achieved some compounds that had the potency comparable to or better than that of the marketed drug clofarabine.[67, 68] But there is still a need to design and synthesize new modified nucleosides, due to drug-resistant tumors and newly emerging viruses. For this reason, we started to build up compound library of (hetero)aromatic rings-fused 7-deazapurine nucleosides and to study their biological activities and fluorescent properties in the last few years.

Figure 8 provides a glimpse of the fused 7-deazapurine nucleosides developed in our group recently. The earliest examples were benzo-fused 7-deazapurine nucleosides **X7** and **X8**, developed in 2012.[70] In this study, the 6-chloro series with 4-(furan-2-yl), 4-(thiophen-2-yl), or 4-(benzofuran-2-yl) group had significant anti-DENV activity. Interestingly, the benzo-fused modifications displayed submicromolar antiviral activity, but they were inactive against several cancer cell

lines, which could be a useful strategy for designing selective compounds with anti-RNA virus activities and decreased cytotoxicities.

Since the benzo-fused modifications led to weak or no cytotoxic activity, smaller 5-membered heterocycle-fused 7-deazapurine nucleosides were investigated (Figure 8). Thieno-fused modification was designed and two isomeric series of thieno-fused 7-deazapurine nucleosides **X9** and **X10** were prepared.[71] The synthetic approach included Negishi coupling, azidation, photo or thermal cyclization, modified Vorbrüggen glycosylation, followed by cross-couplings or nucleophilic substitutions and deprotection. Later on this classic approach was also used for the preparation of several other series of fused 7-deazapurine nucleosides. This type of fused modification was approved to be very successful that most compounds from these two series showed submicromolar cytotoxic/cytostatic activity against a wide range of cancer cell lines and leukemia cell lines. What's more, the methyl derivatives demonstrated good therapeutic index that they were highly active against cancer cell lines and much less active to fibroblasts.

Inspired by the positive results from the thieno-fused series, the 5-membered nitrogenous heterocycles were designed to fuse with 7-deazapurine and the biological activities of resulting nucleoside analogues were tested.[72] First, two isomeric series of pyrrolo-fused 7-deazapurine ribonucleosides **X11** and **X12** were prepared by the above-mentioned classic approach. Interestingly, the series **X11** with the *N*-methyl group pointed up showed not only potent cytostatic and anti-HCV activities but also good selectivity and promising therapeutic index, while the isomeric series **X12** with the *N*-methyl group pointed down were inactive. The most active ones in series **X11** were the methyl, methoxy, and methylsulfanyl derivatives. The study of mechanism of action revealed that the modified nucleoside was first phosphorylated to form the corresponding nucleotide that got incorporated to DNA and RNA, and the resulting DNA eventually led to double-strand breaks and apoptosis. The furo-fused series **X13** was also synthesized and its biological activities were similar with that of pyrrolo-fused series **X11**.

In order to comprehensively understand the SAR of these small heterocycle-fused modifications, pyrazolo-fused 7-deazapurine nucleosides were designed and synthesized.[73] Similar with the *N*-methyl pyrrolo-fused modification, methyl, amino, and methylsulfanyl derivatives in series **X14** displayed potent cytotoxic and anti-HCV activities at submicromolar concentrations. Different methods of glycosylation were compared in this study, and the Vorbrüggen procedure was proved to be the most efficient one and gave the desired configuration.

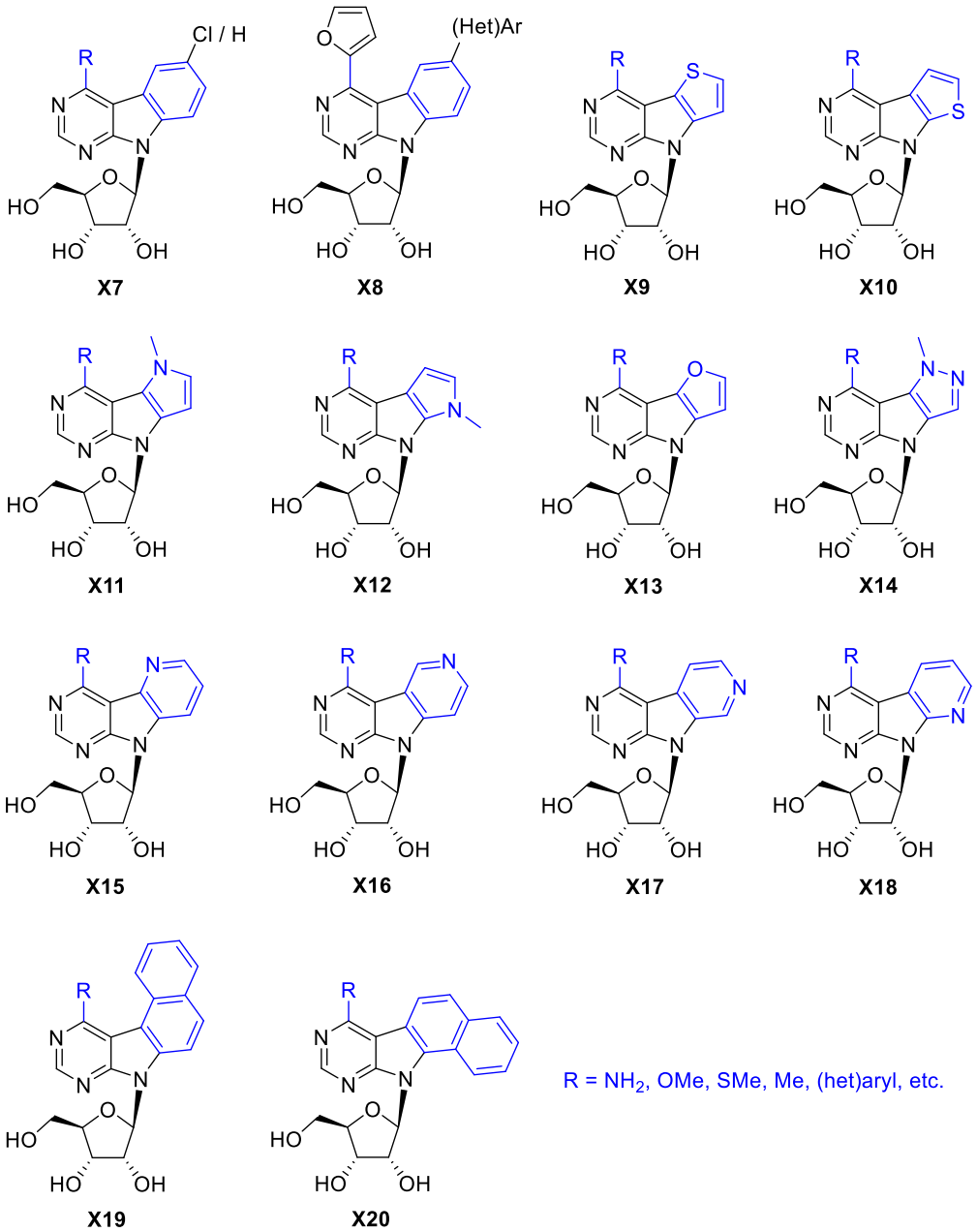


Figure 8. Fused 7-deazapurine nucleosides developed in our group recently

Except for the 5-membered heterocycle-fused modifications, the biological activities of pyrido-fused 7-deazapurine ribonucleosides **X15** – **X18** were investigated. Lucia Veselovská et al. had done a very comprehensive and systematic work that four isomeric series of pyrido-fused 7-deazapurine ribonucleosides with the pyridine nitrogen at different positions were synthesized.[74] All compounds from the four series were tested for cytostatic and antiviral activities. The nucleoside analogues with small substituents at position 4 in series **X17** were very active against a panel of cancer cell lines and HCV, while the other three series showed low or no cytotoxic activities. This comprehensive study revealed that the positions of the pyridine nitrogen were very important for the anti-cancer and anti-viral activities of this type of modified nucleosides.

Later, naphtho-fused modifications were conducted to further investigate the influence of the size of the fused rings on biological activities. Two series of naphtho-fused 7-deazapurine nucleosides were synthesized to study their biological activities and SAR analysis.[75] Compared with the thieno-fused series and pyrrolo-fused series, the naphtho-fused modifications resulted in much lower activities against cancer cell lines and HCV. The biological profiling indicated that naphtho moiety were too bulky for intracellular phosphorylation. Although the the naphtho-fused modifications led to poor activities, the corresponding nucleosides showed strong fluorescence that was promising for the general usage in fluorescent labeling.

Therefore, we conclude that small 5-menbered heterocycles are the most suitable moieties to fuse with 7-deazapurine to increase the anti-cancer and anti-HCV activities and the positions of the hetero atoms are also of great importance.

1.2.3 Other types of bulky fused nucleosides

There are also some other types of bulky fused nucleosides reported in the literatures. Several examples are shown in Figure 9 to make a general comparison with the

modified nucleosides developed in our group.

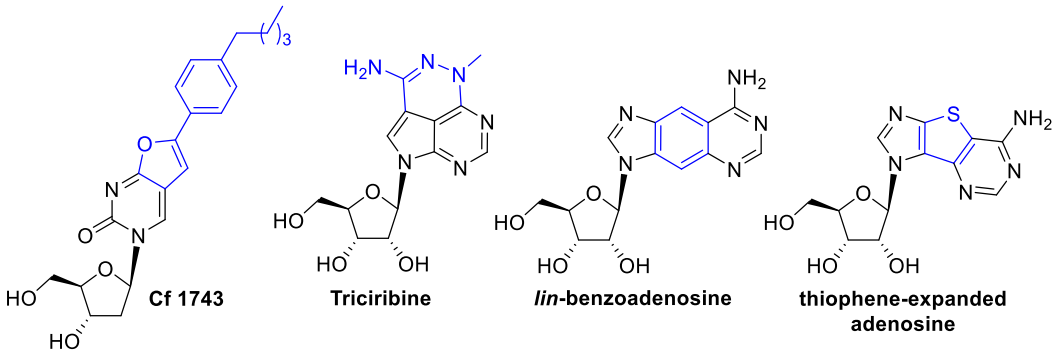


Figure 9. Other types of bulky fused nucleoside analogues reported in literatures

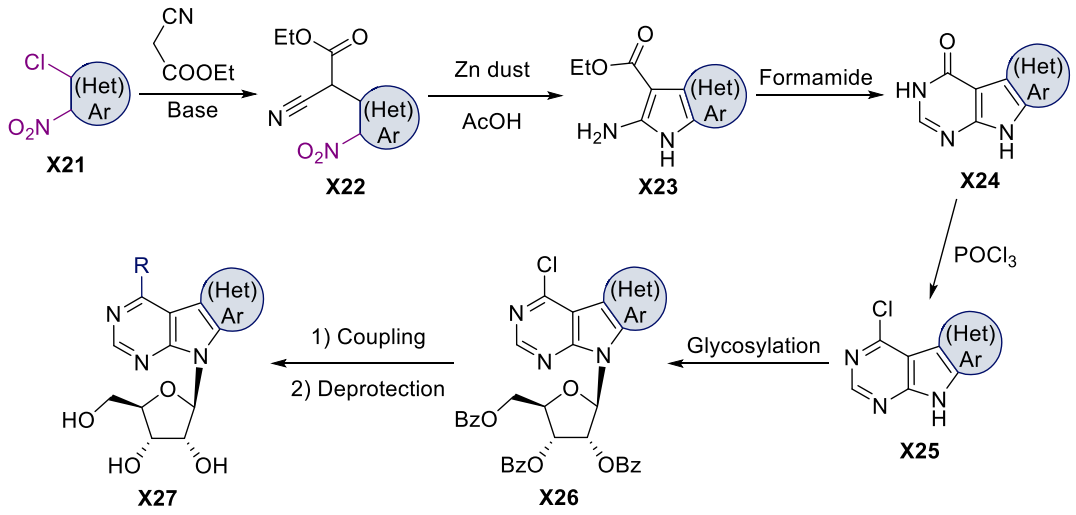
Compound Cf 1743, one example of the bicyclic pyrimidine nucleoside analogues (BCNAs), is extremely potent against VZV with EC_{50} values of 0.2 nM.[76] The long alkyl chain was proved to be essential for increasing the activity. Triciribine is a tricyclic nucleoside analogue that the third pyridazine ring is fused on the northern side of 7-deazapurine. This analogue was first synthesized for development of anti-cancer drug, but the clinical trial failed as being toxic with limited efficacy. However, it was reported in the early 2000s that triciribine would be effective against tumours with hyperactivated Akt.[77] *Lin*-benzoadenosine and thiophene-expanded adenosine are tricyclic (hetero)aromatics-expanded purine ribonucleosides, which were designed to investigate the influence of the size of nucleosides on biological activities. The size of *lin*-benzoadenosine is too big to be active enough for further clinical research.[78] Thiophene-expanded adenosine has relatively smaller size that it displays some interesting biological activities especially against HCV and cancer. However, the study of it did not go further due to difficult synthesis and low total yield.[79, 80]

1.3 Synthesis of Polycyclic Hetero-Fused 7-Deazapurine Nucleosides

Base-modified nucleosides are a privileged class of compounds with a broad spectrum of biological effects, most importantly antiviral[8, 81, 82] and antineoplastic activities.[83-85] In section 1.2, we have described many types of nucleobase modifications and the biological activities of corresponding nucleoside analogues, especially extended fused deazapurine nucleosides. This section introduces several synthetic approaches to building fused deazapurine nucleobases and nucleosides and discusses the advantages and disadvantages of each approach.

1.3.1 Synthetic approach via multi-heterocyclization

The synthesis of fused 7-deazapurine bases is always a challenging and tedious task. One general procedure is based on multi-heterocyclization, starting from *o*-chloronitroheterocycles.[70, 74, 86] The nucleophilic substitution of the starting material **X21** with cyanoacetate in basic conditions gives intermediate **X22**. Then this intermediate undergoes reduction and spontaneous cyclization in the presence of zinc dust and acetic acid to form the bicyclic compound **X23**. Compound **X23** then reacts with formamide under high temperature to afford the tricyclic intermediate **X24**. After treating this tricyclic intermediate with $POCl_3$, the key chlorinated nucleobase **X25** is finally obtained. Later, the protected nucleoside **X26** is prepared by a modified Vorbrüggen glycosylation of the fused nucleobase. With the protected nucleoside in hand, different coupling reactions are conducted to introduce a series of substituents to position 6 of 7-deazapurine moiety, which is followed by deprotection with sodium methoxide to eventually give the target free nucleosides. Other derivatives, like amino, methoxy and methylsulfanyl, are obtained by nucleophilic substitution of the protected nucleoside **X26**, during which the benzoyl group is simultaneously removed.

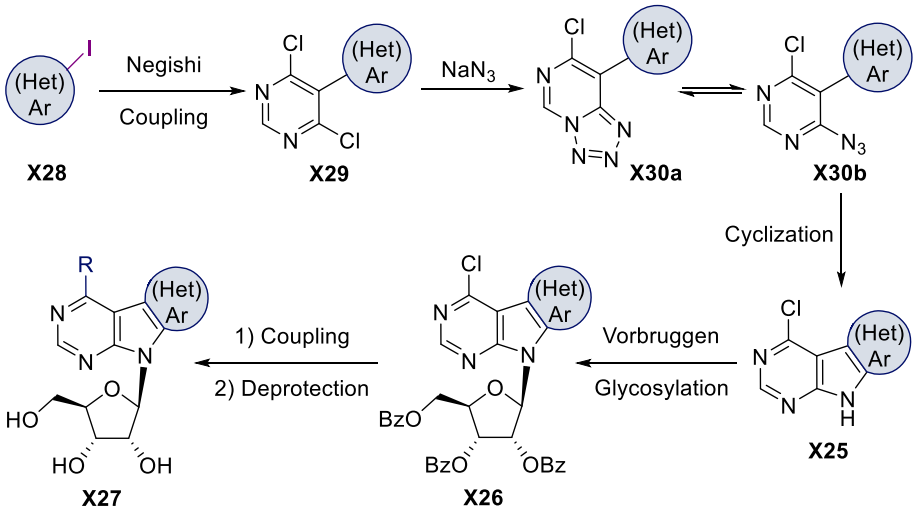


Scheme 1. Multi-heterocyclization approach via chloronitro-heterocycles

Previously, we have successfully prepared series **X7**,[70] **X8**,[86] **X14**,[73] **X15** – **X18**[74] by this multi-heterocyclization approach in our laboratory. These positive examples proved the procedure could have a broad usage. Besides, the starting material **X21** bears chloro and nitro groups, which avoid the regioselective problem of cyclization. On the other hand, there are some disadvantages as well. The cyclocondensation with formamide needs very harsh condition, heating to 170 or 190 °C. For some nucleoside derivatives, the corresponding starting materials, *o*-chloronitroheterocycles, are inaccessible, expensive or unreactive.

1.3.2 Synthetic approach via Negishi coupling and cyclization

Since there are some disadvantages and limitations in the multi-heterocyclization approach, other methods have been developed. Another synthetic procedure to fused 7-deazapurine nucleosides starts from Negishi coupling of zincated dichloropyrimidine, generated in situ, and iodoheterocycles.[71-73, 75] The resulting compound **X29** is treated with sodium azide to form tetrazole **X30a** and azide **X30b** in equilibrium. The subsequent cyclization of the azide affords the fused nucleobases **X25**, and this step can be performed under photochemical or thermal conditions, or with transition metal catalysts. The next glycosylation and further reactions to obtain the final free nucleosides are similar with the first approach.

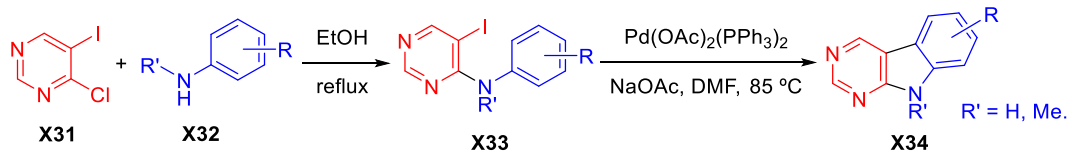


Scheme 2. Synthetic approach of fused 7-deazapurine nucleosides through Negishi coupling and cyclization

This procedure has been applied in the synthesis of different types of fused 7-deazapurine nucleosides in our laboratory, such as series **X9** – **X14**,[71-73] **X19**, **X20**.[75] The fused moieties of these analogues can range from small polar heterocycles to bulky nonpolar aromatics, which demonstrates the versatility of this procedure. However, some of the starting materials iodoheterocycles **X28** are not commercially available or not able to be prepared through easy procedures. For example, the iodination of some heterocycles end up with inseparable mixture with iodine substituted at different positions. During the cyclization step, if there are two chemically equivalent or similar CH on (hetero)aromatics, it may has regioselectivity problems. Besides, sodium azide is very acutely poisonous and has explosion hazard.

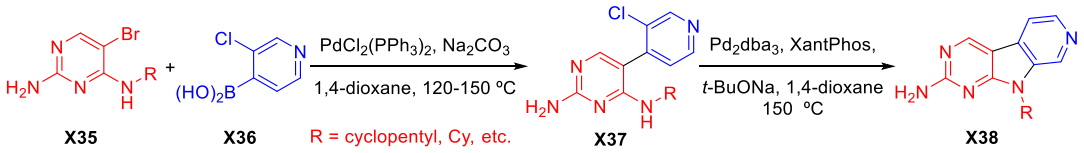
1.3.3 Other synthetic approachs to fused 7-deazapurine nucleobases

The above two approaches are commonly used in our laboratory. Since both methods have some disadvantages or limitations, here some other representative synthetic approachs reported in literatures to fused 7-deazapurine nucleobases are introduced and discussed.



Scheme 3. Construction of the tricyclic scaffold via nucleophilic substitution and intramolecular arylation

In 2002, Zhang et al. reported a method for construction of tricyclic pyrimido[4,5-*b*]indoles.[87] In presence of $Pd(OAc)_2(PPh_3)_2$ and base at 85 °C, the tricyclic compounds **X34** were obtained by intramolecular arylation of 4-anilino-5-iodopyrimidines **X33**, which were prepared from 4-chloro-5-iodopyrimidine **X31** and anilines **X32** in refluxing EtOH. This two-step sequence used relatively mild conditions, comparing with the previous two approaches. And the scope of substrates applied in the reaction was broad. On the other hand, the palladium catalyst was not cheap.

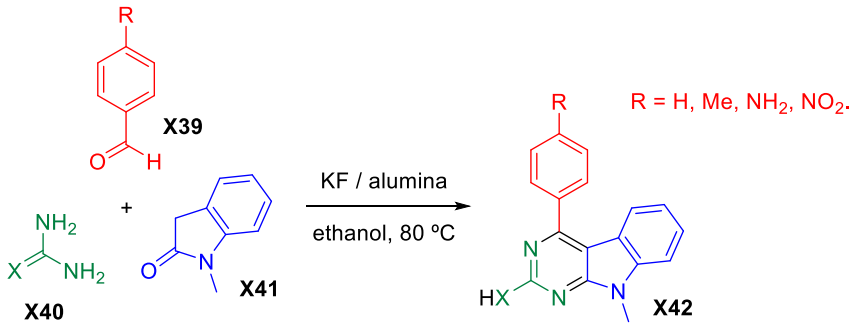


Scheme 4. Synthesis of pyrido[4′,3′:4,5]pyrrolo[2,3-*d*]pyrimidine via Suzuki coupling and Buchwald–Hartwig amination

Similar tricyclic compounds pyrido[4′,3′:4,5]pyrrolo[2,3-*d*]pyrimidine can also be prepared by a two-step approach, which features Suzuki cross-coupling and Buchwald–Hartwig amination.[88] This method can quickly provide a series of special tricyclic scaffolds with 2-amino group, which appears in guanosine derivatives. The same problem of this sequence is the price of the palladium catalysts.

A multi-component synthesis of pyrimido[4,5-b]indole scaffold **X42** was reported in 2008. The desired pyrimidine derivatives were prepared by the three-component reaction of aryl aldehyde, urea or guanidine, and 1-methylindolin-2-one **X41** in ethanol catalyzed by $KF–Al_2O_3$ at 80 °C.[89] The biggest advantage is that this reaction provides a convenient way to synthesize a large series

of pyrimido[4,5-b]indoles with diverse substituents, which is suitable for building up a compound library for biological activity screening. However, only aryl aldehydes **X39** were investigated in this reaction, which led to relatively monotonous products bearing 4-aryl groups.



Scheme 5. Synthesis of pyrimido[4,5-*b*]indole scaffold via three-component reaction

In conclusion, we have introduced two approaches commonly used in our lab and some other methods reported in the literatures to fused 7-deazapurine nucleosides or nucleobases and discussed their usage and limits. Although these procedures have successfully applied in the preparation of many series of nucleoside analogues, developing alternative methods is still necessary due to the great demand of novel fused nucleosides for drug-resistant tumor and newly emerging viruses.

1.4 Chemistry of Sulfonium Salts

In section 1.3, we have discussed the advantages and disadvantages of different approaches to fused nucleobases and nucleosides. Since each method has particular limitations, like inaccessible starting materials, regioselectivity, expensive or hazardous reagents, harsh reaction conditions, developing other alternative synthetic methods to fused 7-deazapurine nucleosides is always under our consideration. In my second Ph.D. project, the aim was to synthesize 7-deazapurine nucleosides fused with thiophene-based heterocycles. The preparation of starting materials, iodothiophene derivatives, could be problematic according to the literatures, if the approach via Negishi coupling and cyclization was used. So we were keen to develop a new method for synthesis of the target molecules.

Sulfonium salts are versatile tools in organic synthesis, which have usually been used in cross-coupling chemistry[90] or as sources of organic radicals[91] for decades. Recently Ritter et al. reported convenient preparation and versatile application of aryl sulfonium salts from thianthrene sulfoxides.[92-95] Later on a similar chemical tool for C-H functionalization, 5-(aryl)dibenzothiophenium salts, was reported, and its synthesis, structure, and reactivity was investigated.[96]

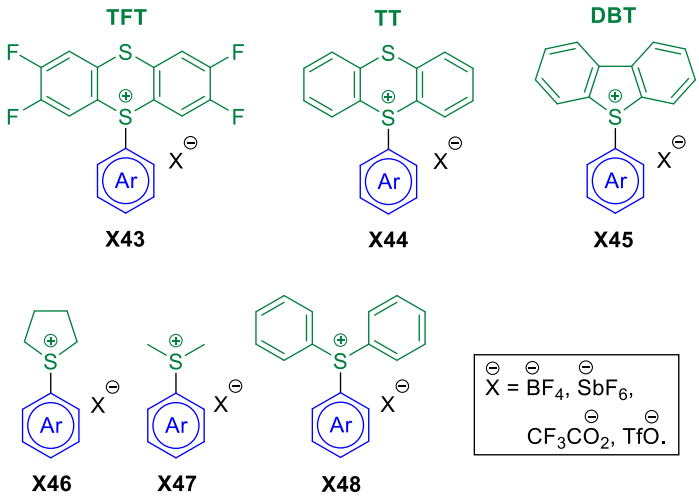


Figure 10. Commonly used sulfonium salts in organic chemistry

The structures of representative sulfonium salts are displayed in Figure 10. The sulfonium salt has a positively charged sulfur ion, which is linked with an aryl moiety. And the sulfonium moiety could be in different patterns, such as tetrafluorothianthrenium **X43** (TFT),[92] thianthrenium **X44** (TT),[93] dibenzothiophenium **X45** (DBT),[96] tetramethylenesulfonium **X46**,[97] dimethylsulfonium **X47**,[98] diphenylsulfonium **X48**.[99] The difference of sulfonium moieties lead to different reactivity and selectivity. When these sulfonium reagents are applied to same type of reaction, the scope of substrates can be quite different. The anions of sulfonium salts include BF_4^-, SbF_6^-, $CF_3CO_2^-$, TfO^-, etc.

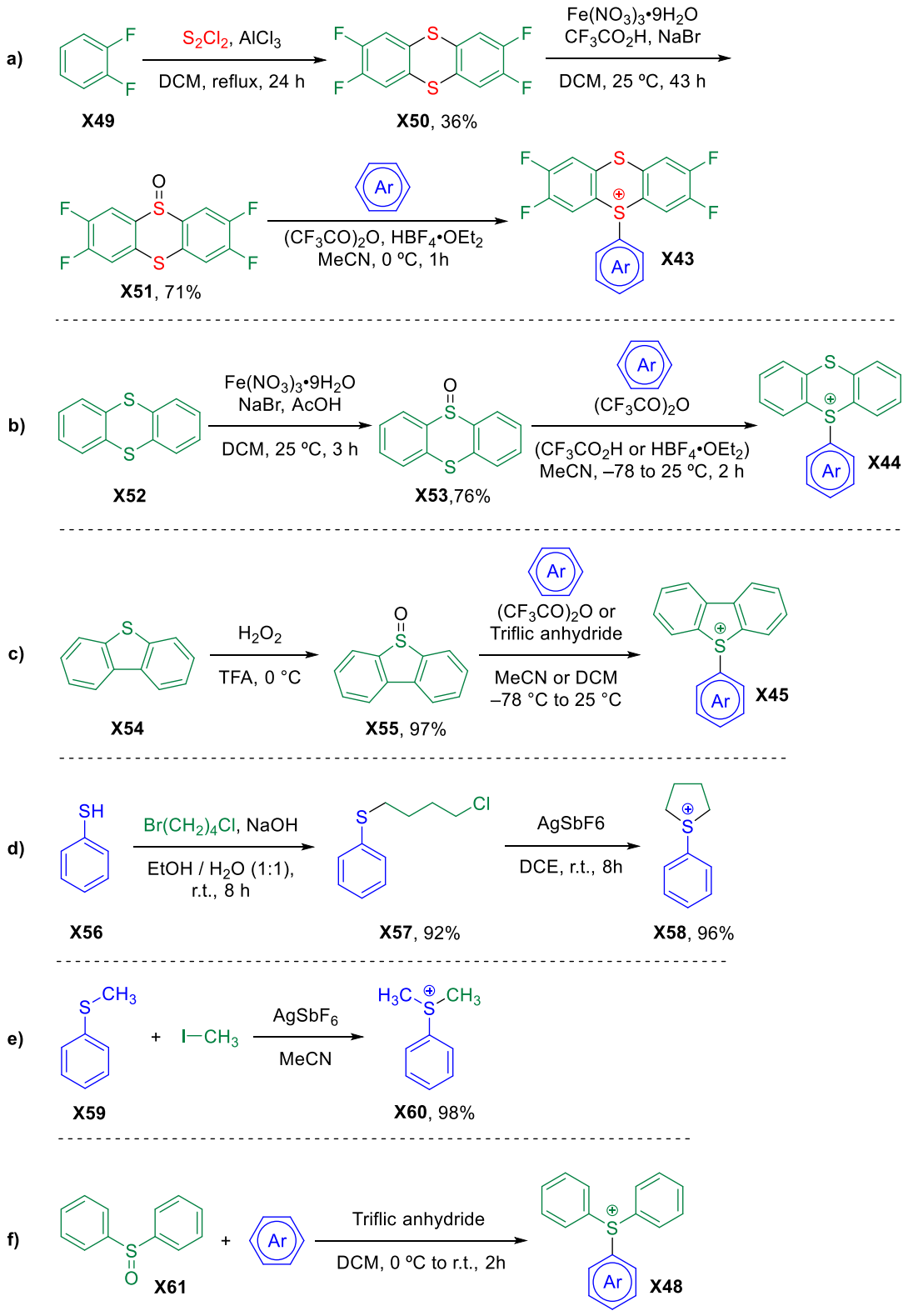
1.4.1 Preparation of arylsulfonium salts

It is necessary to have some basic knowledge of the preparation of arylsulfonium salts, as the reagents have diverse applications in organic synthesis, especially late-stage functionalization. The early procedures of the preparation of arylsulfonium salts involve nucleophilic substitution of aryl thiols and haloalkanes. Nowadays chemists tend to use a more direct reaction of sulfonium *S*-oxides and aromatics in presence of carboxylic anhydrides.

Tetrafluorothianthrenium salt **X43** can be prepared by a three-step procedure (Scheme 6a).[92] First, 1,1-difluorobenzene reacts with disulfur dichloride under reflux in DCM to afford tetrafluorothianthrene **X50**, followed by oxidation with iron(III) nitrate nonahydrate. The resulting tetrafluorothianthrene-*S*-oxide **X51** is treated with a broad series of aromatics (most are substituted benzenes) under trifluoroacetic anhydride to obtain the desired sulfonium reagents **X43**. The tetrafluorothianthrenium salts are proved to be versatile linchpin electrophiles that can be successfully used in both palladium-catalysed cross-coupling chemistry and photoredox catalysis.

A similar variant of tetrafluorothianthrenium salt is thianthrenium salt **X44**, which can be easily prepared through oxidation of thianthrene and treatment of carboxylic anhydrides (Scheme 6b).[94] Aryl thianthrenium and aryl

tetrafluorothianthrenium salts perform similarly in many reactions, but the former gives higher yields is some Pd-catalyzed chemistry.



Scheme 6. Preparation of arylsulfonium salts

5-(Aryl)dibenzothiophenium salt **X45** is now widely used in the study of cross-coupling reactions and many new methodologies, since the concise preparation procedure and the cheap starting material dibenzothiophene. The preparation includes the oxidation of dibenzothiophene via H_2O_2, and the treatment of the resulting dibenzothiophene *S*-oxide **X55** with simple arenes in presence of anhydrides.[96] The scope of substrates can be moderate electron-rich arenes and heteroarenes such as anisole derivatives, alkyl- or aryl-substituted benzenes, haloarenes, and thiophenes, with which the reaction gives good yields and high site-selectivity. But it becomes problematic with electron-poor rings or electron-rich (hetero)aromatic rings. Interestingly, the DBT unit is more electron-poor than the thianthrenium one that the insertion of [Pd] to the C–DBT bond is more favored, which enables dibenzothiophenium salts to be ideal substrates for Pd- catalyzed coupling reactions.

Cyclic tetramethylenesulfonium salt **X58** is obtained by a two-step procedure: *S*-alkylation of thiophenol, followed by intramolecular cyclization with silver hexafluoroantimonate(V), as shown in Scheme 6d.[98] It is reported that the Pd_2dba_3-catalyzed coupling reaction using cyclic arylsulfonium salts has very broad scope of substrates, including electron-rich and electron-poor arenes.[97]

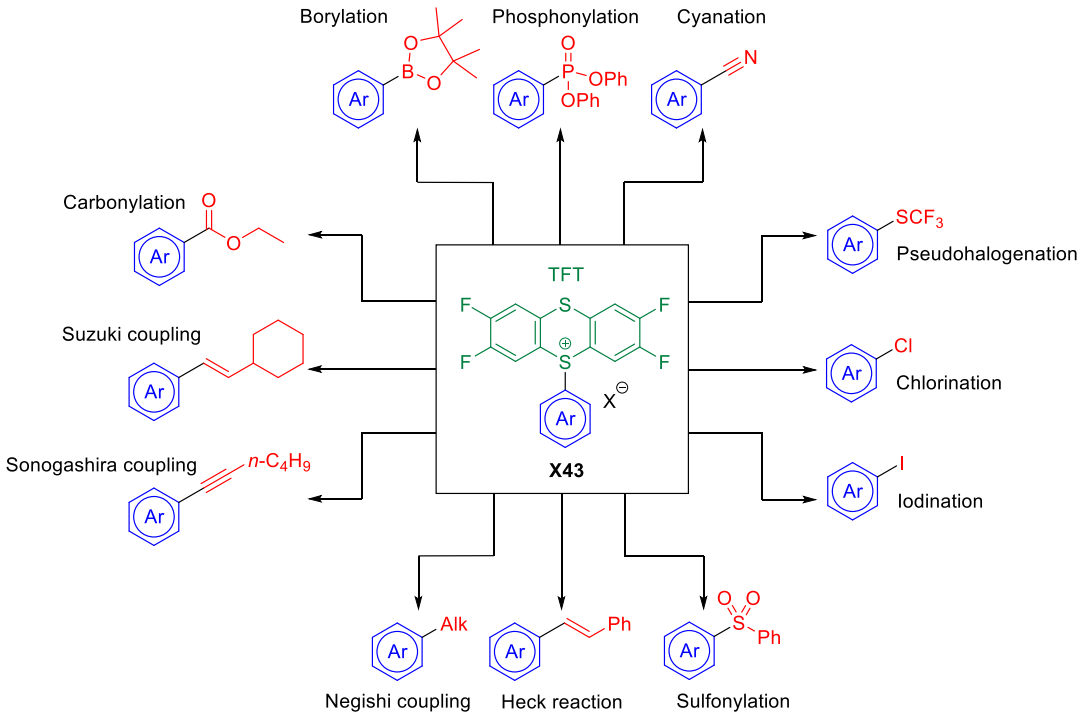
Another example is dimethyl(phenyl)sulfonium salt **X60**, which is prepared from thioanisole through simple *S*-methylation with MeI and $AgSbF_6$ (Scheme 6e).[98] One disadvantage is that dimethyl sulfide, releasing from the reactions of dimethylsulfonium salts, has disgusting odor.

Diphenylsulfonium salt **X48** is the easiest one to be prepared among the sulfonium salts shown in Scheme 6. Diphenyl sulfoxide is treated with arenes in presence of trifluoromethanesulfonic anhydride to form sulfonium salt **X48**.[99]

To sum up, there are two typical procedures for the preparation of sulfonium salts: one is engaging sulfonium *S*-oxides and anhydrides, the other one is *S*-alkylation with haloalkanes.

1.4.2 Reactions of arylsulfonium salts

Sulfonium salts have shown to be highly versatile reagents and have been broadly applied in organic chemistry, such as synthesis of three-membered rings via sulfur ylides, formation of C-C bonds through cross-coupling reactions, redox catalysis, and the umpolung of the cyano group.[100] There are numerous applications of different types of sulfonium salts reported in the last two decades. In this section, we are only focusing on 1) reactions of arylsulfonium salts, and 2) selected up-to-date reactions of sulfonium salts.



Scheme 7. Organic chemistry applications of arylsulfonium salts (I)

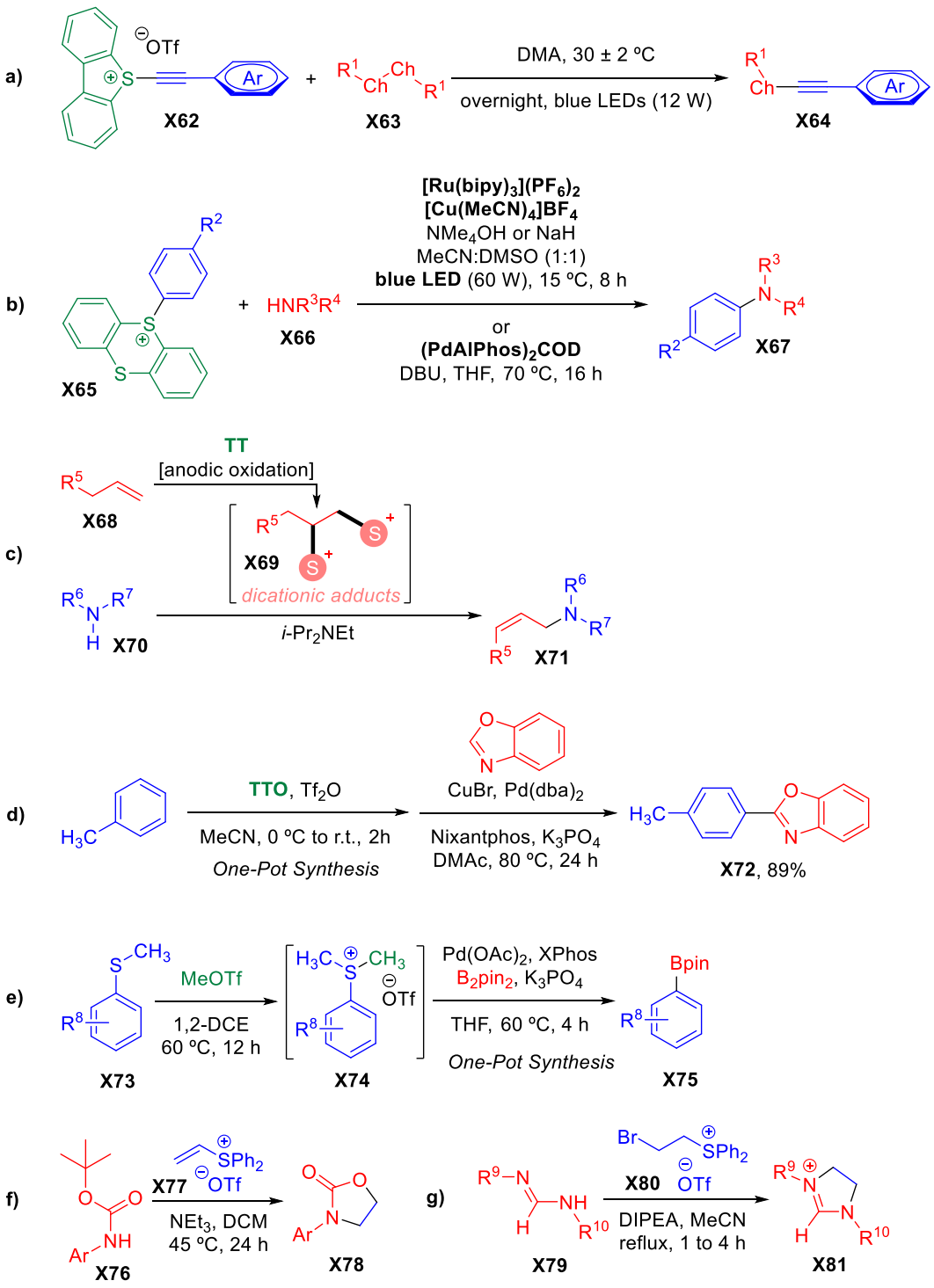
Recently, Ritter group developed a concise synthetic approach of arylsulfonium salts via thianthrenation, and these thianthrenium salts were successfully applied in several coupling reactions and photoredox catalysis.[92] Tetrafluorothianthrenium salts were synthesized and used as standard substrates for studying the reactivity. Compound **X43** was used in a series of palladium-catalysed reactions as useful cross-coupling partners due to the faster oxidative addition reactivity. Sulfonylation, Heck reaction, Negishi coupling, Sonogashira coupling, Suzuki coupling, and

carbonylation of the tetrafluorothianthrenium salt were investigated and proved to be successful with high yields. These methods demonstrated the powerful capacity of thianthrenium salts for forming C-C bonds. Several photoredox catalyses were also tried with this thianthrenium salt, such as borylation, phosphonylation, cyanantion, pseudohalogenation, chlorination, iodination. And these photoredox reactions were able to form C-X bonds that could introduce different reactive functional groups to arenes for further synthesis. Most of the reactions gave moderate to good yields.

In the last few years, some new types of reactions using sulfonium salts have been developed and thus further broaden the range of their applications.

Chen et al. have recently developed a chalcogen bonding (ChB) catalysis of alkynylsulfonium salts **X62** and dichalcogenides **X63** under photochemical conditions to form a variety of chalcogenoacetylenes **X64**, which are versatile intermediates in organic synthesis and have a broad range of applications in material and medicinal chemistry (Scheme 8a).[101] The simple selenation method was enabled by alkynyl radicals, generating from alkynylsulfonium salts under blue light irradiation. This novel reaction can be used to prepare not only alkynyl selenides but also alkynyl tellurides with high yields. The reaction exhibits a broad substrate scope and can tolerate phenyl rings bearing electron-donating or electron-withdrawing groups.

The first C−N cross-couplings for site-selective late-stage diversification via aryl sulfonium salts were reported in 2019.[94] The reactions were performed under photoredox conditions or with palladium catalysts (Scheme 8b) and provided the desired products with a broad range of Nnucleophiles, includeing alkyl and aryl amines, and N-containing heterocycles. These methods provided a way to achieve molecular diversity via selective late-stage C−N bond formation, thus avoiding protective group manipulations or interferences with other synthetic steps.



Scheme 8. Organic chemistry applications of sulfonium salts (II)

The sulfonium salts are also proved to be a versatile tool in electrochemistry. In 2021, an electrochemical synthesis of allylic amines from unactivated terminal alkenes and secondary amines was reported.[102] The anodic oxidation of thianthrene and alkenes generated dicationic vinylthianthrenium salts **X69** that are the key

intermediates for this transformation (Scheme 8c). This electrochemical method was able to be applied to the synthesis of not only simple chemical intermediates but also complex bio-active molecules.

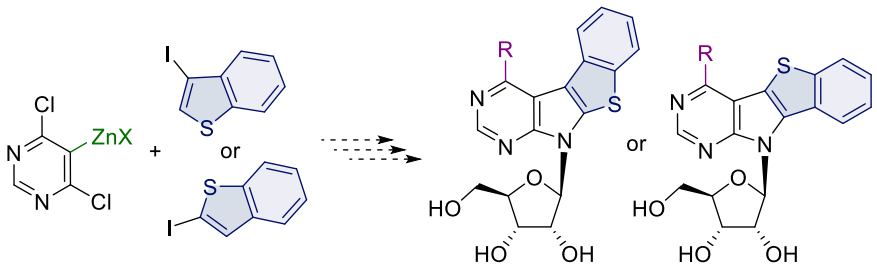
Although the sulfonium salts are versatile tools in organic synthesis and in other chemistry, using them usually involves two-step procedure: thianthrenation by thianthrene-S-oxide and further reactions to form C-C bonds or C-X bonds. In order to simplify the procedures, chemists attempted to develop alternative methods of one-pot synthesis with thianthrene-S-oxide. A selective formal Pd/Cu-catalyzed C–H/C–H cross coupling of arenes with azoles through sulfonium intermediates was reported in 2021.[103] And importantly this method can be carried out with one-pot procedures, which efficiently gives a wide range of 2-(hetero)aryl azole products **X72** (Scheme 8d). Another earlier example of one-pot synthesis through sulfonium intermediates was developed by Yorimitsu and co-workers in 2018.[104] The reaction went through key intermediates arylsulfonium ions, which were formed from methylation of sulfides (Scheme 8e). The aryl dimethylsulfonium ions **X74** underwent subsequent cross-coupling with bis(pinacolato)diboron, affording arylboronate esters.

Additionally, the vinylsulfonium salts can be used to build up various heterocycles (Scheme 8f, 8g). Xie and co-workers reported an efficient reaction of vinyl sulfonium salt **X77** with carbamates towards the syntheses of N-aryloxazolidin-2-ones **X78**.[105] Similarly, a concise synthesis of imidazolinium salts **X81** from formamidines and (2-bromoethyl)diphenylsulfonium triflate was developed.[106] The vinyl sulfonium salt intermediate was generated in situ as the key intermediate.

As mentioned in section 1.3, the current approaches towards the synthesis of fused modified nucleosides have the problems of poor site-selectivity of cyclization and inaccessibility of starting materials. Given the fact that sulfonium salts are versatile reagents for building up diverse organic scaffolds, I was encouraged to apply sulfonium chemistry to overcome the synthetic problems.

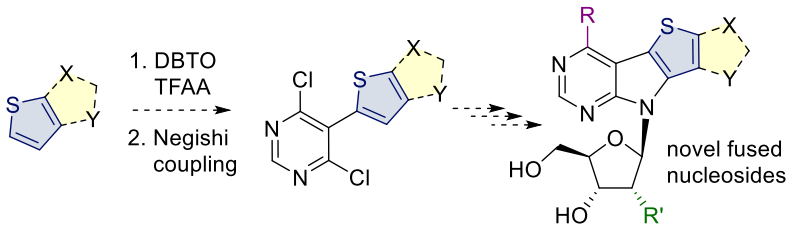
2 Specific Aims of the Thesis

1) To synthesize two isomeric series of benzothieno-fused 7-deazapurine ribonucleosides and study their fluorescent properties in DNA.



Rationale: Base-modified nucleosides are a privileged class of compounds with a broad spectrum of biological effects, most importantly antiviral[8, 81, 82] and antineoplastic activities.[83-85] As mentioned in section 1.2, the second generation of deazapurine nucleosides was based on fused deazapurine nucleobases. Previously, we reported benzo-[70, 86] and naphtho-fused[75] deazapurine nucleosides which were non-cytotoxic but exerted some anti-RNA-virus activities. On the other hand, isomeric thieno-,[71] as well as furo- and methylpyrrolo-fused[72] deazapurine ribonucleosides were found to exert cytotoxic effect at submicromolar concentrations with good selectivity for cancer and leukemia cell lines. Encouraged by the promising biological activities, we designed the two isomeric series of new benzothieno-fused deazapurine ribonucleosides as the **extended analogues** to the cytotoxic **thienopyrrolopyrimidine** nucleosides and hetero-analogues of antiviral **naphthopyrrolopyrimidine** nucleosides. The aim of this work was to synthesize the two series of modifies nucleosides and to study their fluorescent properties in DNA. And the resulting free ribonucleosides were also sent to Olomouc for biological activity screening.

2) To develop a new approach for synthesizing polycyclic hetero-fused 7-deazapurine heterocycles and synthesize the corresponding nucleosides, and to study enzymatic incorporation of the modified nucleotide.



Rationale: Although modified nucleoside and nucleotide analogues have been studied and developed as important cytostatic and antiviral drugs for more than three decades, there is still a great need for novel types of them in development of antiviral drugs against newly emerging viruses or anticancer agents against drug resistant tumors or leukemias. Recently, we designed and synthesized novel types of tri- and tetracyclic fused 7-deazapurine ribonucleosides and some of these derivatives showed strong cytostatic effects or antiviral activities.[70-74] These tri- and tetracyclic fused nucleobases can be synthesized either by multistep heterocyclization approach[70, 74, 86] or through Negishi cross-coupling of in situ generated 4,6-dichloropyrimidine-5-zinc reagent with hetaryl halides,[71, 72, 75] but for some heterocycles, the corresponding halides are inaccessible, expensive or unreactive. Therefore, there is a need of an alternative general approach that would enable to synthesize wide range of novel fused deazapurine bases for further applications. The goal of the second part of my work was to develop a new approach for preparing the target compounds using sulfonium chemistry,[92, 95, 96] followed by **novel Negishi coupling**. The implications of this research, if successful, could overcome the above-mentioned synthetic problems and be applied to the synthesis of a portfolio of novel tri-, tetra- and even pentacyclic fused 7-deazapurine bases. More importantly, the corresponding **extended bulky nucleosides and nucleotides** were unknown and unique, thus their photophysical properties and **biochemical and biological profiling** were worth studying.

3 Results and Discussion

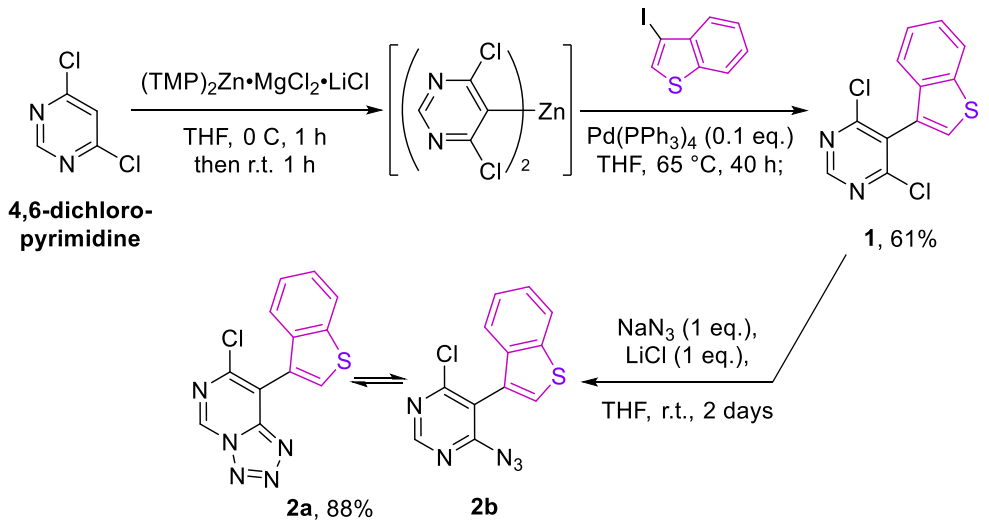
3.1 Benzothieno-Fused 7-Deazapurine Ribonucleosides

Two isomeric series of benzothieno-fused 7-deazapurine (benzo[4',5']thieno[3',2':4,5]- and benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine) ribonucleosides were synthesized as the extended analogues to the cytotoxic thienopyrrolopyrimidine nucleosides[71] and hetero-analogues of antiviral naphthopyrrolopyrimidine nucleosides.[75] The synthesis was accomplished by a similar approach as our previous works.[71, 72] The published paper related to this work was written in a logic order to show our readers the rational design. For the result part of this thesis, I decided to write in chronological order as I did the experiments, in order to present more experimental details.

3.1.1 Synthesis of benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*] pyrimidine ribonucleosides (syn-Series)

The chemistry journey of my Ph.D. study started from Negishi coupling of 4,6-dichloropyrimidine. First, the commercially available 4,6-dichloropyrimidine was treated with $(TMP)_2Zn{\cdot}MgCl_2{\cdot}LiCl$ (TMP = 2,2,6,6-tetramethylpiperidyl) to get an active organozinc intermediate,[107] and then this intermediate underwent a $Pd(PPh_3)_4$-catalyzed Negishi cross-coupling reaction[108] with 3-iodobenzothiophene to give the desired 4,6-dichloro-5-(benzothienyl)pyrimidines **1** (Scheme 9). The yield of benzothiophen-3-yl derivative **1** was 61%.

In the second step, the azidation of dichloropyrimidine **1** with sodium azide in THF was performed to get azidopyrimidine **2** in excellent 88% yield, though the reaction was very slow taking two days. The NMR spectra in DMSO showed that compounds **2** existed as the equilibrium of both tetrazolopyrimidines **2a** and azidopyrimidines **2b** (in ratio 4:1).

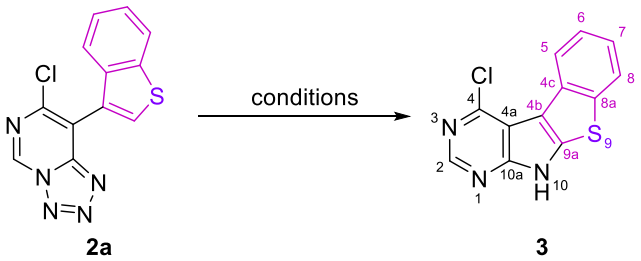


Scheme 9. Negishi coupling of 3-iodobenzothiophene and azidation

The azidopyrimidine form is needed for the next step of the heterocyclization, which can be achieved either thermally or photochemically (Table 1). The cyclization is one of the crucial steps in this approach, so the desired nucleobase was eventually obtained after I tried a lot of different reaction conditions. For compound **2**, heating at 180 °C resulted in decomposition, while the irradiation with 254 nm UV light in TFA gave the desired tricyclic nucleobase **3** in good yield of 75%. I also tried rhodium-catalyzed cyclization of **2**.[109] After screening of several Rh-catalysts and conditions, I found that the reaction using $Rh_2(esp)_2$ catalyst in presence of molecular sieves in toluene/TFA also gave the desired product **3** in 49% yield (Table 1). The use of TFA was important for this transformation. Since the Rh-catalyst was not cheap, the photochemical condition was chosen for large-scale preparation.

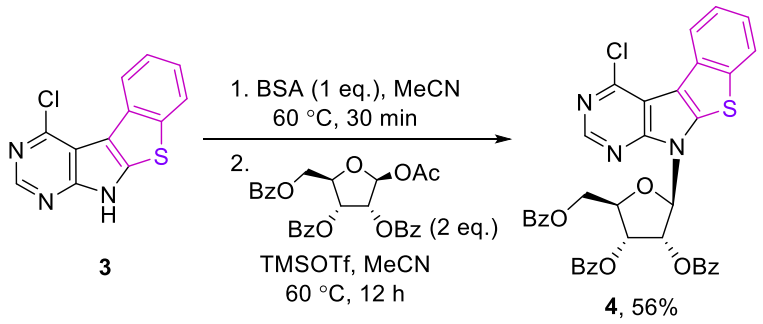
The next step was the glycosylation of benzothienopyrrolopyrimidine base **3**, using a modified procedure of classic Vorbrüggen glycosylation as in our previously published study.[70, 75] A MeCN solution of nucleobase **3** and BSA was heated to 60 °C for 30 min. After adding TMSOTf and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose, the mixture was stirred at the same temperature overnight. The protected benzothienopyrrolopyrimidine nucleoside **4** was obtained in good yields of 56% as pure *β*-anomers (Scheme 10).

Table 1. Optimization of cyclization of azide/tetrazole **2**



2a **3**

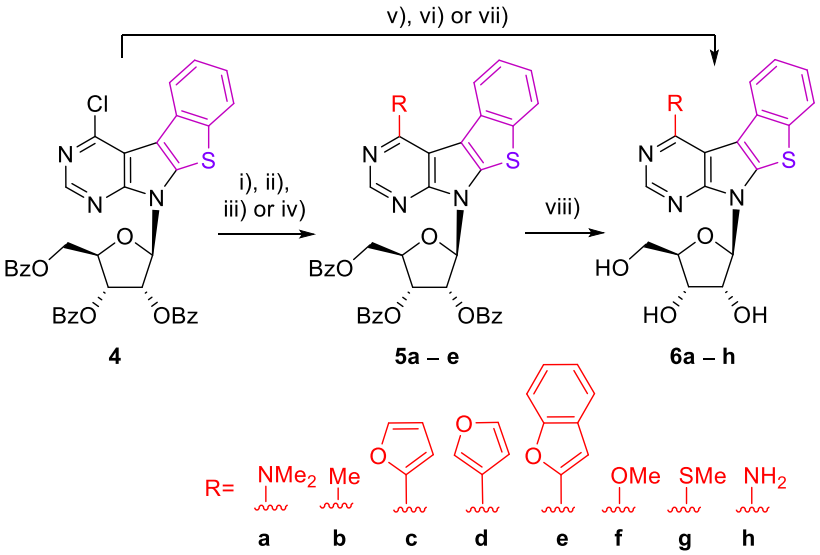
Entry	Conditions/Catalyst	Solvent	Additive	Temp (°C)	Time (h)	Yield (%)	Note
1	**UV (254 nm, 4 W)**	**TFA**	**--**	**r.t.**	**48**	**75**	--
2	1,4-dibromobenzene	--	--	180	0.5	15	decomp.
3	$Rh_2(esp)_2$	PhMe	--	90	16	traces	SM
4	$Rh_2(O_2CC_3F_7)_4$	PhMe	--	90	16	traces	SM
5	$Rh_2(O_2CC_7H_{15})_4$	PhMe	--	90	16	traces	SM
6	$Rh_2(O_2CC_7H_{15})_4$	PhMe/TFA (1:1)	--	70	24	traces	inseparable byproduct
7	$Rh_2(OOCCH_3)_4$	PhMe/TFA (1:1)	--	70	24	traces	inseparable byproduct
8	$Rh_2(esp)_2$	PhMe/TFA (1:1)	--	70	24	traces	inseparable byproduct
9	$Rh_2(O_2CC_3F_7)_4$	PhMe/TFA (1:1)	--	70	24	traces	inseparable byproduct
10	$Rh_2(O_2CC_7H_{15})_4$	PhMe/TFA (1:1)	4 Å mol. sieves	70	24	28	--
11	**$Rh_2(esp)_2$**	**PhMe/TFA (1:1)**	**4 Å mol. sieves**	**70**	**24**	**49**	**--**



Scheme 10. Glycosylation of benzo[4′,5′]thieno[3′,2′:4,5]pyrrolo[2,3-*d*]pyrimidine nucleobase

The protected 4-chlorobenzothienopyrrolopyrimidine nucleoside **4** was used as key intermediate for the further steps to functionalize the position 4 followed by deprotection in analogy with our previous works (Scheme 11, Table 2).[71, 72, 75] Dimethylamino derivative **5a** was prepared in good yield (60%) from protected nucleoside **4** by reaction with Me_2NH. The introduction of methyl group to positon 4 was performed through cross-coupling reactions with trimethylaluminum and $Pd(PPh_3)_4$ in THF to obtain nucleoside **5b** (in 62% yield). 4-(2-Furyl) derivative **5c** was obtained in good yield by the Stille cross-coupling with 2-furyl$SnBu_3$ in presence of $PdCl_2(PPh_3)_2$ in DMF at 100 °C. The $Pd(PPh_3)_4$-catalyzed Suzuki−Miyaura cross-coupling with the corresponding hetarylboronic acid in presence of potassium carbonate in toluene gave desired products **5d**, **5e** in moderate to good yields. Shorter reaction time gave lower conversions whereas longer reaction times led to partial decomposition. Protected nucleosides **5a** – **5e** were then deprotected by MeONa in MeOH to obtain free nucleosides **6a** – **6e** in moderate to good yields. Due to the poor solubility of the intermediates **5**, the mixture of 1,4-dioxane and methanol and refluxing at 65 °C were used to accelerate the reaction and increase the yield. Nucleosides **6f** – **6h** bearing MeO, MeS or NH_2 groups at position 4 were prepared directly from the key intermediate **4** using nucleophilic substitution with aqueous ammonia, sodium methoxide, or sodium methylthiolate, respectively. In all of these cases, the benzoyl groups at the sugar moiety were cleaved simultanelously to give directlty the desired free nucleosides. Importantly, 1,4-dioxane must be used as the solvent for introducing methylsulfanyl group using sodium methanethiolate and the

reactions took 4 days at 100 °C to obtain the desired methylsulfanyl nucleoside **6g**. When sodium methanethiolate was used in MeOH, the reaction led to 4-methoxy derivatives **6f**. The target free nucleosides **6f** – **6h** were obtained in acceptable yields and good purity (Scheme 11, Table 2).



Scheme 11. Late-stage diversification of protected syn benzothieno-fused nucleoside Reagents and conditions: (i) Me_2NH in THF (3 equiv), propan-2-ol/DCM 1:1, 40 °C, 24 h; (ii) Me_3Al (2 equiv), $Pd(PPh_3)_4$ (0.1 equiv), THF, 65 °C, 20 h; (iii) 2-tributylstannylfuran (1.2 equiv), $PdCl_2(PPh_3)_2$ (0.15 equiv), DMF, 100 °C, 30 min; (iv) R-boronic acid (1.5 equiv), $Pd(PPh_3)_4$ (0.1 – 0.2 equiv), K_2CO_3 (2–2.5 equiv), toluene, 100 °C, 8–24 h; (v) MeONa (2–5 equiv), MeOH, 65 °C, 2 days; (vi) MeSNa (5 or 8 equiv), dioxane, 100 °C, 4 days; (vii) NH_3 (aq), dioxane, 120 °C, 24 – 48 h; (viii) MeONa in MeOH (3 – 6 equiv), MeOH/dioxane, 60 – 65 °C, 2 days.

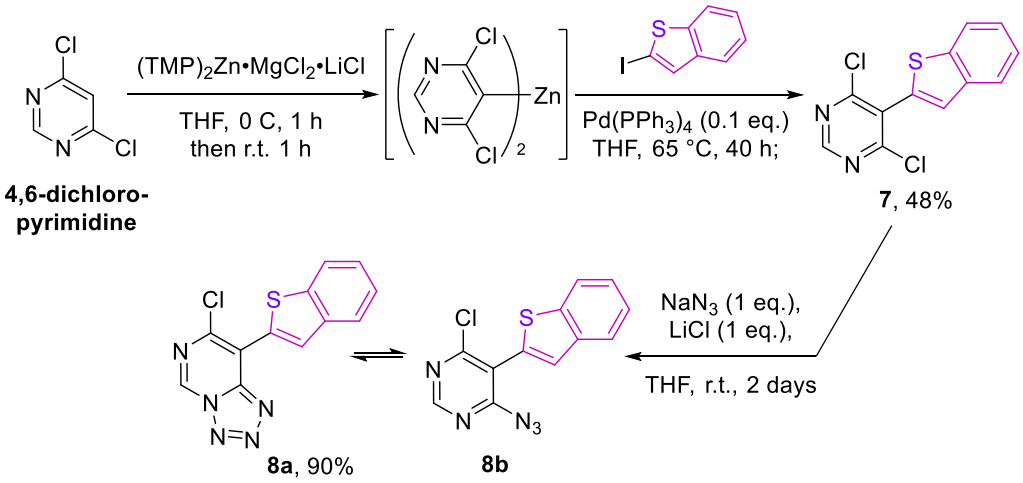
Table 2. Synthesis of 4-substituted nucleosides **5** and **6**

Entry	Reaction	R	Protected nucleoside	Yield [%]	Final nucleoside	Yield [%]
1	i)	dimethylamino	**5a**	60	**6a**	80
2	ii)	methyl	**5b**	62	**6b**	82
3	iii)	2-furyl	**5c**	67	**6c**	71
4	iv)	3-furyl	**5d**	70	**6d**	84
5	iv)	2-benzofuryl	**5e**	30	**6e**	81
6	v)	methoxy			**6f**	57
7	vi)	methylsulfanyl			**6g**	68
8	vii)	amino			**6h**	51

3.1.2 Synthesis of benzo[4′,5′]thieno[2′,3′:4,5]pyrrolo[2,3-*d*] pyrimidine ribonucleosides (anti-Series)

The synthesis of the anti-series is similar to that of the syn-series. The steps that used the same reaction conditions as syn-series will be described briefly, and the steps that used different conditions will be introduced with more details.

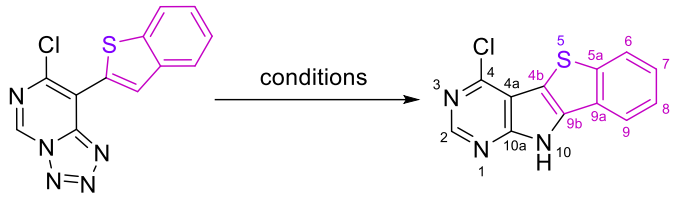
The synthesis of anti-series also stared with Negishi coupling, but with 2-iodobenzothiophene. The active organozinc intermediate was treated with 2-iodobenzothiophene in presence of $Pd(PPh_3)_4$ to afford benzothiophen-2-yl derivative **7** with 48% yield. Next, the azidation of dichloropyrimidine **7** with sodium azide in THF was performed to get azidopyrimidine **8** in excellent 90% yield. In DMSO, compound **8** existed as the equilibrium of both tetrazolopyrimidine **8a** and azidopyrimidine **8b** (in ratio 6:1).



Scheme 12. Negishi coupling of 2-iodobenzothiophene and azidation

Same as syn series, the cyclization of the azidopyrimidine **8** was problematic. Different conditions were tried with a lot of patience (Table 3). The robust thermal cyclization at 180°C successively gave us the cyclic product **9** in acceptable 60% yield. On the other hand, the attempted photocyclization of **8** gave the desired compound **9** in an inseparable mixture with other byproducts.

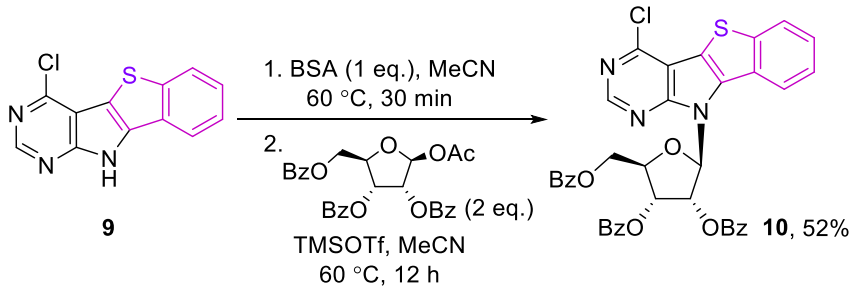
Table 3. Optimization of cyclization of azide/tetrazole **8**



8a **9**

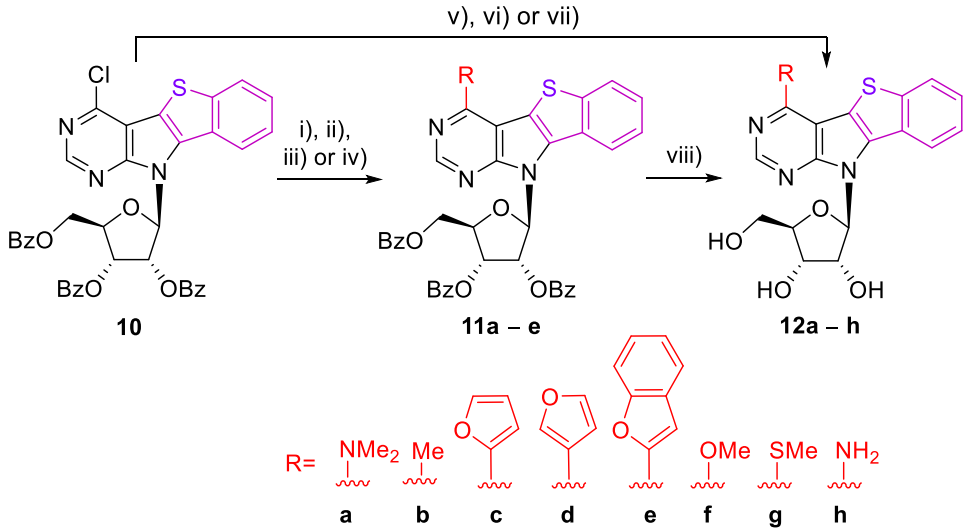
Entry	Conditions	Type	Yield	Note
1	UV (254 nm, 4 W), TFA, r.t.	photo	--	Inseparable byproduct
2	**1,4-dibromobenzene, 180 °C, 30 min**	**thermal**	**60 %**	**--**

The reaction condition for glycosylation of nucleobase **9** was the same as that of syn series. The nucleobase was treated with BSA, followed by addition of TMSOTf and ribofuranose, to give protected benzothienopyrrolopyrimidine nucleoside **10** as pure *β*-anomer with 52% yield.



Scheme 13. Glycosylation of benzo[4′,5′]thieno[2′,3′:4,5]pyrrolo[2,3-*d*]pyrimidine) nucleobase

The late-stage diversification of protected anti benzothieno-fused nucleoside **10** was performed in analogy with syn series. Dimethylamino derivative **11a** was prepared in good yields (84%) from protected nucleosides **10** by reaction with Me_2NH. The introduction of methyl group to positon 4 was performed through cross-coupling reactions with trimethylaluminum and $Pd(PPh_3)_4$ in THF to obtain nucleosides **11b** in 59% yield.



Scheme 14. Late-stage diversification of protected anti benzothieno-fused nucleoside Reagents and conditions: (i) Me_2NH in THF (3 equiv), propan-2-ol/DCM 1:1, 40 °C, 24 h; (ii) Me_3Al (2 equiv), $Pd(PPh_3)_4$ (0.1 equiv), THF, 65 °C, 20 h; (iii) 2-tributylstannylfuran (1.2 equiv), $PdCl_2(PPh_3)_2$ (0.15 equiv), DMF, 100 °C, 30 min; (iv) R-boronic acid (1.5 equiv), $Pd(PPh_3)_4$ (0.1–0.2 equiv), K_2CO_3 (2–2.5 equiv), toluene, 100 °C, 8–24 h; (v) MeONa (2–5 equiv), MeOH, 65 °C, 2 days; (vi) MeSNa (5 or 8 equiv), dioxane, 100 °C, 4 days; (vii) NH_3 (aq), dioxane, 120 °C, 24–48 h; (viii) MeONa in MeOH (3–6 equiv), MeOH/dioxane, 60–65 °C, 2 days.

Table 4. Synthesis of 4-Substituted Nucleosides **11** and **12**

Entry	Reaction	R	Protected nucleoside	Yield [%]	Final nucleoside	Yield [%]
1	i)	dimethylamino	**11a**	84	**12a**	78
2	ii)	methyl	**11b**	59	**12b**	77
3	iii)	2-furyl	**11c**	90	**12c**	73
4	iv)	3-furyl	**11d**	51	**12d**	51
5	iv)	2-benzofuryl	**11e**	25	**12e**	72
6	v)	methoxy			**12f**	55
7	vi)	methylsulfanyl			**12g**	58
8	vii)	amino			**12h**	47

4-(2-Furyl) derivative **11c** was obtained in good yield by the Stille coupling with 2-furyl$SnBu_3$ in presence of $PdCl_2(PPh_3)_2$ in DMF at 100 °C. The Pd-catalyzed Suzuki coupling with the corresponding hetarylboronic acid in presence of potassium carbonate in toluene gave desired products **11d** and **11e** in low yields. Protected

nucleosides **11a** – **11e** were then deprotected by MeONa in MeOH to obtain free nucleosides **12a** – **12e** in moderate to good yields. Due to the poor solubility of the intermediates **11**, the mixture of 1,4-dioxane and methanol and refluxing at 65 °C were used to accelerate the reaction and increase the yields. Nucleosides **12f** – **12h** bearing MeO, MeS or NH_2 groups at position 4 were prepared directly from the key intermediates **10** using nucleophilic substitution with aqueous ammonia, sodium methoxide, or sodium methylthiolate, respectively. Importantly, 1,4-dioxane must be used as the solvent for introducing methylsulfanyl group using sodium methanethiolate and the reactions took 4 days at 100 °C to obtain the desired methylsulfanyl nucleoside **12g**. The target free nucleosides **12f** – **12h** were obtained in acceptable yields and good purity (Scheme 14 and Table 4).

In the synthetic parts, the biggest challenge is the purification of those compounds that have very low solubility in organic solvents. The two nucleobases are fused tetracyclic aromatics, and this structural feature leads to low solubility in all commonly used organic solvents. Thus the column chromatography of the nucleobases and some of the free nucleosides are very time-consuming. In some cases, the crude products are used directly for the next step to avoid the tedious separation.

3.1.3 Fluorescence properties of benzothieno-fused deazapurine nucleosides

Previously reported benzo-[110] and naphtho-fused[75, 111] 7-deazapurine nucleosides exerted useful fluorescence properties and were used for construction of fluorescent DNA probes. And the modified nucleosides I prepared also have polycyclic aromatic systems. Therefore, I decided to study the photophysical properties of the new benzothieno-fused deazapurine nucleosides. Table 5 shows the results of UV-vis and fluorescence measurement of the final compounds **6a** – **6h** and **12a** – **12h** in MeOH, 1,4-dioxane and water. The UV absorption maximum of all derivatives was observed below wavelength 400 nm, which would limit using these nucleosides in living cells.

Table 5. UV absorption and fluorescence properties of nucleosides **6a** – **6h** and **12a** – **12h**.

		absorption	emission	
Compd	solvent	λ_{abs} [nm] (ε [10^3 M^{-1} cm^{-1}])	λ_{em} [nm]	Φ_f
	MeOH	243 (25.5), 305 (13.4)	414	0.03
6a	Dioxane	241 (22.7), 306 (13.4)	384	0.02
	H_2O	244 (21.3), 307 (11.6)	425	0.01
	MeOH	246 (25.7), 261 (21.6)	428	0.20
6b	Dioxane	248 (30.1), 271 (18.5)	412	0.10
	H_2O	246 (22.5)	450	0.09
	MeOH	270 (23.2), 353 (6.2)	499	0.19
6c	Dioxane	270 (23.2), 348 (7.0)	478	0.57
	H_2O	270 (17.8), 348 (5.3)	530	0.03
	MeOH	266 (22.1), 326 (3.7)	475	0.28
6d	Dioxane	263(20.8), 328 (4.4)	456	0.52
	H_2O	283 (16.5)	435	0.19
	MeOH	275 (21.4), 366 (7.9)	523	0.16
6e	Dioxane	277 (20.8), 368 (8.5)	503	0.49
	H_2O	284 (14.8), 378 (5.7)	533	0.14
	MeOH	247 (27.4), 290 (12.4)	397	0.08
6f	Dioxane	249 (31.2), 291 (13.5)	369	0.06
	H_2O	249 (6.5), 293 (4.2)	374	0.08
	MeOH	277 (14.2), 300 (9.9)	471	0.12
6g	Dioxane	277 (13.7), 302 (10.1)	438	0.22
	H_2O	280 (9.3)	477	0.04
	MeOH	251 (28.3), 295 (13.3)	390	0.03
6h	Dioxane	253 (28.6), 296 (14.3)	340	0.02
	H_2O	251 (24.9), 295 (12.0)	412	0.05
	MeOH	236 (35.0), 329 (23.8), 345 (23.7)	370	0.18
12a	Dioxane	241 (30.6), 331 (24.0), 348 (24.6)	373	0.22
	H_2O	236 (27.3), 329 (18.0), 345 (16.5)	391	0.10
	MeOH	264 (28.4), 314 (12.0), 337 (6.3)	422	0.53
12b	Dioxane	264 (26.7), 314 (13.3), 336 (7.0)	399	0.76
	H_2O	265 (10.6), 313 (5.2), 335 (3.7)	432	0.28
	MeOH	276 (25.5), 342 (12.9), 375 (10.2)	500	0.29
12c	Dioxane	276 (26.7), 341 (13.8), 375 (11.3)	473	0.75
	H_2O	276 (17.0), 346 (8.0)	506	0.04
	MeOH	267 (21.4), 328 (11.0), 356 (8.2)	469	0.52
12d	Dioxane	266 (22.8), 328 (12.4), 356 (9.5)	444	0.72
	H_2O	270 (13.3), 328 (6.7), 352 (5.3)	488	0.10
	MeOH	283 (18.2), 354 (10.4), 389 (8.0),	526	0.18
12e	Dioxane	252 (11.1), 284 (18.9), 390 (9.1)	493	0.57
	H_2O	295 (4.9), 363 (4.8)	554	0.05

	MeOH	261 (25.7), 308 (17.0), 323 (10.6), 335 (10.1)	381	0.37
12f	Dioxane	262 (25.0), 309 (17.8), 324 (12.2), 335 (11.7)	369	0.28
	H_2O	269 (14.4), 352 (19.6), 321 (13.5), 336 (14.6)	373	0.24
	MeOH	275 (17.4), 321 (15.0), 339 (11.8)	472	0.29
12g	Dioxane	276 (17.2), 322 (15.9), 341 (13.1)	432	0.68
	H_2O	286 (11.2), 374 (11.4)	457	0.18
	MeOH	266 (15.4), 313 (14.6), 323 (15.4), 337 (14.5)	363	0.11
12h	Dioxane	267 (15.2), 314 (14.1), 324 (16.7), 338 (16.0)	362	0.05
	H_2O	265 (15.2), 313 (13.9), 322 (13.7), 334 (12.6)	384	0.21

Fluorescence quantum yields were measured by using quinine sulfate in 0.5 M H_2SO_4 (Φ_f = 0.55) as a reference (excitation wavelength is 310 nm).

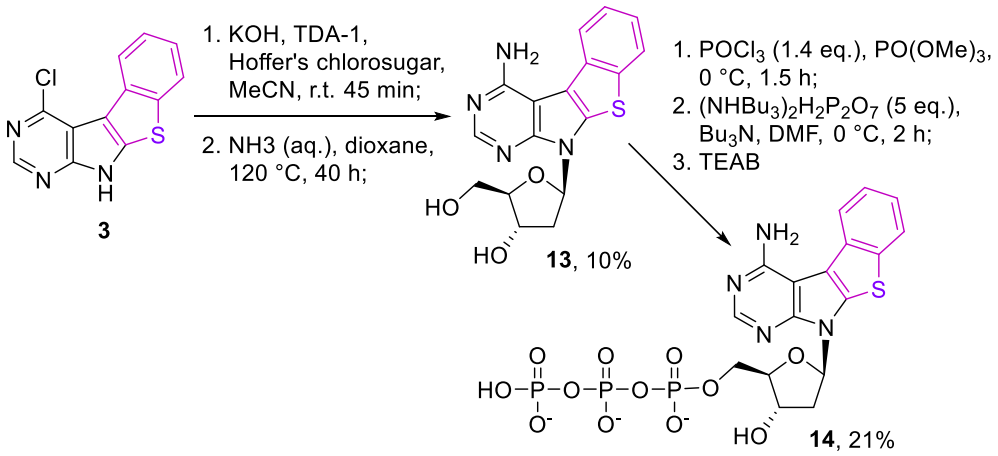
All compounds exerted fluorescence with emission maxima at 340–554 nm. The furyl and benzofuryl derivatives in syn-series (benzo[4′,5′]thieno[3′,2′:4,5]pyrrolo [2,3-*d*]pyrimidine derivatives) (**6c** – **6e**) and most derivatives in anti-series (benzo[4′,5′]thieno[2′,3′:4,5]pyrrolo[2,3-*d*]pyrimidine derivatives) (**12a** – **12g**) exerted medium to high fluorescence quantum yields in methanol and 1,4-dioxane, whereas in water the quantum yields were generally lower (with the exception of compound **12h**). This indicated that the benzo[4′,5′]thieno[2′,3′:4,5]pyrrolo[2,3-d] pyrimidine core was crucial for stronger fluorescence. The largest Stokes shifts were found in 2-furyl (**6c**, **12c**) and 2-benzofuryl derivatives (**6e**, **12e**). Positive solvatochromic effect was observed in methyl, 2-furyl, 2-benzofuryl and amino derivatives in syn-series (**6b**, **6c**, **6e** and **6h**) and methyl, furyl and benzofuryl derivatives in anti-series (**12b**, **12c**, **12d** and **12e**) when measured in 1,4-dioxane, methanol and water (see experimental section 5.2.2).

3.1.4 Biochemistry of benzothieno-fused nucleotide

1) Enzymatic incorporation of benzothieno-fused 7-deazapurine nucleotides into nucleic acids

Previously, a few benzo- and naphtho-fused deazapurine nucleotides were applied to fluorescent labelling of DNA.[75, 111] I was also curious about the fluorescence properties of oligo-2′-deoxyribonucleotides containing the benzothieno-fused 7-deazaadenine base. For this reason, I prepared the corresponding

2′-deoxynucleoside and its triphosphate as a substrate for polymerase synthesis of modified DNA. The glycosylation of nucleobase **3** with the Hoffer's chlorosugar under standard conditions followed by amination using aqueous ammonia in dioxane gave the nucleoside **13** (**dA^{BT}**) in 10% yield over two steps. The low yield was caused by poor solubility of the nucleobase and the difficult purification. The triphosphorylation of **13** by the standard procedure furnished the desired benzothieno-fused 7-deazapurine 2′-deoxynucleoside triphosphate **14** (**dA^{BT}TP**) in acceptable 21% yield (Scheme 15).



Scheme 15. Synthesis of benzothieno-fused 2′-deoxynucleoside and its triphosphate

The enzymatic incorporation was investigated via primer extension (PEX) in presence of KOD XL DNA polymerase with a template (Temp1A, 19 bp; for sequences of oligonucleotides, see Table 6) that allows single incorporation of modified **dA^{BT}**. A 5′-FAM-labeled primer (PrimFAM, 15 bp) was used in the PEX to visualize the extension on denaturing polyacrylamide gel electrophoresis (PAGE). Figure 11A shows that the PEX reaction using **dA^{BT}TP** was successful giving the full-length product **ON_1A** which was also characterized by MALDI-TOF analysis (Table 7). However, the incorporation with four modifications using template Temp4A was unsuccessful (Figure 11B), which indicated that **dA^{BT}TP** was not a good substrate of DNA polymerase.

In 2004, Saito and and co-workers reported that nahphtho-fused deazaadenine

modifications in DNA showed fluorescence properties sensitive to changes of secondary structure and sequence.[111] In order to study the fluorescence of **dA^{BT}**-modified oligonucleotides (ONs), I designed and synthesized several modified ONs containing one **dA^{BT}** within a loop of a hairpin structure in different sequence context (Table 6). A non-hairpin sequence ON_Ctrl was also prepared and used as a control. Both PAGE (Figure 11B) and MALDI-TOF MS analysis (Table 7) proved that the enzymatic preparation of the four modified ONs was successful.

Table 6. Sequences of oligonucleotides used in the study

Oligonucleotide	Sequence[a]
Prim	5′-CATGGGCGGCATGGG
Prim^{FAM}	5′-(FAM)-CATGGGCGGCATGGG
PrimHair	5′-CATCCGCGGCAAGGG
PrimHair^{FAM}	5′-(FAM)-CATCCGCGGCAAGGG
Temp1A	5′-CCCTCCCATGCCGCCCATG
Temp1A^{bio}	5′-(biotin)-CCCTCCCATGCCGCCCATG
Temp4A	5’-CTAGCATGAGCTCAGTCCCATGCCGCCCATG
Temp4A^{bio}	5’-(biotin)-CTAGCATGAGCTCAGTCCCATGCCGCCCATG
TempTAT	5′-ACAGAGCAAGGGATAGCCCTTGCCGCGGATG
TempTAT^{bio}	5′-(biotin)-ACAGAGCAAGGGATAGCCCTTGCCGCGGATG
TempCAC	5′-ACAGAGCAAGGGGTGGCCCTTGCCGCGGATG
TempCAC^{bio}	5′-(biotin)-ACAGAGCAAGGGGTGGCCCTTGCCGCGGATG
TempGAG	5′-ACAGAGCAAGGGCTCGCCCTTGCCGCGGATG
TempGAG^{bio}	5′-(biotin)-ACAGAGCAAGGGCTCGCCCTTGCCGCGGATG
TempCtrl	5′-ACAGAAAGACACATAGCCCTTGCCGCGGATG
TempCtrl^{bio}	5′-(biotin)-ACAGAAAGACACATAGCCCTTGCCGCGGATG
ON_1A	5′-CATGGGCGGCATGGG**A*GGG**
ON_4A	5’-CATGGGCGGCATGGG**A***CTG**A***GCTCA*TGCTA*G[b]
ON_TAT	5′-CATCCGCGGCAAGGGCT**A***TCCCTTGCTCTGT
ON_CAC	5′-CATCCGCGGCAAGGGCC**A***CCCCTTGCTCTGT
ON_GAG	5′-CATCCGCGGCAAGGGCG**A***GCCCTTGCTCTGT
ON_Ctrl	5′-CATCCGCGGCAAGGGCT**A***TGTGTCTTTCTGT

[a] Asterisk: position of a modified nucleotide in the product. [b] not obtained.

Table 7. MALDI data of **dA^{BT}**-modified oligonucleotides.

ssDNA	M (calcd.) [Da]	M (found) [Da]
ON_1A	6079.9	6080.8
ON_TAT	9544.1	9545.0
ON_CAC	9514.1	9515.2
ON_GAG	9594.1	9595.1
ON_Ctrl	9614.2	9515.4
ON_4A	10037.3	9854.4[a]

[a] Unsuccessful enzymatic incorporation with template *Temp4A*.

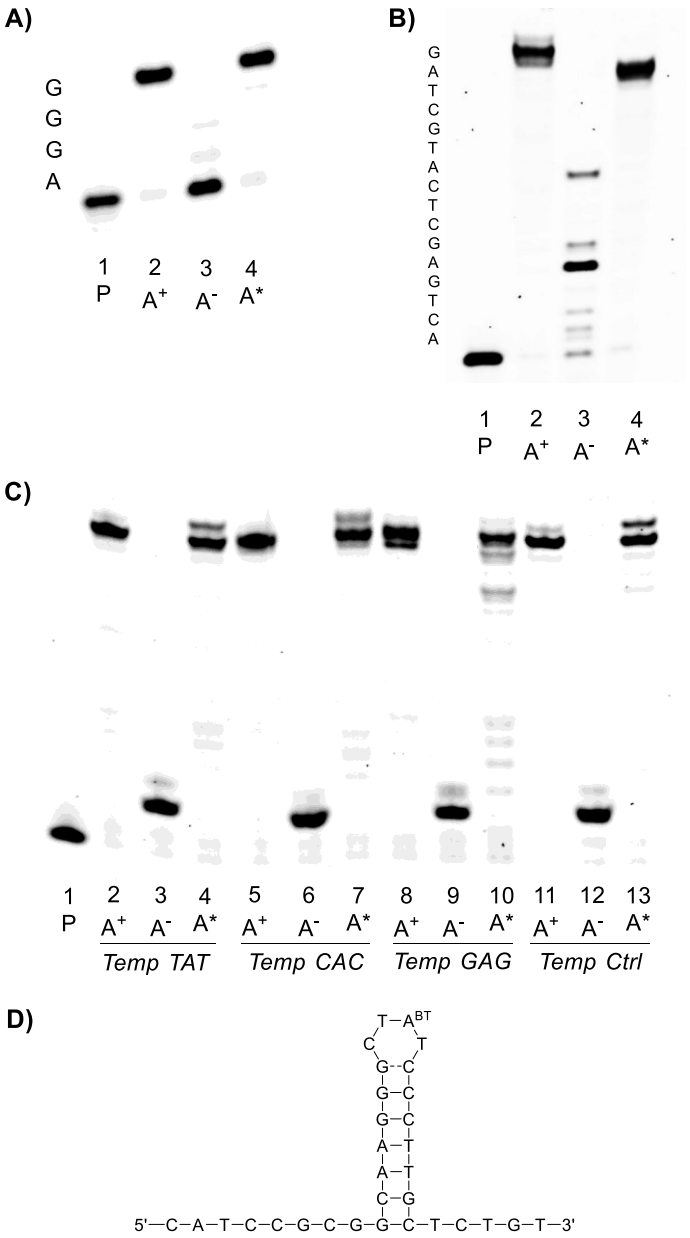


Figure 11. PEX with benzothieno-fused 7-deazapurine deoxynucleotide **14 (dA^{BT}TP)** and KOD XL DNA polymerase with templates encoding for incorporation of one or

four modified nucleotides. (A) Template *Temp1A*; (B) Template *Temp4A* (unsuccessful enzymatic incorporation); (C) hairpin-forming templates *TempTAT, TempCAC* and *TempGAG* and an unstructured template *TempCtrl*. (D) schematic structure of hairpin sequence **ON_TAT**. Key for (A): P: FAM-labeled primer; A^+: dATP, dGTP; A^-:dGTP; A^{BT}: **dA^{BT}TP**, dGTP. Key for (B) and (C): P: FAM-labeled primer; A^+: dATP, dGTP, dCTP, dTTP; A^-: dGTP, dCTP, dTTP; A^{BT}: **dA^{BT}TP**, dGTP, dCTP, dTTP.

2) Fluorescence properties of modified DNA oligonucleotides

With the **dA^{BT}**-modified ONs and duplexes in hand, fluorescence properties were finally studied with an excitation wavelength of 305 nm. Although the fluorescence of the parent nucleoside **6h** was rather weak with quantum yeild of 5%, all the oligonucleotides showed fluorescence with emission maxima at 415 nm (Figure 12). The fluorescence intensities in double-stranded DNA (dsDNA) were slightly higher than that in the single stranded ON, which is opposite to the reported naphtho-fused 7-deazapurine 2′-deoxynucleotide.[111] Unfortunately, the difference in fluorescence intensity on hybridization was not high enough for application of this base in hybridization probes. Also the fluorescence of different sequences of the hairpin **ON_TAT**, **ON_CAC**, **ON_GAG** as well as the non-hairpin sequence **ON_Ctrl** gave similar intensities, which indicated that the hairpin structure and the different neighboring nucleobases did not affect the fluorescence intensities. Unlike the previous naphtho-fused 7-deazapurine 2′-deoxynucleotide,[111] we did not observe fluorescence quenching by neighboring G/C pairs. The fluorescence of these **dA^{BT}**-modified ONs was stable but weak, which may limit the applications in DNA labelling.

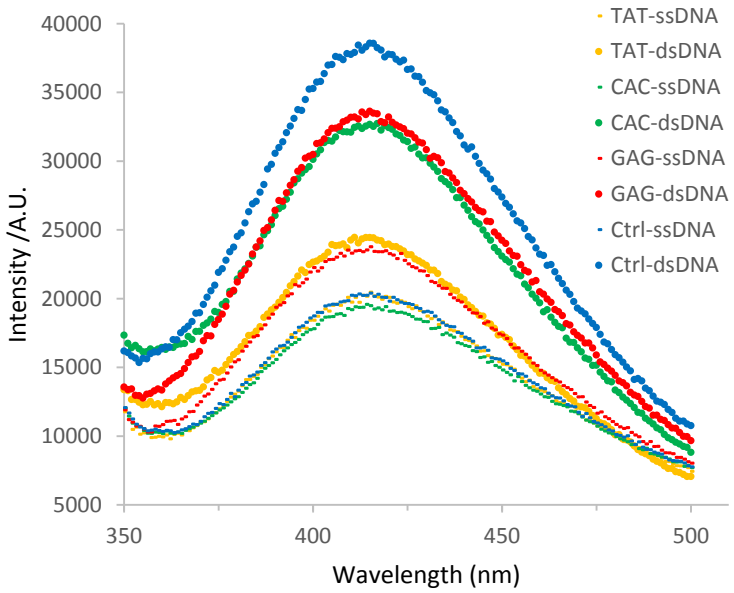


Figure 12. Fluorescence emission spectra of hairpin-forming oligonucleotides **ON_TAT**, **ON_CAC** and **ON_GAG** and unstructured oligonucleotide **ON_Ctrl** (1 μM in medium salt buffer: 100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0) and corresponding duplexes with complementary strands *TempTAT, TempCAC, TempGAG* and *TempCtrl*. Excitation wavelength: 305 nm. Background fluorescence was subtracted in all spectra.

3.1.5 Biological activity profiling

In this section, the anti-HCV activity of all modified nucleosides **6a** – **6h** and **12a** – **12h** was tested by Gilead Science, all these compounds were also sent to Palacky University and University Hospital in Olomouc for testing *in vitro* cytotoxic activity, and all title nucleosides were also screened for other antiviral activities against herpes simplex, influenza, coxsackie, human immunodeficiency virus (HIV), and dengue viruses in Dr. Weber's group at IOCB.

Unfortunately, almost all final nucleosides were inactive against most of these viruses. Only the anti-HCV screening (performed as described previously)[112] showed activities of most of the nucleosides against both HCV replicons 1B and 2A in micromolar concentrations (Table 8). Compound **6e** was the most active one exerting activity against 2A replicon at 60 nM concentration.

Table 8. Antiviral activities of ribonucleosides **6a** – **6h** and **12a** – **12h**

	HCV replicon 1B		HCV replicon 2A	
Compd	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50}
6a	44.0	>44.4	31.0	>44.4
6b	2.1	43.9	7.5	25.9
6c	24.8	>44.4	13.1	>44.4
6d	17.9	>44.4	>44.4	>44.4
6e	1.4	2.1	0.06	30.6
6f	4.3	>44.4	28.3	>44.4
6g	5.3	18.7	13.2	18.7
6h	4.2	24.3	20.2	30.6
12a	9.5	38.7	14.1	17.3
12b	10.6	>44.4	>44.4	>44.4
12c	22.7	41.6	23.0	>44.4
12d	33.9	37.8	24.5	31.7
12e	>44.4	>44.4	>44.4	>44.4
12f	36.8	>44.4	>44.4	>44.4
12g	12.5	>44.4	>44.4	43.4
12h	34.0	44.0	43.2	44.0

Nucleoside analogues **6a** – **6h** and **12a** – **12h** were also tested for *in vitro* cytotoxic activity on several cancer cell lines (A549 – human lung adenocarcinoma, HCT116 and HCT116p53$^{-/-}$ – colon cancer cells with/without p53 gene, U2OS – human osteosarcoma), as well as leukemic cell lines (CCRF-CEM – acute lymphoblastic leukemia, CEM-DNR – CCRF-CEM cells resistant to daunorubicin, K562 – myelogenous leukemia, K562-TAX – K562 cells resistant to paclitaxel)[113] using a 3-days MTS (3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.[114] In addition, testing on HeLaS3 – human HPV positive cervical carcinoma, HepG2 – hepatocellular carcinoma and HL60 – acute promyelocytic leukemia cell lines were performed using XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay.[115] Non-malignant fibroblast cell lines MRC-5 and BJ were used in the MTS assay for comparison. Table 9 summarizes the cytotoxic activities of compounds in MTS assay. Most compounds showed moderate effects at micromolar concentrations with little

selectivity to cancer cells. In XTT assay, the compounds did not show significant cytotoxic effect at 10 μM concentration with the exception of compound **6e**, which showed submicromolar activity on HL60 and HeLaS3 cells and a 50 nM effect on HepG2 cells (Table 10).

Table 9. Cytotoxic activities of nucleosides **6a** – **6h** and **12a** – **12h**

	MTS, IC_{50} [μM]									
Compd	BJ	MRC-5	A549	CCRF-CEM	CEM-DNR	HCT116	HCT116 $p53^{-/-}$	K562	K562-Tax	U2OS
6a	>50	>50	>50	39.7	40.0	>50	>50	>50	26.5	>50
6b	27.5	34.2	27.8	9.2	5.1	14.1	21.6	25.9	3.2	28.6
6c	>50	47.3	>50	20.0	22.3	49.5	>50	48.1	17.7	49.8
6d	>50	>50	>50	43.0	49.2	>50	>50	>50	41.5	>50
6e	25.7	20.9	6.4	7.7	25.4	29.0	19.0	9.0	7.7	9.7
6f	>50	>50	>50	12.8	21.0	>50	>50	>50	10.1	>50
6g	>50	44.8	>50	18.3	5.7	40.3	>50	23.9	4.5	38.6
6h	>50	>50	36.1	12.4	29.0	24.9	26.1	41.9	26.7	25.6
12a	>50	45.4	12.2	6.9	6.3	9.8	16.0	10.6	6.0	12.8
12b	>50	>50	>50	11.8	4.5	31.1	48.5	44.3	3.4	>50
12c	36.9	23.7	14.2	5.2	2.00	9.16	14.2	9.5	1.51	16.3
12d	49.2	36.3	31.2	9.2	3.5	14.0	27.5	14.9	2.8	24.3
12e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
12f	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
12g	>50	>50	>50	17.3	2.33	>50	>50	>46.8	2.22	>50
12h	>50	>50	49.5	28.7	35.6	41.0	38.6	46.5	23.5	>50

Table 10. Cytotoxic activity of nucleosides **6a** – **6h** and **12a** – **12h** in XTT assy

	XTT, IC50 [μM]			
Compd	HL60	HepG2	HeLaS3	CEM
6a	>10	>10	>10	>10
6b	>10	>10	>10	5.3
6c	>10	>10	>10	>10
6d	>10	>10	>10	>10
6e	0.24	0.05	0.34	8.8
6f	>10	>10	>10	>10
6g	>10	>10	>10	>10
6h	>10	>10	>10	>10
12a	9.0	>10	>10	>10
12b	>10	>10	>10	>10
12c	>10	>10	>10	>10
12d	>10	>10	>10	>10
12e	>10	0.30	>10	>10
12f	>10	>10	>10	>10
12g	>10	>10	>10	>10
12h	>10	>10	>10	>10

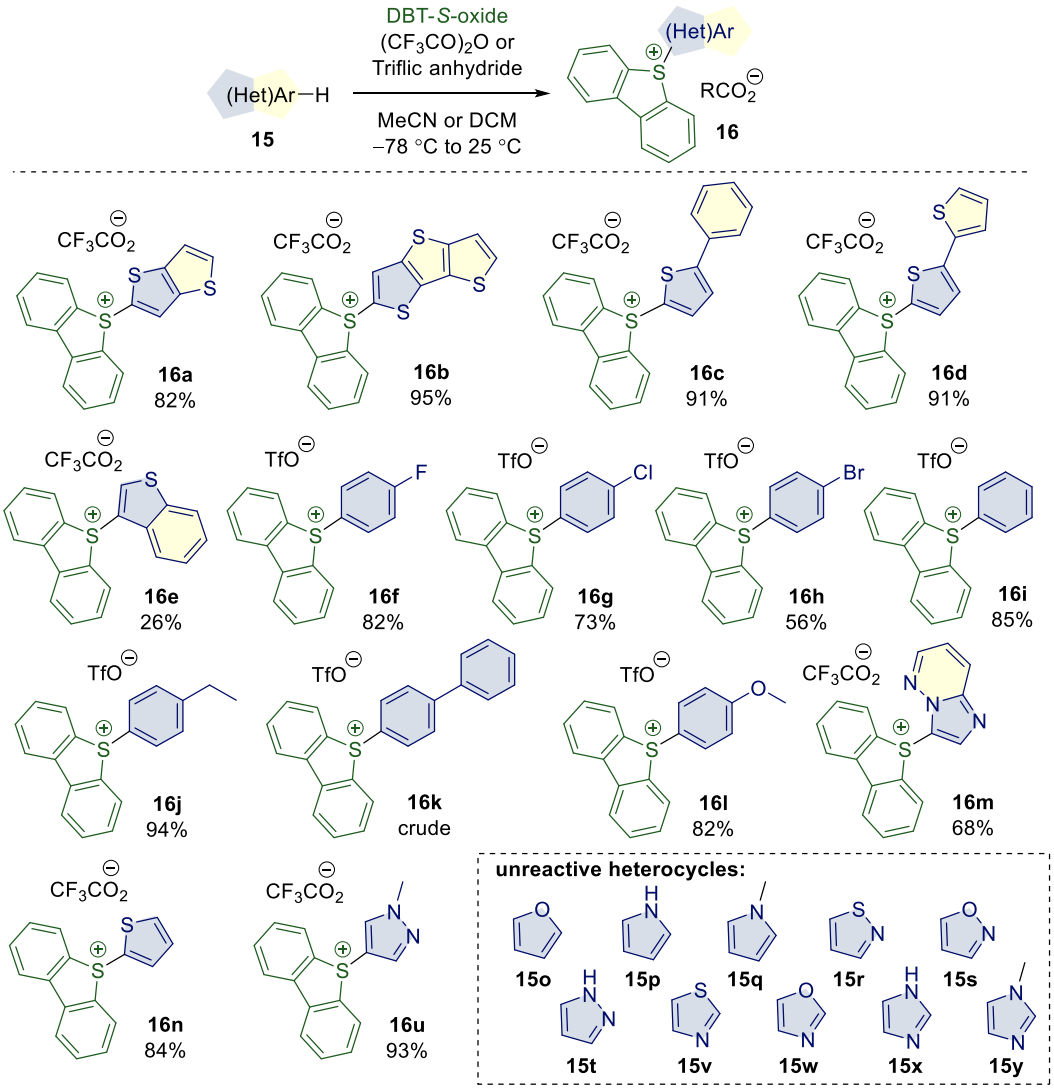
3.2 Polycyclic Hetero-Fused 7-Deazapurine Nucleosides

In Section 1.3, we have discussed the advantages and disadvantages of each approach for synthesis of fused 7-deazapurine nucleosides, the starting materials for each approach, chloronitro-heterocycles and iodo-heterocycles, are not always easy to be obtained due to the regioselectivity of halogenation or nitrozation of the heterocycles. Thus, there is a need of an alternative general approach that would enable to synthesize wide range of novel fused deazapurine bases for further applications. The recently developed C-H functionalization of (hetero)aromatics with sulfur heterocycles and further cross-coupling of the resulting sulfonium intermediates showed wide applicability to form C-C bonds. Inspired by the high activity and good site selectivity of sulfonium salt chemistry, I decided to apply this versatile reagent to develop new methodology for the synthesis of polycyclic hetero-fused 7-deazapurine nucleosides. This section is written in chronological order as I did the experiments, different from the logical order in my paper.

3.2.1 New methodology of Negishi coupling via (het)aryl sulfonium salts

In order to study the new method, the first task is to prepare a series of aryl sulfonium salts as substrates. So I tested the C-H functionalization of a wide range of substituted benzenes and substituted and fused thiophenes, as well as imidazo[1,2-*b*]pyridazine and a set of other five-membered heterocycles with dibenzothiophene-*S*-oxide (that was reported[96, 116, 117] to perform better on thiophenes compared to thianthrene). The reactions were performed in presence of trifluoroacetic or triflic anhydride according to literature protocols (Scheme 16).[92, 96, 116-118] In case of all thiophenes, benzenes, imidazopyridazine and *N*-methylpyrazole, we obtained the desired (het)arylsulfonium salts in good yields. Only in case of benzothiophene, the yield was lower and in case of biphenyl the dibenzothiophenium salt was inseparable and was used directly for the Negishi coupling. On the other hand, the other five-membered heterocycles **15o** – **15y**

including furan and diverse azoles did not give any detectable sulfonium products. In all these cases, we also tried C-H thianthrenation[91, 97, 119, 120] that also did not provide the desired products. The thianthrenation and dibenzothiophenation of the small five membered rings were tried by my colleague Marianne Fleuti, and most of them gave negative results. It seems that this C-H functionalization has a severe limitation for most five-membered heterocycles beyond thiophene.

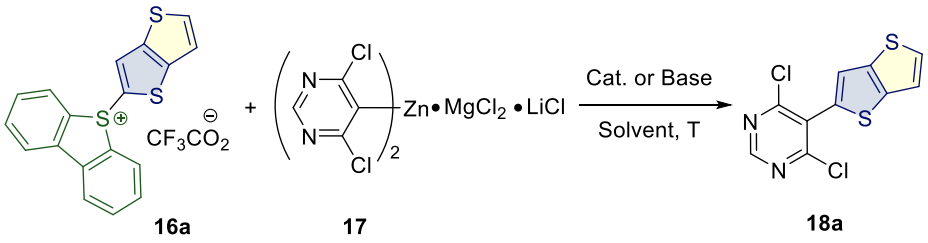


Scheme 16. Preparation of (het)aryl sulfonium salts

Next I needed to try the envisaged Negishi coupling of sulfonium salts with zincated dichloropyrimidine, which is most important task in this project. If the Negishi coupling can work, this project is 80% succeeded. With the portfolio of (het)arylsulfonium salts in hand, I then tested the feasibility of the Negishi

cross-coupling reaction. Previously, only two examples of Negishi coupling of a substituted phenyl-tetrafluorothianthrenium salt with alkylzinc halides was reported.[92] Initially, we tested the model reaction of thienothiophene-derived dibenzothiophenium salt **16a** with *in situ* generated zincated dichloropyrimidine **17**[71] under different conditions. Reaction with a 0.5 equiv of **17** in presence of $Pd(PPh_3)_2Cl_2$ did not proceed, whereas the same reaction in presence of $Pd(PPh_3)_4$ overnight led to complex inseparable mixture containing the desired product **18a** (TLC-MS). However, simple prolonging of the reaction time to 40 h afforded the desired product **18a** with 42% isolated yields. The yield was improved to 60% when using 1 equiv of organozinc **17** (that offers two equivalents of the arylorganometallic moiety for transmetallation[121]).

Table 11. Optimization of Negishi cross-coupling with **17**



Entry	Cat. or Base	**17** (eq.)	Solvent	T / °C	Time	Yield or Result
1	$Pd(PPh_3)_2Cl_2$	0.5	THF	65	overnight	no product
2	$Pd(PPh_3)_4$	0.5	THF	65	overnight	mixture[a]
3	$Pd(PPh_3)_4$	0.5	THF	65	40 h	42%
4	$Pd(PPh_3)_4$	1	THF	65	40 h	60%
5	$Pd(PPh_3)_4$	1	THF/MeCN	65	40 h	64%
6	K_2CO_3	0.5	THF	65	48 h	no reaction
7	K_2CO_3	0.5	MeCN	65	48 h	no reaction

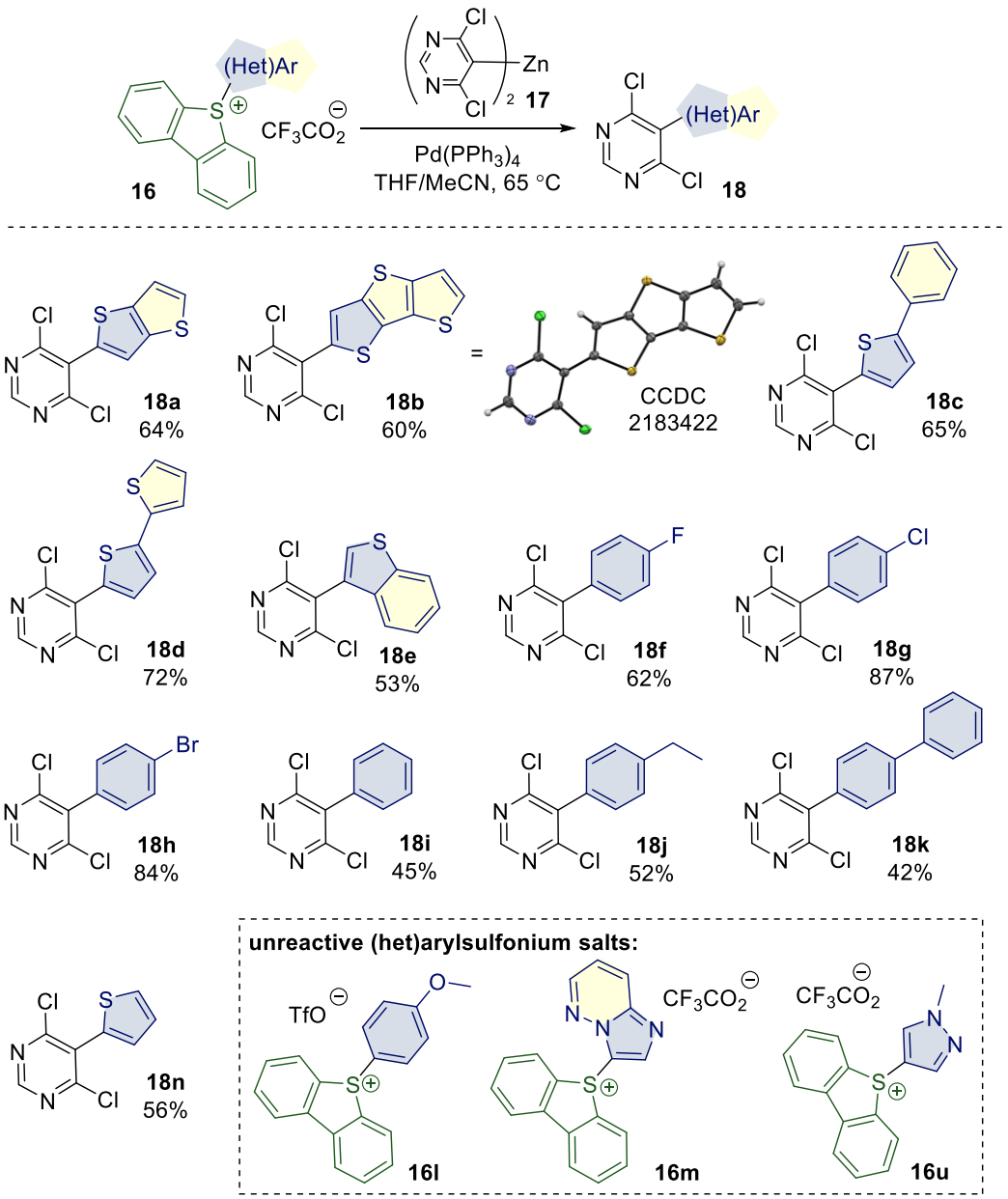
Reaction conditions: Sulfonium salt (0.23−0.45 mmol, 1.0 equiv), catalyst (0.05−0.08 equiv), zincated pyrimidine (0.5−1 equiv) and the solvent (THF or MeCN; c = 0.2 M) were stirred at 65 °C for 12−48 h. [a] Inseparable mixture containing product **18a** (TLC).

Moreover, using THF/MeCN mixture as the solvent further slightly improved the yield to 64%, since the thienothiophene-derived dibenzothiophenium salt **16a** has better solubility in MeCN. Then the *cine*-substitution of thienothiophene-derived dibenzothiophenium salt and zincated dichloropyrimidine was tested, as this type of substitution was reported with exquisite site selectivity. Unfortunately, the reaction did not proceed at 65 °C in presence of K_2CO_3. Therefore, the condition outlined in Table 11, entry 5 was chosen as the optimized condition for subsequent investigations.

Encouraged by the positive results of Negishi coupling with the standard substrate, I then examined the substrate scope of this new reaction (Scheme 17). All the dibenzothiophenium salts derived from thiophene-based heterocycles (thienothiophene, dithienothiophene, phenylthiophene, bithiophene, benzothiophene and thiophene) **16a** – **16e** and **16n** reacted very well to produce the desired products **18a** – **18e** and **18n** in moderate to good yields (53% – 72%).

Similarly, most of the sulfonium salts derived from substituted benzenes **16f** – **16k** were amenable to this Negishi coupling reaction with zincated dichloropyrimidine **17** to form the corresponding products **18f** − **18k** in acceptable yields (42% − 87%) with excellent site selectivity. On the other hand, no reaction was observed with strongly electron-rich methoxybenzene- and imidazopyridazine-derived sulfonium salts **16l** and **16m**.

Since the site selectivity of iodination of some (hetero)aromatics, such as thienothiophene and benzothiophene, is poor, the traditional Negishi coupling using (het)aryl iodides can be quite problematic. Therefore, the regioselective C-H functionalization followed by the Negishi coupling of sulfonium salts and aryl-zinc reagents can be an excellent complementary strategy for the synthesis of complex heterocyclic biaryls.



Scheme 17. Investigation of the substrate scope of the Negishi cross-coupling of (het)arylsulfonium salts **16a** – **16u** with dichloropyrimidine-zinc **17**

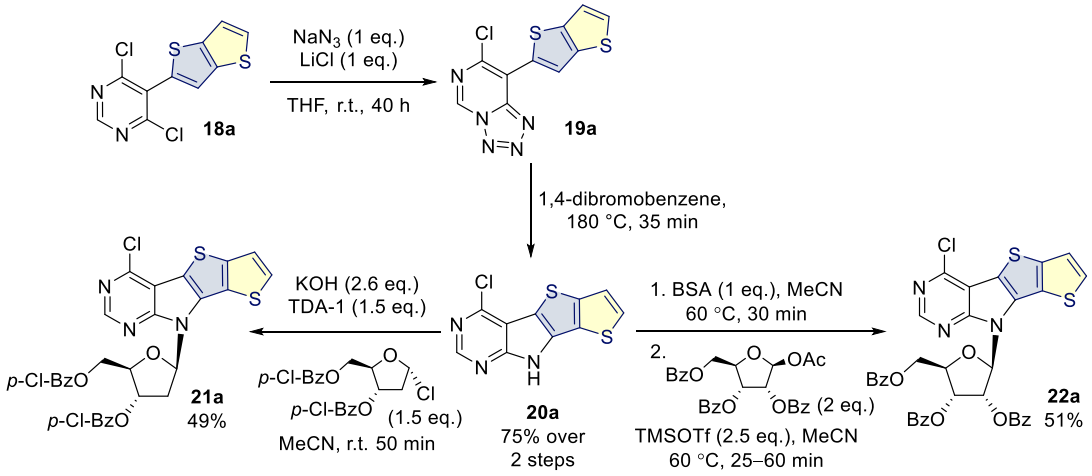
3.2.2 Synthesis of polycyclic hetero-fused 7-deazapurine nucleosides

Based on the biological activity of benzo-, thieno-, benzothieno- and naphtho-fused deazapurine nucleosides, we designed novel tetracyclic thienothieno- and pentacyclic thienothienothieno-fused deazapurines **20a** and **20b**, as well as phenyl- and thienyl-substituted thieno-fused 7-deazapurines **20c** and **20d** as key intermediates in the synthesis of the corresponding nucleosides and their synthesis started from the

new thienyl-pyrimidines **18a** − **18d**. The synthesis of these nucleosides is very similar as reported before,[71, 72, 122] and the steps using different conditions are described in details.

1) Thienothieno series

First, the azidation of dichloropyrimidines **18a** with sodium azide in THF was performed to get tetrazolopyrimidine **19a**, which have poor solubility in organic solvents and were used directly for the next step without chromatographic purification. The NMR spectra in DMSO showed that crude compound **19a** exist mainly as the form of tetrazolopyrimidine. Based on our previous experience with the synthesis of heteroaryl-fused 7-deazapurine nucleobases, the thermal condition was applied for the heterocyclization of tetrazolopyrimidine to nucleobase. The robust thermal cyclization at 180 °C successively gave us the desired fused deazapurine product **20a** in 75% yield.

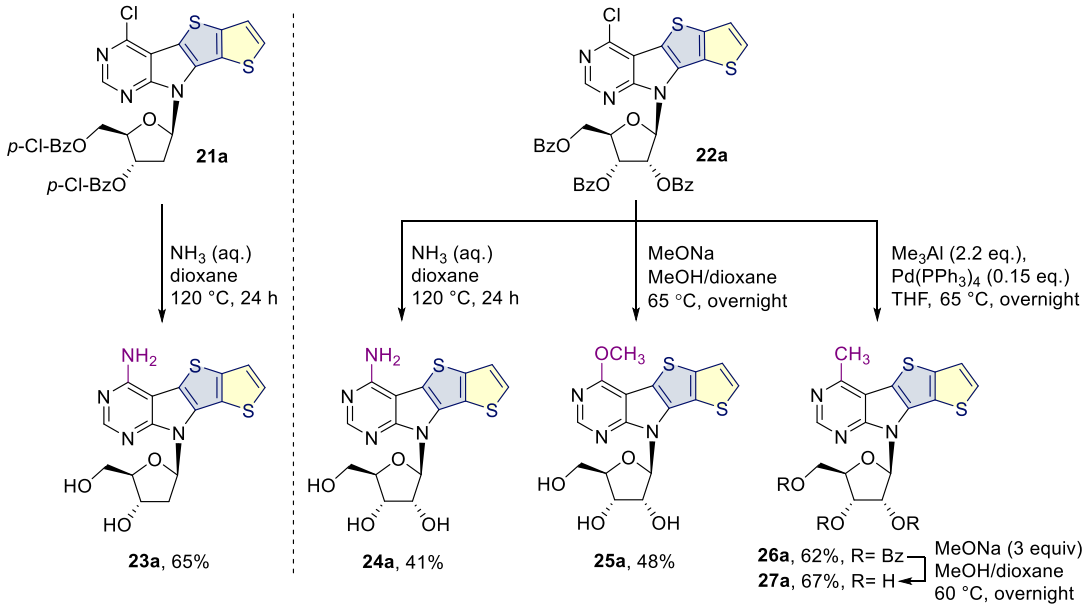


Scheme 18. Synthesis of thienothieno-fused deazapurine and nucleosides

The glycosylation of nucleobase **20a** with the 1-chloro-3,5-bis-*O*-(4-chlorobenzoyl)-2-deoxy-*α*-D-ribofuranose (Hoffer's chlorosugar) under basic conditions gave the desired protected deoxynucleosides **21a** in moderate yield of 49% and with exclusive stereoselectivity to form *β*-anomer. Next we performed the glycosylation of nucleobase **20a** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose to prepare the corresponding ribonucleoside. I used a modified

procedure of Vorbrüggen glycosylation as in our previous works.[71, 72, 122] A MeCN solution of nucleobase **20a** and BSA was heated to 60 °C for 30 min. After adding TMSOTf and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose, the mixture was stirred at the same temperature for 25–60 min. The protected ribonucleoside **22a** was obtained in good yield of 51% as pure *β*-anomer (Scheme 18).

The protected chloro derivative of deoxyribonucleoside **21a** was converted to the corresponding analogue of 2'-deoxydeazaadenosine (Scheme 19, Left). The nucleophilic substitution reaction with aqueous ammonia at 120 °C in dioxane substituted the chlorine at position 4 with amino group with concomitant deprotection of the sugar part to form the desired fused 2'-deoxy-7-deazaadenosine derivative **23a** in good yield of 65%.



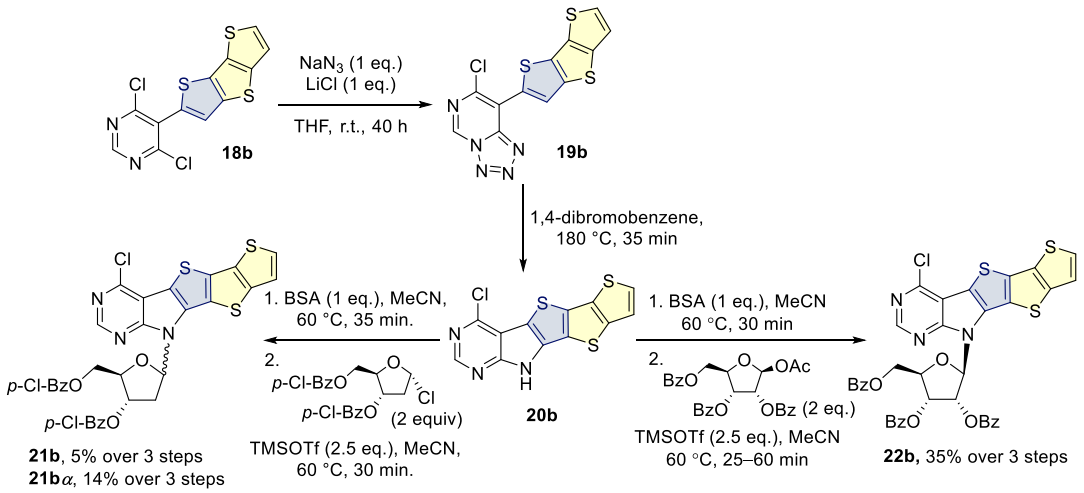
Scheme 19. Late-stage diversification of thienothieno-fused nucleosides

In the ribonucleoside series, we designed 4-amino-, 4-methoxy- and 4-methyl derivatives in each type of fused deazapurine nucleosides, as these were the most active in the related tricyclic fused nucleosides in our previous works. Starting from the protected 4-chloro nucleoside **22a**, the amino group was introduced to position 4 using same procedure and conditions as for the 2′-deoxynucleosides with aqueous ammonia in dioxane to give the desired product **24a** (fused analogue of adenosine) in

41% yield. Reaction of intermediate **22a** with sodium methoxide displaced the chloro group with methoxy at the position 4 and simultaneously cleaved the benzoyl protecting groups to give 4-methoxy nucleoside **25a** in moderate yield. Due to the poor solubility of intermediate **22a**, the mixture of 1,4-dioxane and methanol and refluxing at 65 °C were used to accelerate the reaction and increase the yield. The introduction of methyl group to positon 4 was achieved through cross-coupling reaction of **22a** with trimethylaluminum and $Pd(PPh_3)_4$ in THF to obtain protected 4-methyl nucleoside **26a** in 62% yield that were deprotected by MeONa in MeOH/dixane to give free nucleoside **27a** in 67% yield (Scheme 19, Right).

2) Thienothienothieno series

The synthesis of thienothienothieno-fused deazapurine nucleosides was definitely a nightmare. The tetrazole **19b**, base **20b**, and even some of the free nucleosides are nearly insoluble in all common organic solvents, which makes it difficult to purify these compounds.



Scheme 20. Synthesis of thienothienothieno-fused deazapurine and nucleosides

The azidation of dichloropyrimidine **18b** was performed under the same conditions as the first series, and the crude product **19b** was used directly for the next cyclization, as the poor solubility did not allow chromatographic purification. Luckily, the robust thermal cyclization of crude tetrazole **19b** worked. The crude product of cyclization **20b**, obtained by simple precipitation and filtration, looked like black

graphite powder. I first tried to purify it with column chromatography, but the nucleobase can never be eluted out even with huge amount of mobile phase. What is worse, the reaction can't be monitored with TLC, as the product spot never move up on TLC plate. I thought my reaction failed the first time I did it, as there was nothing on the TLC plate. Luckily, I did a NMR measurement of crude base **20b** in DMSO-d_6, which confirmed the formation of the desired nucleobase.

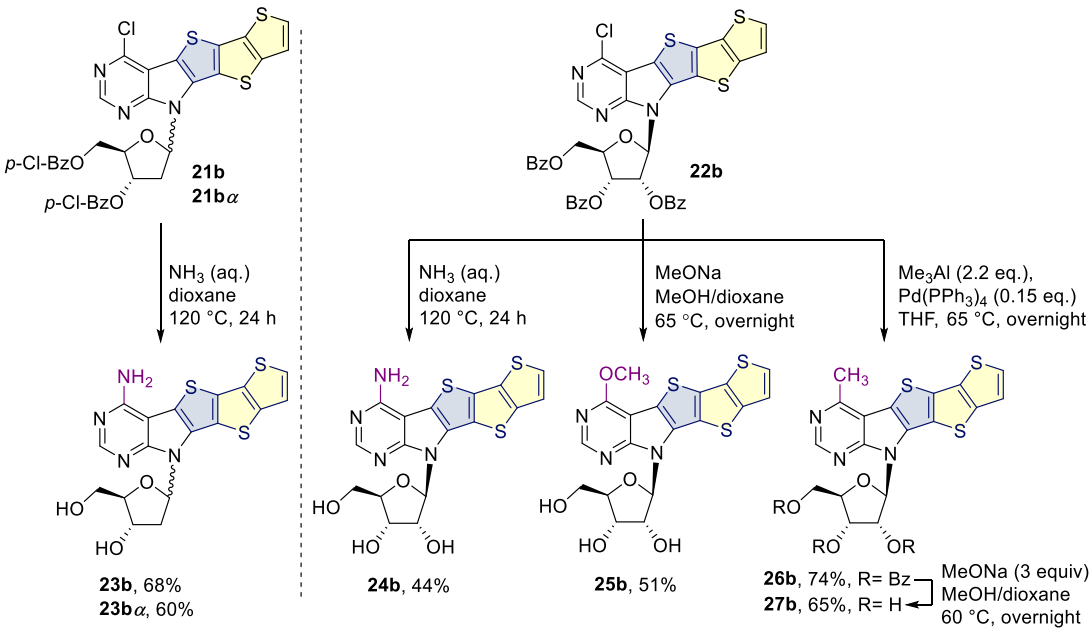
The glycosylation of crude nucleobase **20b** to deoxynucleoside was also problematic, probably because the pentacyclic base is too bulky. Using Hoffer's chlorosugar under basic conditions did not give the targeted deoxynucleoside. Suggested by my supervisor Michal Hocek, I also tried the acidic conditions using BSA, TMSOTf and chlorosugar, which resulted in the formation of the desired *β*-anomeric nucleoside **21b** in low yield of 5% over 3 steps and its *α*-anomer **21b*α*** in 14% yield over 3 steps.

Next I performed the glycosylation of nucleobase **20b** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose to prepare the corresponding ribonucleoside. The same modified procedure of Vorbrüggen glycosylation as in the first series was used. The protected ribonucleoside **22b** was synthesized from crude **20b** in acceptable yield of 35% over 3 steps as pure *β*-anomer (Scheme 20). Finally this protected ribonucleoside was able to be purified by column chromatography.

The protected chloro derivatives of deoxyribonucleosides **21b** and **21b*α*** were converted to the corresponding analogues of 2'-deoxyadenosine (Scheme 21, Left). Treatment with aqueous ammonia at 120 °C in dioxane substituted the chlorine at position 4 with amino group with concomitant deprotection of the sugar part to form the desired fused 2'-deoxy-7-deazaadenosine derivative **23b** and **23b*α*** in good yields (68%, 60% respectively).

4-Amino-, 4-methoxy- and 4-methyl ribonucleosides was designed and synthesized in the thienothienothieno series. Starting from the protected 4-chloro nucleoside **22b**, the amino group was introduced using aqueous ammonia in dioxane

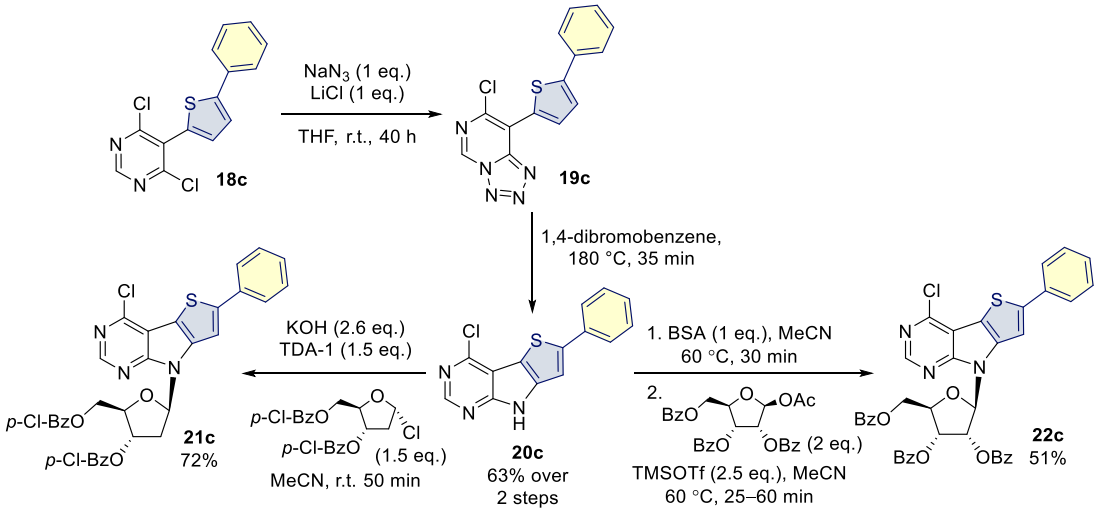
to give the desired 2'-deoxynucleoside **24b** in 44% yield. Reaction of intermediate **22b** with sodium methoxide in the mixture of 1,4-dioxane and methanol at 65 °C displaced the chloro group with methoxy at the position 4 and simultaneously cleaved the benzoyl protecting groups to give 4-methoxy nucleoside **25b** in moderate yield. The 4-methyl nucleoside **27b** was achieved through cross-coupling reaction with trimethylaluminum and $Pd(PPh_3)_4$, followed by deprotection with MeONa in MeOH/dixane. The methyl intermediate **26b** was obtained in 74% yield and the deprotection gave 65% yield.



Scheme 21. Late-stage diversification of thienothienothieno-fused nucleosides

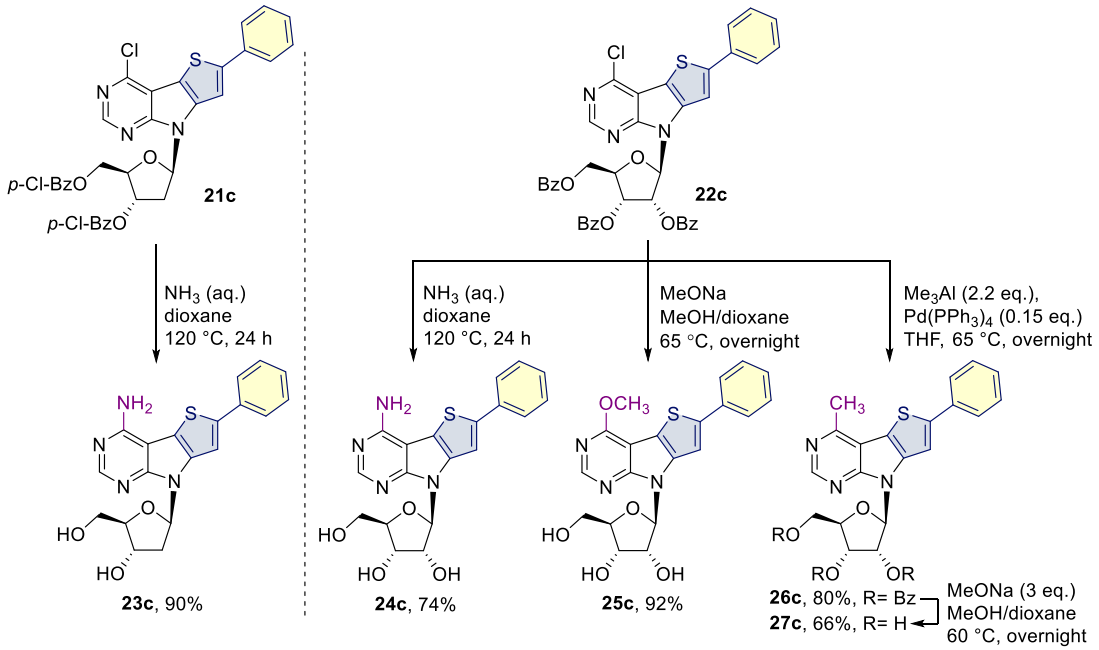
3) Phenylthieno series

The synthesis of the last two series was kind of routine works. The azidation of dibbchloropyrimidine **18c** gave tetrazolopyrimidine **19c**, which was used directly for the next step without purification. The NMR spectra in DMSO showed that crude compound **19c** exists mainly as the form of tetrazolopyrimidine. Then the robust thermal cyclization of **19c** at 180 °C successively formed the desired fused deazapurine product **20c** in 63% yield over 2 steps. The glycosylation of nucleobase **20c** with Hoffer's chlorosugar under basic condition gave the desired protected deoxynucleosides **21c** in good 72% yield as exclusive *β*-anomer.



Scheme 22. Synthesis of phenylthieno-fused deazapurine and nucleosides

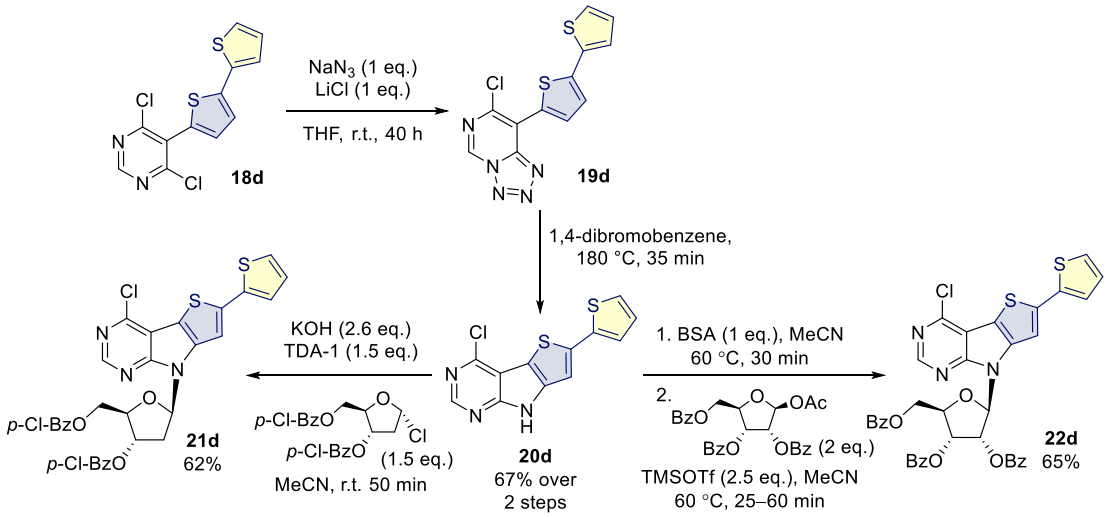
Next we performed the glycosylation of nucleobase **20c** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose to prepare the corresponding ribonucleoside. A MeCN solution of nucleobase **20c** and BSA was heated to 60 °C for 30 min. After adding TMSOTf and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- *β*-D-ribofuranose, the mixture was stirred at the same temperature for 25–60 min. The protected ribonucleoside **22c** was obtained in 51% yield as pure *β*-anomer.



Scheme 23. Late-stage diversification of phenylthieno-fused nucleosides

The protected chloro derivative of deoxyribonucleoside **21c** was converted to the corresponding analogue of 2′-deoxyadenosine (Scheme 23, Left). The nucleophilic substitution reaction with aqueous ammonia at 120 °C in dioxane replaced the chlorine at position 4 with amino group with concomitant deprotection of the sugar moiety to form the desired fused 2′-deoxy-7-deazaadenosine derivative **23c** in high yield. In the ribonucleoside series, 4-amino-, 4-methoxy- and 4-methyl derivatives of fused deazapurine nucleoside were synthesized. Starting from the protected 4-chloro nucleoside **22c**, the amino group was introduced to position 4 using aqueous ammonia in dioxane to give the desired product **24c** in 74% yield. Reaction of intermediate **22c** with sodium methoxide displaced the chlorine with methoxy at the position 4 and simultaneously cleaved the benzoyl protecting groups to give 4-methoxy nucleoside **25c** in high yield. 4-Methyl free nucleoside **27c** was achieved through cross-coupling reaction with trimethylaluminum and $Pd(PPh_3)_4$, followed by deprotection with MeONa (in 80%, 66% yields respectively).

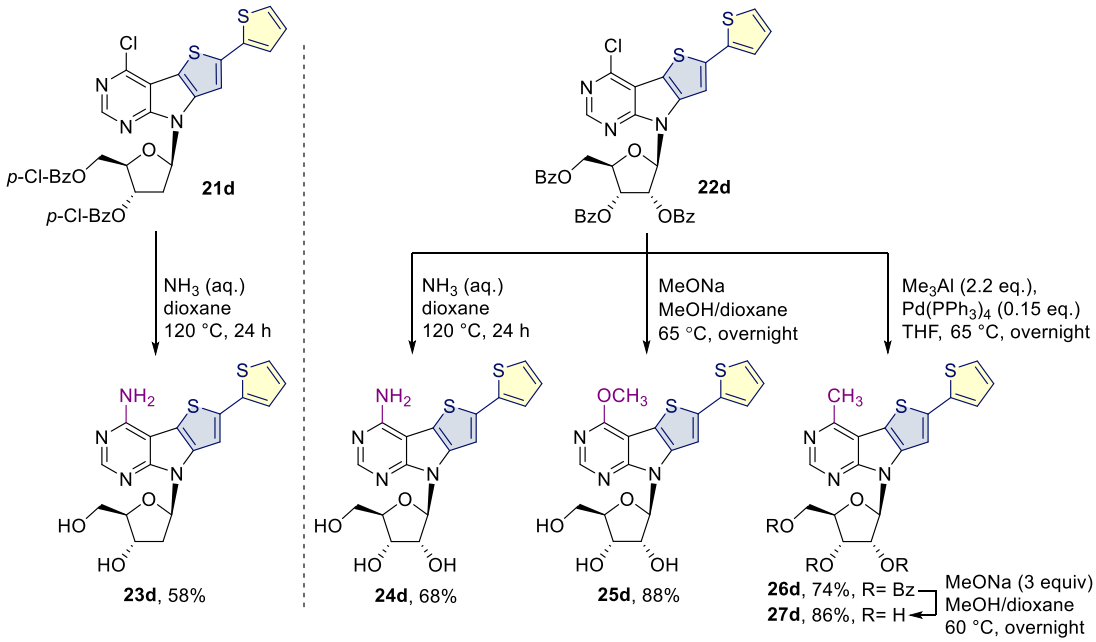
4) Thiophenylthieno series



Scheme 24. Synthesis of thiophenylthieno-fused deazapurine and nucleosides

The synthesis of the thiophenylthieno series was fairly similar to the last phenylthieno series. First, the azidation of dichloropyrimidine **18d** with sodium azide in THF formed tetrazolopyrimidine **19d**. Without any purification, the crude **19d** was used for the next thermal cyclization to obtain nucleobase **20d** in 67% yield over two steps

(Scheme 24). The glycosylation of nucleobase **20d** with the 1-chloro-3,5-bis-*O*-(4-chlorobenzoyl)-2-deoxy-*α*-D-ribofuranose under basic condition gave the desired protected deoxynucleoside **21d** in moderate yield as exclusive *β*-anomer. Next I used the modified procedure of Vorbrüggen glycosylation to prepare the corresponding ribonucleoside. A MeCN solution of nucleobase **20d** and BSA was heated to 60 °C for 30 min. After adding TMSOTf and 1-*O*-acetyl-2,3,5-tri-*O*- benzoyl-*β*-D-ribofuranose, the mixture was stirred at the same temperature for 25–60 min. The protected ribonucleoside **22d** was obtained in good yield of 65%. All ribonucleosides **22a** – **22d** were obtained as pure *β*-anomers.



Scheme 25. Late-stage diversification of thiophenylthieno-fused nucleosides

The nucleophilic substitution reaction of protected deoxyribonucleoside **21d** with aqueous ammonia at 120 °C in dioxane substituted the chlorine at position 4 with amino group with concomitant deprotection of the sugar part to form the desired fused 2′-deoxy-7-deazaadenosine derivative **23d** in 58% yield (Scheme 25, Left). In the ribonucleoside series, 4-amino-, 4-methoxy- and 4-methyl derivatives of fused deazapurine nucleoside were synthesized (Scheme 25, Right). With the protected 4-chloro nucleoside **22d**, the amino group was introduced using aqueous ammonia in dioxane to give the desired product **24d** in 68% yield. Reaction of intermediate **22d**

with sodium methoxide displaced the chlorine with methoxy group and simultaneously cleaved the benzoyl protecting groups to give 4-methoxy nucleoside **25d** in high yield. 4-Methyl free nucleoside **27d** was achieved through cross-coupling reaction with trimethylaluminum and $Pd(PPh_3)_4$, followed by deprotection with MeONa (in 74%, 86% yields respectively). All the target free nucleosides **24a** – **24d**, **25a** – **25d** and **27a** – **27d** were obtained in sufficient amounts and purity for further biological profiling.

3.2.3 Fluorescence properties of polycyclic fused deazapurine deoxynucleosides

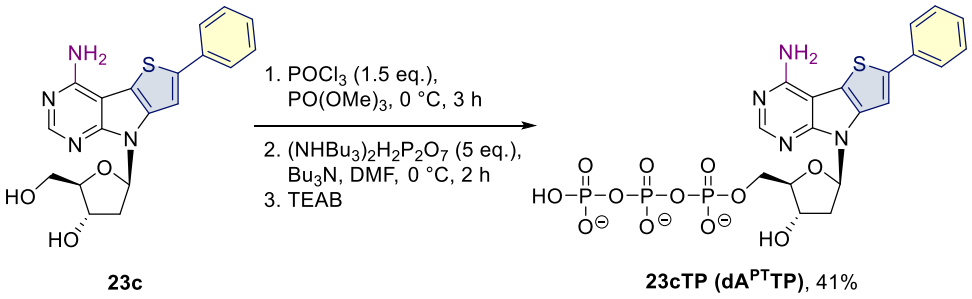
Earlier works reported that benzo-[110, 123] and naphtho-fused[75, 111] 7-deazapurine nucleosides showed useful fluorescence properties in the construction of fluorescent DNA probes. To this end, I investigated the photophysical properties of the new polycyclic fused 7-deazapurine nucleosides. Table 12 shows the results of measurement of UV-vis and fluorescence of the fused 2'-deoxyadenosine analogues **23a** – **23d** in three different solvents. All of the four amino derivatives exerted fluorescence with emission maxima at 359–424 nm. Among these amino derivatives, the phenyl-thieno-fused 7-deazapurine nucleoside **23c** showed the strongest fluorescence with high quantum yields. Interestingly, it exerted the highest fluorescence quantum yield of 48% in water. The thienyl-thieno-fused 7-deazapurine nucleoside **23d** also exhibited relatively strong fluorescence with 16–25% quantum yields. The fluorescence emission maxima and quantum yields of **23c** and **23d** did not significantly change in different solvents. Surprisingly, the extended tetra- and pentacyclic thienothieno- and thienothienothieno-fused nucleosides **23a** and **23b** showed only very weak fluorescence.

Table 12. UV absorption and fluorescence properties of nucleosides **23a** – **23d**

Compd	solvent	absorption	emission	
		λ_{abs} [nm] (ε [10^3 M^{-1} cm^{-1}])	λ_{em} [nm]	Φ_f
	MeOH	323 (21.1), 338 (19.8)	359	0.02
23a	Dioxane	325 (21.7), 340 (20.2)	367	0.01
	H_2O	322 (18.2), 337 (16.4)	367	0.04
	MeOH	341 (29.6), 358 (28.1)	385	0.03
23b	Dioxane	343(29.9), 360 (28.0)	392	0.02
	H_2O	341 (21.6), 357 (17.1)	386	0.04
	MeOH	344 (23.3)	398	0.40
23c	Dioxane	348 (23.0)	405	0.32
	H_2O	342 (19.8)	406	0.48
	MeOH	268 (12.4), 357 (22.9)	417	0.16
23d	Dioxane	268 (12.3), 362 (22.3)	424	0.19
	H_2O	268 (10.3), 356 (21.3)	419	0.25

Fluorescence quantum yields were measured by using anthracene in EtOH (Φ_f = 0.27) as a reference (excitation wavelength is 320 nm).

3.2.4 Biochemistry of phenylthieno-fused deoxynucleoside triphosphate



Scheme 26. Synthesis of phenylthieno-fused 2′-deoxynucleoside triphosphate

As phenyl-thieno-fused nucleoside **23c** showed strong fluorescence in water, we planned to study enzymatic incorporation of the corresponding nucleotide and investigate the photophysical properties of the resulting oligo-2′-deoxyribonucleotides. Triphosphorylation of **23c** by the standard procedure formed the desired phenylthieno-fused 7-deazapurine 2′-deoxynucleoside triphosphate **23cTP** (**dA^{PT}TP**) in 41% yield after HPLC purification (Scheme 26). First, the enzymatic synthesis was

performed using **dA^{PT}TP** (**23cTP**) as a substrate in primer extension (PEX) in presence of KOD XL DNA polymerase with a 19-nt template Temp1A encoding for incorporation of one **dA^{PT}**-modified nucleotide into the extended primer (for sequences of oligonucleotides, see Table 13). A FAM-labeled primer (Prim^{FAM}, 15-nt) was used in the PEX to visualize the extension on denaturing polyacrylamide gel electrophoresis (PAGE). Figure 13A confirms that the PEX reaction using **dA^{PT}TP** was successful giving the full-length product **19ON_1A^{PT}** which was also characterized by MALDI-TOF mass analysis (found: 6107.4 Da, calculated: 6105.9 Da, Table 15). When the PEX reaction with template Temp4A was conducted in lower concentrations, the full-length product **ON_4A** (**31ON_4A^{PT}**) was obtained, which was proved by both denaturing PAGE (Figure 13 B) and MALDI-TOF mass (Table 15), but it was accompanied by shorter products of incomplete primer extension. However, the same reaction at higher concentrations gave the products with one and two modified **dA^{PT}** (5′-CATGGGCGGCATGGG**A^{PT}**CTG, 5′-CATGGGCGGCATGGG**A^{PT}**CTG**A^{PT}**G CTC; see Figure 14, Table 15).

Table 13. List of sequences of primers and templates used in this study

Name	Sequence[a]	Length (nt)
Prim	5′-CATGGGCGGCATGGG-3′	15
Prim^{FAM}	5′-(FAM)-CATGGGCGGCATGGG-3′	15
Temp1A	5′-CCCTCCCATGCCGCCCATG-3′	19
Temp1A^{bio}	5′-(biotin)-CCCTCCCATGCCGCCCATG-3′	19
Temp4A	5′-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′	31
Temp4A^{bio}	5′-(biotin)-CTAGCATGAGCTCAGTCCCATGCCGCCCATG-3′	31
Temp^{termA}	5′-TCCCATGCCGCCCATG-3′	16
Temp$^{termA\ bio}$	5′-(biotin)-TCCCATGCCGCCCATG-3′	16
Temp1C	5′-CCCGCCCATGCCGCCCATG-3′	19
Temp1T	5′-CCCACCCATGCCGCCCATG-3′	19

[a] in the template ONs the segments forming duplex with the primer are underlined

Table 14. List of synthesized modified ssONs

Oligonucleotide[a]	Sequence[b]	Length (nt)
19ON_1A_natural	5′-CATGGGCGGCATGGGAGGG-3′	19
19ON_1A^{H}	5′-CATGGGCGGCATGGG**A^{H}**GGG-3′	19
19ON_1A^{PT}	5′-CATGGGCGGCATGGG**A^{PT}**GGG-3′	19
31ON_4A_natural	5′-CATGGGCGGCATGGGACTGAGCTCATGCTAG-3′	31
31ON_4A^{H}	5′-CATGGGCGGCATGGG**A^{H}**CTG**A^{H}**GCTC**A^{H}**TGCT**A^{H}**G-3′	31
31ON_4A^{PT}	5′-CATGGGCGGCATGGG**A^{PT}**CTG**A^{PT}**GCTC**A^{PT}**TGCT**A^{PT}**G-3′	31
16ON_1A_natural	5′-CATGGGCGGCATGGGA-3′	16
16ON_1A^{H}	5′-CATGGGCGGCATGGG**A^{H}**-3′	16
16ON_1A^{PT}	5′-CATGGGCGGCATGGG**A^{PT}**-3′	16

[a] ON: single-stranded DNA; [b] primer regions underlined.

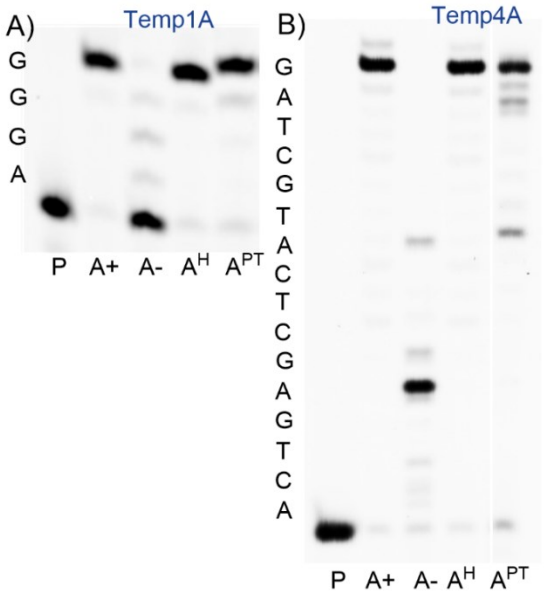


Figure 13. PEX with **23cTP (dA^{PT}TP)** and KOD XL DNA polymerase with templates encoding for incorporation of one (A) or four (B) modified nucleotide(s). A) template Temp1A, (P): FAM-labeled primer; (A+): dATP, dGTP; (A–): dGTP; (A^{H}): **dA^{H}TP**, dGTP; (A^{PT}): **dA^{PT}TP**, dGTP. B) template Temp4A^{bio} (for original uncut gel, see Figure 14), (P): FAM-labeled primer; (A+): dATP, dGTP, dCTP, dTTP; (A–):

dGTP, dCTP, dTTP; (A^H): **$dA^{H}TP$**, dGTP, dCTP, dTTP; (A^{PT}): **$dA^{PT}TP$**, dGTP, dCTP, dTTP.

To shed light into it, we performed kinetic experiments of single nucleotide extension with **$dA^{PT}TP$** in comparison with dATP and 7-deaza-dATP (**$dA^{H}TP$**) (Figure 16), which showed that indeed the incorporation of the very bulky tricyclic nucleotide was significantly slower, in particular when it was positioned against the 5′-terminal nucleotide in the template. Nevertheless, the fact that the polymerase was able to incorporate even such bulky nucleotide is remarkable.

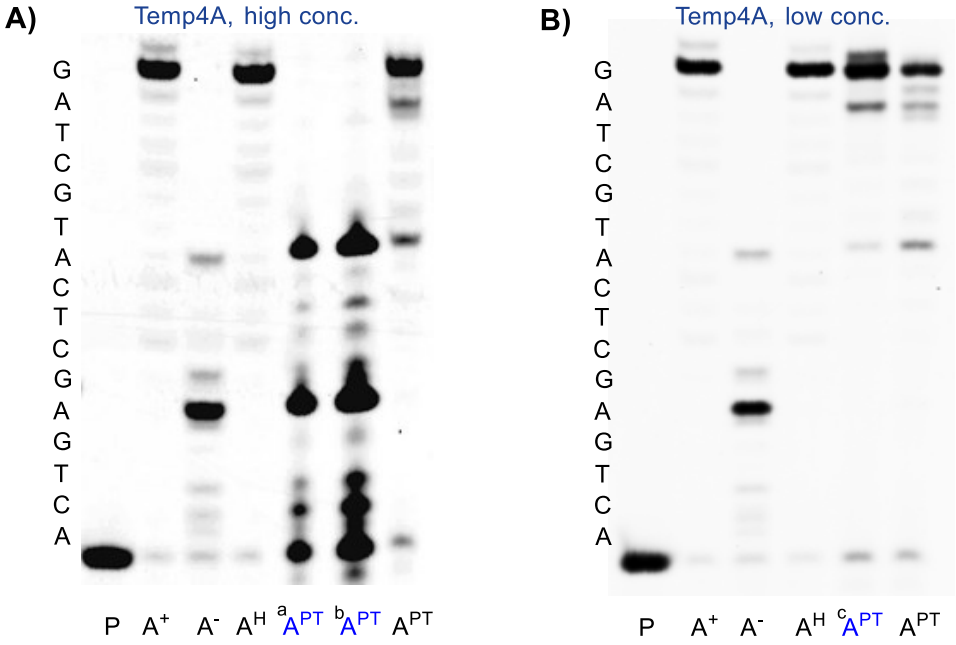


Figure 14. Denaturing PAGE analysis of PEX experiments for comparison of the **23cTP** (**$dA^{PT}TP$**) incorporation at different concentrations (in blue) using *Temp4A*: (A) High concentration of the reaction mixture; (B) Low concentration of the reaction mixture. *P*: FAM-labeled primer; A^+: dATP, dGTP, dCTP, dTTP; A^-: dGTP, dCTP, dTTP; A^H: 7-deaza-dATP, dGTP, dCTP, dTTP; A^{PT}: **$dA^{PT}TP$**, dGTP, dCTP, dTTP. a: 0.5µL aliquot was loaded onto denaturing PAGE; b: 1.5µL aliquot was loaded onto denaturing PAGE; c: reaction mixture was also used for MALDI-TOF measurements.

The rate of incorporation of **$dA^{PT}TP$** with KOD XL DNA Polymerase was studied, using the natural dATP and 7-deaza-dATP as references. The incorporation

of dATP and 7-deaza-dATP with template Temp^{termA}, which can introduce one dNTP at the 3′-end, were finished within 2 min at 60 °C. The incorporation of **dA^{PT}TP** was much slower that the reaction was not completely finished after 2 h incubation (Figure 15, 16A). The resulting oligonucleotide **ON_termA** (**16ON_1A^{PT}**) was also confirmed by MALDI-TOF mass (Table 15).

Table 15. MALDI data of **dA^{PT}**-modified oligonucleotides.

ssDNA	Mw (calcd) [Da]	Mw (found) [Da]	Δ [Da]	Figure number
19ON_1A^{PT}	6105.9	6107.4	1.5	Fig. 20
16ON_1A^{PT} [a]	5118.2	5119.1	0.9	Fig. 21
16ON_1A^{PT} [b]	5118.2	5119.2	1.0	Fig. 22
31ON_4A^{PT} [c] [d]	10678.2	10680.2	2.0	Fig. 23
31ON_4A^{PT} [e]	10141.2	6041.5[f], 7697.4[g]	0.7, 0.6	Fig. 24

Note: a, prepared with *Temp$^{termA\ bio}$*; b, prepared with *Temp1A^{bio}* and only **dA^{PT}TP**; c, 5′-(6-FAM)-labelled; d, low concentrations of PEX mixture; e, high concentrations of PEX mixture; f, 5′-CATGGGCGGCATGGG**A^{PT}**CTG, Mw (calcd) 6040.8 Da; g, 5′-CATGGGCGGCATGGG**A^{PT}**CTG**A^{PT}**GCTC Mw (calcd) 7696.8 Da.

Since the Temp^{termA} had no extra dNTPs after the incorporation site (T), the slow incorporation of **dA^{PT}TP** may be caused by the unstable binding of KOD XL DNA Polymerase. Thus, the rate of incorporation of **dA^{PT}TP** was also conducted with template Temp1A which has 5′-CCC after the incorporation site. As shown in Figure 16B, when only dATP, 7-deaza-dAPT or **dA^{PT}TP** were added in the PEX reaction mixture, one dATP or one 7-deaza-dATP could be incorporated into the DNA within 1 min, then one extra dATP or 7-deaza-dAPT were also added starting from 2 min. The incorporation of one **dA^{PT}TP** with Temp1A took about 30 min which was still slower than that of dATP and 7-deaza-dATP. But the incorporation rate with Temp1A was much faster than with Temp^{termA}. The PEX reaction with both **dA^{PT}TP** and dGTP was then investigated. The oligonucleotide product with full-length was obtained in approximate 5 min. These results indicated that both the length of the template and the addition of dGTP could fasten the speed of the incorporation of **dA^{PT}TP**.

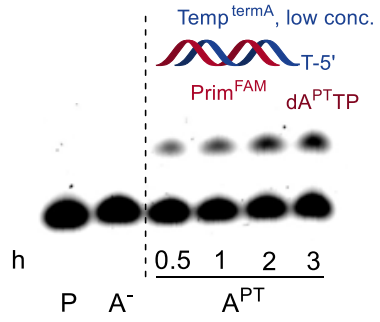


Figure 15. Denaturing PAGE analysis of PEX experiments for study of the rate of **23cTP** (**dA^{PT}TP**) incorporation (0.5, 1, 2, 3 h) using *Temp^{termA}*: *P*: FAM-labeled primer; *A*$^{-}$: H_2O; *A^{PT}*: **dA^{PT}TP**.

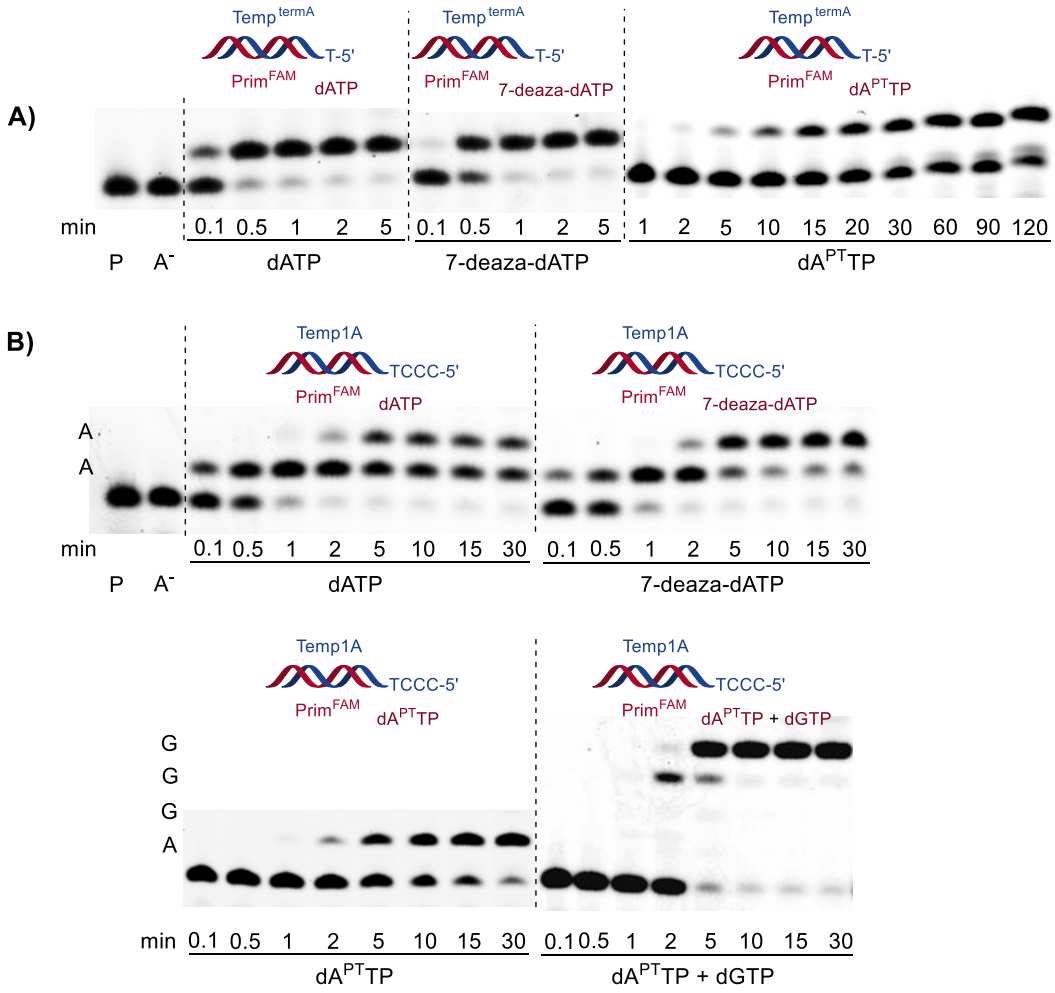
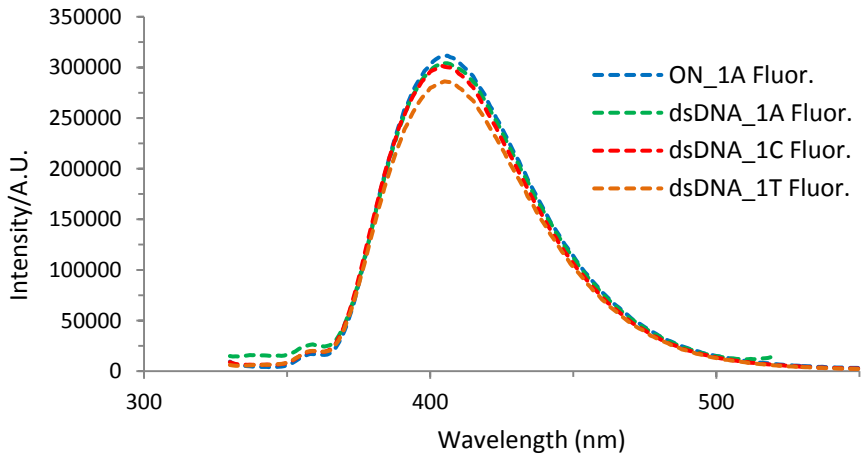


Figure 16. Primer extension experiments of deoxynucleotide **23cTP** (**dA^{PT}TP**) with KOD XL DNA polymerase, FAM-labeled primer and templates: (A) Comparison of the rate of incorporation with template *Temp^{termA}*; (B) Comparison of the rate of incorporation with template *Temp1A*. Key for (A) *P*: FAM-labeled primer; *A*$^{-}$: H_2O;

dATP: dATP; *7-deaza-dATP*: 7-deaza-dATP; *dA^{PT}TP*: **dA^{PT}TP**; (B) *P*: FAM-labeled primer; *A*$^{-}$: H_2O; *dATP*: dATP; *7-deaza-dATP*: 7-deaza-dATP; *dA^{PT}TP*: **dA^{PT}TP**; *dA^{PT}TP + dGTP*: **dA^{PT}TP**, dGTP.

In order to study the fluorescence properties of **dA^{PT}**-modified oligonucleotides (ONs), we first measured the fluorescence emission and quantum yields of the **19ON_1A^{PT}**. Fluorescence properties of the resulting ONs and duplexes were studied with an excitation wavelength of 320 nm. **ON_1A** in medium salt buffer showed fluorescence with emission maxima at 406 nm and with quantum yield of 10.2%. Hybridization with matched or mismatched ONs did not show any significant difference either in emission maxima or in quantum yields (Figure 17). Therefore, we believe that this stable fluorescence property makes **dA^{PT}** promising for DNA labelling and quantification but it is not an environment sensitive label for studying changes of secondary structures or hybridization.[124]



5'—C–A–T–G–G–G–C–G–G–C–A–T–G–G–G–A*–G–G–G **ON_1A**

5'—C–A–T–G–G–G–C–G–G–C–A–T–G–G–G–A*–G–G–G
G–T–A–C–C–C–G–C–C–G–T–A–C–C–C–T–C–C–C–5' **dsDNA_1A**

5'—C–A–T–G–G–G–C–G–G–C–A–T–G–G–G–A*–G–G–G
G–T–A–C–C–C–G–C–C–G–T–A–C–C–C–G–C–C–C–5' **dsDNA_1C**

5'—C–A–T–G–G–G–C–G–G–C–A–T–G–G–G–A*–G–G–G
G–T–A–C–C–C–G–C–C–G–T–A–C–C–C–A–C–C–C–5' **dsDNA_1T**

Figure 17. Fluorescence emission spectra of oligonucleotides **ON_1A** (1 μM in medium salt buffer: 100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0) and corresponding duplexes with complementary strands *Temp1A, Temp1C, Temp1T*. Excitation wavelength: 320 nm. Background fluorescence was subtracted in all spectra.

Table 16. Quantum yields of A^{PT}-modified oligonucleotides.

Oligonucleotide	Quantum Yield
ssDNA	10.2%
dsDNA-1A	13.0%
dsDNA-1C	10.4%
dsDNA-1T	13.9%

3.2.5 Biological activity profiling

In this section, all nucleosides **23a–23d**, **24a–24d**, **25a–25d** and **27a–27d** were sent to Palacky University and University Hospital in Olomouc for testing *in vitro* cytotoxic activity, and all title nucleosides were also screened for other antiviral activities against herpes simplex, influenza, human immunodeficiency virus (HIV), and dengue and SARS-CoV-2 viruses in Dr. Weber's group at IOCB.

Nucleosides **23a–23d**, **24a–24d**, **25a–25d** and **27a–27d** were tested for *in vitro* cytotoxic activity on the panel of leukemic cell lines (CCRF-CEM – acute lymphoblastic leukemia, K562 – myelogenous leukemia,)[125-128] and solid tumor cells (A549 – human lung adenocarcinoma, HCT116 and HCT116p53$^{-/-}$ – colon cancer cells with/without p53 gene, U2OS – human osteosarcoma)[113] using a 3-days MTS (3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetra zolium) assay.[114] Non-malignant fibroblast cell lines MRC-5 and BJ were used in the MTS assay to assess the cancer cell selectivity. Table 17 summarizes the cytotoxic activities of compounds in the MTS assay in comparison with known parent compounds: strongly and non-selectively cytotoxic tubercidin (7-deazaadenosine)[129] and 2'-deoxytubercidin (2'-deoxy-7-deazaadenosine).[130]

Ribonucleoside **24b** showed low micromolar activities against most of cancer cell lines but not non-malignant fibroblasts in the MTS assay. Ribonucleosides **24a**, **24c**, **24d**, **27b** and **27d** showed only moderate effects at micromolar concentrations with little selectivity to cancer cells. The rest of the ribonucleosides are inactive against cancer cells. On the other hand, most of the deoxyribonucleosides **23b**–**23d** and the alpha-anomer **23b*α*** displayed good anti-tumor activities in micromolar or submicromolar (for CCRF-CEM cell line) concentrations. Compounds **23b*α***, **23c** and **23d** show good selectivity toward cancer cell lines and are non-toxic to fibroblasts as compared to toxic tubercidin. These rather surprising results are interesting because in the previous examples of tubercidin,[129] 7-substituted[131] or fused deazapurine nucleosides,[71, 72] the ribonucleosides were always more active whereas 2′-deoxytubercidin[130] was inactive. This suggests that the mode of action of this class of nucleosides will probably be different from the previously reported tubercidin or tricyclic thieno-, furo- and pyrrolo-fused 7-deazapurine ribonucleosides that get phosphorylated and incorporated to DNA causing DNA damage and apoptosis. The mechanism of action of these new polycyclic 2'-deoxyribonuclesides will need a separate study in the future. The biological activities of these polycyclic deoxynucleosides were more potent than I had expected, and I felt happy with the results.

All the title nucleosides were also screened for their antiviral activity against herpes simplex, influenza, human immunodeficiency virus (HIV), dengue and SARS-CoV-2 viruses using previously published protocols.[74, 132] None of the final nucleosides showed any significant activity at 25 μM concentration.

Table 17. Cytotoxic activities of nucleosides.

Compd	MTS, IC_{50} [μM]							
	BJ	MRC-5	CCRF-CEM	K562	A549	HCT116	HCT116 $p53^{-/-}$	U2OS
23a	>50	50	6.6	20.9	32.7	18.8	16.2	20.4
23b	18.1	23.8	0.48	1.9	14.2	1.91	1.46	2.8
23bα	>50	>50	0.73	3.7	>50	3.2	2.3	4.7
23c	50	50	0.74	2.2	12.6	2.5	2.7	2.1
23d	50	49.9	0.77	3.2	27.2	2.9	2.8	2.5
24a	48.1	43.8	14.2	20.4	27.2	17.2	19.1	16.6
24b	50	>50	1.4	4.2	8.8	2.3	2.5	4.5
24c	>50	>50	3.3	13.6	>50	14.8	14.3	10.6
24d	>50	>50	3.2	20.7	>50	12.5	9.6	13.5
25a	>50	>50	>50	>50	>50	>50	>50	>50
25b	>50	>50	>50	>50	>50	>50	>50	>50
25c	>50	>50	>50	>50	>50	>50	>50	>50
25d	>50	>50	>50	>50	>50	>50	>50	>50
27a	>50	>50	15.5	48.5	>50	35.2	46.2	41.7
27b	>50	>50	1.8	3.5	>50	5.5	n.d.	44.5
27c	>50	>50	2.0	>50	>50	>50	>50	>50
27d	>50	>50	2.0	12.3	>50	25.9	14.9	21.5
tubercidin	0.73	0.74	0.017	0.36	0.52	0.08	0.15	0.089
2′-deoxy tubercidin	>50	>50	>50	>50	>50	>50	>50	>50

4 Conclusions

My doctoral work consists of two parts: 1. Synthesis of two isomeric series of benzothieno-fused 7-deazapurine nucleosides as extended analogues of previously reported cytotoxic thieno-fused nucleosides[71] and heteroaryl-analogues of fluorescent and antiviral naphtho-fused deazapurine nucleosides,[75, 111] 2. Development of a new approach for the synthesis of polycyclic hetero-fused 7-deazapurine heterocycles and nucleosides via C-H functionalization of diverse (hetero)aromatics with dibenzothiophene-*S*-oxide followed by the Negishi cross-cooupling with bis(4,6-dichloropyrimidin-5-yl)zinc.

First, we have designed and synthesized two isomeric series of benzothieno-fused 7-deazapurine nucleosides. The synthesis of the novel heterocyclic scaffolds (benzo[4′,5′]thieno[3′,2′:4,5]- and benzo[4′,5′]thieno[2′,3′:4,5]pyrrolo[2,3-*d*] pyrimidine) was based on *in situ* generation of 4,6-dichloropyrimidine-5-zinc reagent followed by the Negishi coupling with iodo-benzothiophenes, azidation and either thermal, photochemical or Rh-catalyzed cyclization. The subsequent glycosylation, functionalization at position 4 and deprotection provided the title nucleosides. Compared with the potent parent thieno-fused nucleosides, most of the nucleosides only exerted moderate cytotoxic activities, which indicated that the extended heterocyclic system might be too bulky for some of the steps in the intracellular activation of the nucleosides. The biological activities of these nucleoside analogues were very frustrating. Compound **6e** exerted significant anti-HCV activity, but poor selectivity. Then, fluorescent properties of the novel extended nucleosides were studied. We also prepared a 2′-deoxyribonucleoside **13** and the corresponding triphosphate (**14**, as analogue of dATP) and successfully used it for enzymatic synthesis of fluorescently-labelled DNA, which showed weak environment sensitivity to secondary structures or sequence changes.

The second part of my work began with the development of a new approach for synthesizing fused 7-deazapurine heterocycles and nucleosides in order to overcome the synthetic problems occurring in the old procedures. This new approach relied on C-H functionalization of (hetero)aromatics with DBTO followed by the Negishi cross-coupling of the resulting sulfonium salts with zincated pyrimidine giving a series of 5-(het)aryl-4,6-dichloropyrimidines in good yields and excellent regioselectivity. But it is problematic with small nitrogenous and oxygenous heterocycles. It is the first example of the Negishi cross-coupling of (het)arylsulfonium salts with hetarylzinc reagent and this cross-coupling has a very promising potential in synthesis of other complex heterocyclic biaryls. Depending on the biological activities of previous fused nucleosides, four thiophene-containing biaryls were then azidated and cyclized to form novel substituted or extended thieno-fused 7-deazapurine heterocycles. The purification of these tetrazoles and nucleobases were very difficult, due to the poor solubility. These extended nucleobase analogues, that would be hardly accessible by previously known synthetic approaches, were then glycosylated to form 2'-deoxy- or ribonucleosides and series of derivatives were prepared by nucleophilic substitutions at position 4. Most of modified ribonucleosides were inactive or moderately active against a panel of cancer cell lines in cytotoxic MTS assay, whereas deoxyribonucleosides **23b**–**23d** displayed high anticancer activities, especially for T-lymphoblastic leukemia cells CCRF-CEM. On the other hand, the extended fused nucleosides did not show any significant antiviral activity. Fluorescent property study showed that phenylthieno-fused 2′-deoxynucleoside displayed strong fluorescence in water, thus its triphosphate **23cTP** was prepared as analogue of dATP and used for enzymatic synthesis of fluorescently-labelled DNA, which showed stable fluorescence in different sequences and was promising for DNA labeling and quantification. These polycyclic fused nucleosides and nucleotides also might have potential for construction of new types of expanded nucleic acids.[133, 134]

5 Experimental Section

5.1 General Information

1) For organic chemistry

All reactions except for the amination were performed under argon atmosphere. For reactions that require heating, the heat source is oil bath. Thin-layer chromatography (TLC) was conducted on TLC Silica gel 60 F254 (Merck) and detected by UV (254 nm and 366 nm) or by solution of 4-anisaldehyde in ethanol and 10% of sulphuric acid. Melting points were recorded using a Stuart SMP40 melting point apparatus. Infrared spectra were recorded by a Bruker Alpha FTIR spectrometer with attenuated total reflection (ATR). Optical rotations of the free nucleosides were measured on an Autopol IV polarimeter (Rudolph Research Analytical) in DMSO. ^{1}H and ^{13}C NMR were measured by a Bruker Avance III 500 MHz spectrometer (499.8 MHz for 1H, 125.7 MHz for 13C, and 202.3 MHz for 31P) or a Bruker Avance III HD 400 MHz spectrometer (400 MHz for 1H, 101 MHz for 13C). The spectral data were reported in ppm and referenced to the residual solvent signal [δ(^{1}H) = 2.50 ppm, δ(^{13}C) = 39.52 ppm] in DMSO-*d6*, or referenced to the residual solvent signal [δ(^{1}H) = 7.26 ppm, δ(^{13}C) = 77.16 ppm] in $CDCl_3$. The full assignment of all NMR signals was determined by using a combination of DFQ-COSY, H,C-HSQC, and H,C-HMBC experiments. Low resolution mass spectra were recorded on LCQ Fleet (Thermo Fisher Scientific) using electrospray ionization (ESI), and high resolution mass was measured on LTQ Orbitrap XL (Thermo Fisher Scientific) using APCI or ESI. UV absorption was measured by a Cary 100 UV/Vis spectrometer (Agilent Technologies) and fluorescence emission spectra were recorded using a Fluoromax 4 spectrofluorimeter (HORIBA Scientific). All measurements on UV spectrometer and Fluoromax were conducted under room temperature. Purification with high-performance flash chromatography (HPFC) was conducted by an ISCO Combiflash Rf system on RediSep Rf Gold Silica Gel Disposable columns or Reverse

Phase (C18) RediSep Rf column. Purification of nucleoside triphosphate was performed using HPLC (Waters modular HPLC system) on a column packed with 10 μm C18 reversed phase (Phenomenex, Luna C18 (2) 100 Å). Purity of all final compounds (>95%) was determined by analytical HPLC and by clean NMR spectra. HPLC analysis was performed on a Waters 600 HPLC system (Waters 600 Controller, Waters 2996 Photodiode Array Detector) with a Gemini 5μ C18 110A column (250 × 4.60 mm, 5 μm) and 1 mL/min flow. 3-Iodobenzothiophene was prepared according to published procedure.[135] Photocyclization of the azide was performed using a 4 W germicidal ultraviolet GTL3 bulb, model EUV-13B. The sulfonium salts (**16a**, **16c**, **16d**, **16f**, **16g**, **16h**, **16i**, **16j**, **16l**, **16m**) were prepared according to the published procedures.[95, 96]

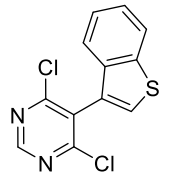
2) For biochemistry

All PAGE gels were analyzed by fluorescence imaging using Typhoon FLA 9500 (GE Healthcare Life Sciences). Mass spectra of oligonucleotides were measured on UltrafleXtreme MALDI-TOF/TOF (Bruker) mass spectrometer with 1 kHz smartbeam II laser technology. UV-Vis spectra (concentration of products) were measured at room temperature on NanoDrop1000 (ThermoFisher Scientific). Samples were concentrated on CentriVap Vacuum Concentrator system (Labconco). Synthetic oligonucleotides (unmodified primers, 5′-end labelled primers with 6-carboxyfluorescein (6-FAM), unmodified templates and 5′-biotinylated templates; were purchased from Generi Biotech (Czech Republic). Natural nucleoside triphosphates (dATP, dGTP, dTTP, dCTP) were purchased from either ThermoFisher Scientific or New England Biolabs. KOD XL DNA polymerases and corresponding polymerase reaction buffer were purchased from Merck Life Science. Streptavidin magnetic particles (SMB) (Roche) were obtained from Merck Life Science. Milli-Q water was used for all experiments. Reactions after PEX were stopped by addition of PAGE stop solution (20 μL; 95 % [v/v] formamide, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.025 % [w/v] SDS, 0.025 % [w/v]

bromophenol blue, 0.025 % [w/v] xylene cyanol FF in water) and the mixtures were heated to 95 °C for 5 min. Aliquots of PEX reaction mixtures (4 μL) were separated by 12.5% denaturing PAGE (acrylamide/bisacrylamide 19:1, 25 % urea) under denaturing conditions (1 h, 42 mA, 50 °C, 1X TBE buffer) and visualized by fluorescence imaging. Biological activity screening was performed as described in previous works.[112-115]

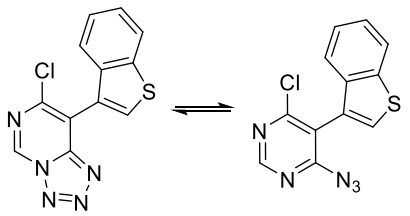
5.2 Synthesis and Photophysical Properties of Benzothieno-Fused 7-Deazapurine Ribonucleosides

5.2.1 Synthesis of benzothieno-fused 7-deazapurine ribonucleosides

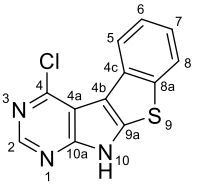


5-(Benzo[*b*]thiophen-3-yl)-4,6-dichloropyrimidine (1)

TMPMgCl·LiCl (1 M, 25 mL, 25 mmol) was added to $ZnCl_2$ (1.7 g, 12.5 mmol) under argon atmosphere, the mixture was stirred at r.t. for 12 h to give a tetramethylpiperidinylzinc complex. 4,6-Dichloropyrimidine (3.1 g, 20.8 mmol) was dissolved in THF (16 mL) and added dropwise to an ice-cooled solution of a tetramethylpiperidinylzinc complex. The mixture was stirred at 0 °C for 1 h and then at r.t. for 1 h to give a zincated pyrimidine. A solution of 3-iodobenzothiophene (5.4 g, 20.8 mmol) and $Pd(PPh_3)_4$ (2.4 g, 2 mmol) in THF (16 mL), which was prestirred at r.t. for 20 min, was added to the solution of zincated pyrimidine, and stirred at 65 °C for 40 h. After that, solvent was evaporated under reduced pressure. Purification by HPFC (SiO_2, hexane/EtOAc 0→1%) gave **1** (3.5 g, 61%) as a white solid. m.p. 150–152 °C. IR (ATR): ν = 1504, 1430, 1396, 1364, 1257, 1220, 1100, 797, 753, 711, 690 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 7.35 (m, 1H, H-4′); 7.41 (m, 1H, H-5′); 7.44 (m, 1H, H-6′); 7.56 (s, 1H, H-2′); 7.96 (m, 1H, H-7′); 8.87 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 122.0 (CH-4′); 123.00 (CH-7′); 124.8 (CH-5′); 125.1 (CH-6′); 127.4 (C-3′); 128.0 (CH-2′); 128.7 (C-5); 136.7 (C-3′a); 139.7 (C-7′a); 157.3 (CH-2); 162.6 (C-4,6). MS (EI): *m/z* (rel. %): 281 (22) $[M + H]^+$. HRMS (EI): calcd. for $C_{12}H_6Cl_2N_2S$ $[M]^+$ 279.9629; found 279.9630.

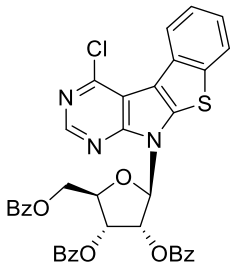


8-(Benzo[*b*]thiophen-3-yl)-7-chlorotetrazolo[1,5-*c*]pyrimidine (2a), 4-azido-5-(benzo[*b*]thiophen-3-yl)-6-chloropyrimidine (2b): Compound **1** (410 mg, 1.46 mmol) was dissolved in THF (6 mL), NaN_3 (95 mg, 1.46 mmol) and LiCl (61.3 mg, 1.46 mmol) were added. The mixture was stirred at r.t. for 2 days. Solvent was removed under vacuum, and the crude product was purified by flash chromatography (SiO_2, hexane/EtOAc 6:1) to give the desired product **2** (332 mg, 88%) as a light yellow solid. According to NMR in DMSO, **2** was present as a mixture of tautomers **2a** and **2b** in ≈ 4:1 ratio. m.p. 130 °C (decomposition). IR (ATR): ν = 2144, 2046, 1680, 1525, 1507, 1395, 1366, 1259, 1231, 1152, 939, 729 cm^{-1}. ^1H NMR for tautomer **2a** (500.0 MHz, DMSO-d_6): 7.40 (ddd, 1H, $J_{5',4'}$ = 8.1, $J_{5',6'}$ = 7.1, $J_{5',7'}$ = 1.2, H-5′); 7.48 (ddd, 1H, $J_{6',7'}$ = 8.1, $J_{6',5'}$ = 7.1, $J_{6',4'}$ = 1.4, H-6′); 7.57 (ddd, 1H, $J_{4',5'}$ = 8.1, $J_{4',6'}$ = 1.4, $J_{4',7'}$ = 0.8, H-4′); 8.15 (ddd, 1H, $J_{7',6'}$ = 8.1, $J_{7',5'}$ = 1.2, $J_{7',4'}$ = 0.8, H-4′); 8.17 (s, 1H, H-2′); 10.34 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 117.3 (C-5); 123.3 (CH-7′); 123.4 (CH-4′); 124.8 (CH-5′); 125.18 (CH-6′); 125.24 (C-3′); 130.8 (CH-2′); 137.2 (C-3′a); 139.4 (C-7′a); 139.6 (CH-2); 147.0, 151.5 (C-4,6). ^1H NMR for tautomer **2b** (500.0 MHz, $CDCl_3$): 7.35–7.45 (m, 3H, H-4′,5′,6′); 7.50 (s, 1H, H-2′); 7.95 (m, 1H, H-7′); 8.77 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 118.0 (C-5); 122.3 (CH-4′); 123.0 (CH-7′); 124.7 (CH-5′); 124.9 (CH-6′); 126.1 (C-3′); 127.8 (CH-2′); 137.1 (C-3′a); 139.7 (C-7′a); 157.2 (CH-2); 161.7, 163.0 (C-4,6). MS (APCI): *m/z* (rel. %): 288 (100) $[M + H]^+$. HRMS (APCI): calcd. for $C_{12}H_7N_5ClS$ $[M + H]^+$ 288.01052; found 288.01023.



4-Chloro-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (3):

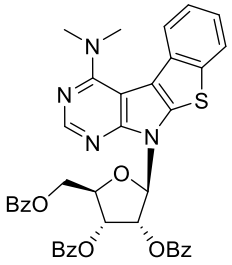
Tetrazole **2a** (443 mg, 1.54 mmol) was suspended in TFA (40 mL), and the solution was stirred under 254 nm UV light (4 W) for 2 days. Then TFA was evaporated under vacuum and the crude product was purified by HPFC (SiO_2, hexane/EtOAc, 10→50%). Nucleobase **3** (300 mg, 75%) was obtained as a brown solid. m.p. 246 °C (decomp.). IR (ATR): ν = 3045, 2958, 2852, 2656, 2365, 1669, 1483, 1199, 1142, 1119, 725 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 7.38 (ddd, 1H, $J_{7,8}$ = 8.3, $J_{7,6}$ = 7.1, $J_{7,5}$ = 1.4, H-7); 7.52 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.1, $J_{6,8}$ = 1.4, H-6); 8.07 (ddd, 1H, $J_{8,7}$ = 8.3, $J_{8,6}$ = 1.4, $J_{8,5}$ = 0.8, H-8); 8.59 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.4, $J_{5,8}$ = 0.8, H-5); 8.67 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d6): 112.4 (C-4a); 113.4 (C-4b); 122.4 (CH-5); 124.0 (CH-7); 124.5 (CH-8); 125.9 (CH-6); 130.9 (C-4c); 138.1 (C-8a); 142.9 (C-9a); 148.0 (C-4); 150.4 (CH-2); 156.6 (C-10a). MS (CI): *m*/*z* (rel. %): 260 (100) $[M + H]^+$. HRMS (CI): calcd. for $C_{12}H_7N_3SCl$ $[M + H]^+$ 260.0049; found 260.0052. Important note: The purification of nucleobase **3** is difficult due to its poor solubility in most organic solvents. Separation in gram scale is quite time consuming so that the reaction mixture was directly used for next step after a filtration on short silica gel column and removal of solvent. Nucleoside **4** (2.93 g, 40%) was obtained from tetrazole **2a** (3.0 g, 10.4 mmol) over two steps.



4-Chloro-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (4)

Nucleobase **3** (450 mg, 1.7 mmol) was dissolved in MeCN (47 mL), and BSA (424 µL, 1.7 mmol) was added. The mixture was heated to 60 °C for 30 min, and then TMSOTf (782 µL, 4.3 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (1.75 g, 3.5 mmol) were added. The mixture was stirred at the same temperature overnight. The mixture was extracted with EtOAc and water, and the organic layer was washed with $NaHCO_3$ and again with water, dried over $MgSO_4$, and evaporated

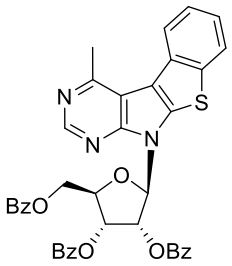
under reduced pressure. Crude product was purified using column chromatography (SiO_2, hexane/EtOAc, 15→35%) to give nucleoside **4** (682 mg, 56%) as a white solid. m.p. 131–134 °C. IR (ATR): ν = 1721, 1537, 1292, 1260, 1082, 1066, 710, 687 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.77 (dd, 1H, J_{gem} = 12.3, $J_{5'b,4'}$ = 4.1, H-5′b); 4.86 (ddd, 1H, $J_{4',3'}$ = 4.6, $J_{4',5'}$ = 4.1, 3.1, H-4′); 5.04 (dd, 1H, J_{gem} = 12.3, $J_{5'a,4'}$ = 3.1, H-5′a); 6.19 (dd, 1H, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 4.6, H-3′); 6.29 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.1, H-2′); 6.99 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 7.32 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.33–7.37 (m, 2H, H-*m*-Bz); 7.39–7.50 (m, 5H, H-6, H-*m*-Bz); 7.51–7.63 (m, 4H, H-8, H-*p*-Bz); 7.89–7.92, 8.00–8.04, 8.12–8.15 (3 × m, 3 × 2H, H-*o*-Bz); 8.65 (s, 1H, H-2); 8.70 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.4 (CH_2-5′); 71.0 (CH-3′); 72.5 (CH-2′); 80.7 (CH-4′); 86.5 (CH-1′); 114.0 (C-4a); 115.6 (C-4b); 123.3 (CH-8); 123.4 (CH-5); 124.2 (CH-7); 125.6 (CH-6); 128.4 (C-*i*-Bz); 128.48, 128.50, 128.56 (CH-*m*-Bz); 128.7, 129.5 (C-*i*-Bz); 129.81, 129.84, 129.89 (CH-*o*-Bz); 130.7 (C-4c); 133.4, 133.7, 133.8 (CH-*p*-Bz); 138.6 (C-8a); 140.9 (C-9a); 149.5 (C-4); 150.1 (CH-2); 155.9 (C-10a); 165.0, 165.4, 166.2 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 704 (51) $[M + H]^+$, 726 (28) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{38}H_{27}ClN_3O_7S$ $[M + H]^+$ 704.1253; found 704.1241.



4-*N,N*-Dimethylamino-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (5a)

Dimethylamine (2 M in THF, 1 mL) was added to a solution of nucleoside **4** (400 mg, 0.57 mmol) in mixture of propan-2-ol/DCM (1:1, 20 mL) and the reaction mixture was stirred at 40 °C for 20 h. The volatiles were removed under reduced pressure and the crude product was purified by HPFC (SiO_2, hexane/EtOAc10→35%) to give a nucleoside **5a** (242 mg, 60%) as a white solid. m.p. 120–124 °C. IR (ATR): ν = 2953, 1720, 1566, 1550, 1450, 1259, 1090, 1066, 1023, 704, 685 cm^{-1}. ^1H NMR (500.0

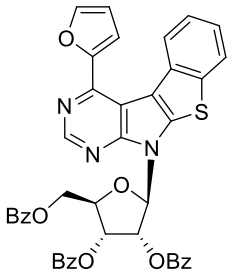
MHz, $CDCl_3$): 3.18 (s, 6H, $(CH_3)_2N$); 4.78 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.3, H-5′b); 4.82 (ddd, 1H, $J_{4',3'}$ = 4.5, $J_{4',5'}$ = 4.3, 3.0, H-4′); 5.00 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.0, H-5′a); 6.20 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 4.5, H-3′); 6.28 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.2, H-2′); 7.02 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 7.26 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.33–7.40 (m, 4H, H-*m*-Bz); 7.42–7.47 (m, 3H, H-6, H-*m*-Bz); 7.50–7.61 (m, 4H, H-8, H-*p*-Bz); 7.93–7.96, 7.97–8.01 (2 × m, 2 × 2H, H-*o*-Bz); 8.15 (bd, 1H, $J_{5,6}$ = 8.2, H-5); 8.16–8.19 (m, 2H, H-*o*-Bz); 8.50 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 41.9 (CH_3N); 63.7 (CH_2-5′); 71.0 (CH-3′); 72.4 (CH-2′); 80.3 (CH-4′); 86.0 (CH-1′); 103.5 (C-4a); 116.6 (C-4b); 123.0 (CH-5); 123.1 (CH-7); 123.4 (CH-8); 125.0 (CH-6); 128.42, 128.45, 128.48 (CH-*m*-Bz); 128.6, 128.8, 129.7 (C-*i*-Bz); 129.8, 129.9, 130.0 (CH-*o*-Bz); 132.1 (C-4c); 133.3, 133.58, 133.63 (CH-*p*-Bz); 136.1 (C-9a); 138.7 (C-8a); 150.3 (CH-2); 156.4 (C-10a); 161.2 (C-4); 165.1, 165.4, 166.3 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 713 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{40}H_{33}O_7N_4S$ $[M + H]^+$ 713.2064; found 713.2054.



4-Methyl-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (5b)

Trimethylaluminum (2 M in toluene, 966 µL, 1.93 mmol) was added to a solution of nucleoside **4** (680 mg, 0.97 mmol) and $Pd(PPh_3)_4$ (112 mg, 97 µmol) in THF (15 mL) and the reaction mixture was stirred at 65 °C for 20 h. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 15→50%) to give **5b** (410 mg, 62%) as a white solid. m.p. 95–102 °C. IR (ATR): ν = 1720, 1450, 1434, 1313, 1260, 1176, 1066, 1023, 1001, 750, 705 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.10 (s, 3H, CH_3); 4.73 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.1, H-5′b); 4.94 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.0, H-5′a); 5.03 (ddd, 1H, $J_{4',3'}$ = 4.9, $J_{4',5'}$ = 4.1, 3.0, H-4′); 6.16 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ =

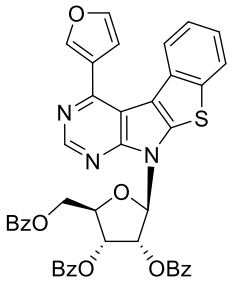
4.9, H-3′); 6.30 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 5.8, H-2′); 6.99 (d, 1H, $J_{1',2'}$ = 5.8, H-1′); 7.27 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.39–7.43 (m, 2H, H-*m*-Bz); 7.48–7.54 (m, 5H, H-6, H-*m*-Bz); 7.59–7.72 (m, 3H, H-*p*-Bz); 7.77 (bd, 1H, $J_{8,7}$ = 8.2, H-8); 7.78–7.81, 7.99–8.05 (2 × m, 6H, H-*o*-Bz); 8.32 (bd, 1H, $J_{5,6}$ = 8.0, H-5); 8.68 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 26.7 (CH_3); 63.5 (CH_2-5′); 70.8 (CH-3′); 72.4 (CH-2′); 79.7 (CH-4′); 86.1 (CH-1′); 113.6 (C-4a); 115.9 (C-4b); 122.1 (CH-5); 123.99, 124.01 (CH-7,8); 126.0 (CH-6); 128.4, 128.8 (C-*i*-Bz); 128.96, 128.98, 129.08 (CH-*m*-Bz); 129.50, 129.56 (CH-*o*-Bz); 129.60 (C-*i*-Bz); 129.7 (CH-*o*-Bz); 131.0 (C-4c); 133.8, 134.21, 134.22 (CH-*p*-Bz); 138.3 (C-8a); 139.4 (C-9a); 150.9 (CH-2); 154.7 (C-10a); 157.5 (C-4); 164.6, 165.0, 165.7 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 684 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{39}H_{30}O_7N_3S$ $[M + H]^+$ 684.1799; found 684.1790.



4-(Furan-2-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno [3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (5c)

Nucleoside **4** (400 mg, 0.57 mmol), 2-furyl(tributyl)stannane (0.22 mL, 0.68 mmol) and $PdCl_2(PPh_3)_2$ (60 mg, 85 μmol) were dissolved in anhydrous DMF (30 mL) and heated to 100 °C for 30 min. The volatiles were removed in vacuo and the residue was loaded onto a silica column that contained 15% KF. The column was washed with hexane (2 L), then with a gradient of EtOAc in hexane (0→30% EtOAc). Desired product **5c** (280 mg, 67%) was obtained as a yellow solid. m.p. 118–121 °C. IR (ATR): ν = 1719, 1435, 1256, 1095, 1067, 1024, 703, 628 cm^{-1}. ^{1}H NMR (500.0 MHz, $CDCl_3$): 4.79 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 4.2, H-5′b); 4.86 (ddd, 1H, $J_{4',3'}$ = 4.5, $J_{4',5'}$ = 4.2, 3.0, H-4′); 5.04 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.0, H-5′a); 6.22 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 4.5, H-3′); 6.32 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.2, H-2′); 6.74 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.8, H-4-furyl); 7.09 (d, 1H, $J_{1',2'}$ = 6.2, H-1′); 7.17 (dd, 1H, $J_{3,4}$ = 3.4, $J_{3,5}$ = 0.8, H-3-furyl);

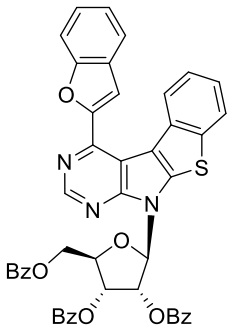
7.26 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.33–7.37 (m, 3H, H-6, H-*m*-Bz); 7.39–7.43, 7.43–7.47 (2 × m, 2 × 2H, H-*m*-Bz); 7.50–7.61 (m, 4H, H-8, H-*p*-Bz); 7.68 (dd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, H-5); 7.79 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H-5-furyl); 7.91–7.94, 8.00–8.03, 8.16–8.19 (3 × m, 3 × 2H, H-*o*-Bz); 8.90 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.6 (CH_2-5′); 71.0 (CH-3′); 72.4 (CH-2′); 80.6 (CH-4′); 86.1 (CH-1′); 110.8 (C-4a); 112.6 (CH-4-furyl); 114.0 (CH-3-furyl); 116.1 (C-4b); 123.3 (CH-8); 123.5 (CH-7); 123.8 (CH-5); 125.0 (CH-6); 128.45, 128.49, 128.55 (CH-*m*-Bz); 128.7, 129.6 (C-*i*-Bz); 129.83, 129.84, 139.95 (C-*i*-Bz, CH-*o*-Bz); 131.7 (C-4c); 133.3, 133.65, 133.70 (CH-*p*-Bz); 138.5 (C-9a); 141.5 (C-8a); 144.8 (CH-5-furyl); 146.5 (C-4); 150.3 (CH-2); 151.8 (C-2-furyl); 156.9 (C-10a); 165.1, 165.4, 166.3 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 736 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{42}H_{30}O_8N_3S$ $[M + H]^+$ 736.1748; found 736.1736.



4-(Furan-3-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno [3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (5d)

Protected nucleoside **4** (400 mg, 0.57 mmol), furan-3-boronic acid (96 mg, 0.86 mmol), K_2CO_3 (157 mg, 1.14 mmol), and $Pd(PPh_3)_4$ (66 mg, 0.06 mmol) were dissolved in toluene (15 mL) and heated to 100 °C overnight. Then, the reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with saturated NH_4Cl, and then with water and was dried over $MgSO_4$. After evaporation of solvent, the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 0→20%). Product **5d** (295 mg, 70%) was obtained as a yellow solid. m.p. 159–162 °C. IR (ATR): ν = 3063, 2926, 1720, 1260, 1088, 1067, 1023, 1001, 705 cm^{-1}. ^{1}H NMR (500.0 MHz, $CDCl_3$): 4.79 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 4.2, H-5′b); 4.87 (ddd, 1H, $J_{4',3'}$ = 4.5, $J_{4',5'}$ = 4.2, 3.0, H-4′); 5.04 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 3.0, H-5′a); 6.23 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 4.5, H-3′); 6.33 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.2, H-2′); 6.96 (dd, 1H, $J_{4,5}$ = 1.9,

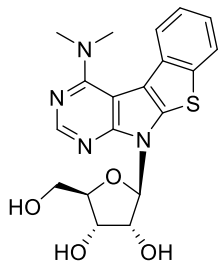
$J_{4,2}$ = 0.9, H-4-furyl); 7.09 (d, 1H, $J_{1',2'}$ = 6.2, H-1′); 7.25 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 6.4, $J_{7,5}$ = 2.1, H-7); 7.29–7.37 (m, 4H, H-5,6, H-*m*-Bz); 7.39–7.47 (m, 4H, H-*m*-Bz); 7.51–7.61 (m, 4H, H-8, H-*p*-Bz); 7.69 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.6, H-5-furyl); 7.88 (dd, 1H, $J_{2,5}$ = 1.6, $J_{2,4}$ = 0.9, H-2-furyl); 7.92–7.95, 8.01–8.04, 8.16–8.19 (3 × m, 3 × 2H, H-*o*-Bz); 8.92 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.6 (CH_2-5′); 71.0 (CH-3′); 72.5 (CH-2′); 80.5 (CH-4′); 86.1 (CH-1′); 111.5 (CH-4-furyl); 113.2 (C-4a); 116.0 (C-4b); 123.35 (CH-8); 123.44 (CH-7); 123.7 (CH-5); 125.1 (CH-6); 125.5 (C-3-furyl); 128.47, 128.49, 128.54 (CH-*m*-Bz); 128.7, 129.6 (C-*i*-Bz); 129.8, 129.9, 130.0 (C-*i*-Bz, CH-*o*-Bz); 131.2 (C-4c); 133.34, 133.68, 133.71 (CH-*p*-Bz); 138.6 (C-8a); 140.9 (C-9a); 143.5 (CH-5-furyl); 143.9 (CH-2-furyl); 150.3 (C-4); 150.7 (CH-2); 156.3 (C-10a); 165.1, 165.4, 166.3 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 736 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{42}H_{30}O_8N_3S$ $[M + H]^+$ 736.1748; found 736.1738.



4-(Benzofuran-2-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (5e)

Protected nucleoside **4** (400 mg, 0.57 mmol), benzofurane-2-boronic acid (138 mg, 0.85 mmol), K_2CO_3 (157 mg, 1.14 mmol), and $Pd(PPh_3)_4$ (66 mg, 0.06 mmol) were dissolved in toluene (15 mL) and heated to 100 °C for 8 h. Then, the reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with saturated NH_4Cl, and then with water and was dried over $MgSO_4$. After evaporation of solvent, the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 0→20%). Compound **5e** (136 mg, 30%) was obtained as a yellow solid. m.p. 136–138 °C. IR (ATR): ν = 2922, 2853, 1724, 1437, 1252, 1124, 1084, 1023, 711, 701 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 4.76 (dd, 1H, J_{gem} = 12.4, $J_{5'b,4'}$ = 4.2, H-5′b); 4.97 (dd, 1H, J_{gem} = 12.4, $J_{5'a,4'}$ = 3.0, H-5′a); 5.06 (ddd, 1H,

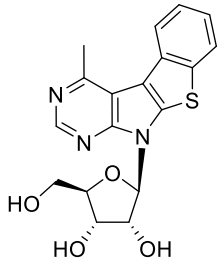
$J_{4',3'}$ = 5.0, $J_{4',5'}$ = 4.2, 3.0, H-4′); 6.19 (dd, 1H, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 5.0, H-3′); 6.33 (dd, 1H, $J_{2',3'}$ = 6.3, $J_{2',1'}$ = 5.7, H-2′); 7.09 (d, 1H, $J_{1',2'}$ = 5.7, H-1′); 7.27 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.3, H-6); 7.31 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.3, H-7); 7.39–7.44 (m, 3H, H-5-benzofuryl, 2 H-*m*-Bz); 7.47–7.55 (m, 5H, H-6-benzofuryl, 4 H-*m*-Bz); 7.59–7.72 (m, 5H, H-5, H-7-benzofuryl, H-*p*-Bz); 7.74 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.78 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.6, H-8); 7.80–7.83 (m, 2H, H-*o*-Bz); 7.87 (ddd, 1H, $J_{4,5}$ = 7.8, $J_{4,6}$ = 1.2, $J_{4,7}$ = 0.7, H-4-benzofuryl); 8.01–8.06 (m, 4H, H-*o*-Bz); 8.88 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 63.5 (CH_2-5′); 70.8 (CH-3′); 72.6 (CH-2′); 79.9 (CH-4′); 86.4 (CH-1′); 109.9 (CH-3-benzofuryl); 110.6 (C-4a); 111.9 (CH-7-benzofuryl); 115.1 (C-4b); 122.9 (CH-4-benzofuryl); 123.3 (CH-5); 123.9 (CH-8); 124.1 (CH-7); 124.3 (CH-5-benzofuryl); 125.4 (CH-6); 126.6 (CH-6-benzofuryl); 128.3 (C-3a-benzofuryl); 128.5, 128.8 (C-*i*-Bz); 128.99, 129.01, 129.1 (CH-*m*-Bz); 129.55, 129.59 (CH-*o*-Bz); 129.61 (C-*i*-Bz); 129.7 (CH-*o*-Bz); 131.4 (C-4c); 133.8, 134.2 (CH-*p*-Bz); 138.2 (C-8a); 142.5 (C-9a); 145.7 (C-4); 150.3 (CH-2); 153.4 (C-2-benzofuryl); 155.1 (C-7a-benzofuryl); 156.5 (C-10a); 164.7, 165.0, 165.8 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 786 (82) $[M + H]^+$, 808 (18) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{46}H_{32}O_8N_3S$ $[M + H]^+$ 786.1905; found 786.1896.



4-*N,N*-Dimethylamino-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6a)

Solution of MeONa in MeOH (25 wt%, 380 µL) was added to a solution of protected nucleoside **5a** (200 mg, 0.28 mmol) in methanol/1,4-dioxane (24 mL, 1:1). The mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10→90%) to give compound **6a** (89 mg, 80%) as a white solid. m.p. 129–135 °C. $[\alpha]_D$ = +24.0 (c = 0.267 in DMSO). IR (ATR): *v* = 3220, 2913, 2851, 2791, 1570,

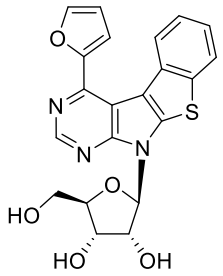
1544, 1481, 1416, 1049, 1022, 746 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.12 (s, 6H, CH_3N); 3.67, 3.71 (2 × dd, 2 × 1H, J_{gem} = 11.6, $J_{5',4'}$ = 5.1, H-5′); 3.98 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 2.9, H-4′); 4.13 (dd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 2.9, H-3′); 4.59 (dd, 1H, $J_{2',1'}$ = 7.1, $J_{2',3'}$ = 5.4, H-2′); 5.15, 5.47 (2 × bs, 3H, OH-2′,3′,5′); 6.40 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.35 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.52 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.2, H-6); 8.07 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.17 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.44 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 41.7 (CH_3N); 62.4 (CH_2-5′); 70.9 (CH-3′); 71.8 (CH-2′); 85.7 (CH-4′); 87.2 (CH-1′); 102.3 (C-4a); 115.3 (C-4b); 122.9 (CH-5); 123.6 (CH-7); 124.2 (CH-8); 125.6 (CH-6); 131.7 (C-4c); 136.6 (C-9a); 138.6 (C-8a); 150.3 (CH-2); 156.4 (C-10a); 160.9 (C-4). MS (ESI): *m*/*z* (rel. %): 401 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{19}H_{21}O_4N_4S$ $[M + H]^+$ 401.1278; found 401.1274.



4-Methyl-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6b)

A solution of MeONa in MeOH (25 wt%, 520 µL) was added to a solution of protected nucleoside **5b** (310 mg, 0.45 mmol) in methanol/1,4-dioxane (30 mL, 2:1). The reaction mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (H_2O/MeOH, 10→90%) to give compound **6b** (138 mg, 82%) as a white solid. m.p. 225–228 °C. $[\alpha]_D$ = –45.1 (c = 0.317 in DMSO). IR (ATR): ν = 3207, 3048, 2918, 1483, 1474, 1036, 1021, 749, 721 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.16 (s, 3H, CH_3); 3.70, 3.73 (2 × dd, 2 × 1H, J_{gem} = 11.7, $J_{5',4'}$ = 5.1, H-5′); 4.01 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.1, H-4′); 4.14 (dd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 3.1, H-3′); 4.58 (dd, 1H, $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 5.4, H-2′); 5.05, 5.46 (2 × bs, 3H, OH-2′,3′,5′); 6.47 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 7.39 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.54 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.2, H-6);

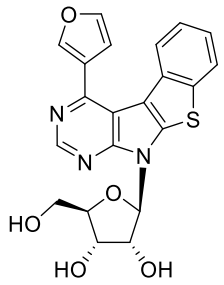
8.09 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.39 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.76 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 25.6 (CH_3); 62.2 (CH_2-5′); 70.7 (CH-3′); 71.7 (CH-2′); 85.7 (CH-4′); 87.0 (CH-1′); 113.3 (C-4a); 115.5 (C-4b); 121.9 (CH-5); 123.8 (CH-7); 124.3 (CH-8); 125.9 (CH-6); 131.1 (C-4c); 138.6 (C-8a); 139.6 (C-9a); 150.8 (CH-2); 155.1 (C-10a); 157.1 (C-4). MS (ESI): *m*/*z* (rel. %): 372 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_4N_3S$ $[M + H]^+$ 372.1012; found 372.1009.



4-(Furan-2-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6c)

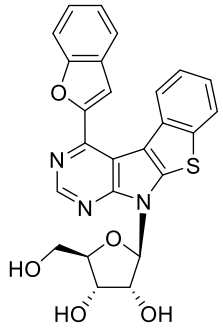
A solution of MeONa in MeOH (25 wt%, 340 µL) was added to a solution of protected nucleoside **5c** (220 mg, 0.30 mmol) in methanol/1,4-dioxane (30 mL, 2:1). The reaction mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10→90 %) to give a compound **6c** (90 mg, 71%) as a yellow solid. m.p. 164–167 °C. $[\alpha]_D$ = +30.8 (c = 0.299 in DMSO). IR (ATR): ν = 3247, 3099, 3052, 2928, 1554, 1468, 1436, 1308, 1242, 1029, 750 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.72, 3.75 (2 × dt, 2 × 1H, J_{gem} = 11.6, $J_{5',4'}$ = $J_{5',OH}$ = 5.3, H-5′); 4.03 (td, 1H, $J_{4',5'}$ = 5.3, $J_{4',3'}$ = 3.0, H-4′); 4.16 (ddd, 1H, $J_{3',2'}$ = 5.6, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 3.0, H-3′); 4.62 (ddd, 1H, $J_{2',1'}$ = 7.0, $J_{2',OH}$ = 6.2, $J_{2',3'}$ = 5.6, H-2′); 5.10 (t, 1H, $J_{OH,5'}$ = 5.3, OH-5′); 5.41 (d, 1H, $J_{OH,3'}$ = 4.7, OH-3′); 5.58 (d, 1H, $J_{OH,2'}$ = 6.2, OH-2′); 6.55 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 6.93 (dd, 1H, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.8, H-4-furyl); 7.28 (dd, 1H, $J_{3,4}$ = 3.4, $J_{3,5}$ = 0.9, H-3-furyl); 7.36 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.41 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.2, H-6); 7.58 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 8.09 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.17 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.9, H-5-furyl); 8.91 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.2 (CH_2-5′); 70.8 (CH-3′); 71.7 (CH-2′); 85.9

(CH-4′); 87.1 (CH-1′); 109.8 (C-4a); 113.3 (CH-4-furyl); 113.8 (CH-3-furyl); 114.9 (C-4b); 122.9 (CH-5); 123.8 (CH-7); 124.2 (CH-8); 125.5 (CH-6); 131.4 (C-4c); 138.5 (C-8a); 141.9 (C-9a); 146.01 (C-4); 146.02 (CH-5-furyl); 150.4 (CH-2); 151.7 (C-2-furyl); 156.9 (C-10a). MS (ESI): *m*/*z* (rel. %): 424 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{21}H_{18}O_5N_3S$ $[M + H]^+$ 424.0962; found 424.0960.



4-(Furan-3-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6d)

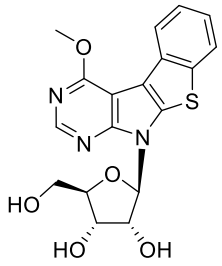
A solution of MeONa in MeOH (25 wt%, 150 μL) was added to a solution of protected nucleoside **5d** (168 mg, 0.23 mmol) in methanol (15 mL). The reaction mixture was stirred at 65 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10→90 %) to give a compound **6d** (81 mg, 84%) as a yellow solid. m.p. 229–232 °C. $[\alpha]_D$ = –7.1 (c = 0.238 in DMSO). IR (ATR): *v* = 3327, 3101, 2927, 1470, 1436, 1028, 992, 726, 715 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.71, 3.74 (2 × dd, 2 × 1H, J_{gem} = 11.6, $J_{5',4'}$ = 5.1, 2 H-5′); 4.03 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.0, H-4′); 4.16 (dd, 1H, $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 3.0, H-3′); 4.62 (dd, 1H, $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 5.4, H-2′); 4.91–5.68 (br, 3H, OH-2′,3′,5′); 6.52 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 7.00 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.16 (m, 1H, H-5); 7.31–7.37 (m, 2H, H-6,7); 8.00 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl); 8.05 (m, 1H, H-8); 8.15 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 8.91 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 62.4 (CH_2-5′); 70.9 (CH-3′); 72.0 (CH-2′); 86.0 (CH-4′); 87.3 (CH-1′); 112.1 (CH-4-furyl); 112.6 (C-4a); 115.0 (C-4b); 123.1 (CH-5); 124.0 (CH-7); 124.4 (CH-8); 125.6 (CH-6, C-3-furyl); 131.0 (C-4c); 138.9 (C-8a); 141.5 (C-9a); 144.57, 144.59 (CH-2,5-furyl); 150.2 (C-4); 150.8 (CH-2); 156.4 (C-10a). MS (ESI): *m*/*z* (rel. %): 424 (100) $[M + H]^+$, 446 (19) $[M + Na]^+$. HRMS (ESI) calcd. for $C_{21}H_{18}O_5N_3S$ $[M + H]^+$ 424.0962; found 424.0958.



4-(Benzofuran-2-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6e)

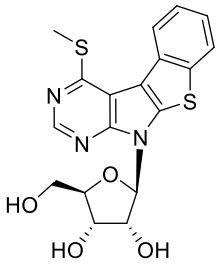
A solution of MeONa in MeOH (25 wt%, 75 μL) was added to a solution of protected nucleoside **5e** (91 mg, 0.12 mmol) in methanol (10 mL). The reaction mixture was stirred at 65 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 0–80 %) to give compound **6e** (44 mg, 81%) as a yellow solid. m.p. 145–149 °C. $[\alpha]_D$ = +32.0 (c = 0.425 in DMSO). IR (ATR): ν = 3344, 2918, 2850, 1540, 1464, 1438, 1092, 1043, 749 cm^{-1}. ^{1}H NMR (500.2 MHz, DMSO-d_6): 3.71–3.78 (m, 2H, H-5′); 4.05 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.1, H-4′); 4.17 (bm, 1H, H-3′); 4.62 (bm, 1H, H-2′); 5.22 (t, 1H, $J_{OH,5'}$ = 5.5, OH-5′); 5.46 (bd, 1H, $J_{OH,3'}$ = 4.6, OH-3′); 5.63 (bd, 1H, $J_{OH,2'}$ = 5.5, OH-2′); 6.55 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 7.23 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.2, H-6); 7.33 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.41 (ddd, 1H, $J_{5,4}$ = 8.2, $J_{5,6}$ = 7.2, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.49 (ddd, 1H, $J_{6,7}$ = 8.4, $J_{6,5}$ = 7.2, $J_{6,4}$ = 1.4, H-6-benzofuryl); 7.56 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5); 7.60 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,5}$ = $J_{7,4}$ = $J_{7,3}$ = 1.0, H-7-benzofuryl); 7.71 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 7.87 (ddd, 1H $J_{4,5}$ = 8.2, $J_{4,6}$ = 1.4, $J_{4,7}$ = 1.0, H-4-benzofuryl); 8.04 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.95 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 62.5 (CH_2-5′); 71.0 (CH-3′); 72.1 (CH-2′); 86.1 (CH-4′); 87.6 (CH-1′); 110.1 (CH-3-benzofuryl); 110.8 (C-4a); 112.2 (CH-7-benzofuryl); 115.2 (C-4b); 123.25 (CH-4-benzofuryl); 123.31 (CH-5); 124.4 (CH-7); 124.5 (CH-5-benzofuryl); 124.7 (CH-8); 125.6 (CH-6); 126.6 (C-3a-benzofuryl); 127.0 (CH-6-benzofuryl); 131.6 (C-4c); 138.9 (C-8a); 143.0 (C-9a); 145.8 (C-4); 150.6 (CH-2); 153.7 (C-2-benzofuryl); 155.4 (C-7a-benzofuryl); 157.3

(C-10a). MS (ESI): *m*/*z* (rel. %): 474 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{25}H_{20}O_5N_3S$ $[M + H]^+$ 474.1118; found 474.1110.



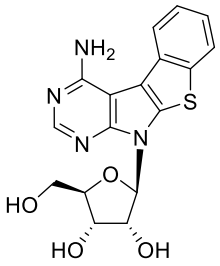
4-Methoxy-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6f)

Protected nucleoside **4** (400 mg, 0.57 mmol) was suspended in methanol (37 mL), and sodium methoxide (25 wt% in MeOH, 0.26 mL) was added. The reaction mixture was stirred at 65 °C for two days, and then solvent was evaporated and the crude material was purified by RP-HPFC (C18, gradient water/MeOH 10→80%) to give nucleoside **6f** (125 mg, 57%) as a white solid. m.p. 253–257 °C. $[\alpha]_D$ = –48.9 (c = 0.276 in DMSO). IR (ATR): ν = 3460, 3057, 3010, 2928, 1609, 1596, 1499, 1433, 1046, 1021, 750 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.68, 3.71 (2 × ddd, 2 × 1H, J_{gem} = 11.7, $J_{5',OH}$ = 5.5, $J_{5',4'}$ = 5.1, H-5′); 4.00 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.0, H-4′); 4.13 (bddd, 1H, $J_{3',2'}$ = 5.6, $J_{3',OH}$ = 3.4, $J_{3',4'}$ = 3.0, H-3′); 4.25 (s, 3H, CH_3O); 4.57 (bddd, 1H, $J_{2',1'}$ = 7.0, $J_{2',3'}$ = 5.6, $J_{2',OH}$ = 3.9, H-2′); 5.08 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.41 (bd, 1H, $J_{OH,3'}$ = 3.4, OH-3′); 5.57 (d, 1H, $J_{OH,2'}$ = 3.9, OH-2′); 6.42 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 7.37 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.3, H-7); 7.52 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.1, H-6); 8.06 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.6, H-8); 8.36 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.3, $J_{5,8}$ = 0.6, H-5); 8.57 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 54.5 (CH_3O); 62.3 (CH_2-5′); 70.8 (CH-3′); 71.9 (CH-2′); 85.7 (CH-4′); 87.2 (CH-1′); 100.2 (C-4a); 115.2 (C-4b); 122.2 (CH-5); 123.9 (CH-7); 124.0 (CH-8); 125.7 (CH-6); 130.9 (C-4c); 136.9 (C-9a); 138.7 (C-8a); 151.1 (CH-2); 156.6 (C-10a); 161.5 (C-4). MS (ESI): *m*/*z* (rel. %): 388 (100) $[M + H]^+$, 410 (74) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_5N_3S$ $[M + H]^+$ 388.0962; found 388.0957.



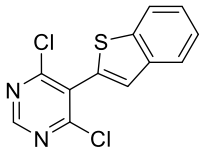
4-(Methylsulfanyl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6g)

Protected nucleoside **4** (400 mg, 0.57 mmol) was suspended in 1,4-dioxane (37 mL), and sodium thiomethoxide (318 mg, 4.54 mmol) was added. The reaction mixture was stirred at 100 °C for four days, and then solvent was evaporated and the crude material was purified by RP-HPFC (C18, gradient water/MeOH 10→80 %) to give nucleoside **6g** (155 mg, 68%) as a white solid. m.p. 108–113 °C. $[\alpha]_D$ = –33.5 (c = 0.277 in DMSO). IR (ATR): ν = 3282, 3158, 3081, 2921, 2851, 2687, 1542, 1041, 1024, 746 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 2.80 (s, 3H, CH_3S); 3.70, 3.73 (2 × dt, 2 × 1H, J_{gem} = 11.7, $J_{5',OH} = J_{5',4'}$ = 5.1, H-5′); 4.01 (td, 1H, $J_{4',5'}$ = 5.1, $J_{4',3'}$ = 3.1, H-4′); 4.14 (ddd, 1H, $J_{3',2'}$ = 5.4, $J_{3',OH}$ = 4.8, $J_{3',4'}$ = 3.1, H-3′); 4.57 (ddd, 1H, $J_{2',1'}$ = 6.9, $J_{2',OH}$ = 6.2, $J_{2',3'}$ = 5.4, H-2′); 5.04 (t, 1H, $J_{OH,5'}$ = 5.1, OH-5′); 5.33 (bd, 1H, $J_{OH,3'}$ = 4.8, OH-3′); 5.49 (d, 1H, $J_{OH,2'}$ = 6.2, OH-2′); 6.45 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 7.39 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.2, H-7); 7.55 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.2, H-6); 8.08 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.75 (s, 1H, H-2); 8.83 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (125.8 MHz, DMSO-d_6): 12.7 (CH_3S); 62.2 (CH_2-5′); 70.7 (CH-3′); 71.7 (CH-2′); 85.7 (CH-4′); 87.1 (CH-1′); 111.1 (C-4a); 115.1 (C-4b); 122.4 (CH-5); 123.9 (CH-7); 124.2 (CH-8); 125.6 (CH-6); 130.5 (C-4c); 138.7 (C-8a); 138.8 (C-9a); 150.4 (CH-2); 153.4 (C-10a); 158.9 (C-4). MS (ESI): *m*/*z* (rel. %): 404 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_4N_3S_2$ $[M + H]^+$ 404.0733; found 404.0735.



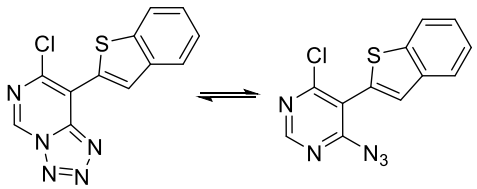
4-Amino-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (6h)

Protected nucleoside **4** (420 mg, 0.60 mmol) was dissolved in 1,4-dioxane (2 mL) and ammonia solution in water (30%, 6 mL) was added. The mixture was stirred and heated to 120 °C in a pressure tube for 24 h. After that, the solvent was removed under vacuum and the crude product was purified by RP-HPFC (C18, gradient water/MeOH 10→80 %) to give **6h** (113 mg, 51%) as a white solid. m.p. 280–282 °C. $[\alpha]_D$ = –37.3 (c = 0.316 in DMSO). IR (ATR): ν = 3501, 3350, 3213, 3081, 2941, 2783, 1634, 1480, 1045, 743 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 3.66, 3.70 (2 × ddd, 2 × 1H, J_{gem} = 11.5, $J_{5',OH}$ = 5.7, $J_{5',4'}$ = 5.0, H-5′); 3.97 (td, 1H, $J_{4',5'}$ = 5.0, $J_{4',3'}$ = 2.9, H-4′); 4.11 (ddd, 1H, $J_{3',2'}$ = 5.5, $J_{3',OH}$ = 4.7, $J_{3',4'}$ = 2.9, H-3′); 4.57 (ddd, 1H, $J_{2',1'}$ = 7.0, $J_{2',OH}$ = 6.4, $J_{2',3'}$ = 5.5, H-2′); 5.16 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.33 (d, 1H, $J_{OH,3'}$ = 4.7, OH-4′); 5.49 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2′); 6.33 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 6.87 (s, 2H, NH_2); 7.33 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.2, $J_{7,5}$ = 1.1, H-7); 7.47 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.2, $J_{6,8}$ = 1.1, H-6); 8.03 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.1, $J_{8,5}$ = 0.6, H-8); 8.23 (s, 1H, H-2); 8.32 (ddd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.1, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (125.7 MHz, DMSO-d_6): 62.4 (CH_2-5′); 70.9 (CH-3′); 71.7 (CH-2′); 85.6 (CH-4′); 87.2 (CH-1′); 98.7 (C-4a); 115.5 (C-4b); 122.4 (CH-5); 123.3 (CH-7); 124.1 (CH-8); 125.4 (CH-6); 131.1 (C-4c); 135.5 (C-9a); 138.3 (C-8a); 151.8 (CH-2); 155.7 (C-10a); 156.9 (C-4). MS (ESI): *m*/*z* (rel. %): 373 (100) $[M + H]^+$, 395 (11) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{17}H_{17}O_4N_4S$ $[M + H]^+$ 373.0965; found 373.0962.



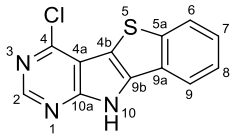
5-(Benzo[*b*]thiophen-2-yl)-4,6-dichloropyrimidine (7)

TMPMgCl·LiCl (1 M, 18.4 mL, 18.4 mmol) was added to $ZnCl_2$ (1.26 g, 9.26 mmol) under an argon atmosphere, the mixture was stirred at r.t. for 12 h to give a tetramethylpiperidinylzinc complex. 4,6-Dichloropyrimidine (2.3 g, 15.4 mmol) was dissolved in THF (12 mL) and added dropwise to an ice-cooled solution of tetramethylpiperidinylzinc complex. The mixture was stirred at 0 °C for 1 h and then at r.t. for 1 h to give a zincated pyrimidine. A solution of 2-iodobenzothiophene (4.0 g, 15.4 mmol) and $Pd(PPh_3)_4$ (1.78 g, 1.54 mmol) in THF (12 mL), which was prestirred at r.t. for 20 min, was added to the zincated pyrimidine, and stirred at 65 °C for 16 h. After that, solvent was evaporated under reduced pressure. Purification by HPFC (SiO_2, hexane/EtOAc 0→1%) gave **7** (2.1 g, 48%) as a white solid. m.p. 97–100 °C. IR (ATR): ν = 3059, 3026, 2933, 2857, 1736, 1497, 1455, 1365, 1235, 1220, 932, 799 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 7.38 (d, 1H, $J_{3',7'}$ = 0.7, H-3′); 7.41–7.46 (m, 2H, H-5′,6′); 7.87 (s, 1H, H-4′); 7.91 (m, 1H, H-7′); 8.82 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 122.3 (CH-7′); 124.4 (CH-4′); 124.8 (CH-5′); 125.4 (CH-6′); 126.7 (CH-3′); 128.3 (C-5); 132.4 (C-2′); 139.1 (C-3′a); 140.9 (C-7′a); 157.4 (CH-2); 162.5 (C-4,6). MS (EI): *m/z* (rel. %): 281 (15) $[M + H]^+$. HRMS (EI): calcd. for $C_{12}H_6N_2SCl_2$ $[M]^+$ 279.9629; found 279.9627.



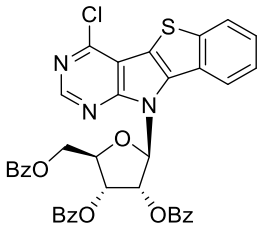
8-(Benzo[*b*]thiophen-2-yl)-7-chlorotetrazolo[1,5-*c*]pyrimidine (8a), 4-azido-5-(benzo[*b*]thiophen-2-yl)-6-chloropyrimidine (8b): Compound **8** was synthesized as described for compound **2** from derivative **7** (1.01 g, 3.59 mmol). Compound **8** (930 mg, 90%) was obtained as a light yellow solid and according to NMR spectra in DMSO it was present as a mixture of tautomers **8a** and **8b** in 6:1 ratio.

m.p. 145–147 °C. IR (ATR): ν = 3085, 2931, 2856, 2151, 2120, 1736, 1584, 1462, 1248, 990, 746 cm^{-1}. ^{1}H NMR for tautomer **8a** (500.0 MHz, DMSO-d_6): 7.48–7.55 (m, 2H, H-5′,6′); 8.10–8.15 (m, 2H, H-4′,7′); 8.71 (d, 1H, $J_{3',7'}$ = 0.8, H-3′); 10.22 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 116.6 (C-5); 122.5 (CH-7′); 125.1 (CH-5′); 125.3 (CH-4′); 126.5 (CH-6′); 130.51 (C-2′); 130.55 (CH-3′); 138.4 (CH-2); 138.6 (C-3′a); 140.4 (C-7′a); 144.1, 150.6 (C-4,6). ^{1}H NMR for tautomer **8b** (500.0 MHz, $CDCl_3$): 7.37–7.42 (m, 3H, H-3′,5′,6′); 7.85 (m, 1H, H-4′); 7.89 (m, 1H, H-7′); 8.71 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 117.5 (C-5); 122.2 (CH-7′); 124.3 (CH-4′); 124.7 (CH-5′); 125.3 (CH-6′); 127.0 (CH-3′); 131.0 (C-2′); 139.1 (C-3′a); 140.9 (C-7′a); 157.1 (CH-2); 161.4, 162.6 (C-4,6). MS (APCI): m/z (rel. %): 288 (18) $[M + H]^+$. HRMS (APCI): calcd. for $C_{12}H_7N_5ClS$ $[M + H]^+$ 288.01052; found 288.01072.



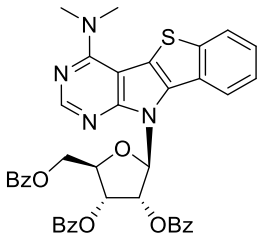
4-Chloro-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (9):

Tetrazole **8a** (100 mg, 0.35 mmol) and 1,4-dibromobenzene (3.0 g) were charged in a pressure tube and stirred at 180 °C for 30 min. The mixture was purified by HPFC (SiO_2, hexane/EtOAc, 10→50%) to give the nucleobase **9** (54 mg, 60%) as a brown solid. m.p. 302–307 °C. IR (ATR): ν = 3056, 2963, 2910, 2863, 2795, 2736, 1553, 1257, 1229, 752, 719 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.51 (ddd, 1H, $J_{7,6}$ = 8.4, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.4, H-7); 7.57 (ddd, 1H, $J_{8,9}$ = 8.1, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.13 (m, 1H, H-6); 8.15 (ddd, 1H, $J_{9,8}$ = 8.1, $J_{9,7}$ = 1.4, $J_{9,6}$ = 0.9, H-9); 8.71 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 110.6 (C-4b); 112.3 (C-4a); 121.4 (CH-9); 124.8 (CH-6); 125.4 (CH-8); 125.8 (C-9a); 126.2 (CH-7); 137.9 (C-9b); 142.8 (C-5a); 148.9 (C-4); 151.4 (CH-2); 155.4 (C-10a). MS (CI): m/z (rel. %): 260 (100) $[M + H]^+$. HRMS (CI): calcd. for $C_{12}H_7N_3SCl$ $[M + H]^+$ 260.0049; found 260.0046.



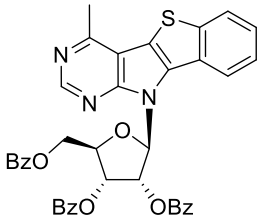
4-Chloro-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (10)

Nucleobase **9** (100 mg, 0.38 mmol) was dissolved in MeCN (12 mL), and BSA (94 μL, 0.38 mmol) was added. The mixture was heated to 60 °C for 30 min, and then TMSOTf (173 μL, 0.96 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (388 mg, 0.77 mmol) were added. The mixture was stirred at 60 °C overnight. The mixture was extracted with EtOAc and water, and the 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 (SiO_2, hexane/EtOAc, 15→35%) to give nucleoside **10** (140 mg, 52%) as a white solid. m.p. 153–156 °C. IR (ATR): ν = 1716, 1601, 1315, 1249, 1094, 1067, 1023, 704, 684 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.67 (dd, 1H, J_{gem} = 11.9, $J_{5'b,4'}$ = 3.8, H-5′b); 4.91 (ddd, 1H, $J_{4',3'}$ = 7.2, $J_{4',5'}$ = 3.8, 3.3, H-4′); 4.94 (dd, 1H, J_{gem} = 11.9, $J_{5'a,4'}$ = 3.3, H-5′a); 6.61 (dd, 1H, $J_{3',4'}$ = 7.2, $J_{3',2'}$ = 6.1, H-3′); 6.95 (d, 1H, $J_{1',2'}$ = 3.8, H-1′); 7.06 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 3.8, H-2′); 7.32–7.41 (m, 6H, H-*m*-Bz); 7.41–7.46 (m, 2H, H-7,8); 7.51–7.60 (m, 3H, H-*p*-Bz); 7.84–7.87 (m, 2H, H-*o*-Bz); 7.93–7.98 (m, 3H, H-6, H-*o*-Bz); 8.00–8.03 (m, 2H, H-*o*-Bz); 8.18 (m, 1H, H-9); 8.66 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 62.9 (CH_2-5′); 71.0 (CH-3′); 73.6 (CH-2′); 80.2 (CH-4′); 88.9 (CH-1′); 113.8 (C-4a); 114.1 (C-4b); 121.2 (CH-9); 124.6 (CH-6); 125.2, 125.6 (CH-7,8); 125.7 (C-9a); 128.29, 128.50, 128.51 (CH-*m*-Bz); 128.7, 128.9, 129.3 (C-*i*-Bz); 129.5, 129.79, 129.80 (CH-*o*-Bz); 133.2, 133.6, 133.7 (CH-*p*-Bz); 137.0 (C-9b); 143.9 (C-5a); 150.8 (C-4); 151.3 (CH-2); 154.7 (C-10a); 165.2, 165.3, 166.0 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 704 (44) $[M + H]^+$. HRMS (ESI): calcd. for $C_{38}H_{27}ClN_3O_7S$ $[M + H]^+$ 704.1253; found 704.1248.



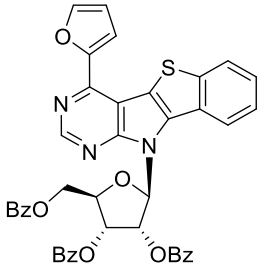
4-*N,N*-Dimethylamino-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (11a)

Dimethylamine (2 M in THF, 1 mL) was added to a solution of nucleoside **10** (450 mg, 0.64 mmol) in a mixture of propan-2-ol and DCM (20 mL, 1:1) and the reaction mixture was stirred at 40 °C for 24 h. The volatiles were removed under reduced pressure and the crude product was purified by HPFC (SiO_2, DCM/EtOAc 5→10%) to give nucleoside **11a** (381 mg, 84%) as a white solid. m.p. 218–219 °C. IR (ATR): ν = 1728, 1717, 1702, 1566, 1544, 1273, 1251, 1120, 709 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 3.49 (s, 6H, $(CH_3)_2N$); 4.73 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.8, H-5′b); 4.85 (ddd, 1H, $J_{4',3'}$ = 6.9, $J_{4',5'}$ = 4.8, 3.4, H-4′); 4.90 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.4, H-5′a); 6.65 (dd, 1H, $J_{3',4'}$ = 6.9, $J_{3',2'}$ = 6.4, H-3′); 6.94 (dd, 1H, $J_{2',3'}$ = 6.4, $J_{2',1'}$ = 4.0, H-2′); 7.01 (d, 1H, $J_{1',2'}$ = 4.0, H-1′); 7.28–7.38 (m, 8H, H-7,8, H-*m*-Bz); 7.49–7.56 (m, 3H, H-*p*-Bz); 7.81 (m, 1H, H-6); 7.94–7.99 (m, 6H, H-*o*-Bz); 8.06 (m, 1H, H-9); 8.41 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 39.5 ($(CH_3)_2N$); 63.6 (CH_2-5′); 71.0 (CH-3′); 73.8 (CH-2′); 79.8 (CH-4′); 88.5 (CH-1′); 99.9 (C-4a); 116.0 (C-4b); 120.2 (CH-9); 123.5 (CH-6); 124.0 (CH-7); 124.9 (CH-7); 126.7 (C-9a); 128.27, 128.38, 128.41 (CH-*m*-Bz); 128.97, 129.01, 129.6 (C-*i*-Bz); 129.76, 129.78 (CH-*o*-Bz); 132.5 (C-9b); 133.0, 133.4, 133.5 (CH-*p*-Bz); 141.2 (C-5a); 151.8 (CH-2); 155.4 (C-10a); 157.1 (C-4); 165.2, 165.3, 166.2 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 713 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{40}H_{33}O_7N_4S$ $[M + H]^+$ 713.2064; found 713.2062.



4-Methyl-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (11b)

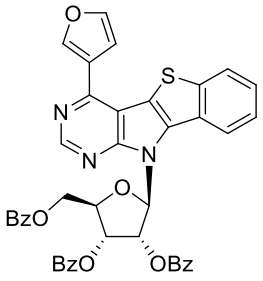
Trimethylaluminum (2 M in toluene, 703 µL, 1.41 mmol) was added to a solution of nucleoside **10** (450 mg, 0.64 mmol) and $Pd(PPh_3)_4$ (111 mg, 96 µmol) in THF (15 mL) and the reaction mixture was stirred at 65 °C for 20 h. The volatiles were removed under reduced pressure and the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 20→33%) to give **11b** (260 mg, 59%) as a light yellow solid. m.p. 153–155 °C. IR (ATR): ν = 1720, 1450, 1262, 1177, 1093, 1067, 1024, 1000, 973, 705 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 2.85 (s, 3H, CH_3); 4.69 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.2, H-5′b); 4.89 (ddd, 1H, $J_{4',3'}$ = 6.8, $J_{4',5'}$ = 4.2, 3.3, H-4′); 4.92 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.3, H-5′a); 6.64 (dd, 1H, $J_{3',4'}$ = 6.8, $J_{3',2'}$ = 6.2, H-3′); 6.99 (d, 1H, $J_{1',2'}$ = 3.9, H-1′); 7.04 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.9, H-2′); 7.31–7.43 (m, 8H, H-7,8, H-*m*-Bz); 7.50–7.58 (m, 3H, H-*p*-Bz); 7.89–7.92 (m, 2H, H-*o*-Bz); 7.94–7.96 (m, 3H, H-6, H-*o*-Bz); 7.99–8.01 (m, 2H, H-*o*-Bz); 8.17 (m, 1H, H-9); 8.82 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 22.3 (CH_3); 63.2 (CH_2-5′); 71.0 (CH-3′); 73.7 (CH-2′); 80.0 (CH-4′); 88.6 (CH-1′); 114.0 (C-4a); 114.8 (C-4b); 121.1 (CH-9); 124.5 (CH-6); 125.0 (CH-7); 125.1 (CH-7); 126.2 (C-9a); 128.3, 128.46, 128.47 (CH-*m*-Bz); 128.8, 128.9, 129.4 (C-*i*-Bz); 129.6, 129.78, 129.79 (CH-*o*-Bz); 133.1, 133.5, 133.6 (CH-*p*-Bz); 135.8 (C-9b); 143.4 (C-5a); 152.0 (CH-2); 154.6 (C-10a); 158.1 (C-4); 165.2, 165.3, 166.1 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 684 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{39}H_{30}O_7N_3S$ $[M + H]^+$ 684.1799; found 684.1793.



4-(Furan-2-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (11c)

Nucleoside **10** (400 mg, 0.57 mmol), 2-furyl(tributyl)stannane (0.22 mL, 0.68 mmol) and $PdCl_2(PPh_3)_2$ (60 mg, 85 µmol) were dissolved in anhydrous DMF (30 mL) and

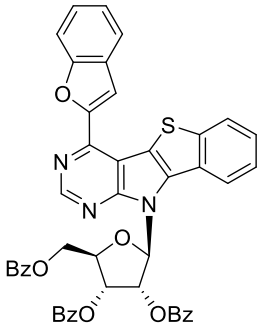
heated to 100 °C for 30 min. The volatiles were removed in vacuo and the residue was loaded onto a silica column that contained 15% KF. The column was washed with hexane (2 L), then with a gradient of EtOAc in hexane (0→60% EtOAc). Desired product **11c** (376 mg, 90%) was obtained as a yellow solid. m.p. 223–225 °C. IR (ATR): ν = 2923, 2853, 1716, 1703, 1600, 1555, 1269, 1253, 1118, 703 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.70 (m, 1H, H-5′b); 4.87–4.94 (m, 2H, H-4′,5′a); 6.66 (dd, 1H, $J_{3',4'}$ = 6.8, $J_{3',2'}$ = 6.2, H-3′); 6.72 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.03 (d, 1H, $J_{1',2'}$ = 3.9, H-1′); 7.09 (dd, 1H, $J_{2',3'}$ = 6.2, $J_{2',1'}$ = 3.9, H-2′); 7.28–7.33 (m, 2H, H-*m*-Bz); 7.35–7.58 (m, 10H, H-7,8, H-3-furyl, H-m,*p*-Bz); 7.87–7.91 (m, 3H, H-5-furyl, H-*o*-Bz); 7.93–7.98 (m, 3H, H-6, H-*o*-Bz); 7.99–8.02 (m, 2H, H-*o*-Bz); 8.17 (m, 1H, H-9); 8.87 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.2 (CH_2-5′); 71.0 (CH-3′); 73.7 (CH-2′); 80.0 (CH-4′); 88.6 (CH-1′); 109.1 (C-4a); 112.7 (CH-4-furyl); 113.0 (CH-3-furyl); 116.0 (C-4b); 120.9 (CH-9); 124.1 (CH-6); 124.8 (CH-8); 125.2 (CH-7); 126.2 (C-9a); 128.2, 128.45, 128.47 (CH-*m*-Bz); 128.8, 128.9, 129.4 (C-*i*-Bz); 129.6, 129.8 (CH-*o*-Bz); 133.1, 133.5, 133.6 (CH-*p*-Bz); 136.9 (C-9b); 143.7 (C-5a); 145.0 (CH-5-furyl); 146.1 (C-4); 151.8 (CH-2); 152.7 (C-2-furyl); 156.1 (C-10a); 165.2, 165.3, 166.1 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 736 (100) $[M + H]^+$, 758 (9) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{42}H_{30}O_8N_3S$ $[M + H]^+$ 736.1748; found 736.1738.



4-(Furan-3-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (11d)

Protected nucleoside **10** (306 mg, 0.43 mmol), furan-3-boronic acid (88 mg, 0.79 mmol), K_2CO_3 (157 mg, 1.1 mmol), and $Pd(PPh_3)_4$ (100 mg, 0.09 mmol) were dissolved in toluene (20 mL) and heated to 100 °C overnight. Then, the reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with saturated NH_4Cl, and then with water and was dried over $MgSO_4$. After

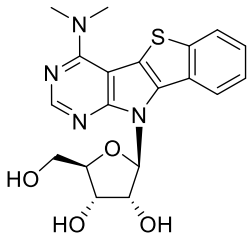
evaporation of solvent, the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 0→25%). Product **11d** (163 mg, 51%) was obtained as a yellow solid. m.p. 204–208 °C. IR (ATR): ν = 1735, 1721, 1705, 1267, 1250, 1120, 1093, 702 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.71 (m, 1H, H-5′b); 4.89–4.96 (m, 2H, H-4′,5′a); 6.66 (dd, 1H, $J_{3',4'}$ = 6.8, $J_{3',2'}$ = 6.1, H-3′); 7.03 (d, 1H, $J_{1',2'}$ = 3.9, H-1′); 7.07 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 3.9, H-2′); 7.24 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.7, H-4-furyl); 7.30–7.45 (m, 8H, H-7,8, H-*m*-Bz); 7.48, 7.55, 7.57 (3 × m, 3 × 1H, H-*p*-Bz); 7.66 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl); 7.89–7.93 (m, 2H, H-6, H-*o*-Bz); 7.94–7.97, 8.00–8.03 (2 × m, 2 × 2H, H-*o*-Bz); 8.17 (m, 1H, H-9); 8.43 (bdd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.7, H-2-furyl); 8.92 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.2 (CH_2-5′); 71.0 (CH-3′); 73.7 (CH-2′); 80.0 (CH-4′); 88.6 (CH-1′); 109.7 (CH-4-furyl); 111.5 (C-4a); 114.3 (C-4b); 121.1 (CH-9); 124.2 (CH-6); 124.9 (C-3-furyl); 125.2 (CH-8); 125.5 (CH-7); 126.0 (C-9a); 128.3, 128.47, 128.48 (CH-*m*-Bz); 128.8, 128.9, 129.4 (C-*i*-Bz); 129.6, 129.79, 129.80 (CH-*o*-Bz); 133.1, 133.5, 133.6 (CH-*p*-Bz); 136.9 (C-9b); 142.5 (C-5a); 143.7 (CH-2-furyl); 144.3 (CH-5-furyl); 150.3 (C-4); 151.8 (CH-2); 155.6 (C-10a); 165.3, 165.3, 166.1 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 736 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{42}H_{30}O_8N_3S$ $[M + H]^+$ 736.1748; found 736.1736.



4-(Benzofuran-2-yl)-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (11e)

Protected nucleoside **10** (450 mg, 0.64 mmol), benzofurane-2-boronic acid (155 mg, 0.97 mmol), K_2CO_3 (176 mg, 1.28 mmol), and $Pd(PPh_3)_4$ (147 mg, 0.13 mmol) were dissolved in toluene (18 mL) and heated to 100 °C for 24 h. Then, the reaction mixture was diluted with water and extracted with chloroform. The organic layer was washed with saturated NH_4Cl, and then with water and was dried over $MgSO_4$. After

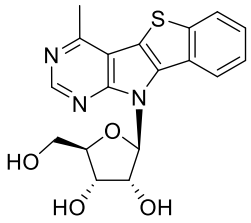
evaporation of solvent, the crude product was purified by column chromatography (SiO_2, hexane/EtOAc, 0−40% EtOAc). Compound **11e** (126 mg, 25%) was obtained as a yellow solid. m.p. 228–230 °C. IR (ATR): ν = 2922, 2853, 1723, 1450, 1263, 1107, 1094, 1068, 1025, 749, 706, 686, 666 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.72 (m, 1H, H-5′b); 4.89–4.97 (m, 2H, H-4′,5′a); 6.69 (dd, 1H, $J_{3',4'}$ = 6.7, $J_{3',2'}$ = 6.1, H-3′); 7.03 (d, 1H, $J_{1',2'}$ = 3.8, H-1′); 7.15 (dd, 1H, $J_{2',3'}$ = 6.1, $J_{2',1'}$ = 3.8, H-2′); 7.27–7.31 (m, 2H, H-m-Bz); 7.34 (ddd, 1H, $J_{5,4}$ = 8.0, $J_{5,6}$ = 7.2, $J_{5,7}$ =1.0, H-5-benzofuryl); 7.36–7.43 (m, 7H, H-7,8, H-*m*,*p*-Bz); 7.46 (ddd, 1H, $J_{6,7}$ = 8.4, $J_{6,5}$ = 7.2, $J_{6,4}$ =1.3, H-6-benzofuryl); 7.53–7.59 (m, 2H, H-*p*-Bz); 7.74 (ddd, 1H, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.3, $J_{4,7}$ =1.0, H-4-benzofuryl); 7.81 (d, 1H, $J_{3,7}$ =1.0, H-3-benzofuryl); 7.86 (dq, 1H, $J_{7,6}$ = 8.4, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ =1.0, H-7-benzofuryl); 7.87–7.90 (m, 2H, H-*o*-Bz); 7.95–8.03 (m, 5H, H-6, H-*o*-Bz); 8.17 (m, 1H, H-9); 8.91 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, $CDCl_3$): 63.2 (CH_2-5′); 71.1 (CH-3′); 73.8 (CH-2′); 80.0 (CH-4′); 88.6 (CH-1′); 108.6 (CH-3-benzofuryl); 110.2 (C-4a); 111.8 (CH-7-benzofuryl); 116.0 (C-4b); 121.0 (CH-9); 122.3 (CH-4-benzofuryl); 123.7 (CH-5-benzofuryl); 124.0 (CH-6); 124.8 (CH-8); 125.3 (CH-7); 126.1 (C-9a); 126.4 (CH-6-benzofuryl); 128.1 (C-3a-benzofuryl); 128.2, 128.45, 128.49 (CH-*m*-Bz); 128.8, 128.9, 129.3 (C-*i*-Bz); 129.6, 129.8 (CH-*o*-Bz); 133.0, 133.5, 133.6 (CH-*p*-Bz); 137.3 (C-9b); 144.0 (C-5a); 146.0 (C-4); 151.7 (CH-2); 153.9 (C-2-benzofuryl); 155.8 (C-7a-benzofuryl); 156.2 (C-10a); 165.2, 165.3, 166.1 (CO-Bz). MS (ESI): *m*/*z* (rel. %): 786 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{46}H_{32}O_8N_3S$ $[M + H]^+$ 786.1905; found 786.1896.



4-*N*,*N*-Dimethylamino-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12a)

A solution of MeONa in MeOH (25 wt%, 415 µL) was added to a solution of protected nucleoside **11a** (324 mg, 0.45 mmol) in methanol/1,4-dioxane (30 mL, 1:1).

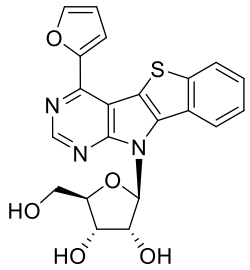
The reaction mixture was stirred at 60 °C for 40 h. Solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (SiO_2, DCM/MeOH 3→15%) to give the desired product **12a** (142 mg, 78%) as a white solid. m.p. 213–216 °C. $[\alpha]_D$ = –16.8 (c = 0.303 in DMSO). IR (ATR): ν = 3345, 2927, 2892, 1572, 1428, 1403, 1334, 1116, 1034, 891, 748, 720 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.47 (s, 6H, CH_3N); 3.68 (bd, 1H, J_{gem} = 12.0, H-5′b); 3.79 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.3, H-5′a); 4.02 (m, 1H, H-4′); 4.29 (bm, 1H, H-3′); 4.91 (dt, 1H, $J_{2',1'}$ = 7.0, $J_{2',OH}$ = 6.5, $J_{2',3'}$ = 5.3, H-2′); 5.21 (bd, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 5.29 (bd, 1H, $J_{OH,2'}$ = 6.5, OH-2′); 5.47 (bs, 1H, OH-5′); 6.55 (d, 1H, $J_{1',2'}$ = 7.0, H-1′); 7.40 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.2, H-7); 7.48 (ddd, 1H, $J_{8,9}$ = 8.2, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.02 (m, 1H, H-6); 8.23 (m, 1H, H-9); 8.28 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 39.3 (CH_3N); 61.8 (CH_2-5′); 69.7 (CH-3′); 71.6 (CH-2′); 86.1 (CH-4′); 89.1 (CH-1′); 99.0 (C-4a); 114.9 (C-4b); 121.7 (CH-9); 123.9 (CH-6); 124.5 (CH-7); 125.2 (CH-8); 126.5 (C-9a); 132.8 (C-9b); 140.5 (C-5a); 151.3 (CH-2); 155.2 (C-10a); 156.8 (C-4). MS (ESI): *m*/*z* (rel. %): 401 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{19}H_{21}O_4N_4S$ $[M + H]^+$ 401.1278; found 401.1274.



4-Methyl-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12b)

A solution of MeONa in MeOH (25 wt%, 430 µL) was added to a solution of protected nucleoside **11b** (215 mg, 0.32 mmol) in methanol/1,4-dioxane (24 mL, 1:1). The reaction mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10–80%) to give compound **12b** (90 mg, 77%) as a white solid. m.p. 247–250 °C. $[\alpha]_D$ = –14.1 (c = 0.290 in DMSO). IR (ATR): ν = 3450, 3314, 3051, 2947, 2923, 2873, 1586, 1433, 1399, 1112, 1086, 751, 721 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6):

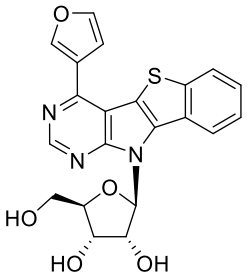
2.81 (s, 3H, CH_3); 3.69 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.3, $J_{5'b,4'}$ = 4.6, H-5′b); 3.80 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.7, H-5′a); 4.04 (ddd, 1H, $J_{4',5'}$ = 4.6, 3.7, $J_{4',3'}$ = 4.0, H-4′); 4.34 (ddd, 1H, $J_{3',2'}$ = 6.4, $J_{3',OH}$ = 5.6, $J_{3',4'}$ = 4.0, H-3′); 4.96 (dt, 1H, $J_{2',1'}$ = 6.9, $J_{2',OH}$ = $J_{2',3'}$ = 6.4, H-2′); 5.23 (dd, 1H, $J_{OH,5'}$ = 6.3, 5.0, OH-5′); 5.29 (d, 1H, $J_{OH,3'}$ = 5.6, OH-3′); 5.38 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2′); 6.56 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 7.50 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.2, H-7); 7.56 (ddd, 1H, $J_{8,9}$ = 8.2, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.17 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.2, $J_{6,9}$ = 0.7, H-6); 8.39 (ddd, 1H, $J_{9,8}$ = 8.2, $J_{9,7}$ = 1.2, $J_{9,6}$ = 0.7, H-9); 8.81 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 22.1 (CH_3); 61.7 (CH_2-5′); 69.6 (CH-3′); 71.8 (CH-2′); 86.1 (CH-4′); 88.9 (CH-1′); 113.1 (C-4a); 113.9 (C-4b); 122.7 (CH-9); 124.9 (CH-6); 125.5, 125.6 (CH-7,8); 126.1 (C-9a); 136.1 (C-9b); 142.7 (C-5a); 151.9 (CH-2); 154.6 (C-10a); 158.2 (C-4). MS (ESI): *m*/*z* (rel. %): 372 (100) $[M + H]^+$, 394 (14) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_4N_3S$ $[M + H]^+$ 372.1012; found 372.1008.



4-(Furan-2-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12c)

A solution of MeONa in MeOH (25 wt%, 500 µL) was added to a solution of protected nucleoside **11c** (300 mg, 0.41 mmol) in methanol/1,4-dioxane (30 mL, 2:1). The reaction mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10→90%) to give compound **12c** (125 mg, 73%) as a yellow solid. m.p. 248–251 °C. $[\alpha]_D$ = –5.5 (c = 0.253 in DMSO). IR (ATR): ν = 3343, 3068, 2988, 2968, 2942, 2922, 1598, 1552, 1416, 1226, 1074, 735 cm^{-1}. ^{1}H NMR (500.2 MHz, DMSO-d_6): 3.71 (bdt, 1H, J_{gem} = 11.7, $J_{5'b,4'}$ = $J_{5'b,OH}$ = 4.6, H-5′b); 3.82 (bdt, 1H, J_{gem} = 11.7, $J_{5'a,4'}$ = $J_{5'a,OH}$ = 3.3, H-5′b); 4.06 (ddd, 1H, $J_{4',5'}$ = 4.6, 3.3, $J_{4',3'}$ = 4.4, H-4′); 4.29 (dd, 1H, $J_{3',2'}$ = 6.3, $J_{3',4'}$ = 4.4, H-3′); 4.99 (dd, 1H, $J_{2',1'}$ = 6.8, $J_{2',3'}$ = 6.3, H-2′); 5.19 (bdd, 1H, $J_{OH,5'}$ = 4.6,

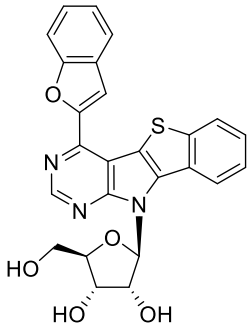
3.3, OH-5′); 5.33 (bs, 1H, OH-3′); 5.43 (bs, 1H, OH-2′); 6.62 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 6.91 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.7, H-4-furyl); 7.51–7.58 (m, 3H, H-7, 8, H-3-furyl); 8.16 (m, 1H, H-6); 8.28 (dd, 1H, $J_{5,4}$ = 1.7, $J_{5,3}$ = 0.8, H-5-furyl); 8.40 (m, 1H, H-9); 8.91 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 61.6 (CH_2-5′); 69.4 (CH-3′); 71.7 (CH-2′); 86.0 (CH-4′); 88.9 (CH-1′); 107.9 (C-4a); 113.4 (CH-4-furyl); 113.6 (CH-3-furyl); 114.6 (C-4b); 122.7 (CH-9); 124.3 (CH-6); 125.2 (CH-8); 125.8 (CH-7); 126.1 (C-9a); 137.3 (C-9b); 142.8 (C-5a); 145.4 (C-4); 146.7 (CH-5-furyl); 151.7 (CH-2); 152.2 (C-2-furyl); 156.2 (C-10a). MS (ESI): *m*/*z* (rel. %): 424 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{21}H_{18}O_5N_3S$ $[M + H]^+$ 424.0962; found 424.0959.



4-(Furan-3-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12d)

A solution of MeONa in MeOH (25 wt%, 180 μL) was added to a solution of protected nucleoside **11d** (196 mg, 0.27 mmol) in methanol (30 mL). The reaction mixture was stirred at 60 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 10–90%) to give compound **12d** (57 mg, 51%) as a yellow solid. m.p. 214–218 °C. $[\alpha]_D$ = –5.4 (c = 0.279 in DMSO). IR (ATR): ν = 3355, 3132, 3069, 2945, 2922, 2874, 1573, 1554, 1376, 1110, 873 cm^{-1}. ^{1}H NMR (500.2 MHz, DMSO-d_6): 3.71 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 4.6, H-5′b); 3.83 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 3.7, H-5′a); 4.06 (ddd, 1H, $J_{4',5'}$ = 4.6, 3.7, $J_{4',3'}$ = 4.3, H-4′); 4.37 (dd, 1H, $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 4.3, H-3′); 4.99 (dd, 1H, $J_{2',1'}$ = 6.8, $J_{2',3'}$ = 6.4, H-2′); 5.19 (bs, 1H, OH-5′); 5.36 (bs, 2H, OH-2′,3′); 6.63 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 7.26 (dd, 1H, $J_{4,5}$ = 1.9, $J_{4,2}$ = 0.9, H-4-furyl); 7.54 (ddd, 1H, $J_{7,6}$ = 8.1, $J_{7,8}$ = 7.2, $J_{7,9}$ = 1.3, H-7); 7.57 (ddd, 1H, $J_{8,9}$ = 8.4, $J_{8,7}$ = 7.2, $J_{8,6}$ = 1.3, H-8); 8.05 (dd, 1H, $J_{5,4}$ = 1.9, $J_{5,2}$ = 1.5, H-5-furyl); 8.18 (m, 1H, H-6); 8.42 (m, 1H, H-9); 8.63 (dd, 1H, $J_{2,5}$ = 1.5, $J_{2,4}$ = 0.9, H-2-furyl); 8.95 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 61.5

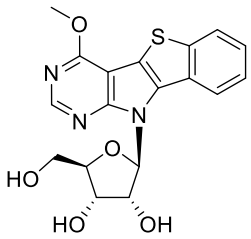
(CH_2-5′); 69.4 (CH-3′); 71.7 (CH-2′); 86.0 (CH-4′); 88.9 (CH-1′); 109.8 (CH-4-furyl); 110.7 (C-4a); 113.3 (C-4b); 122.8 (CH-9); 124.6 (CH-6); 124.9 (C-3-furyl); 125.6 (CH-8); 125.9 (C-9a); 126.0 (CH-7); 137.2 (C-9b); 141.8 (C-5a); 144.0 (CH-2-furyl); 145.5 (CH-5-furyl); 149.9 (C-4); 151.9 (CH-2); 155.7 (C-10a). MS (ESI): *m*/*z* (rel. %): 424 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{21}H_{18}O_5N_3S$ $[M + H]^+$ 424.0962; found 424.0960.



4-(Benzofuran-2-yl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (12e)

A solution of MeONa in MeOH (25 wt%, 105 µL) was added to a solution of protected nucleoside **11e** (120 mg, 0.15 mmol) in methanol (20 mL). The reaction mixture was stirred at 65 °C for 2 days. Solvent was evaporated under reduced pressure, and the crude product was purified by RP-HPFC (C18, H_2O/MeOH, 20→100%) to give compound **12e** (52 mg, 72%) as a yellow solid. m.p. 272–276 °C. $[\alpha]_D$ = –10.7 (c = 0.253 in DMSO). IR (ATR): ν = 3258, 3055, 2919, 2850, 1590, 1358, 1307, 1198, 1109, 1021, 998, 949 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.72 (bdt, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 4.6, H-5′b); 3.83 (bdt, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.1, $J_{5'a,4'}$ = 3.7, H-5′b); 4.06 (ddd, 1H, $J_{4',5'}$ = 4.6, 3.7, $J_{4',3'}$ = 4.4, H-4′); 4.37 (ddd, 1H, $J_{3',2'}$ = 6.5, $J_{3',OH}$ = 5.8, $J_{3',4'}$ = 4.4, H-3′); 5.00 (ddd, 1H, $J_{2',1'}$ = 6.8, $J_{2',3'}$ = 6.5, $J_{2',OH}$ = 6.3, H-2′); 5.21 (dd, 1H, $J_{OH,5'}$ = 6.1, 5.1, OH-5′); 5.31 (d, 1H, $J_{OH,3'}$ = 5.8, OH-3′); 5.44 (d, 1H, $J_{OH,2'}$ = 6.3, OH-2′); 6.65 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 7.44 (ddd, 1H, $J_{5,4}$ = 7.7, $J_{5,6}$ = 7.3, $J_{5,7}$ = 1.0, H-5-benzofuryl); 7.55–7.62 (m, 3H, H-7,8, H-6-benzofuryl); 7.90 (dt, 1H, $J_{4,5}$ = 7.7, $J_{4,6}$ = $J_{4,7}$ = 1.0, H-4-benzofuryl); 8.00 (d, 1H, $J_{3,7}$ = 1.0, H-3-benzofuryl); 8.03 (dq, 1H, $J_{7,6}$ = 8.3, $J_{7,3}$ = $J_{7,4}$ = $J_{7,5}$ = 1.0, H-7-benzofuryl); 8.23 (m, 1H, H-6); 8.44 (m, 1H, H-9); 9.02 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, DMSO-d_6): 61.5 (CH_2-5′); 69.4 (CH-3′); 71.7

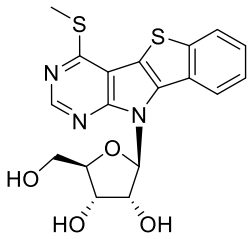
(CH-2′); 86.1 (CH-4′); 88.8 (CH-1′); 109.1 (CH-3-benzofuryl); 109.3 (C-4a); 111.9 (CH-7-benzofuryl); 114.9 (C-4b); 123.0 (CH-9); 123.1 (CH-4-benzofuryl); 124.48 (CH-6); 124.50 (CH-5-benzofuryl); 125.4 (CH-8); 126.08 (C-9a); 126.11 (CH-7); 127.2 (CH-6-benzofuryl); 127.9 (C-3a-benzofuryl); 138.0 (C-9b); 143.3 (C-5a); 145.3 (C-4); 151.8 (CH-2); 153.7 (C-2-benzofuryl); 155.5 (C-7a-benzofuryl); 156.6 (C-10a). MS (ESI): *m*/*z* (rel. %): 474 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{25}H_{20}O_5N_3S$ $[M + H]^+$ 474.1118; found 474.1113.



4-Methoxy-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12f)

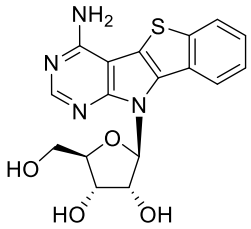
Protected nucleoside **10** (65 mg, 0.09 mmol) was suspended in methanol (6 mL), and treated with solution of sodium methoxide (25 wt% in MeOH, 0.1 mL). The reaction mixture was stirred overnight at 65 °C, and then solvent was evaporated and the crude material was purified by RP-HPFC (C18, water/MeOH 10→80%) to give nucleoside **12f** (19 mg, 55%) as a white solid. m.p. 258–260 °C. $[\alpha]_D$ = –14.3 (c = 0.280 in DMSO). IR (ATR): ν = 3356, 3077, 2944, 2920, 2872, 1608, 1551, 1338, 1118, 1077, 893 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.65 (ddd, 1H, J_{gem} = 11.9, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 4.0, H-5′b); 3.76 (dt, 1H, J_{gem} = 11.9, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 4.0, H-5′a); 4.00 (q, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 4.0, H-4′); 4.14 (s, 3H, CH_3O); 4.29 (dd, 1H, $J_{3',2'}$ = 6.2, $J_{3',4'}$ = 4.0, H-3′); 4.90 (dd, 1H, $J_{2',1'}$ = 6.9, $J_{2',3'}$ = 6.2, H-2′); 5.19 (bdd, 1H, $J_{OH,5'}$ = 6.1, 4.0, OH-5′); 5.29 (bs, 1H, OH-3′); 5.38 (bs, 1H, OH-2′); 6.50 (d, 1H, $J_{1',2'}$ = 6.9, H-1′); 7.44 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.2, H-7); 7.50 (ddd, 1H, $J_{8,9}$ = 8.2, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.10 (dd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.2, H-6); 8.23 (ddd, 1H, $J_{9,8}$ = 8.2, $J_{9,7}$ = 1.2, $J_{9,6}$ = 0.8, H-9); 8.56 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 54.4 (CH_3O); 61.6 (CH_2-5′); 69.5 (CH-3′); 71.9 (CH-2′); 86.1 (CH-4′); 89.1 (CH-1′); 100.6 (C-4a); 112.8 (C-4b); 122.2 (CH-9); 124.8 (CH-6); 125.1 (CH-7); 125.3 (CH-8); 126.1 (C-9a); 134.8 (C-9b); 142.2

(C-5a); 151.8 (CH-2); 155.9 (C-10a); 161.9 (C-4). MS (ESI): *m*/*z* (rel. %): 388 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_5N_3S$ $[M + H]^+$ 388.0962; found 388.0959.



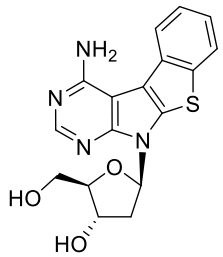
4-(Methylsulfanyl)-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12g)

Protected nucleoside **10** (300 mg, 0.43 mmol) was suspended in 1,4-dioxane (20 mL), and sodium thiomethoxide (149 mg, 2.13 mmol) was added. The reaction mixture was stirred at 100 °C for four days, and then solvent was evaporated and the crude material was purified by RP-HPFC (C18, water/MeOH 10→80%) to give nucleoside **12g** (99 mg, 58%) as a white solid. m.p. 246–249 °C. $[\alpha]_D$ = –16.4 (c = 0.291 in DMSO). IR (ATR): ν = 3374, 3264, 3062, 2945, 2923, 2851, 1542, 1438, 1380, 1107, 1076, 891, 746 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 2.79 (s, 3H, CH_3S); 3.69 (ddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.2, $J_{5'b,4'}$ = 4.6, H-5′b); 3.80 (ddd, 1H, J_{gem} = 12.0, $J_{5'a,OH}$ = 5.0, $J_{5'a,4'}$ = 3.7, H-5′a); 4.04 (ddd, 1H, $J_{4',5'}$ = 4.6, 3.7, $J_{4',3'}$ = 4.2, H-4′); 4.34 (ddd, 1H, $J_{3',2'}$ = 6.4, $J_{3',OH}$ = 5.6, $J_{3',4'}$ = 4.2, H-3′); 4.94 (dt, 1H, $J_{2',1'}$ = 6.8, $J_{2',3'}$ = $J_{2',OH}$ = 6.4, H-2′); 5.18 (dd, 1H, $J_{OH,5'}$ = 6.2, 5.0, OH-5′); 5.26 (d, 1H, $J_{OH,3'}$ = 5.6, OH-3′); 5.37 (d, 1H, $J_{OH,2'}$ = 6.4, OH-2′); 6.54 (d, 1H, $J_{1',2'}$ = 6.8, H-1′); 7.51 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.2, H-7); 7.56 (ddd, 1H, $J_{8,9}$ = 8.2, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.19 (m, 1H, H-6); 8.39 (m, 1H, H-9); 8.80 (s, 1H, H-2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 11.8 (CH_3S); 61.5 (CH_2-5′); 69.4 (CH-3′); 71.8 (CH-2′); 86.1 (CH-4′); 88.9 (CH-1′); 111.1 (C-4a); 113.2 (C-4b); 122.6 (CH-9); 124.9 (CH-6); 125.4 (CH-7); 125.5 (CH-8); 126.0 (C-9a); 135.4 (C-9b); 142.8 (C-5a); 151.5 (CH-2); 152.6 (C-10a); 159.9 (C-4). MS (ESI): *m*/*z* (rel. %): 404 (100) $[M + H]^+$. HRMS (ESI): calcd. for $C_{18}H_{18}O_4N_3S_2$ $[M + H]^+$ 404.0733; found 404.0728.



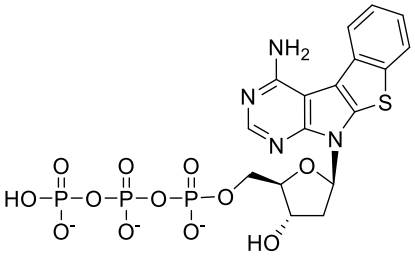
4-Amino-10-(*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (12h)

Protected nucleoside **10** (400 mg, 0.57 mmol) was dissolved in 1,4-dioxane (8 mL) and 30% aqueous ammonia (8 mL) was added. The mixture was stirred and heated to 120 °C in a pressure tube for 2 days. After that, the solvent was removed under vacuum and the crude product was purified by RP-HPFC (C18, gradient water/MeOH 10−65 %) to give **12h** (100 mg, 47%) as a white solid. m.p. 230–232 °C. $[\alpha]_D = -17.2$ (c = 0.274 in DMSO). IR (ATR): ν = 3458, 3347, 3226, 2947, 2919, 2869, 2820, 1643, 1596, 1468, 1382, 1122, 750 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.66 (bddd, 1H, J_{gem} = 12.0, $J_{5'b,OH}$ = 6.1, $J_{5'b,4'}$ = 4.0, H-5′b); 3.76 (dt, 1H, J_{gem} = 12.0, $J_{5'a,OH} = J_{5'a,4'}$ = 2.5, H-5′a); 4.00 (ddd, 1H, $J_{4',3'}$ = 4.3, $J_{4',5'}$ = 4.0, 2.5, H-4′); 4.27 (m, 1H, H-3′); 4.93 (ddd, 1H, $J_{2',1'}$ = 7.1, $J_{2',1'}$ = 6.6, $J_{2',3'}$ = 5.9, H-2′); 5.22 (bd, 1H, $J_{OH,3'}$ = 5.2, OH-3′); 5.32 (d, 1H, $J_{OH,2'}$ = 6.6, OH-2′); 5.58 (bs, 1H, OH-5′); 6.49 (d, 1H, $J_{1',2'}$ = 7.1, H-1′); 7.33 (bs, 2H, NH_2); 7.41 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.1, $J_{7,9}$ = 1.2, H-7); 7.50 (ddd, 1H, $J_{8,9}$ = 8.2, $J_{8,7}$ = 7.1, $J_{8,6}$ = 1.2, H-8); 8.10 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.2, $J_{6,9}$ = 0.8, H-6); 8.21 (s, 1H, H-2); 8.24 (ddd, 1H, $J_{9,8}$ = 8.2, $J_{9,7}$ = 1.2, $J_{9,6}$ = 0.8, H-9). ^{13}C NMR (125.8 MHz, DMSO-d_6): 61.9 (CH_2-5′); 70.00 (CH-3′); 71.8 (CH-2′); 86.2 (CH-4′); 89.2 (CH-1′); 98.2 (C-4a); 113.8 (C-4b); 121.4 (CH-9); 124.1 (CH-7); 124.5 (CH-6); 124.9 (CH-8); 126.1 (C-9a); 132.8 (C-9b); 141.9 (C-5a); 152.6 (CH-2); 154.7 (C-10a); 156.7 (C-4). MS (ESI): *m*/*z* (rel. %): 373 (100) $[M + H]^+$, 395 (18) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{17}H_{17}O_4N_4S$ $[M + H]^+$ 373.0965; found 373.0961.



4-Amino-10-(2'-deoxy-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine (13)

Nucleobase **3** (330 mg, 1.27 mmol) was suspended in dry MeCN (25 mL), the mixture was stirred for 15 min. Then KOH (178 mg, 3.18 mmol) and TDA-1 (29 μL, 0.09 mmol) were added and stirred for another 30 min. Hoffer's chlorosugar[72] (592 mg, 1.52 mmol) was then added, and the mixture was stirred at r.t. for 45 min. The mixture was treated with aqueous NH_4Cl and concentrated under reduced pressure. Then the crude product was dissolved in 1,4-dioxane (2 mL) and 30% aqueous ammonia (6 mL) was added. The mixture was stirred and heated to 120 °C in a pressure tube for 40 h. After that, the solvent was removed under vacuum and the crude product was purified by RP-HPFC (C18, water/MeOH 10→80 %) to give **13** (45 mg, 10%) as a white solid. ^{1}H NMR (600.1 MHz, DMSO-d_6): 2.27 (ddd, 1H, J_{gem} = 13.3, $J_{2'b,1'}$ = 5.8, $J_{2'b,3'}$ = 2.7, H-2′b); 2.51 (ddd, 1H, J_{gem} = 13.3, $J_{2'a,1'}$ = 8.6, $J_{2'a,3'}$ = 6.3, H-2′a); 3.66, 3.68 (2 × dt, 2 × 1H, J_{gem} = 11.3, $J_{5',OH}$ = $J_{5',4'}$ = 5.6, H-5′); 3.91 (td, 1H, $J_{4',5'}$ = 5.6, $J_{4',3'}$ = 2.7, H-4′); 4.38 (ddt, 1H, $J_{3',2'}$ = 6.3, 2.7, $J_{3',OH}$ = 4.2, $J_{3',4'}$ = 2.7, H-3′); 5.00 (t, 1H, $J_{OH,5'}$ = 5.6, OH-5′); 5.43 (d, 1H, $J_{OH,3'}$ = 4.2, OH-3′); 6.77 (dd, 1H, $J_{1',2'}$ = 8.6, 5.8, H-1′); 6.84 (s, 2H, NH_2); 7.32 (ddd, 1H, $J_{7,8}$ = 8.2, $J_{7,6}$ = 7.1, $J_{7,5}$ = 1.2, H-7); 7.46 (ddd, 1H, $J_{6,5}$ = 8.2, $J_{6,7}$ = 7.1, $J_{6,8}$ = 1.2, H-6); 8.02 (ddd, 1H, $J_{8,7}$ = 8.2, $J_{8,6}$ = 1.2, $J_{8,5}$ = 0.6, H-8); 8.24 (s, 1H, H-2); 8.31 (ddd, 1H, $J_{5,6}$ = 8.2, $J_{5,7}$ = 1.2, $J_{5,8}$ = 0.6, H-5). ^{13}C NMR (150.9 MHz, DMSO-d_6): 38.1 (CH_2-2′); 62.4 (CH_2-5′); 71.1 (CH-3′); 83.6 (CH-1′); 87.5 (CH-4′); 98.4 (C-4a); 115.3 (C-4b); 122.3 (CH-5); 123.1 (CH-7); 123.9 (CH-8); 125.2 (CH-6); 131.0 (C-4c); 134.8 (C-9a); 138.4 (C-8a); 151.7 (CH-2); 154.9 (C-10a); 156.7 (C-4). MS (ESI): *m*/*z* (rel. %): 357 (100) $[M + H]^+$, 379 (49) $[M + Na]^+$. HRMS (ESI): calcd. for $C_{17}H_{16}O_3N_4NaS$ $[M + Na]^+$ 379.0835; found 379.0832.



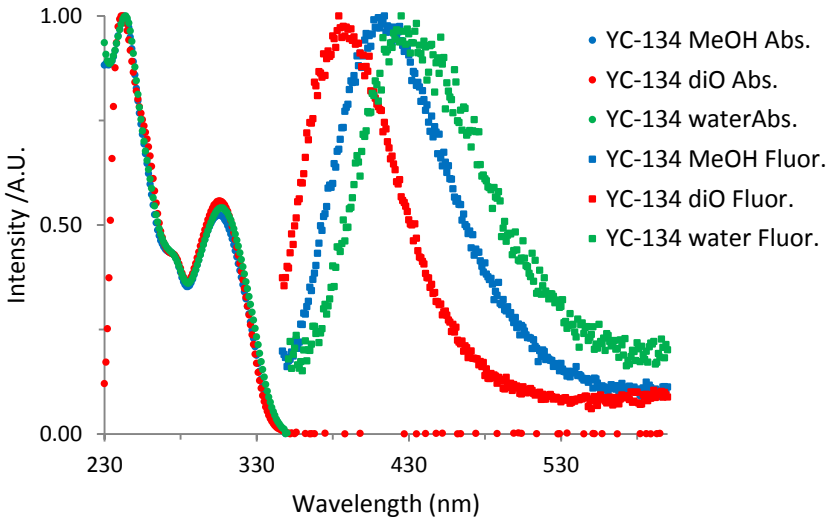
4-Amino-10-(2'-deoxy-*β*-D-ribofuranosyl)-10*H*-benzo[4',5']thieno[3',2':4,5]pyrrolo[2,3-*d*]pyrimidine-5′-*O*-Triphosphate Sodium Salt (14)

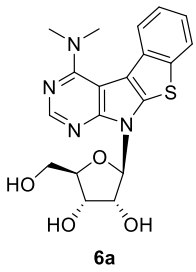
Nucleoside **13** (20 mg, 0.056 mmol) was dissolved in trimethyl phosphate (0.2 mL), cooled to 0 °C and treated with freshly distilled $POCl_3$ (7.3 μL, 0.08 mmol). The mixture was stirred at 0 °C for 3 h. A solution of $(NHBu_3)_2H_2P_2O_7$ (170 mg, 0.30 mmol) and Bu_3N (89 μL, 0.38 mmol) in anhydrous DMF (0.5 mL), which was prestirred at 0 °C for 5 min, was added to the former mixture, and stirred at 0 °C for 2 h. Then the mixture was treated with aqueous TEAB (2 M, 2 mL). The reaction mixture was evaporated under reduced pressure and co-evaporated four times with water. The crude product was purified by HPLC (C-18, 5–100 % MeOH in 0.1 M TEAB) and coevaporated four times with water. The residue was converted to sodium salt on an ion-exchange column (Dowex 50WX8 in Na^+ cycle) and freeze-dried. The product **14** (8 mg, 21%) was obtained as a white solid. ^{1}H NMR (500.0 MHz, D_2O, ref(dioxane) = 3.75 ppm): 2.26–2.41 (bm, 2H, H-2′); 4.21–4.30 (m, 3H, H-4′,5′); 4.61 (bm, 1H, H-3′); 6.19 (bt, 1H, $J_{1',2'}$ = 6.6, H-1′); 7.01 (bt, 1H, $J_{7,8} = J_{7,6}$ = 7.4, H-7); 7.07 (bt, 1H, $J_{6,5} = J_{6,7}$ = 7.4, H-6); 7.18 (bd, 1H, $J_{5,6}$ = 7.4, H-5); 7.54 (bd, 1H, $J_{8,7}$ = 7.4, H-8); 7.67 (bs, 1H, H-2). ^{13}C NMR (125.7 MHz, D_2O, ref(dioxane) = 69.3 ppm): 40.4 (CH_2-2′); 68.4 (d, $J_{C,P}$ = 4.4, CH_2-5′); 73.5 (CH-3′); 86.3 (CH-1′); 87.5 (d, $J_{C,P}$ = 8.9, CH-4′); 100.2 (C-4a); 117.6 (C-4b); 122.7 (CH-5); 125.8 (CH-7); 126.0 (CH-8); 127.6 (CH-6); 131.3 (C-4c); 137.6 (C-9a); 141.1 (C-8a); 149.9 (CH-2); 154.1 (C-10a); 155.6 (C-4). ^{31}P NMR (202.4 MHz, D_2O): –20.70 (bt, J =18.6, P_β); –10.22 (d, J = 18.6, P_α); –7.07 (bd, J = 18.6, P_γ). MS (ESI): *m*/*z* (rel. %): 595 (24) $[M + 3H – 4Na]^–$, 617 (30) $[M + 2H – 3Na]^–$. HRMS (ESI): calcd. for $C_{17}H_{18}O_{12}N_4P_3S$ $[M + 3H – 4Na]^-$ 594.9860; found 594.9853.

5.2.2 UV/Vis and fluorescence measurements with nucleosides

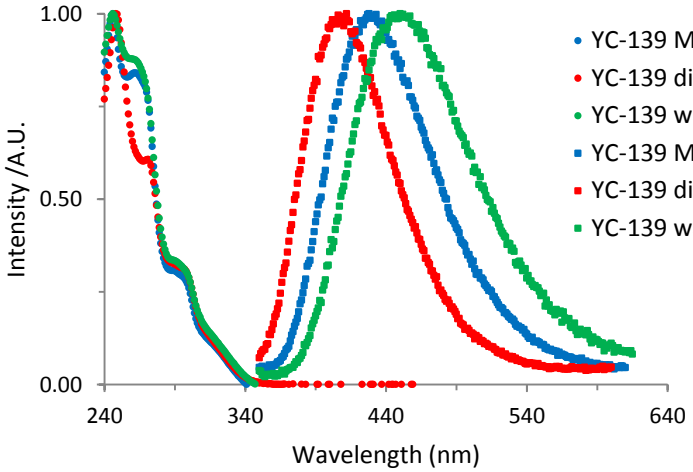
UV/Vis absorption and fluorescence emission spectra of nucleosides **6a**–**6h** and **12a**–**12h** were measured and their fluorescence quantum yields were determined as described previously.[136] The spectra were recorded in 1,4-dioxane, MeOH or water.

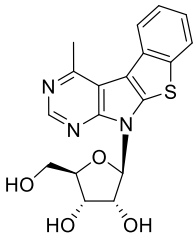
The excitation wavelength was 310 nm and the integration range was 330–650 nm.



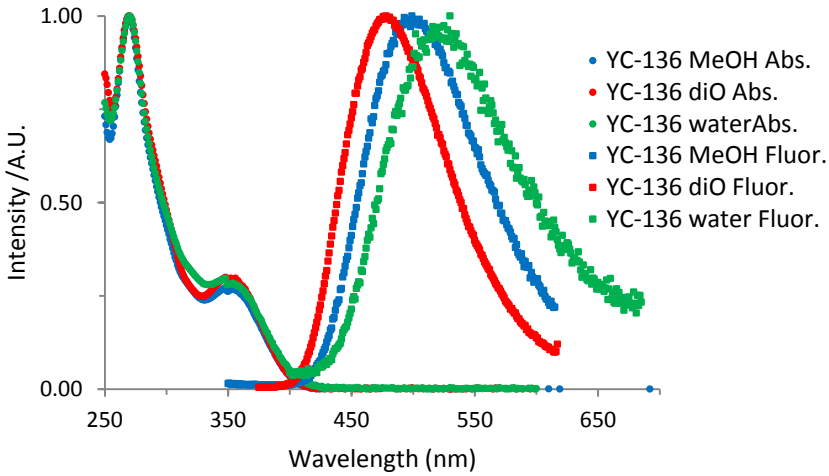


6a





6b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0.50
0.00
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250
350
450
550
650
Wavelength (nm)
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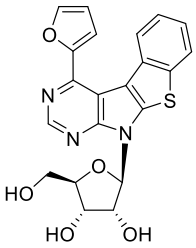
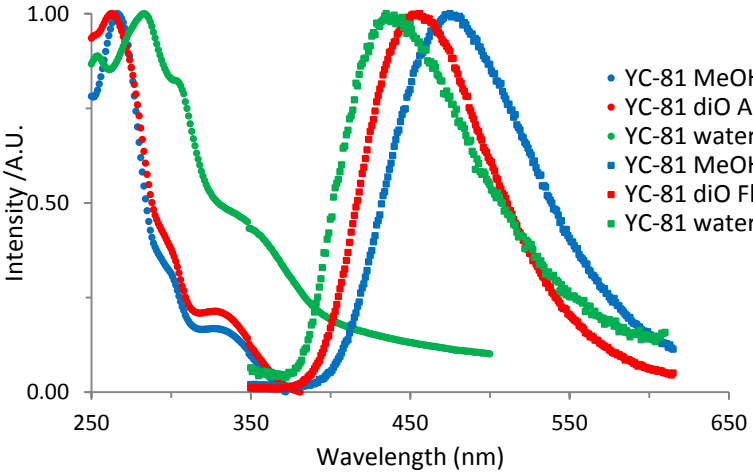
YC-136 diO Abs.
YC-136 waterAbs.
YC-136 MeOH Fluor.
YC-136 diO Fluor.
YC-136 water Fluor.


O
N
N
S
N
O
HO
HO
OH

6c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550
650
Wavelength (nm)
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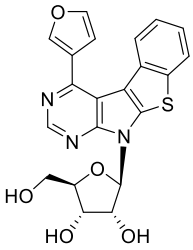
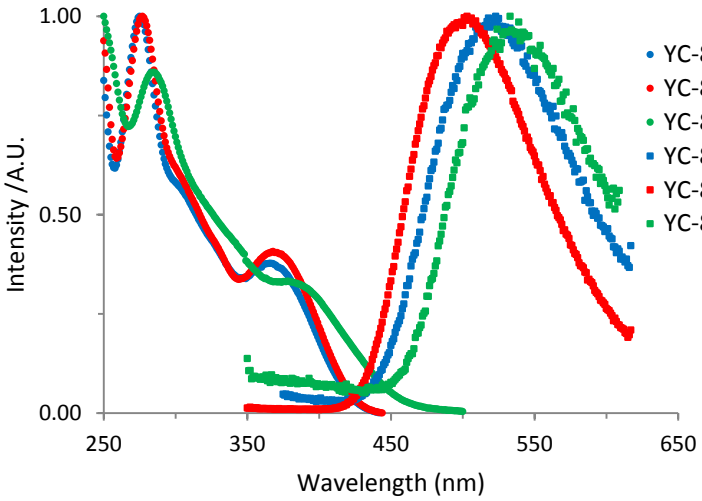
YC-81 water Abs.
YC-81 MeOH Fluor.
YC-81 diO Fluor.
YC-81 water Fluor.


O
N
N
S
N
O
HO
HO
OH

6d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Intensity /A.U.
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450
550
650
Wavelength (nm)
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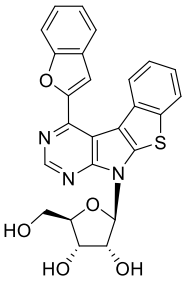
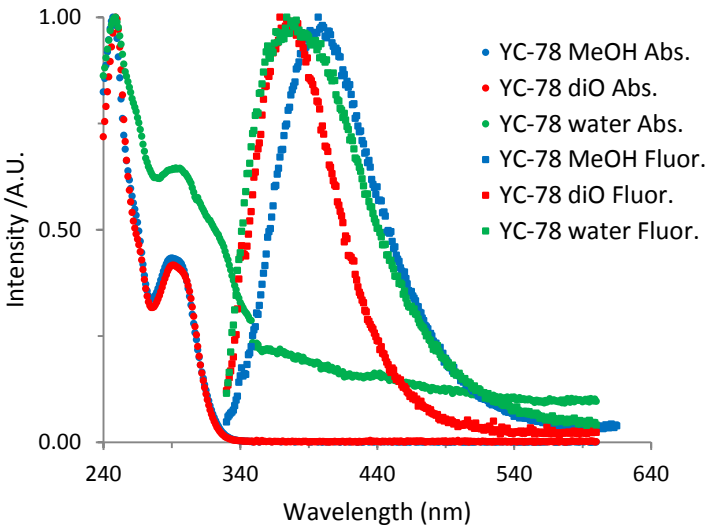
YC-82 water Abs.
YC-82 MeOH Fluor.
YC-82 diO Fluor.
YC-82 water Fluor.


O
N
N
S
N
O
HO
HO
OH

6e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0.50
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Intensity /A.U.
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440
540
640
Wavelength (nm)
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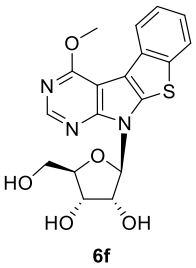
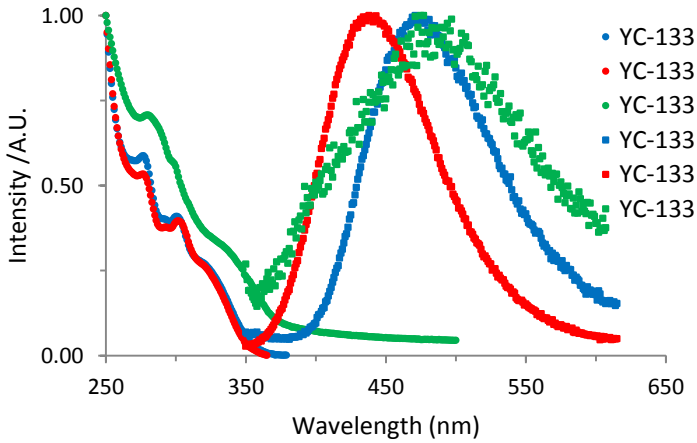
YC-78 diO Abs.
YC-78 water Abs.
YC-78 MeOH Fluor.
YC-78 diO Fluor.
YC-78 water Fluor.


O
N
N
S
N
O
HO
HO
OH

6f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Intensity /A.U.
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350
450
550
650
Wavelength (nm)
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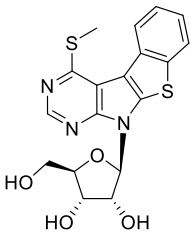
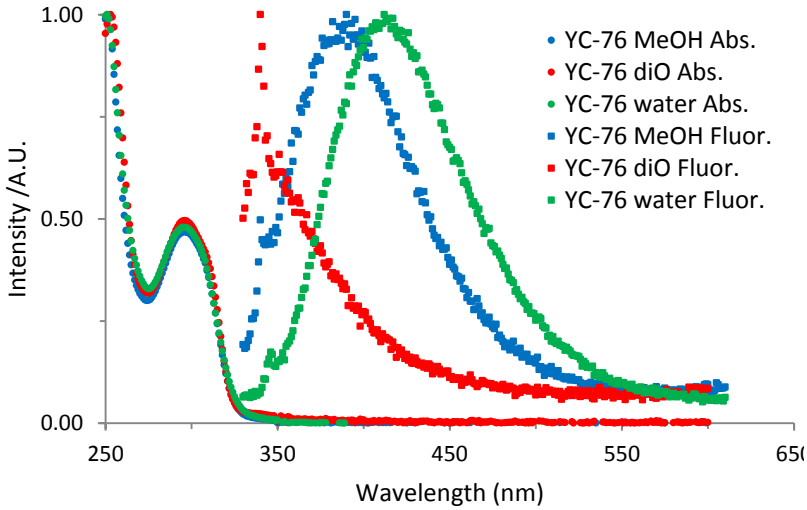
YC-133 diO Abs.
YC-133 water Abs.
YC-133 MeOH Fluor.
YC-133 diO Fluor.
YC-133 water Fluor.


S
N
N
S
N
O
HO
HO
OH

6g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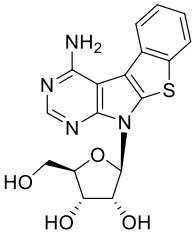
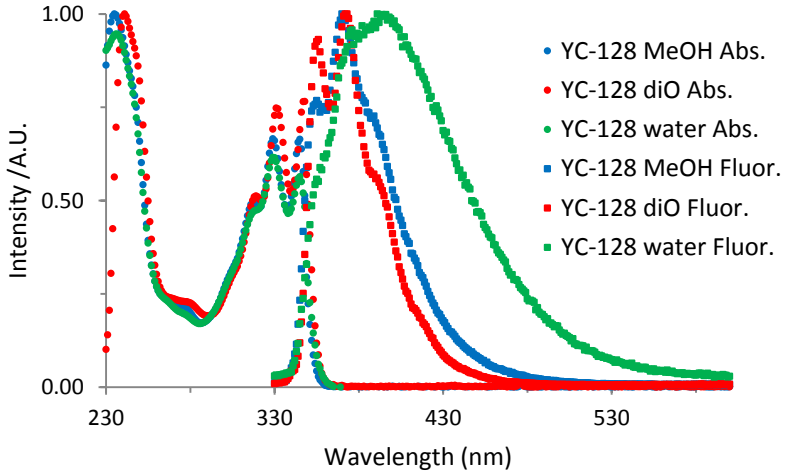
YC-76 water Fluor.


NH2
N
N
S
N
O
HO
HO
OH

6h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330
430
530
Wavelength (nm)
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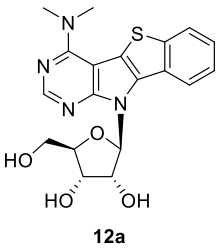
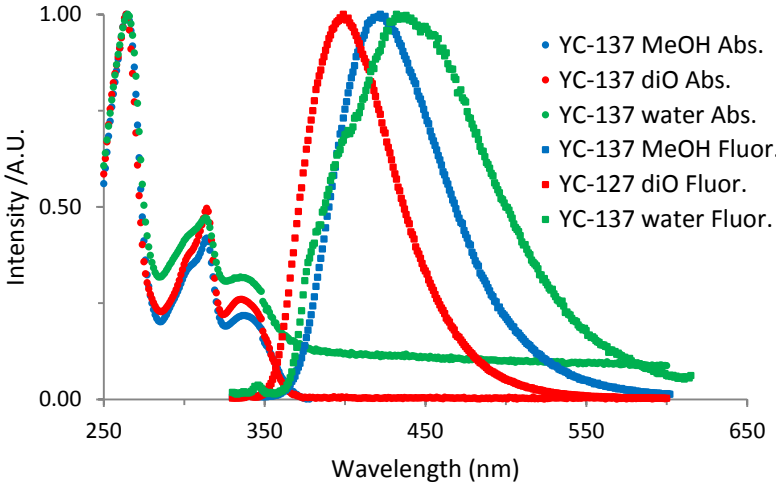
YC-128 diO Abs.
YC-128 water Abs.
YC-128 MeOH Fluor.
YC-128 diO Fluor.
YC-128 water Fluor.


N
S
N
N
O
HO
HO
OH

12a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Intensity /A.U.
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Wavelength (nm)
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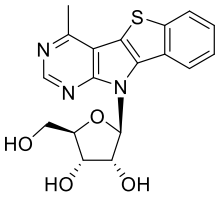
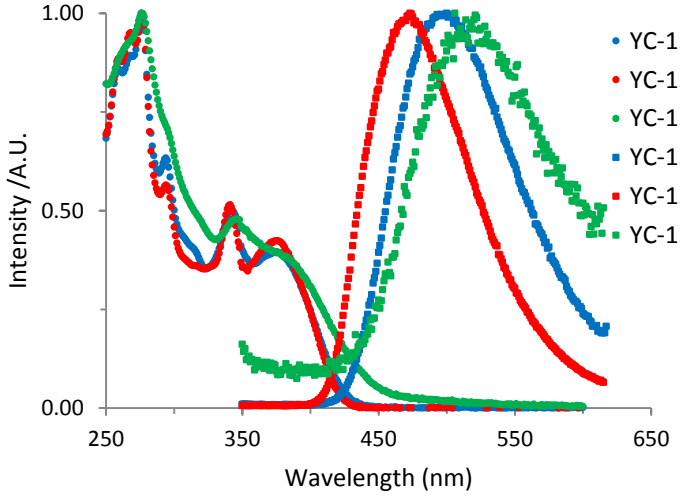
YC-137 water Fluor.


S
N
N
N
O
HO
HO
OH

12b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0.00
Intensity /A.U.
250
350
450
550
650
Wavelength (nm)
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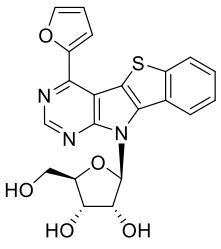
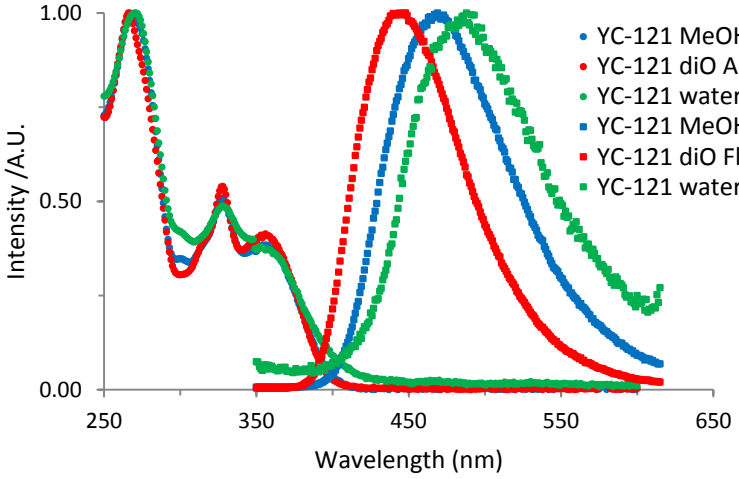
YC-138 diO Abs.
YC-138 water Abs.
YC-138 MeOH Fluor.
YC-138 diO Fluor.
YC-138 water Fluor.


O
S
N
N
N
O
HO
HO
OH

12c


1.00
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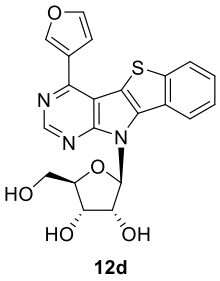
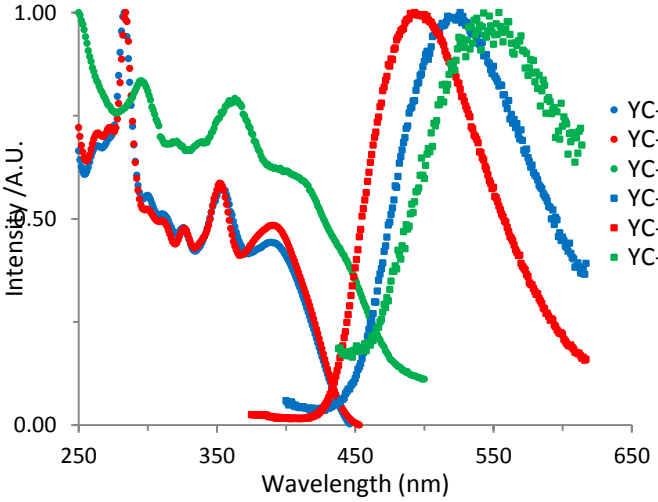
0.00
Intensity /A.U.
250
350
450
550
650
Wavelength (nm)
YC-121 MeOH Abs.
YC-121 diO Abs.
YC-121 water Abs.
YC-121 MeOH Fluor.
YC-121 diO Fluor.
YC-121 water Fluor.


O
S
N
N
N
O
HO
HO
OH

12d


1.00
0.50
0.00
Intensity /A.U.
250
350
450
550
650
Wavelength (nm)
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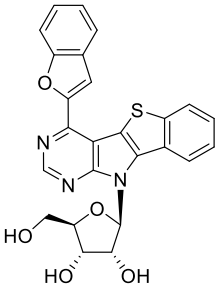
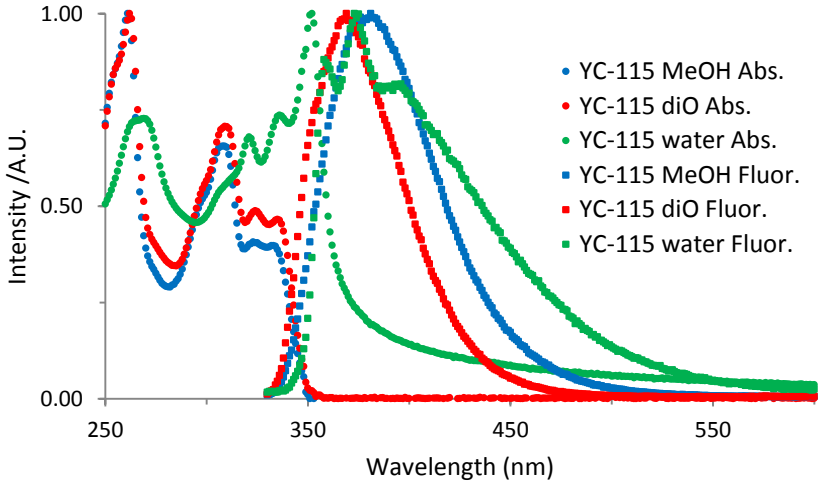
YC-122 diO Abs.
YC-122 water Abs.
YC-122 MeOH Fluor.
YC-122 diO Fluor.
YC-122 water Fluor.


O
S
N
N
N
O
HO
HO
OH

12e


1.00
0.50
0.00
Intensity /A.U.
250
350
450
550
Wavelength (nm)
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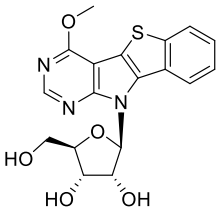
YC-115 diO Abs.
YC-115 water Abs.
YC-115 MeOH Fluor.
YC-115 diO Fluor.
YC-115 water Fluor.


O
S
N
N
N
O
HO
HO
OH

12f

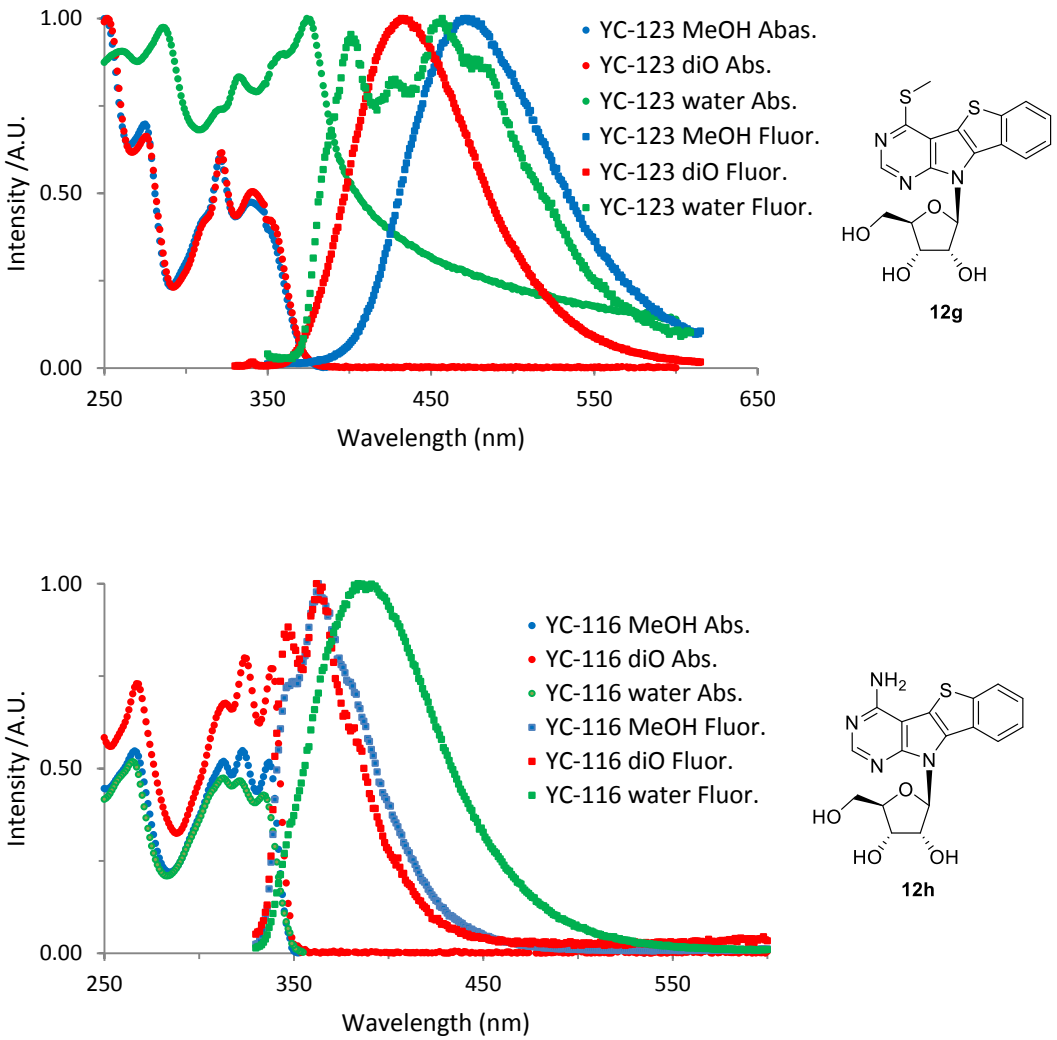


Figure 18. Normalized UV and fluorescence spectra of nucleosides **6a–6h** and **12a–12h**

5.2.3 Biochemistry of benzothieno-fused 7-deazapurine nucleotide

Primer Extension Experiments with KOD XL DNA Polymerase: The primer extension experiments were performed as described previously.[75] The reaction mixtures (20 µL) contained a FAM labeled primer (0.15 µM; *Prim*^{FAM} used in combination with *Temp1A* and *Temp4A* or *PrimHair*^{FAM} used in combination with *TemprTAT*, *TempCAC*, *Temp-GAG* and *TempCtrl*), a template (0.225 µM), KOD XL DNA polymerase (0.0625 U) and KOD XL DNA polymerase buffer (2 µL) supplied by the manufacturer. For template *Temp1A*: Reaction mixtures contained also dGTP (15 µM) and either dATP or modified **dA^{BT}TP** (30 µM). Reactions were incubated at

60 °C for 15 min. For template *Temp4A*: Reaction mixtures contained also dGTP (30 μM), dCTP (30 μM), dTTP (30 μM) and either dATP or modified **dA^{BT}TP** (120 μM). Reactions were incubated at 60 °C for 30 min. For templates *TempTAT*, *TempCAC*, *TempGAG* and *TempCtrl*: Reaction mixtures contained also dGTP (10 μM), dCTP (10 μM), dTTP (10 μM) and either dATP or modified **dA^{BT}TP** (120 μM). Reaction mixtures were incubated at 60 °C for 15 min. Reactions were stopped by addition of PAGE stop solution [20 μL; 95 % (v/v) formamide, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.025 % (w/v) SDS, 0.025 % (w/v) bromophenol blue, 0.025 % (w/v) xylene cyanol in water] and the mixtures were heated to 95 °C for 5 min. Aliquots (4 μL) were separated by denaturing PAGE (12.5 %) with urea and visualized by fluorescence imaging.

Preparation of ONs for MALDI-TOF Analysis and Fluorescence Studies: The ONs were prepared as described previously.[75] The reaction mixtures (100 μL) contained a primer (3.2 μM; *Prim* used in combination with *Temp1A^{bio}* and *Temp4A^{bio}* or *PrimHair* used in combination with *TempTAT^{bio}*, *TempCAC^{bio}*, *TempGAG^{bio}* and *TempCtrl^{bio}*), a biotinylated template (3.2 μM), KOD XL DNA polymerase buffer (10 μL) supplied by the manufacturer. For template *Temp1A^{bio}*: Reaction mixtures contained also KOD XL DNA polymerase (2.5 U) dGTP (300 μM) and modified **dA^{BT}TP** (600 μM). Reaction mixtures were incubated at 60 °C for 40 min. For template *Temp4A^{bio}*: Reaction mixtures contained also KOD XL DNA polymerase (5 U), dGTP (300 μM), dCTP (300 μM), dTTP (300 μM) and modified **dA^{BT}TP** (1.2 mM). Reaction mixtures were incubated at 60 °C for 30 min. For templates *TempTAT^{bio}*, *TempCAC^{bio}, TempGAG^{bio}, TempCtrl^{bio}*: Reaction mixtures contained also KOD XL DNA polymerase (2.5 U), dGTP (300 μM), dCTP (300 μM), dTTP (300 μM) and modified **dA^{BT}TP** (0.6 mM). Reaction mixtures were incubated at 60 °C for 30 min.

A stock solution of streptavidin magnetic particles (Roche, 50 μL) was washed with buffer TEN_{100} (3× 200 μL; 10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5). The PEX solution and buffer TEN_{100} (50 μL) were added. The mixture was vigorously shaken at 15 °C for 30 min. The magnetic particles were collected on a magnet and

washed with buffer TEN_{500} (3× 200 μL; 10 mM Tris, 1 mM EDTA, 500 mM NaCl, pH 7.5) and water (5× 200 μL). Water (50 μL) was added and the suspension was heated to 55 °C for 2 min. The magnetic particles were then collected on a magnet and the supernatant was transferred into a clean microtube. The concentration of the ON product was determined by Nanodrop1000 spectrophotometer.

Fluorescence Measurements with Oligonucleotides: Fluorescence emission spectra of ONs were measured as described previously.[75] ONs (1 μM or 1 μM of each strand for DNA duplexes) were mixed in medium salt buffer (100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0) and heated to 95 °C for 5 min and cooled slowly to 25 °C over a period of 60 min. Fluorescence emission spectra were recorded by using the excitation wavelength of 305 nm and the integration range of 330–600 nm. Measurements were performed at 20 °C. Background spectra of the buffer solutions were recorded and subtracted from the spectra.

5.2.4 HPLC purity of final compounds

compd	method	t_r [min]	purity [%]	method	t_r [min]	purity [%]
6a	A	24.45	99.80	B	16.72	99.96
6b	A	23.20	98.40	B	14.73	98.36
6c	A	24.65	98.05	B	16.98	98.49
6d	A	24.42	99.75	B	16.71	99.86
6e	A	27.71	95.15	B	20.37	95.12
6f	A	24.90	98.98	B	17.02	99.43
6g	A	26.50	97.08	B	18.58	96.96
6h	A	20.80	95.25	B	10.64	95.32
12a	A	25.61	99.39	B	17.58	99.59
12b	A	24.47	99.69	B	15.88	99.77
12c	A	26.44	99.64	B	18.54	99.57
12d	A	26.35	98.70	B	18.48	99.11
12e	A	29.67	95.35	B	22.48	95.32
12f	A	25.89	99.80	B	18.02	99.77
12g	A	27.27	98.44	B	19.41	98.72
12h	A	21.36	99.04	B	12.02	98.65

Methods: A = 20% MeOH in H_2O to 100% MeOH in 25 min, 100% MeOH in 10 min, UV 280 nm; B = 10% MeCN in H_2O to 100% MeCN in 25 min, 100% MeCN in 10 min, UV 280 nm.

5.3 Synthesis and Biochemistry of Polycyclic Hetero-Fused 7-Deazapurine Nucleosides

5.3.1 General procedures

General Procedure for Novel Negishi Coupling: TMPMgCl·LiCl (1 M, 2.4 equiv) was added to $ZnCl_2$ (1.2 equiv) under an argon atmosphere, and the mixture was stirred at r.t. for 12 h to give a tetramethylpiperidinylzinc complex. The solution of 4,6-dichloropyrimidine (2 equiv) in THF (1 M) was added dropwise to an ice-cooled solution of a tetramethylpiperidinylzinc complex. The mixture was stirred at 0 °C for 1 h and then at r.t. for 1 h to give a zincated pyrimidine **17**. A solution of the sulfonium salt (1 equiv) and $Pd(PPh_3)_4$ (0.08 equiv) in THF/MeCN (1:1, 0.2–0.3 M) or THF (0.2–0.3 M), which was prestirred at r.t. for 20 min, was added to the solution of zincated pyrimidine **17** and stirred at 65 °C 40 h. After that, solvent was evaporated under reduced pressure and the crude material was purified by HPFC (SiO_2, hexane/EtOAc, 0−5%).

General Procedure for Azidation: Heteroaryl-dichloropyrimidine **18** (1 equiv), sodium azide (1 equiv), lithium chloride (1 equiv) and THF (0.2–0.3 M) were added into a round-bottom flask and this mixture was stirred at r.t. for 40 h. The crude product **19** was used directly for the next step after removing the solvent under reduced pressure.

General Procedure for Thermal Cyclization (for **20a**, **20c**, **20d**): Tetrazole **19** (crude product) and 1,4-dibromobenzene (8–10 times weight of the tetrazole) were mixed and added into a reaction tube with a rubber septum. The tube was heated to 180 °C for 35 min while a needle was inserted through the rubber septum to release N_2 gas. After cooling down to r.t., the solid mixture was purified by flash chromatography (SiO_2, hexane/EtOAc 10:1–1.5:1) to give the desired product **20**.

General Procedure for Glycosylation (Deoxyribonucleosides 21a, **21c**, **21d**): Nucleobase **20** (1 equiv) was suspended in dry MeCN (0.03 M) and the mixture was stirred for 10 min. Then, KOH (2.6 equiv) and TDA-1 (1.5 equiv) were added and stirred for another 30 min. 1-Chloro-3,5-di-(4-chlorobenzoyl)-2-deoxy-*α*-D-ribose[137] (1.5 equiv) was then added and the mixture was stirred at r.t. for 50 min. The mixture was treated with aqueous NH_4Cl (10−15 mL/mmol) and extracted by EtOAc (300 mL/mmol), then the organic layer was washed by H_2O (2 × 15 mL), brine (15 mL) and dried over N_2SO_4. After removing the solvent, the residue was purified by HPFC [SiO_2, hexane/EtOAc, 5−15%, or (hexane/DCM 4:1)/EtOAc, 0−5%] to obtain the protected deoxyribonucleoside **21**.

General Procedure for Glycosylation (Ribonucleosides 22a–22d): Nucleobase **20** (1 equiv) was suspended in MeCN (0.03 M), and BSA (1 equiv) was added. The mixture was heated to 60 °C for 30 min, and then, TMSOTf (2.5 equiv) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (2 equiv) were added. The mixture was stirred at the same temperature for 25−60 min and treated with $NaHCO_3$ (aq, 10−15 mL/mmol). Most of the organic solvent was evaporated under reduce pressure. The mixture was diluted with EtOAc (200−300 mL/mmol), and the organic layer was collected and washed with water (2 × 15 mL), $NaHCO_3$ (aq, 20 mL) and brine (30 mL), dried over Na_2SO_4. After evaporation, crude product was purified by HPFC [SiO_2, (hexane/DCM 2:1)/EtOAc, 2−6% or hexane/ EtOAc, 15−22%] to give protected ribonucleosides **22**.

General Procedure for Amination: Protected nucleoside **21** or **22**, 1,4-dioxane (16 mL/mmol) and ammonia solution in water (25−29%, 16 mL/mmol) were added into a pressure tube with a screw cap. The tube was sealed and heated to 120 °C for 24 h. After removing the volatiles, the crude product was purified by RP-HPFC (C18, gradient water/MeOH, 15−100%; for **23b**, **23b*α***, **24a**, **24b**) or by HPFC (SiO_2, DCM/MeOH, 3−10%] to give free nucleoside **23** or **24**.

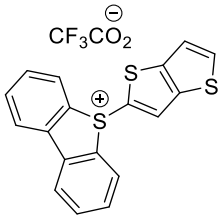
General Procedure for Methoxylation: Protected nucleoside **22** (1 equiv) was suspended in methanol (0.017 M) and 1,4-dioxane (0.034 M), and sodium methoxide

(25 wt % in MeOH, 12 equiv) was added. The reaction mixture was stirred at 65 °C overnight, and then, solvent was evaporated and the crude material was purified by HPFC (SiO_2, DCM/MeOH, 2−10%) to give nucleoside **25**.

General Procedure for Methylation: To the solution of protected nucleoside **22** (1 equiv) and $Pd(PPh_3)_4$ (0.15 equiv) in THF (0.03 M), Me_3Al (2.0 M in toluene, 2.2 equiv) was added. The mixture was stirred and heated to 65 °C overnight. After removing the volatiles under reduced pressure, the residue was purified by HPFC [SiO_2, hexane/EtOAc, 15−50%, or (hexane/DCM 3:1)/EtOAc, 10%−45%] to obtain compound **26**.

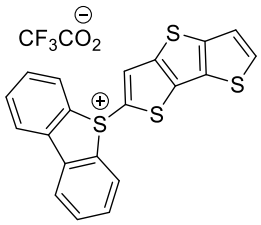
General Procedure for Deprotection: Protected nucleoside **26** (1 equiv) was suspended in methanol (0.017 M) and 1,4-dioxane (0.034 M), and treated with sodium methoxide (25 wt % in MeOH, 3 equiv). The mixture was stirred at 60 °C overnight. The solvent was evaporated till 2−3 mL, and the solid residue, which was collected from filtration, was washed by cold EtOAc (3 × 2 mL) and water (2 × 2 mL) to obtain nucleoside **27**.

5.3.2 Synthesis of polycyclic hetero-fused 7-deazapurine nucleosides



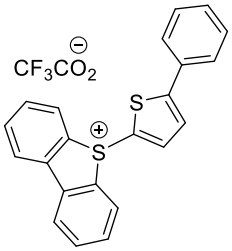
5-(Thieno[3,2-*b*]thiophen-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (16a)

Literature procedure starting from thieno[3,2-*b*]thiophene **15a** (1.2 g, 8.6 mmol) gave **16a** (3.05 g, 82%) as a grey-green foam. ^{1}H NMR is in agreement with literature.[95]



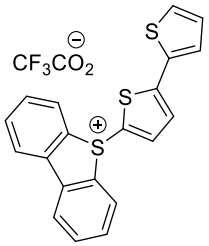
5-(Dithieno[3,2-*b*:2',3'-*d*]thiophen-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoroacetate (16b)

Under an ambient atmosphere, dibenzothiophene-*S*-oxide (500 mg, 2.5 mmol) and dithieno[3,2-*b*:2',3'-*d*]thiophene (490 mg, 2.5 mmol) were dissolved in MeCN (8 mL). The mixture was cooled to –78 °C, and treated with trifluoroacetic acid anhydride (0.53 mL, 3.7 mmol) dropwise. Subsequently, the reaction mixture was moved to room temperature and stirred for 1.5 h at 25 °C. The solvent was removed under reduced pressure. Flash chromatography (SiO_2, DCM/MeOH 0–15%) gave compound **16b** (1.3 g, 95%) as a light yellow foam. mp 102–108 °C. IR (ATR): ν = 3369, 3060, 1678, 1428, 1360, 1195, 1114, 757 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.56 (d, 1H, $J_{5,6}$ = 5.3 Hz, H-5-DTT); 7.77 (ddd, 2H, $J_{2,1}$, $J_{8,9}$ = 8.5, $J_{2,3}$, $J_{8,7}$ = 7.5 Hz, $J_{2,4}$, $J_{8,6}$ = 1.2 Hz, H-2, H-8); 7.84 (d, 1H, $J_{6,5}$ = 5.3 Hz, H-6-DTT); 7.97 (td, 2H, $J_{3,4}$, $J_{7,6}$ = $J_{3,2}$, $J_{7,8}$ = 7.6 Hz, $J_{3,1}$, $J_{7,9}$ = 1.1 Hz, H-3, H-7); 8.51 (dd, 2H, $J_{1,2}$, $J_{9,8}$ = 8.1 Hz, $J_{1,3}$, $J_{9,7}$ = 1.0 Hz, H-1, H-9); 8.53 (dd, 2H, $J_{4,3}$, $J_{6,7}$ = 7.9 Hz, $J_{4,2}$, $J_{6,8}$ = 1.2 Hz, H-4, H-6); 8.86 (s, 1H, H-3-DTT). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 121.3 (CH-5-DTT); 122.3 (C-2-DTT); 124.4 (CH-4, CH-6); 128.2 (CH-1, CH-9); 130.0 (C-7a-DTT); 131.4 (CH-2, CH-8); 132.0 (CH-6-DTT); 134.1 (CH-3, CH-7); 135.3 (CH-3-DTT); 135.5 (C-9a, C-9b); 136.6 (C-7b-DTT); 138.2 (C-4a, C-5a); 140.1 (C-3a-DTT); 145.4 (C-4a-DTT). ^{19}F NMR (376 MHz, DMSO-d_6): δ –73.4. MS (ESI): *m/z* (rel. %): 379 (100) $[M]^+$. HRMS (ESI): *m/z* $[M]^+$ Calcd for $C_{20}H_{11}S_4$ 378.97381; Found 378.97341.



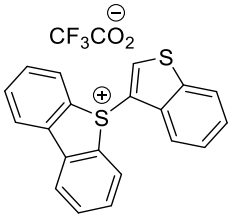
5-(5-Phenylthiophen-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (16c)

Literature procedure starting from 2-phenylthiophene **15c** (640 mg, 4 mmol) gave **16c** (1.65 g, 91%) as a yellow foam. ^{1}H NMR is in agreement with literature.[95]



5-([2,2'-Bithiophen]-5-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (16d)

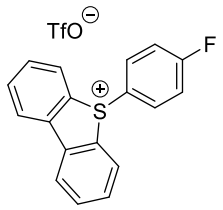
Literature procedure starting from 2,2'-bithiophene **15d** (872 mg, 5.2 mmol) gave **16d** (2.1 g, 91%) as a brown solid. ^{1}H NMR is in agreement with literature.[95]



5-(Benzo[*b*]thiophen-3-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoroacetate (16e)

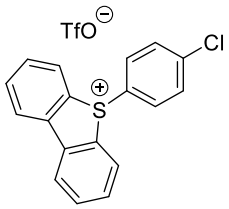
Dibenzothiophene-*S*-oxide (1.0 g, 5.0 mmol) was suspended in MeCN (15 mL) and the solution was cooled down to –78 °C. A solution of benzothiophene (670 mg, 5.0 mmol) in DCM (5 mL) was slowly added. Subsequently trifluoroacetic acid anhydride (1.1 mL, 7.5 mmol) was added dropwise. The mixture was stirred at –78 °C for 15 min and then moved to r.t. for another 1 h. Then the solvent was removed under reduced pressure. Flash chromatography (SiO_2, DCM/MeOH 0–15%) afforded compound **16e** (558 mg, 26%) as a gray foam. mp 127–137 °C. IR (ATR): ν = 3062, 1676, 1449, 1195, 1163, 1115, 750 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.79 (d, 1H, $J_{4,5}$ = 8.3 Hz, H-4-BT); 7.26 (ddd, 1H, $J_{5,4}$ = 8.3 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,7}$ = 1.1 Hz, H-5-BT); 7.44 (ddd, 1H, $J_{6,7}$ = 8.3 Hz, $J_{6,5}$ = 7.1 Hz, $J_{6,4}$ = 1.1 Hz, H-6-BT); 7.73 (ddd, 2H, $J_{2,1}$, $J_{8,9}$ = 8.4, $J_{2,3}$, $J_{8,7}$ = 7.5 Hz, $J_{2,4}$, $J_{8,6}$ = 1.2 Hz, H-2, H-8); 8.00 (td, 2H, $J_{3,4}$, $J_{7,6}$ = $J_{3,2}$, $J_{7,8}$ = 7.6 Hz, $J_{3,1}$, $J_{7,9}$ = 1.0 Hz, H-3, H-7); 8.21 (dt, 1H, $J_{7,6}$ = 8.3 Hz, $J_{7,5}$ =

$J_{7,4}$ = 1.0 Hz, H-7-BT); 8.32 (dd, 2H, $J_{1,2}$, $J_{9,8}$ = 8.1 Hz, $J_{1,3}$, $J_{9,7}$ = 1.0 Hz, H-1, H-9); 8.61 (dd, 2H, $J_{4,3}$, $J_{6,7}$ = 7.9 Hz, $J_{4,2}$, $J_{6,8}$ = 1.2 Hz, H-4, H-6); 9.17 (s, 1H, H-2-BT). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO-d_6): δ 112.1 (C-3-BT); 119.8 (CH-4-BT); 124.4 (CH-4, CH-6); 124.7 (CH-7-BT); 126.3 and 126.4 (CH-5-BT, CH-6-BT); 128.2 (CH-1, CH-9); 130.6 (C-9a, C-9b); 131.4 (CH-2, CH-8); 132.9 (C-3a-BT); 134.1 (CH-3, CH-7); 139.3 (C-4a, C-5a); 139.9 (C-7a-BT); 145.3 (CH-8-BT). ^{19}F NMR (376 MHz, DMSO-d_6) δ –73.4. MS (ESI): *m/z* (rel. %): 317 (100) $[M]^+$. HRMS (ESI): *m/z* $[M]^+$ Calcd for $C_{20}H_{13}S_2$ 317.04532; Found 317.04536.



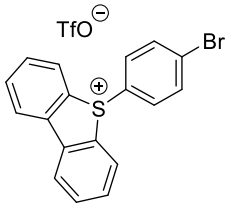
5-(4-Fluorophenyl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16f)

Literature procedure starting from fluorobenzene **15f** (0.38 mg, 4 mmol) gave **16f** (1.4 g, 82%) as a white solid. ^{1}H NMR is in agreement with literature.[96]



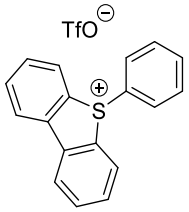
5-(4-Chlorophenyl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16g)

Literature procedure starting from chlorobenzene **15g** (0.41 mL, 4 mmol) gave **16g** (1.3 g, 73%) as a white solid. ^{1}H NMR is in agreement with literature.[96]



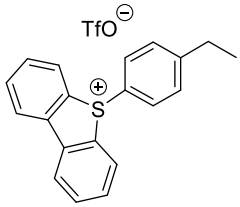
5-(4-Bromophenyl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16h)

Literature procedure starting from bromobenzene **15h** (0.42 mL, 4 mmol) gave **16h** (1.09 mg, 56%) as a light yellow foam. ^{1}H NMR is in agreement with literature.[96]



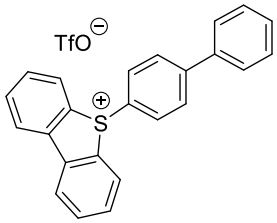
5-Phenyl-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16i)

Literature procedure starting from benzene **15i** (0.36 mL, 4 mmol) gave **16i** (1.4 g, 85%) as a white solid. ^{1}H NMR is in agreement with literature.[96]



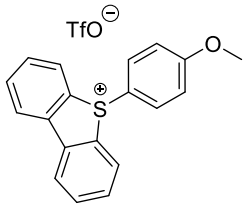
5-(4-Ethylphenyl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16j)

Literature procedure starting from ethylbenzene **15j** (0.47 mL, 3.8 mmol) gave **16j** (1.59 g, 94%) as a yellow foam. ^{1}H NMR is in agreement with literature.[96]



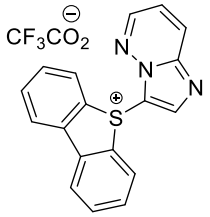
5-([1,1'-Biphenyl]-4-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (**16k**)

Dibenzothiophene-*S*-oxide (800 mg, 4.0 mmol) and biphenyl (616 mg, 4.0 mmol) were dissolved in DCM (30 mL) and this mixture was cooled down to –78 °C, after which triflic anhydride (0.74 mL, 4.4 mmol) was added dropwise. After 10 minutes, the mixture was moved to r.t. for 40 min. The crude **16k** (1.8 g, white foam) was directly used for the next coupling reaction after a filtration on short silica gel column eluting with DCM/MeOH 2:1 and removal of the volatiles.



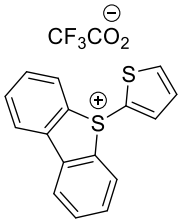
5-(4-Methoxyphenyl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium trifluoromethanesulfonate (16l)

Literature procedure starting from anisole **15l** (0.54 mL, 5 mmol) gave **16l** (1.66 g, 82%) as a colorless glue. ^{1}H NMR is in agreement with literature.[96]



5-(Imidazo[1,2-*b*]pyridazin-3-yl)-5*H*-dibenzo[b,d]thiophen-5-ium 2,2,2-trifluoroacetate (16m)

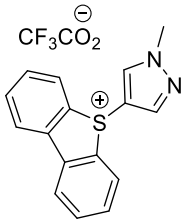
Literature procedure starting from imidazo[1,2-b]pyridazine **15m** (476 mg, 4 mmol) gave **16m** (1.13 g, 68%) as a brown foam. ^{1}H NMR is in agreement with literature.[95]



5-(Thiophen-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (16n)

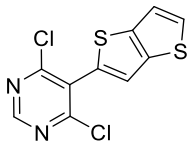
Dibenzothiophene-*S*-oxide (1.0 g, 5 mmol), thiophene (0.40 mL, 5 mmol) and MeCN (15 mL) were added to a 100 mL flask, and the mixture was cooled down to –78 °C. Subsequently trifluoroacetic anhydride (1.1 mL, 7.5 mmol) was added dropwise. The mixture was stirred at –78 °C for 10 min and then slowly warmed to r.t. over 1 h. After the removal of the volatiles under vacuum, the residue was purified by HPFC [SiO_2; EtOAc, DCM and then DCM/MeOH (4:1)] to afford the product **16n** (1.6 g, 84%) as a grey solid. mp 99–102 °C. IR (ATR): ν = 3078, 3005, 1675, 1395, 1160, 1114, 798 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30 (dd, 1H, $J_{4,5}$ = 5.2 Hz, $J_{4,3}$ =

3.9 Hz, H-4-T); 7.76 (ddd, 2H, $J_{2,1}$, $J_{8,9}$ = 8.1, $J_{2,3}$, $J_{8,7}$ = 7.4 Hz, $J_{2,4}$, $J_{8,6}$ = 1.2 Hz, H-2, H-8); 7.95 (td, 2H, $J_{3,4}$, $J_{7,6}$ = $J_{3,2}$, $J_{7,8}$ = 7.6 Hz, $J_{3,1}$, $J_{7,9}$ = 1.1 Hz, H-3, H-7); 8.06 (dd, 1H, $J_{5,4}$ = 5.2 Hz, $J_{5,3}$ = 1.4 Hz, H-5-T); 8.32 (dd, 1H, $J_{3,4}$ = 3.9 Hz, $J_{3,5}$ = 1.4 Hz, H-3-T); 8.39 – 8.45 (m, 2H, H-1, H-9); 8.50 (dd, 2H, $J_{4,3}$, $J_{6,7}$ = 7.8 Hz, $J_{4,2}$, $J_{6,8}$ = 1.3 Hz, H-4, H-6). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO-d_6): δ 124.4 (CH-4, CH-6); 124.6 (C-2-T); 128.0 (CH-1, CH-9); 129.3 (CH-4-T); 131.3 (CH-2, CH-8); 134.0 (CH-3, CH-7); 135.4 (C-9a, C-9b); 137.5 (CH-5-T); 138.2 (C-4a, C-5a); 140.3 (CH-3-T). ^{19}F NMR (376 MHz, DMSO-d_6): δ –73.5. MS (ESI): *m/z* (rel. %): 267 (63) $[M]^+$. HRMS (ESI): *m/z* $[M]^+$ Calcd for $C_{16}H_{11}S_2$ 267.02967; Found 267.02927.



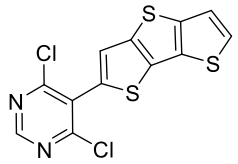
5-(1-Methyl-1*H*-pyrazol-4-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (16u)

Compound **16u** was prepared using same procedure as **16n**. 1-Methyl-1*H*-pyrazole (0.42 mL, 5 mmol) gave **16u** (1.75 g, 93%) as a light yellow solid. mp 147–150 °C. IR (ATR): ν = 3081, 3012, 2951, 1679, 1514, 1195, 1113, 798, 751 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.97 (s, 3H, CH_3); 6.90 (d, 1H, $J_{3,5}$ = 0.7 Hz, H-3-Py); 7.65 (ddd, 2H, $J_{3,4}$, $J_{7,6}$ = 8.1 Hz, $J_{3,2}$, $J_{7,8}$ = 7.5 Hz, $J_{3,1}$, $J_{7,9}$ = 1.2 Hz, H-3, H-7); 7.82 (td, 2H, $J_{2,1}$, $J_{8,9}$ = $J_{2,3}$, $J_{8,7}$ = 7.7 Hz, $J_{2,4}$, $J_{8,6}$ = 1.1 Hz, H-2, H-8); 8.10 (dd, 2H, $J_{1,2}$, $J_{9,8}$ = 7.9 Hz, $J_{1,3}$, $J_{9,7}$ = 1.2 Hz, H-1, H-9); 8.28 (dd, 2H, $J_{4,3}$, $J_{6,7}$ = 8.7 Hz, $J_{4,2}$, $J_{6,8}$ = 1.0 Hz, H-4, H-6); 9.46 (s, 1H, H-5-Py). ^{13}C NMR (101 MHz, $CDCl_3$): δ 40.4 (CH_3); 100.7 (C-4-Py); 123.6 (CH-1, CH-9); 128.8 (CH-4, CH-6); 131.7 (CH-3, CH-7); 133.4 (C-4a, C-5a); 134.0 (CH-2, CH-8); 138.0 (CH-3-Py); 138.3 (C-9a, C-9b); 140.2 (CH-5-Py). ^{19}F NMR (376 MHz, $CDCl_3$): δ –75.2. MS (ESI): *m/z* (rel. %): 265.1 (100) $[M]^+$. HRMS (ESI): *m/z* $[M]^+$ Calcd for $C_{16}H_{13}N_2S$ 265.07940; Found 265.07943.



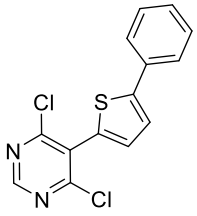
4,6-Dichloro-5-(thieno[3,2-*b*]thiophen-2-yl)pyrimidine (18a)

Following the general procedure for novel Negishi coupling, compound **18a** was obtained as a light yellow solid (85 mg, 64%). mp 123–125 °C. IR (ATR): ν = 1737, 1494, 1384, 1358, 1217, 798, 697 cm^{-1}. ^{1}H NMR (500.0 MHz, $CDCl_3$): 7.32–7.34 (m, 2H, H-3,6-thienothienyl); 7.49 (d, 1H, $J_{5,6}$ = 5.2, H-5-thienothienyl); 8.80 (s, 1H, H-2-pyrimidine). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): 119.4 (CH-6-thienothienyl); 122.2 (CH-3-thienothienyl); 128.2 (C-5-pyrimidine); 128.7 (CH-5-thienothienyl); 133.2 (C-2-thienothienyl); 138.9 (C-3a-thienothienyl); 141.3 (C-6a-thienothienyl); 157.2 (CH-2-pyrimidine); 162.7 (C-4,6-pyrimidine). MS (ESI): *m/z* (rel. %): 286.9 (35) [M + H]$^+$. HRMS (ESI): *m/z* [M + H]$^+$ Calcd for $C_{10}H_5N_2Cl_2S_2$ 286.92657; Found 286.92681.



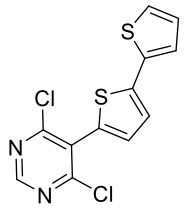
4,6-Dichloro-5-(dithieno[3,2-*b*:2',3'-*d*]thiophen-2-yl)pyrimidine (18b)

Following the general procedure for novel Negishi coupling, compound **18b** was obtained as a light yellow solid (4.6 g, 60%). mp 168–169 °C. IR (ATR): ν = 1494, 1396, 1360, 1323, 1226, 801 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.33 (d, 1H, $J_{5,6}$ = 5.3 Hz, H-5-DTT); 7.35 (s, 1H, H-3-DTT); 7.45 (d, 1H, $J_{6,5}$ = 5.3 Hz, H-6); 8.81 (s, 1H, H-2). ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): 120.9 (CH-5-DTT); 123.8 (CH-3-DTT); 127.4 (CH-6-DTT); 128.1 (C-5); 130.8 (C-7a-DTT); 131.6 (C-2/3a-DTT); 133.2 (C-7b-DTT); 141.1 (C-2/3a-DTT); 142.4 (C-4a-DTT); 157.4 (CH-2); 162.9 (C-4,6). MS (APCI): *m/z* (rel. %): 341.9 (26) [M]$^+$. HRMS (APCI): *m/z* [M]$^+$ Calcd for $C_{12}H_4N_2Cl_2S_3$ 341.89082; Found 341.89090.



4,6-Dichloro-5-(5-phenylthiophen-2-yl)pyrimidine (18c)

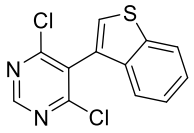
Following the general procedure for novel Negishi coupling, compound **18c** was obtained as a white solid (175 mg, 65%). mp 134–137 °C. IR (ATR): v = 3083, 1504, 1397, 1359, 797, 752 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (d, 1H, $J_{3,4}$ = 3.8 Hz, H-3-thienyl); 7.31–7.36 (m, 1H, H-*p*-Ph); 7.37 (d, 1H $J_{4,3}$ = 3.8 Hz, H-4-thienyl); 7.39–7.46 (m, 2H, H-*m*-Ph); 7.61–7.69 (m, 2H, H-*o*-Ph); 8.78 (s, 1H, H-2). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$): δ 123.2 (CH-4-thienyl); 126.2 (CH-*o*-Ph); 128.3 (C-5); 128.4 (CH-*p*-Ph); 129.2 (CH-*m*-Ph); 131.0 (C-2-thienyl); 131.3 (CH-3-thienyl); 133.6 (C-*i*-Ph); 148.0 (C-5-thienyl); 157.0 (CH-2); 162.6 (C-4, C-6). MS (EI): *m/z* (rel. %): 306 (100) $[M]^+$, 307 (13) $[M + H]^+$. HRMS (EI): *m/z* $[M]^+$ Calcd for $C_{14}H_8Cl_2N_2S$ 305.9780; Found 305.9790.



5-([2,2'-Bithiophen]-5-yl)-4,6-dichloropyrimidine (18d)

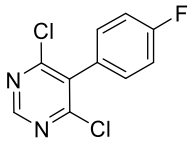
Following the general procedure for novel Negishi coupling, compound **18d** was obtained as a yellow solid (245 mg, 72%). mp 103–105 °C. IR (ATR): v = 3083, 3065, 1498, 1395, 1350, 1222, 793 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.13 (dd, 1H, $J_{4',5'}$= 5.1, Hz, $J_{4',3'}$ = 3.6 Hz, H-4′-thienyl); 7.26 (d, 1H, $J_{4,3}$ = 3.7 Hz, H-4-thienyl); 7.36–7.44 (m, 2H, H-3- thienyl, H-3′-thienyl); 7.59 (dd, 1H, $J_{5',4'}$ = 5.1, Hz, $J_{5',3'}$ = 1.2 Hz, H-5′-thienyl); 8.96 (s, 1H, H-2). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO-d_6): δ 124.0 (CH-3-thienyl); 124.9 (CH-3′-thienyl); 126.3 (CH-5′-thienyl); 127.3 (C-5); 128.5 (CH-4′-thienyl); 130.3 (C-5-thienyl); 131.7 (CH-4-thienyl); 135.4 (C-2′-thienyl); 139.7 (C-2-thienyl); 157.4 (C-2); 161.5 (C-4, C-6). MS (APCI): *m/z*

(rel. %): 311.9 (100) $[M]^+$, 312.9 (30) $[M + H]^+$. HRMS (APCI): *m/z* $[M]^+$ Calcd for $C_{12}H_6N_2Cl_2S_2$ 311.93440; Found 311.93475.



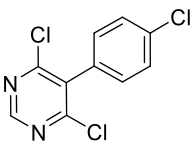
5-(Benzo[*b*]thiophen-3-yl)-4,6-dichloropyrimidine (18e)

Following the general procedure for novel Negishi coupling, compound **18e** was obtained as a yellow solid (138 mg 53%). **18e** is a known compound and ^{1}H NMR of **18e** is in agreement with literature.[122]



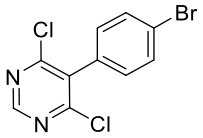
4,6-Dichloro-5-(4-fluorophenyl)pyrimidine (18f)

Following the general procedure for novel Negishi coupling, compound **18f** was obtained as a light yellow solid (177 mg, 62%). mp 92–94 °C. IR (ATR): ν = 1542, 1504, 1396, 1367, 1219, 1155, 802 cm^{-1}. ^{1}H NMR (401 MHz, $CDCl_3$): δ 7.18–7.24 (m, 2H, H-*m*-Ph); 7.28–7.33 (m, 2H, H-*o*-Ph); 8.78 (s, 1H, H-2). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 116.2 (d, J = 21.9 Hz, CH-*m*-Ph), 128.7 (d, J = 3.7 Hz, C-*i*-Ph), 131.4 (d, J = 8.5 Hz, CH-*o*-Ph), 133.3 (C-5), 157.0 (CH-2), 161.7 (C-4, C-6), 163.3 (d, J = 249.9 Hz, C-*p*-Ph). ^{19}F NMR (376 MHz, $CDCl_3$) δ –110.9. MS (EI): *m/z* (rel. %): 242 (100) $[M]^+$. HRMS (EI): *m/z* $[M]^+$ Calcd for $C_{10}H_5Cl_2FN_2$ 241.9808; Found 241.9813.



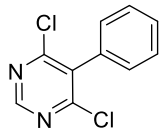
4,6-Dichloro-5-(4-chlorophenyl)pyrimidine (18g)

Following the general procedure for novel Negishi coupling, compound **18g** was obtained as a light yellow solid (254 mg, 87%). **18g** is a known compound and ^{1}H NMR of **18g** is in agreement with literature.[138]



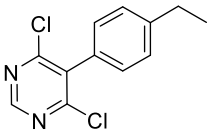
5-(4-Bromophenyl)-4,6-dichloropyrimidine (18h)

Following the general procedure for novel Negishi coupling, compound **18h** was obtained as a white solid (263 mg, 84%). **18h** is a known compound and ^{1}H NMR of **18h** is in agreement with literature.[138]



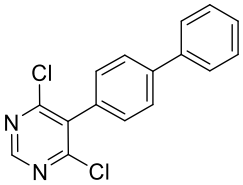
4,6-Dichloro-5-phenylpyrimidine (18i)

Following the general procedure for novel Negishi coupling, compound **18i** was obtained as a yellow solid (100 mg, 45%). **18i** is a known compound and ^{1}H NMR of **18i** is in agreement with literature.[138]



4,6-Dichloro-5-(4-ethylphenyl)pyrimidine (18j)

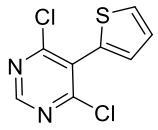
Following the general procedure for novel Negishi coupling, compound **18j** was obtained as a colorless oil (150 mg, 52%). **18j** is a known compound and ^{1}H NMR of **18j** is in agreement with literature.[138]



5-([1,1'-Biphenyl]-4-yl)-4,6-dichloropyrimidine (18k)

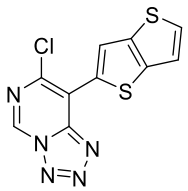
Following the general procedure for novel Negishi coupling, compound **18k** was obtained as a white solid (130 mg, 42% over two steps). mp 130–132 °C. IR (ATR): ν = 1505, 1484, 1396, 1371, 1224, 789, 761 cm^{-1}. ^{1}H NMR (401 MHz, $CDCl_3$): δ 7.36–7.44 (m, 3H, H-3-biphenyl, H-5-biphenyl, H-4′-biphenyl); 7.46–7.51 (m, 2H,

H-3′-biphenyl, H-5′-biphenyl); 7.63–7.69 (m, 2H, H-2′-biphenyl, H-6′-biphenyl); 7.72–7.78 (m, 2H, H-2-biphenyl, H-6-biphenyl); 8.80 (s, 1H, H-2). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$): δ 127.3 (CH-2′-biphenyl, CH-6′-biphenyl); 127.6 (CH-2-biphenyl, CH-6-biphenyl); 128.0 (CH-4′-biphenyl); 129.1 (CH-3′-biphenyl, CH-5′-biphenyl); 129.8 (CH-3-biphenyl, CH-5-biphenyl); 131.6 (C-4-biphenyl); 134.0 (C-5); 140.2 (C-1′-biphenyl); 142.4 (C-1-biphenyl); 156.9 (CH-2); 161.6 (C-4, C-6). MS (EI): *m/z* (rel. %): 300 (100) $[M]^+$, 301 (15) $[M + H]^+$. HRMS (EI): *m/z* $[M]^+$ Calcd for $C_{16}H_{10}Cl_2N_2$ 300.0216; Found 300.0221.



4,6-Dichloro-5-(thiophen-2-yl)pyrimidine (18n)

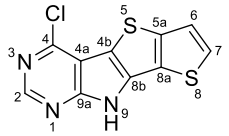
Following the general procedure for novel Negishi coupling, compound **18n** was obtained as a yellow solid (171 mg, 56%). **18n** is a known compound and ^{1}H NMR of **18n** is in agreement with literature.[71]



7-Chloro-8-(thieno[3,2-*b*]thiophen-2-yl)tetrazolo[1,5-*c*]pyrimidine (19a)

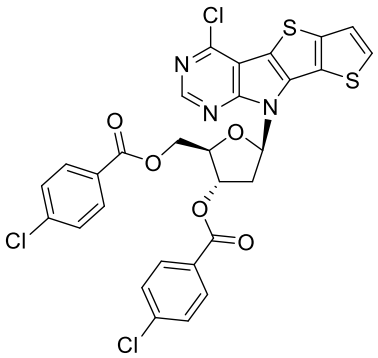
Following the general procedure for azidation, compound **18a** (2.96 g, 10.3 mmol) gave crude product **19a** (3.78 g) as a yellow solid. IR (ATR): ν = 3362, 3084, 1579, 1497, 1377, 1330, 1072, 917 cm^{-1}. ^{1}H NMR (500.0 MHz, DMSO-d_6): 7.60 (dd, 1H, $J_{6,5}$ = 5.2, $J_{6,3}$ = 0.8, H-6-thienothienyl); 7.92 (d, 1H, $J_{5,6}$ = 5.2, H-5-thienothienyl); 8.82 (d, 1H, $J_{3,6}$ = 0.8, H-3-thienothienyl); 10.16 (s, 1H, H-6-tetrazolopyrimidine). $^{13}C\{^{1}H\}$ NMR (125.7 MHz, DMSO-d_6): 116.9 (C-5-tetrazolopyrimidine); 120.2 (CH-6-thienothienyl); 126.2 (CH-3-thienothienyl); 131.6 (C-2-thienothienyl); 132.2 (CH-5-thienothienyl); 137.4 (CH-2-tetrazolopyrimidine); 139.2 (C-3a-thienothienyl); 142.4 (C-7-tetrazolopyrimidine); 142.5 (C-6a-thienothienyl); 150.4

(C-8a-tetrazolopyrimidine). MS (APCI): *m/z* (rel. %): 266 (100) $[M + H–N_2]^+$. HRMS (APCI): *m/z* $[M + H]^+$ Calcd for $C_{10}H_5N_5ClS_2$ 293.96694; Found 293.96734.



4-Chloro-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (20a)

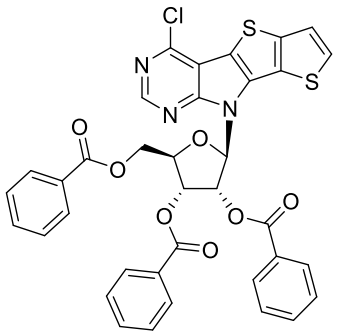
Following the general procedure for thermal cyclization, tetrazole **19a** (2.78 g, crude product) gave nucleobase **20a** (1.52 g, 75% over 2 steps) as a brown solid. mp 261 °C (decomp.). IR (ATR): ν = 3059, 2917, 1563, 1530, 1317, 1231, 863, 728 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 7.66 (d, 1H, $J_{6,7}$ = 5.2, H-6); 7.88 (d, 1H, $J_{7,6}$ = 5.2, H-7); 8.67 (s, 1H, H-2); 13.34 (s, 1H, H-9). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 113.0 (C-4a); 113.4 (C-4b); 122.1 (CH-6); 123.5 (C-8a); 130.1 (CH-7); 135.1 (C-8b); 143.9 (C-5a); 147.7 (C-4); 150.8 (CH-2); 155.2 (C-9a). MS (ESI): *m/z* (rel. %): 266 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{10}H_5N_3ClS_2$ 265.96079; Found 265.96070.



4-Chloro-9-(3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (21a)

Following the general procedure for glycosylation to deoxyribonucleoside, nucleobase **20a** (500 mg, 1.9 mmol) gave product **21a** (602 mg, 49%) as a white solid. mp 84–87 °C. IR (ATR): ν = 3090, 1716, 1591, 1262, 1087, 1013, 756 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 2.86 (ddd, 1H, J_{gem} = 14.5, $J_{2'b,1'}$ = 6.2, $J_{2'b,3'}$ = 2.7, H-2′b); 3.48 (dt, 1H, J_{gem} = 14.5, $J_{2'a,1'} = J_{2'a,3'}$ = 8.0, H-2′a); 4.68 (dt, 1H, $J_{4',5'}$ = 6.1, 4.3, $J_{4',3'}$ = 4.3, H-4′); 4.75 (dd, 1H, J_{gem} = 11.8, $J_{5'b,4'}$ = 6.1, H-5′b); 4.93 (dd, 1H, J_{gem} = 11.8, $J_{5'a,4'}$ =

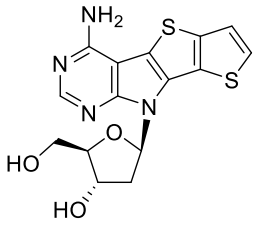
4.3, H-5′a); 5.91 (ddd, 1H, $J_{3',2'}$ = 7.9, 2.7, $J_{3',4'}$ = 4.3, H-3′); 6.88 (dd, 1H, $J_{1',2'}$ = 8.0, 6.2, H-1′); 7.28–7.31 (m, 2H, H-*m*-C_6H_4Cl); 7.41 (d, 1H, $J_{6,7}$ = 5.3, H-6); 7.46–7.49 (m, 2H, H-*m*-C_6H_4Cl); 7.49 (d, 1H, $J_{7,6}$ = 5.3, H-7); 7.79–7.83, 8.02–8.05 (2 × m, 2 × 2H, H-*o*-C_6H_4Cl); 8.66 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 37.6 (CH_2-2′); 64.2 (CH_2-5′); 74.7 (CH-3′); 81.9 (CH-4′); 85.0 (CH-1′); 114.2 (C-4a); 116.2 (C-4b); 121.3 (CH-6); 123.7 (C-8a); 127.6, 127.8 (C-*i*-C_6H_4Cl); 128.5 (CH-7); 128.6, 129.0 (CH-*m*-C_6H_4Cl); 131.0, 131.2 (CH-*o*-C_6H_4Cl); 134.6 (C-8b); 139.7, 140.3 (C-*p*-C_6H_4Cl); 144.5 (C-5a); 149.5 (C-4); 150.6 (CH-2); 154.0 (C-9a); 165.1, 165.2 (CO). MS (APCI): *m/z* (rel. %): 658 (99) $[M + H]^+$. HRMS (APCI): *m/z* $[M + H]^+$ Calcd for $C_{29}H_{19}O_5N_3Cl_3S_2$ 657.98262; Found 657.98256.



4-Chloro-9-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (22a)

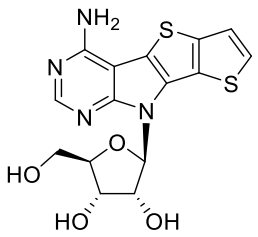
Following the general procedure for glycosylation to ribonucleoside, nucleobase **20a** (1.15g, 4.3 mmol) gave product **22a** (1.56 g, 51%) as a yellow foam. mp 75–79 °C. IR (ATR): ν = 3062, 1721, 1451, 1263, 1113, 1090, 1025, 987, 707 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.79 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'a,4'}$ = 5.5 Hz, H-5′a); 4.88 (bdt, 1H, $J_{4',5'a} = J_{4',3'}$ = 5.9 Hz, $J_{4',3'}$ = 3.4 Hz, H-4′); 5.03 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'b,4'}$ = 3.4 Hz, H-5′b); 6.34 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 6.5 Hz, H-3′); 6.55 (dd, 1H, $J_{2',3'}$ = 6.7 Hz, $J_{2',1'}$ = 5.1 Hz, H-2′); 6.77 (d, 1H, $J_{1',2'}$ = 5.1 Hz, H-1′); 7.33 (m, 2H, H-*m*-Bz-2′); 7.36 (m, 2H, H-*m*-Bz-5′); 7.39 (m, 2H, H-*m*-Bz-3′); 7.40 (d, 1H, $J_{6,7}$ = 5.3 Hz, H-6); 7.47 (d, 1H, $J_{7,6}$ = 5.3 Hz, H-7); 7.48–7.61 (m, 3H, H-*p*-Bz); 7.90 (m, 2H, H-*o*-Bz-2′); 7.96 (m, 2H, H-*o*-Bz-5′); 8.00 (m, 2H, H-*o*-Bz-3′); 8.60 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): 63.5 (CH_2-5′); 70.7 (CH-3′); 73.6 (CH-2′); 80.5 (CH-4′); 88.3 (CH-1′);

114.7 (C-4a); 116.3 (C-4b); 121.4 (CH-6); 123.3 (C-8a); 128.48 (CH-*m*-Bz); 128.56 (CH-7); 128.62 and 128.65 (CH-*m*-Bz); 128.9 and 129.6 (C-*i*-Bz); 129.8 (CH-*o*-Bz-5′); 129.9 (CH-*o*-Bz-2′); 130.0 (CH-*o*-Bz-3′); 133.4 (CH-*p*-Bz-5′); 133.80 and 133.82 (CH-*p*-Bz-2′,3′); 135.0 (C-8b); 144.8 (C-5a); 149.6 (C-4); 150.9 (CH-2); 154.5 (C-9a); 165.2 (CO-2′); 165.4 (CO-3′); 166.2 (CO-5′). MS (ESI): *m/z* (rel. %): 710 (7) $[M + H]^+$, 732 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{36}H_{25}O_7N_3ClS_2$ 710.08170; Found 710.08133.



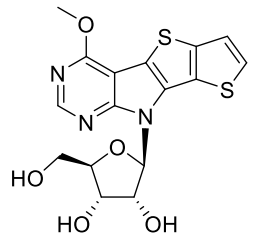
4-Amino-9-(2′-deoxy-*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (23a)

Following the general procedure for amination, protected nucleoside **21a** (320 mg, 0.49 mmol) gave product **23a** (120 mg, 65%) as a white solid. mp 237–239 °C. $[\alpha]_D$ = +13.1 (*c* = 0.335 g/100 mL in DMSO). IR (ATR): *v* = 3394, 3310, 3114, 1596, 1550, 1390, 1095, 982, 703 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 2.27 (ddd, 1H, J_{gem} = 13.6 Hz, $J_{2'a,1'}$ = 6.0 Hz, $J_{2'a,3'}$ = 2.3 Hz, H-2′a); 2.79 (ddd, 1H, J_{gem} = 13.6 Hz, $J_{2'b,1'}$ = 9.0 Hz, $J_{2'b,3'}$ = 7.5 Hz, H-2′b); 3.72 (dd, 1H, J_{gem} = 11.7 Hz, $J_{5'a,4'}$ = 6.3 Hz, H-5′a); 3.76 (dd, 1H, J_{gem} = 11.7 Hz, $J_{5'b,4'}$ = 4.6 Hz, H-5′b); 3.89 (dt, 1H, $J_{4',5'a}$ = 6.3 Hz, $J_{4',5'b}$ = $J_{4',3'}$ = 4.3 Hz, H-4′); 4.40 (m, 1H, H-3′); 5.43 (bs, 1H, OH-3′); 6.68 (dd, 1H, $J_{1',2'b}$ = 9.0 Hz, $J_{1',2'a}$ = 6.0 Hz, H-1′); 7.29 (bs, 2H, NH_2); 7.59 (d, 1H, $J_{6,7}$ = 5.3 Hz, H-6); 7.71 (d, 1H, $J_{7,6}$ = 5.3 Hz, H-7); 8.21 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 39.8 (CH_2-2′); 62.3 (CH_2-5′); 70.9 (CH-3′); 83.4 (CH-1′); 87.4 (CH-4′); 98.2 (C-4a); 116.1 (C-4b); 121.5 (CH-6); 123.5 (C-8a); 127.2 (CH-7); 130.2 (C-8b); 141.2 (C-5a); 151.8 (CH-2); 153.2 (C-9a); 155.6 (C-4). MS (ESI): *m/z* (rel. %): 363 (82) $[M + H]^+$, 385 (7) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{15}H_{15}O_3N_4S_2$ 363.05801; Found 363.05833.



4-Amino-9-(*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (24a)

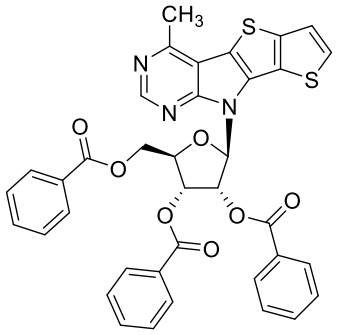
Following the general procedure for amination, protected nucleoside **22a** (330 mg, 0.46 mmol) gave product **24a** (72 mg, 41%) as a light yellow solid. mp 252–255 °C. $[\alpha]_D$ = +17.3 (*c* = 0.323 g/100 mL in DMSO). IR (ATR): *v* = 3431, 3319, 3158, 1644, 1630, 1396, 1061, 1029, 702 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.69 (ddd, 1H, J_{gem} = 12.0 Hz, $J_{5'a,4'}$ =7.0 Hz, $J_{5'a,OH}$ = 5.5 Hz, H-5′a); 3.78 (dt, 1H, J_{gem} = 11.8 Hz, $J_{5'b,4'} = J_{5'b,OH}$ = 4.5 Hz, H-5′b); 3.97 (dt, 1H, $J_{4',5'a}$ = 5.5 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.0 Hz, H-4′); 4.12–4.20 (m, 1H, H-3′); 4.73 (q, 1H, $J_{2',1'} = J_{2',3'} = J_{2',OH}$ = 6.5 Hz, H-2′);5.23 (d, 1H, $J_{OH,3'}$ = 4.8 Hz, OH-3′); 5.31 (dd, 1H, $J_{OH,5'a}$ = 7.0 Hz, $J_{OH,5'b}$ = 4.9 Hz, OH-5′); 5.43 (d, 1H, $J_{OH,2'}$ = 6.3 Hz, OH-2′); 6.17 (d, 1H, $J_{1',2'}$ = 7.2 Hz, H-1′); 7.25 (s, 2H, NH_2), 7.61 (d, 1H, $J_{6,7}$ = 5.2 Hz, H-6);7.71 (d, 1H, $J_{7,6}$ = 5.2 Hz, H-7); 8.19 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 62.4 (CH_2-5′); 70.1 (CH-3′); 72.2 (CH-2′); 86.0 (CH-4′); 88.4 (CH-1 ′); 98.4 (C-4a); 115.8 (C-4b); 121.5 (CH-6); 123.1 (C-8a); 127.0 (CH-7); 130.7 (C-8b); 141.2 (C-5a); 152.0 (CH-2); 153.7 (C-9a); 155.9 (C-4). MS (ESI): *m/z* (rel. %): 379 (67) $[M + H]^+$, 401 (81) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{15}H_{15}O_4N_4S_2$ 379.05292; Found 379.05256.



4-Methoxy-9-(*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (25a)

The reaction of compound **22a** (320 mg, 0.45 mmol) was set up according to the general procedure for methoxylation, the purification was performed as below: The

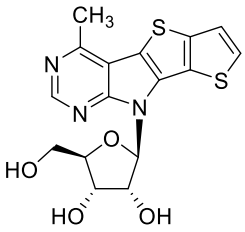
solvent was evaporated till 2−3 mL, and EtOAc (6 mL) was added. The mixture was heated to 60 °C for 10 min, then, filtrated after cooling down on the ice. The solid residue was washed by cold EtOAc (2 × 2 mL) and water (2 × 3 mL) to obtain product **25a** (86 mg, 48%) as a yellow solid. mp 241–244 °C. $[\alpha]_D$ = +14.9 (*c* = 0.322 g/100 mL in DMSO). IR (ATR): *v* = 3413, 3211, 3075, 2921, 1602, 1549, 1341, 1326, 1077, 1056, 891, 877 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.72 (dt, 1H, J_{gem} = 11.8 Hz, $J_{5'a,4'} = J_{5'a,OH}$ = 6.1 Hz, H-5′a); 3.81 (dt, 1H, J_{gem} = 11.8 Hz, $J_{5'b,4'} = J_{5'b,OH}$ = 5.0 Hz, H-5′b); 3.98 (dt, 1H, $J_{4',5'a}$ = 5.9 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.4 Hz, H-4′); 4.17 (s, 3H, CH_3O); 4.18 (m, 1H, H-3′); 4.71 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.6 Hz, H-2′); 5.07 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.8 Hz, OH-5′); 5.33 (bs, 1H, OH-3′); 5.52 (bs, 1H, OH-2′); 6.27 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1′); 7.64 (d, 1H, $J_{6,7}$ = 5.3 Hz, H-6); 7.81 (d, 1H, $J_{7,6}$ = 5.3 Hz, H-7); 8.57 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 54.2 (CH_3O); 62.3 (CH_2-5′); 69.8 (CH-3′); 72.3 (CH-2′); 85.9 (CH-4′); 88.2 (CH-1′); 101.0 (C-4a); 114.8 (C-4b); 121.7 (CH-6); 123.5 (C-8a); 128.5 (CH-7); 132.4 (C-8b); 142.2 (C-5a); 151.1 (CH-2); 155.0 (C-9a); 161.1 (C-4). MS (ESI): *m/z* (rel. %): 394 (4) $[M + H]^+$, 416 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{16}H_{16}O_5N_3S_2$ 394.05259; Found 394.05222.



4-Methyl-9-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5'] thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (26a)

Following the general procedure for methylation, protected nucleoside **22a** (350 mg, 0.49 mmol) gave compound **26a** (210 mg, 62%) as a yellow foam. mp 106–108 °C. IR (ATR): *v* = 3061, 2922, 1720, 1449, 1259, 1090, 1066, 704 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.82 (s, 3H, CH_3); 4.79–4.88 (m, 2H, H-5′a, H-4′); 4.99–5.06 (m, 1H, H-5′b); 6.37 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 6.4 Hz, H-3′); 6.53 (dd, 1H, $J_{2',3'}$ = 6.8 Hz, $J_{2',1'}$ = 5.3

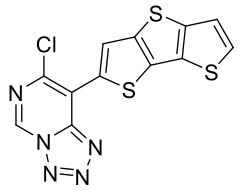
Hz, H-2′); 6.82 (d, 1H, $J_{1',2'}$ = 5.3 Hz, H-1′); 7.29–7.40 (m, 6H, H-*m*-Bz); 7.38 (d, 1H, $J_{6,7}$ = 5.3 Hz, H-6); 7.42 (d, 1H, $J_{7,6}$ = 5.3 Hz, H-7); 7.48–7.59 (m, 3H, H-*p*-Bz); 7.87–7.92 (m, 2H, H-*o*-Bz-2′); 7.96–8.02 (m, 4H, H-*o*-Bz-5′, H-*o*-Bz-3′); 8.76 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 22.6 (CH_3); 63.8 (CH_2-5′); 70.7 (CH-3′); 73.7 (CH-2′); 80.3 (CH-4′); 87.8 (CH-1′); 115.0 (C-4a); 117.3 (C-4b); 121.2 (CH-6); 123.8 (C-8a); 127.6 (CH-7); 128.46, 128.58 and 128.61 (CH-*m*-Bz); 128.8, 129.0 and 129.7 (C-*i*-Bz); 129.9 and 130.0 (CH-*o*-Bz); 133.3 and 133.7 (CH-*p*-Bz); 143.5 (C-5a); 151.8 (CH-2); 154.3 (C-9a); 157.2 (C-4); 165.3 and 165.4 (CO-2′, CO-3′), 166.3 (CO-5′). MS (ESI): *m/z* (rel. %): 690 (100) $[M + H]^+$, 712 (6) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{37}H_{28}O_7N_3S_2$ 690.13632; Found 690.13655.



4-Methyl-9-(*β*-D-ribofuranosyl)-9*H*-thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (27a)

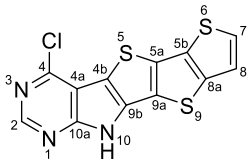
Following the general procedure for deprotection, protected nucleoside **26a** (190 mg, 0.28 mmol) gave the final product **27a** (70 mg, 67%) as a light yellow solid. mp 219–222 °C. $[\alpha]_D$ = +8.3 (*c* = 0.289 g/100 mL in DMSO). IR (ATR): *v* = 3305, 3030, 2947, 2927, 1586, 1446, 1208, 1075, 889 cm^{-1}. ^1H NMR (400 MHz, DMSO) δ 2.77 (s, 3H, CH_3); 3.73 (dt, 1H, J_{gem} = 11.9 Hz, $J_{5'a,4'} = J_{5'a,OH}$ = 6.0 Hz, H-5′a); 3.81 (dt, 1H, J_{gem} = 11.7 Hz, $J_{5'b,4'} = J_{5'b,OH}$ = 4.9 Hz, H-5′b); 3.99 (dt, 1H, $J_{4',5'a}$ = 6.2 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.5 Hz, H-4′); 4.15–4.24 (m, 1H, H-3′); 4.68–4.79 (m, 1H, H-2′); 5.03 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.8 Hz, OH-5′); 5.29 (bs, 1H, OH-3′); 5.47 (bs, 1H, OH-2′); 6.30 (d, 1H, $J_{1',2'}$ = 7.1 Hz, H-1′); 7.67 (d, 1H, $J_{6,7}$ = 5.3 Hz, H-6); 7.86 (d, 1H, $J_{7,6}$ = 5.3 Hz, H-7); 8.78 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO) δ 22.1 (CH_3); 62.2 (CH_2-5′); 69.8 (CH-3′); 72.2 (CH-2′); 85.9 (CH-4′); 87.9 (CH-1′); 113.6 (C-4a); 115.7 (C-4b); 121.7 (CH-6); 123.5 (C-8a); 129.2 (CH-7); 133.5 (C-8b); 142.9 (C-5a), 151.2 (CH-2); 153.8

(C-9a); 156.6 (C-4). MS (ESI): *m/z* (rel. %): 378 (7) $[M + H]^+$, 400 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{16}H_{16}O_4N_3S_2$ 378.05767; Found 378.05728.



7-Chloro-8-(dithieno[3,2-*b*:2',3'-*d*]thiophen-2-yl)tetrazolo[1,5-*c*]pyrimidine (19b)

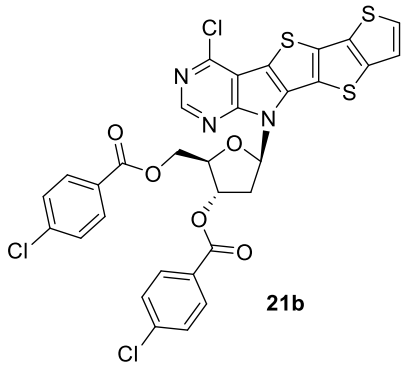
Following the general procedure for azidation, compound **18b** (5.6 g, 16.3 mmol) gave crude product **19b** (6.73 g) as a dark yellow solid. IR (ATR): ν = 3373, 3084, 2122, 1627, 1580, 1387, 1366, 1070 cm^{-1}. ^1H NMR (600.1 MHz, DMSO-d_6): 7.62 (d, 1H, $J_{5,6}$ = 5.2 Hz, H-5-DTT); 7.86 (d, 1H, $J_{6,5}$ = 5.2 Hz, H-6-DTT); 8.98 (s, 1H, H-3-DTT); 10.16 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (150.9 MHz, DMSO-d_6): 116.5 (C-5); 121.7 (CH-5-DTT); 127.7 (CH-3-DTT); 129.7 (C-7a-DTT); 129.9 (CH-6-DTT); 130.1 (C-2/3a-DTT); 133.6 (C-7b-DTT); 137.0 (CH-2); 141.4 (C-2/3a-DTT); 141.9 (C-4/6); 144.0 (C-4a-DTT); 150.0 (C-4/6). MS (ESI): *m/z* (rel. %): 349.9 (8) $[M + H]^+$, 371.9 (24) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{12}H_5N_5ClS_3$ 349.93901; Found 349.93927.



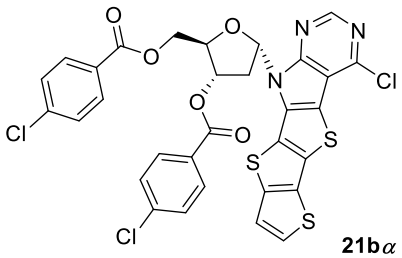
4-Chloro-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (20b)

Tetrazole **19b** (3.5 g, crude) and 1,4-dibromobenzene (30 g) were mixed and added into a reaction tube with a rubber septum. The tube was heated to 180 °C for 35 min while a needle was inserted through the rubber septum to release N_2 gas. After cooling down to r.t., DCM was added to the solid mass with stirring (use as less amount of DCM as possible to dissolve all dibromobenzene). Then further filtration and washing by DCM (2 × 3–5 mL), hexane (2 × 3–5 mL) gave **20b** (2.7 g, crude) as a black solid, which was used directly for the next step. ^1H NMR (500.0 MHz, DMSO-d_6): δ 7.62 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.81 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 8.67 (s,

1H, H-2); 13.82 (s, 1H, NH). $^{13}C\{^{1}H\}$ NMR (125.7 MHz, DMSO-d_6): δ 111.9 (C-4a); 112.7 (C-4b); 121.8 (CH-8); 125.3 (C-5a/9a); 128.9 (CH-7); 131.2 (C-5b); 134.0 (C-5a/9a); 135.9 (C-9b); 142.6 (C-8a); 147.3 (C-4); 150.8 (CH-2); 154.9 (C-10a). MS (EI): *m/z* (rel. %): 320.9 (100) $[M]^+$. HRMS (EI): *m/z* $[M]^+$ Calcd for $C_{12}H_4ClN_3S_3$ 320.9250; Found 320.9250.



21b



21bα

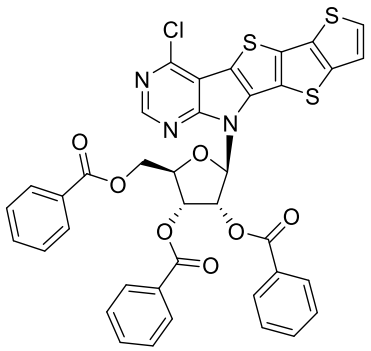
4-Chloro-10-(3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl)-10*H*-thieno [2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (21b) and

4-chloro-10-(3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-*α*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (21b*α*): Nucleobase **20b** (crude, 1.0 g) was suspended in MeCN (97 mL), and BSA (0.77 mL, 3.1 mmol) was added. The mixture was heated to 60 °C for 35 min, and then, TMSOTf (1.4 mL, 7.8 mmol) and 1-chloro-3,5-di-(4-chlorobenzoyl)-2-deoxy-*α*-D-ribose (2.67 g, 6.2 mmol) were added. The mixture was stirred at the same temperature for 30 min and treated with $NaHCO_3$ (aq, 40 mL). Most of the organic solvent was evaporated under reduce pressure. The mixture was diluted with EtOAc (700 mL), and the organic layer was collected and washed with water (2 × 20 mL), $NaHCO_3$ (aq, 20 mL) and brine (30 mL), dried over Na_2SO_4. After evaporation, crude product was purified by HPFC [SiO_2, (hexane/DCM 7:3)/EtOAc, 0−20%] to give compound **21b** (100 mg, 5% over 3 steps) as a yellow solid and **21bα** (325 mg, 14% over 3 steps) as a yellow foam.

For **21b**: mp 203–206 °C. IR (ATR): ν = 2923, 2853, 1714, 1416, 1400, 1267, 1089, 847, 757 cm^{-1}. ^{1}H NMR (500.0 MHz, $CDCl_3$): 2.90 (ddd, 1H, J_{gem} = 14.7 Hz, $J_{2'a,1'}$ =

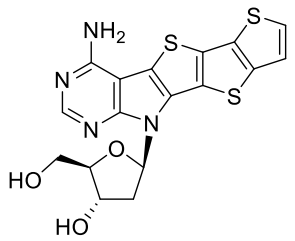
6.2 Hz, $J_{2'a,3'}$ = 2.7 Hz, H-2′a); 3.50 (dt, 1H, J_{gem} = 14.7 Hz, $J_{2'b,1'} = J_{2'b,3'}$ = 7.9 Hz, H-2′b); 4.70 (dt, 1H, $J_{4',5'a}$ = 6.1 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.1 Hz, H-4′); 4.76 (dd, 1H, J_{gem} = 11.8 Hz, $J_{5'a,4'}$ = 6.1 Hz, H-5′a); 4.94 (dd, 1H, J_{gem} = 11.8 Hz, $J_{5'b,4'}$ = 4.1 Hz, H-5′b); 5.92 (ddd, 1H, $J_{3',2'b}$ = 7.9 Hz, $J_{3',4'}$ = 4.1 Hz, $J_{3',2'a}$ = 2.7 Hz, H-3′); 6.85 (dd, 1H, $J_{1',2'b}$ = 7.9 Hz, $J_{1',2'a}$ = 6.2 Hz, H-1′); 7.22 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.26 (m, 2H, H-*m*-Ph); 7.44 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 7.48 (m, 2H, H-*m*-Ph); 7.79 and 8.04 (2×m, 2×2H, H-*o*-Ph); 8.64 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): 38.0 (CH_2-2′); 64.3 (CH_2-5′); 74.8 (CH-3′); 82.2 (CH-4′); 85.3 (CH-1′); 114.3 (C-4a); 114.9 (C-4b); 120.8 (CH-8); 125.4 (C-5a/9a); 127.7 (C-*i*-Ph); 127.8 (CH-7); 127.9 (C-*i*-Ph); 128.8 and 129.2 (CH-*m*-Ph); 131.1 and 131.3 (CH-*o*-Ph); 132.1 (C-5b); 135.5 (C-5a/9a); 135.8 (C-9b); 139.8 and 140.4 (C-*p*-Ph); 142.2 (C-8a); 149.3 (C-4); 150.7 (CH-2); 154.3 (C-10a); 165.3 and 165.3 (CO). MS (APCI): *m/z* (rel. %): 714 (82) $[M + H]^+$. HRMS (APCI): *m/z* $[M + H]^+$ Calcd for $C_{31}H_{19}O_5N_3Cl_3S_3$ 713.95469; Found 713.95487.

For **21bα**: mp 118–120 °C. IR (ATR): ν = 3096, 2925, 1717, 1591, 1416, 1261, 1087, 1013, 847 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 3.01 (dt, 1H, J_{gem} = 14.9 Hz, $J_{2'a,1'} = J_{2'a,3'}$ = 6.3 Hz, H-2′a); 3.67 (dt, 1H, J_{gem} = 14.9 Hz, $J_{2'b,1'} = J_{2'b,3'}$ = 2.6 Hz, H-2′b); 4.72 (dd, 1H, J_{gem} = 11.9 Hz, $J_{5'a,4'}$ = 5.1 Hz, H-5′a); 4.76 (dd, 1H, J_{gem} = 11.9 Hz, $J_{5'b,4'}$ = 4.4 Hz, H-5′b); 5.08 (td, 1H, $J_{4',5'a} = J_{4',5'b}$ = 4.8 Hz, $J_{4',3'}$ = 2.0 Hz, H-4′); 5.63 (dt, 1H, $J_{3',2'a}$ = 6.3 Hz, $J_{3',4'} = J_{3',2'b}$ = 2.2 Hz, H-3′); 6.84 (dd, 1H, $J_{1',2'a}$ = 6.0 Hz, $J_{1',2'b}$ = 2.8 Hz, H-1′); 7.14 (m, 2H, H-*m*-Ph-3′); 7.15 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.20 (m, 2H, H-*o*-Ph-3′); 7.42 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 7.47 (m, 2H, H-*m*-Ph-5′); 8.06 (m, 2H, H-*o*-Ph-5′); 8.57 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): 38.5 (CH_2-2′); 64.6 (CH_2-5′); 74.8 (CH-3′); 84.0 (CH-4′); 88.4 (CH-1′); 114.1 (C-4a); 114.2 (C-4b); 120.6 (CH-8); 126.4 (C-5a/9a); 127.2 (C-*i*-Ph-3′); 127.6 (CH-7); 128.1 (C-*i*-Ph-5′); 128.7 (CH-*m*-Ph-3′); 129.1 (CH-*m*-Ph-5′); 130.5 (CH-*o*-Ph-3′); 131.3 (CH-*o*-Ph-5′); 132.0 (C-5b); 135.4 and 135.5 (C-5a/9a,9b); 140.1 (C-*p*-Ph-3′); 140.2 (C-*p*-Ph-5′); 142.8 (C-8a); 149.0 (C-4); 150.4 (CH-2); 153.5 (C-10a); 164.9 (CO-3′); 165.4 (CO-5′). MS (ESI): *m/z* (rel. %): 714 (74) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{31}H_{19}O_5N_3Cl_3S_3$ 713.95469; Found 713.95591.



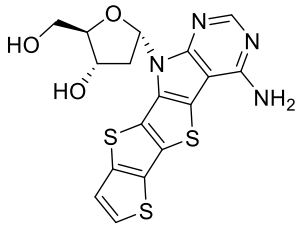
4-Chloro-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (22b)

Following the general procedure for glycosylation to ribonucleoside, nucleobase **20b** (crude, 1.2 g) gave product **22b** (1.02 g, 35% over 3 steps) as a light yellow solid. mp 160–161 °C. IR (ATR): ν = 3061, 2958, 1720, 1417, 1259, 1090, 1066, 893 cm^{-1}. ^1H NMR (500.0 MHz, $CDCl_3$): 4.82 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'a,4'}$ = 5.4 Hz, H-5′a); 4.89 (btd, 1H, $J_{4',5'a} = J_{4',3'}$ = 5.8 Hz, $J_{4',5'b}$ = 3.3 Hz, H-4′); 5.07 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'b,4'}$ = 3.3 Hz, H-5′b); 6.35 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 6.5 Hz, H-3′); 6.54 (dd, 1H, $J_{2',3'}$ = 6.8 Hz, $J_{2',1'}$ = 5.2 Hz, H-2′); 6.76 (d, 1H, $J_{1',2'}$ = 5.2 Hz, H-1′); 7.16 (bd, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.33 (m, 2H, H-*m*-Bz-2′); 7.37 (m, 2H, H-*m*-Bz-5′); 7.39 (m, 2H, H-*m*-Bz-3′); 7.40 (m, 1H, H-7); 7.52, 7.54 and 7.57 (3×m, 3×1H, H-*p*-Bz); 7.90 (m, 2H, H-*o*-Bz-2′); 7.99 (m, 2H, H-*o*-Bz-5′); 8.01 (m, 2H, H-*o*-Bz-3′); 8.59 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): 63.5 (CH_2-5′); 70.6 (CH-3′); 73.6 (CH-2′); 80.6 (CH-4′); 88.1 (CH-1′); 114.7 (C-4a); 115.0 (C-4b); 120.8 (CH-8); 124.8 (C-5a/9a); 127.8 (CH-7); 128.5, 128.6 and 128.7 (CH-*m*-Bz); 128.9 and 129.6 (C-*i*-Bz); 129.88, 129.95 and 129.98 (CH-*o*-Bz); 132.1 (C-5b); 133.4 (CH-*p*-Bz-5′); 133.81 and 133.83 (CH-*p*-Bz-2′,3′); 135.6 (C-9b); 135.9 (C-5a/9a); 142.1 (C-8a); 149.3 (C-4); 150.9 (CH-2); 154.7 (C-10a); 165.2 (CO-2′); 165.4 (CO-3′); 166.2 (CO-5′). MS (ESI): *m/z* (rel. %): 766 (29) $[M + H]^+$, 788 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{38}H_{25}O_7N_3ClS_3$ 766.05377; Found 766.05301.



4-Amino-10-(2′-deoxy-*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (23b)

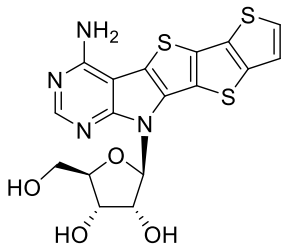
Following the general procedure for amination, protected nucleoside **21b** (125 mg, 0.18 mmol) gave product **23b** (50 mg, 68%) as a light yellow solid. mp 253–254 °C. $[\alpha]_D$ = +17.4 (*c* = 0.310 g/100 mL in DMSO). IR (ATR): *v* = 3324, 3105, 2912, 1643, 1594, 1455, 1392, 1071, 1046, 785 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 2.30 (ddd, 1H, J_{gem} = 13.5 Hz, $J_{2'a,1'}$ = 5.9 Hz, $J_{2'a,3'}$ = 2.2 Hz, H-2′a); 2.76 (ddd, 1H, J_{gem} = 13.5 Hz, $J_{2'b,1'}$ = 9.0 Hz, $J_{2'b,3'}$ = 7.5 Hz, H-2′b); 3.75–3.82 (m, 2H, H-5′); 3.92 (dt, 1H, $J_{4',5'a}$ = 5.9 Hz, $J_{4',5'b}$ = $J_{4',3'}$ = 4.7 Hz, H-4′); 4.42 (m, 1H, H-3′); 5.09 (bs, 1H, OH-5′); 5.45 (bs, 1H, OH-3′); 6.66 (dd, 1H, $J_{1',2'b}$ = 9.0 Hz, $J_{1',2'a}$ = 5.9 Hz, H-1′); 7.31 (bs, 2H, NH_2); 7.59 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.72 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 8.22 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 40.0 (CH_2-2′); 62.3 (CH_2-5′); 70.9 (CH-3′); 83.5 (CH-1′); 87.5 (CH-4′); 98.1 (C-4a); 115.0 (C-4b); 121.6 (CH-8); 125.5 (C-5a/9a); 127.2 (CH-7); 130.8 (C-9b); 131.2 and 131.3 (C-5b, C-5a/9a); 140.4 (C-8a); 152.4 (CH-2); 153.6 (C-10a); 155.7 (C-4). MS (ESI): *m/z* (rel. %): 419 (6) $[M + H]^+$, 441 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{17}H_{15}O_3N_4S_3$ 419.03008; Found 419.02954.



4-Amino-10-(2′-deoxy-*α*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (23b*α*)

Following the general procedure for amination, protected nucleoside **21bα** (320 mg, 0.45 mmol) gave product **23bα** (112 mg, 60%) as a light yellow solid. mp 253–

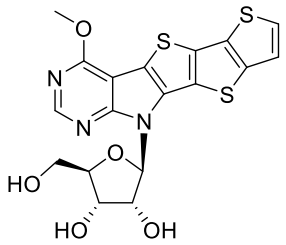
255 °C. $[\alpha]_D$ = +39.2 (*c* = 0.296 g/100 mL in DMSO). IR (ATR): *ν* = 3411, 3308, 3133, 2920, 1646, 1593, 1395, 1077, 1038, 783 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 2.46 (dt, 1H, J_{gem} = 12.9 Hz, $J_{2'a,1'} = J_{2'a,3'}$ = 7.0 Hz, H-2′a); 2.68 (bdt, 1H, J_{gem} = 12.8 Hz, $J_{2'b,1'} = J_{2'b,3'}$ = 6.0 Hz, H-2′b); 3.62 and 3.68 (2×dm, 2×1H, J_{gem} = 11.9 Hz, H-5′); 4.40–4.49 (m, 2H, H-4′,3′); 4.99 (bs, 1H, OH-5′); 5.42 (bs, 1H, OH-3′); 6.61 (dd, 1H, $J_{1',2'a}$ = 7.4 Hz, $J_{1',2'b}$ = 5.7 Hz, H-1′); 7.26 (bs, 2H, NH_2); 7.59 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.71 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 8.21 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 41.3 (CH_2-2′); 61.8 (CH_2-5′); 69.9 (CH-3′); 84.8 (CH-1′); 87.0 (CH-4′); 98.0 (C-4a); 114.6 (C-4b); 121.6 (CH-8); 126.4 (C-5a/9a); 127.0 (CH-7); 130.5 (C-9b); 131.0 and 131.1 (C-5b, C-5a/9a); 140.9 (C-8a); 152.2 (CH-2); 153.1 (C-10a); 155.6 (C-4). MS (ESI): *m/z* (rel. %): 419 (75) $[M + H]^+$, 441 (22) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{17}H_{15}O_3N_4S_3$ 419.03008; Found 419.02971.



4-Amino-10-(*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (24b)

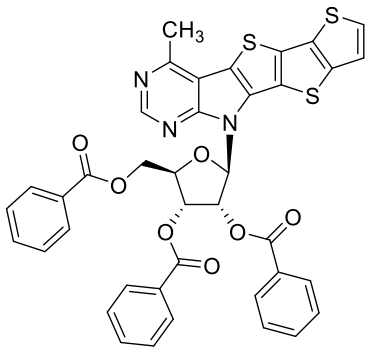
Following the general procedure for amination, protected nucleoside **22b** (320 mg, 0.42 mmol) gave product **24b** (80 mg, 44%) as a light yellow solid. mp 273–277 °C. $[\alpha]_D$ = +9.2 (*c* = 0.325 g/100 mL in DMSO). IR (ATR): *ν* = 3294, 3132, 2919, 1637, 1588, 1387, 1122, 1078, 1034, 891, 876 cm^{-1}. ^1H NMR (500.0 MHz, DMSO-d_6): 3.75 and 3.82 (2×m, 2×1H, H-5′a,5′b); 3.99 (dt, 1H, $J_{4',5'a}$ = 5.5 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.1 Hz, H-4′); 4.18 (dd, 1H, $J_{3',2'}$ = 6.3 Hz, $J_{3',4'}$ = 3.9 Hz, H-3′); 4.70 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.8 Hz, H-2′); 5.20–5.65 (m, 3H, OH-2′,3′,5′); 6.17 (d, 1H, $J_{1',2'}$ = 7.2 Hz, H-1′); 7.36 (bs, 2H, NH_2); 7.60 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.74 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 8.22 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): 62.4 (CH_2-5′); 70.1 (CH-3′); 72.4 (CH-2′); 86.1 (CH-4′); 88.5 (CH-1′); 98.4 (C-4a); 114.9 (C-4b); 121.7 (CH-8); 125.1 (C-5a/9a); 127.4 (CH-7); 131.2, 131.3 and 131.4 (C-9b,5b,5a/9a); 140.3 (C-8a); 152.2

(CH-2); 154.0 (C-10a); 155.8 (C-4). MS (ESI): *m/z* (rel. %): 435 (100) $[M + H]^+$, 457 (34) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{17}H_{15}O_4N_4S_3$ 435.02499; Found 435.02450.



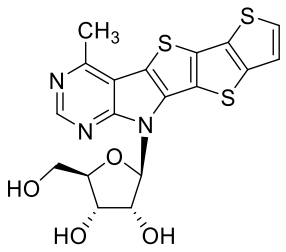
4-Methoxy-10-(*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (25b)

The reaction of compound **22b** (200 mg, 0.26 mmol) was set up according to the general procedure for methoxylation, the purification was performed as below: The solvent was evaporated till 2−3 mL, and the solid residue, which was collected from filtration, was washed by cold EtOAc (2 × 3 mL) and water (2 × 4 mL) to obtain product **25b** (60 mg, 51%) as a white solid. mp 241–243 °C. $[\alpha]_D$ = +5.0 (*c* = 0.253 g/100 mL in DMSO). IR (ATR): *ν* = 3432, 3289, 3065, 2938, 1603, 1379, 1359, 1119, 1053, 1011 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.78, 3.81 (2 × dt, 2 × 1H, J_{gem} = 11.9 Hz, $J_{5',OH} = J_{5',4'}$ =6.2 Hz H-5′a, 5′b); 4.00 (dt, 1H, $J_{4',5'a}$ = 6.2 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.4 Hz, H-4′); 4.18 (s, 3H, CH_3); 4.19 – 4.23 (m, 1H, H-3′); 4.69 (q, $J_{2',1'} = J_{2',3'} = J_{2',OH}$ = 6.3 Hz, H-2′); 5.05 (t, 1H, $J_{OH,5'}$ = 5.8 Hz, OH-5′); 5.31 (d, 1H, $J_{OH,3'}$ = 5.1 Hz, OH-3′); 5.51 (d, 1H, $J_{OH,2'}$ = 6.0 Hz, OH-2′); 6.26 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′); 7.61 (d, 1H, $J_{8,7}$ = 5.3 Hz, H-8); 7.78 (d, 1H, $J_{7,8}$ = 5.3 Hz, H-7); 8.58 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 54.3 (CH_3); 62.2 (CH_2-5′); 69.8 (CH-3′); 72.5 (CH-2′); 86.0 (CH-4′); 88.3 (CH-1′); 100.9 (C-4a); 113.7 (C-4b); 121.6 (CH-8); 125.2 (C-5a/9a); 128.3 (CH-7); 131.1 (C-5b); 132.5 (C-5a/9a); 133.0 (C-9b); 141.2 (C-8a); 151.3 (CH-2); 155.2 (C-10a); 160.9 (C-4). MS (ESI): *m/z* (rel. %): 450 (5) $[M + H]^+$, 472 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{18}H_{16}O_5N_3S_3$ 450.02466; Found 450.02413.



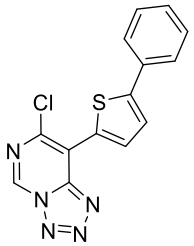
4-Methyl-10-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (**26b**)

Following the general procedure for methylation, protected nucleoside **22b** (360 mg, 0.47 mmol) gave compound **26b** (260 mg, 74%) as a yellow foam. mp 114–116 °C. IR (ATR): ν = 3061, 2923, 1720, 1450, 1260, 1089, 1066, 703 cm^{-1}. ^{1}H NMR (500.0 MHz, $CDCl_3$): 2.83 (s, 3H, CH_3); 4.81–4.89 (m, 2H, H-5′a,4′); 5.07 (m, 1H, H-5′b); 6.37 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 6.5 Hz, H-3′); 6.50 (dd, 1H, $J_{2',3'}$ = 6.9 Hz, $J_{2',1'}$ = 5.4 Hz, H-2′); 6.81 (d, 1H, $J_{1',2'}$ = 5.4 Hz, H-1′); 7.16 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.32 (m, 2H, H-*m*-Bz-2′); 7.37 (m, 2H, H-*m*-Bz-5′); 7.38 (m, 2H, H-*m*-Bz-3′); 7.39 (d, 1H, $J_{7,8}$ = 5.1 Hz, H-7); 7.48–7.59 (m, 3H, H-*p*-Bz); 7.89 (m, 2H, H-*o*-Bz-2′); 8.00 (m, 2H, H-*o*-Bz-3′); 8.00 (m, 2H, H-*o*-Bz-5′); 8.76 (s, 1H, H-2). ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): 22.5 (CH_3); 63.7 (CH_2-5′); 70.5 (CH-3′); 73.6 (CH-2′); 80.4 (CH-4′); 87.6 (CH-1′); 114.9 (C-4a); 116.0 (C-4b); 120.8 (CH-8); 125.3 (C-5a/9a); 127.2 (CH-7); 128.51, 128.59 and 128.63 (CH-*m*-Bz); 128.7, 128.9 and 129.7 (C-*i*-Bz); 129.94, 129.95 and 129.97 (CH-*o*-Bz); 132.1 (C-5b); 133.3 (CH-*p*-Bz-5′); 133.8 (CH-*p*-Bz-2′,3′); 134.5 (C-9b); 134.7 (C-5a/9a); 141.5 (C-8a); 151.5 (CH-2); 154.4 (C-10a); 156.7 (C-4); 165.3 (CO-2′); 165.4 (CO-3′); 166.3 (CO-5′). MS (ESI): *m/z* (rel. %): 746 (100) $[M + H]^+$, 768 (3) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{39}H_{28}O_7N_3S_3$ 746.10839; Found 746.10926.



4-Methyl-10-(*β*-D-ribofuranosyl)-10*H*-thieno[2''',3''':4'',5'']thieno[2'',3'':4',5']thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (27b)

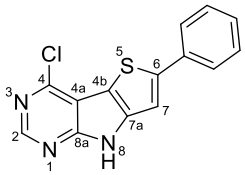
Following the general procedure for deprotection, protected nucleoside **26b** (200 mg, 0.27 mmol) gave final product **27b** (76 mg, 65%) as a light yellow solid. mp 297–299 °C. $[\alpha]_D$ = –3.7 (*c* = 0.298 g/100 mL in DMSO). IR (ATR): *v* = 3393, 3256, 3105, 2925, 1584, 1420, 1380, 1037, 896 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.76 (s, 3H, CH_3); 3.79 (dt, 1H, J_{gem} = 11.9 Hz, $J_{5'a,OH} = J_{5'a,4'}$ = 6.2 Hz, H-5′a); 3.85 (dt, 1H, J_{gem} = 11.7 Hz, $J_{5'b,OH} = J_{5'b,4'}$ = 5.1 Hz, H-5′b); 4.01 (dt, 1H, $J_{4',5'a}$ = 6.2 Hz, $J_{4',5'b} = J_{4',3'}$ = 4.5 Hz, H-4′); 4.17–4.26 (m, 1H, H-3′); 4.72 (q, 1H, $J_{2',1'} = J_{2',3'} = J_{2',OH}$ =6.6 Hz, H-2′); 5.04 (t, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.8 Hz, OH-5′); 5.30 (d, 1H, $J_{OH,3'}$ = 5.3 Hz, OH-3′); 5.49 (d, 1H, $J_{OH,2'}$ = 6.3 Hz, OH-2′); 6.28 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1′); 7.61 (d, 1H, $J_{8,7}$ = 5.2 Hz, H-8); 7.79 (d, 1H, $J_{7,8}$ = 5.2 Hz, H-7); 8.78 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 22.1 (CH_3); 62.2 (CH_2-5′); 69.8 (CH-3′); 72.4 (CH-2′); 86.0 (CH-4′); 88.0 (CH-1′); 113.4 (C-4a); 114.6 (C-4b); 121.6 (CH-8); 125.3 (C-9a); 128.7 (CH-7); 131.1 (C-5b); 133.3 (C-5a); 134.1 (C-9b); 141.6 (C-8a); 151.3 (CH-2); 153.9 (C-10a); 156.5 (C-4). MS (ESI): *m/z* (rel. %): 434 (15) $[M + H]^+$, 456 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{18}H_{16}O_4N_3S_3$ 434.02974; Found 434.02918.



7-Chloro-8-(5-phenylthiophen-2-yl)tetrazolo[1,5-*c*]pyrimidine (19c)

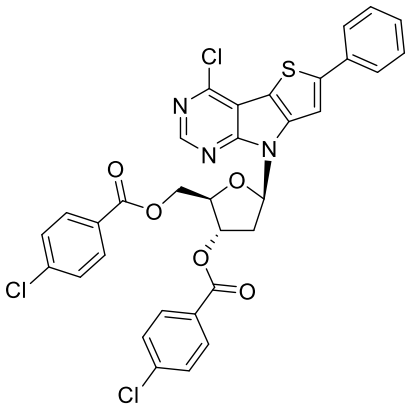
Following the general procedure for azidation, compound **18c** (2.15 g, 7.0 mmol) gave crude product **19c** (2.90 g) as a yellow solid. IR (ATR): *v* = 3363, 3130, 3088, 1635, 1578, 1435, 1250, 938, 757 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.37–7.44 (m, 1H, H-*p*-Ph), 7.46–7.52 (m, 2H, H-*m*-Ph), 7.77–7.84 (m, 3H, H-*o*-Ph, H-4-thienyl), 8.56 (d, 1H, $J_{3,4}$ = 4.1 Hz, H-3-thienyl), 10.15 (s, 1H, H-5). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 116.2 (C-8), 124.4 (CH-4-thienyl), 125.8 (C-*o*-Ph),

128.8 (C-*p*-Ph), 129.3 (C-2-thienyl/C-5-thienyl), 129.4 (C-*m*-Ph), 132.7 (C-*i*-Ph), 135.1 (CH-3-thienyl), 136.9 (CH-5), 141.9 (C-7/C-8a), 147.7 (C-2-thienyl/C-5-thienyl), 150.0 (C-7/C-8a). MS (ESI): *m/z* (rel. %): 314 (38) $[M + H]^+$, 336 (87) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{14}H_9N_5ClS$ 314.02617; Found 314.02667.



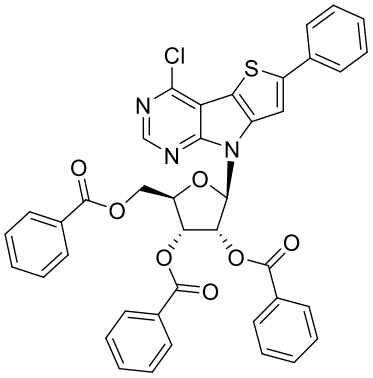
4-Chloro-6-phenyl-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (20c)

Following the general procedure for thermal cyclization, tetrazole **19c** (3.4 g, crude product) gave nucleobase **20c** (1.5 g, 63% over 2 steps) as a brown solid. mp 291 °C (decomp.). IR (ATR): ν = 3092, 1561, 1425, 1229, 1072, 751 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.32–7.42 (m, 1H, H-*p*-Ph); 7.43–7.54 (m, 2H, H-*m*-Ph); 7.78 (s, 1H, H-7); 7.80–7.85 (m, 2H, H-*o*-Ph); 8.64 (s, 1H, H-2); 13.09 (s, 1H, NH). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 109.1 (CH-7); 110.8 (C-4b); 111.9 (C-4a); 125.8 (CH-*o*-Ph); 128.7 (CH-*p*-Ph); 129.4 (CH-*m*-Ph); 133.8 (C-*i*-Ph); 143.8 (C-7a); 147.6 (C-4); 148.9 (C-6); 151.0 (CH-2); 155.8 (C-8a). MS (ESI): *m/z* (rel. %): 286 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{14}H_9N_3ClS$ 286.02002; Found 286.01997.



4-Chloro-6-phenyl-8-(3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (21c)

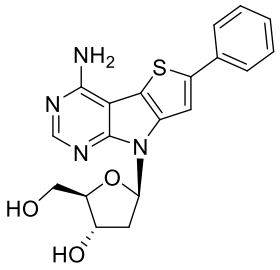
Following the general procedure for glycosylation to deoxyribonucleoside, nucleobase **20c** (500 mg, 1.7 mmol) gave product **21c** (679 mg, 72%) as a light yellow solid. mp 145–148 °C. IR (ATR): v = 3058, 2962, 2921, 1730, 1713, 1592, 1421, 1251, 1091, 750 cm^{-1}. ^{1}H NMR (500.2 MHz, $CDCl_3$): 2.76 (ddd, 1H, J_{gem} = 14.3 Hz, $J_{2'a,1'}$ = 5.9 Hz, $J_{2'a,3'}$ = 2.7 Hz, H-2′a); 3.35 (bdt, 1H, J_{gem} = 14.3 Hz, $J_{2'b,1'} = J_{2'b,3'}$ = 7.6 Hz, H-2′b); 4.63 (q, 1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'}$ = 3.9 Hz, H-4′); 4.74 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'a,4'}$ = 4.5 Hz, H-5′a); 4.81 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'b,4'}$ = 3.7 Hz, H-5′b); 5.86 (dt, 1H, $J_{3',2'b}$ = 7.2 Hz, $J_{3',2'a} = J_{3',4'}$ = 3.0 Hz, H-3′); 6.93 (dd, 1H, $J_{1',2'b}$ = 8.2 Hz, $J_{1',2'a}$ = 5.9 Hz, H-1′); 7.23–7.28 (m, 2H, H-*m*-C_6H_4Cl); 7.32–7.36 (m, 3H, H-*m,p*-Ph); 7.46–7.50 (m, 2H, H-*m*-C_6H_4Cl); 7.52 (s, 1H, H-7); 7.52–7.56 (m, 2H, H-*o*-Ph); 7.86–7.90 (m, 2H, H-*o*-C_6H_4Cl); 8.03–8.07 (m, 2H, H-*o*-C_6H_4Cl); 8.66 (s, 1H, H-2). ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): 36.5 (CH_2-2′); 64.2 (CH_2-5′); 74.7 (CH-3′); 81.7 (CH-4′); 84.5 (CH-1′); 108.1 (CH-7); 113.5 and 113.7 (C-4a,4b); 126.1 (CH-*o*-Ph); 127.6 and 127.7 (C-*i*-C_6H_4Cl); 128.7 (CH-*p*-Ph); 128.8 and 129.0 (CH-*m*-C_6H_4Cl); 129.1 (CH-*m*-Ph); 130.9 and 131.2 (CH-*o*-C_6H_4Cl); 134.0 (C-*i*-Ph); 139.9 and 140.3 (C-*p*-C_6H_4Cl); 142.4 (C-7a); 149.4 (C-4); 150.7 (C-6); 150.8 (CH-2); 154.8 (C-8a); 165.2 and 165.3 (CO). MS (ESI): *m/z* (rel. %): 678 (95) $[M + H]^+$, 700 (82) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{33}H_{23}O_5N_3Cl_3S$ 678.04185; Found 678.04244.



4-Chloro-6-phenyl-8-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (22c)

Following the general procedure for glycosylation to ribonucleoside, nucleobase **20c** (693 mg, 2.4 mmol) gave product **22c** (900 mg, 51%) as a white foam. mp 166–168 °C. IR (ATR): v = 2921, 1722, 1450, 1432, 1262, 1088, 1068 cm^{-1}. ^{1}H NMR

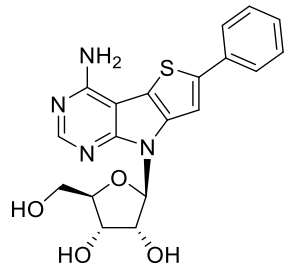
(500.2 MHz, $CDCl_3$): 4.79 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'a,4'}$ = 3.7 Hz, H-5′a); 4.85 (ddd, 1H, $J_{4',3'}$ = 4.6 Hz, $J_{4',5'a}$ = 3.7 Hz, $J_{4',5'b}$ = 2.6 Hz, H-4′); 4.89 (dd, 1H, J_{gem} = 12.1 Hz, $J_{5'b,4'}$ = 2.6 Hz, H-5′b); 6.26 (dd, 1H, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.6 Hz, H-3′); 6.45 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.0 Hz, H-2′); 6.92 (d, 1H, $J_{1',2'}$ = 6.0 Hz, H-1′); 7.26–7.32 (m, 3H, H-*m,p*-Ph); 7.34–7.44 (m, 6H, H-*m*-Bz); 7.45–7.48 (m, 2H, H-*o*-Ph); 7.50–7.54 (m, 2H, H-*p*-Bz); 7.55 (s, 1H, H-7); 7.58 (m, 1H, H-*p*-Bz); 7.92–7.95 (m, 2H, H-*o*-Bz); 8.01–8.04 (m, 2H, H-*o*-Bz); 8.07–8.10 (m, 2H, H-*o*-Bz); 8.65 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): 63.8 (CH_2-5′); 71.0 (CH-3′); 72.9 (CH-2′); 80.3 (CH-4′); 86.3 (CH-1′); 107.7 (CH-7); 113.77 and 113.81 (C-4a,4b); 126.0 (CH-*o*-Ph); 128.49 (CH-*m*-Bz); 128.51 (CH-*p*-Ph); 128.55 and 128.64 (CH-*m*-Bz); 128.8 (C-*i*-Bz); 129.0 (CH-*m*-Ph); 129.3 (C-*i*-Bz); 129.68; 129.81 and 129.84 (CH-*o*-Bz); 133.5 and 133.7 (CH-*p*-Bz); 134.0 (C-*i*-Ph); 142.4 (C-7a); 149.4 (C-4); 150.98 (C-6); 150.99 (CH-2); 155.3 (C-8a); 165.2; 165.5 and 166.2 (CO). MS (ESI): *m/z* (rel. %): 730 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{40}H_{29}O_7N_3ClS$ 730.14093; Found 730.14191.



4-Amino-6-phenyl-8-(2′-deoxy-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (23c)

Following the general procedure for amination, protected nucleoside **21c** (354 mg, 0.52 mmol) gave product **23c** (180 mg, 90%) as a white solid. mp 198–200 °C. $[\alpha]_D$ = +3.6 (*c* = 0.331 g/100 mL in DMSO). IR (ATR): *ν* = 3306, 3116, 2933, 1644, 1595, 1438, 1087, 988, 927 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 2.13 (ddd, 1H, J_{gem} = 13.1 Hz, $J_{2'a,1'}$ = 5.7 Hz, $J_{2'a,3'}$ = 1.9 Hz, H-2′a); 2.73 (ddd, 1H, J_{gem} = 13.1 Hz, $J_{2'b,1'}$ = 9.2 Hz, $J_{2'b,3'}$ = 6.3 Hz, H-2′b); 3.67 (bdt, 1H, J_{gem} = 11.7 Hz, $J_{5'a,OH} = J_{5'a,4'}$ = 4.1 Hz, H-5′a); 3.72 (bdt, 1H, J_{gem} = 11.7 Hz, $J_{5'b,OH} = J_{5'b,4'}$ = 3.7 Hz, H-5′b); 3.90 (td, 1H, $J_{4',5'a} = J_{4',5'b}$ = 3.7 Hz, $J_{4',3'}$ = 2.7 Hz, H-4′); 4.48 (m, 1H, H-3′); 5.26 (bt, 1H, $J_{OH,5'a}$ =

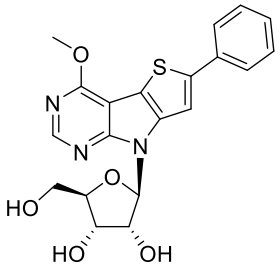
$J_{OH,5'b}$ = 5.0 Hz, OH-5′); 5.32 (bd, 1H, $J_{OH,3'}$ = 3.3 Hz, OH-3′); 6.76 (dd, 1H, $J_{1',2'b}$ = 9.2 Hz, $J_{1',2'a}$ = 5.7 Hz, H-1′); 7.19 (s, 2H, NH_2); 7.32 (m, 1H, H-*p*-Ph); 7.42–7.48 (m, 2H, H-*m*-Ph); 7.68–7.74 (m, 2H, H-*o*-Ph); 8.06 (s, 1H, H-7); 8.18 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO-d_6): 38.9 (CH_2-2′); 62.0 (CH_2-5′); 71.1 (CH-3′); 83.4 (CH-1′); 87.4 (CH-4′); 97.3 (C-4a); 110.2 (CH-7); 113.5 (C-4b); 125.2 (CH-*o*-Ph); 127.9 (CH-*p*-Ph); 129.4 (CH-*m*-Ph); 134.9 (C-*i*-Ph); 139.0 (C-7a); 143.9 (C-6); 152.5 (CH-2); 154.3 (C-8a); 155.9 (C-4). MS (ESI): *m/z* (rel. %): 383 (9) $[M + H]^+$, 405 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{19}H_{19}O_3N_4S$ 383.11724; Found 383.11684.



4-Amino-6-phenyl-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (24c)

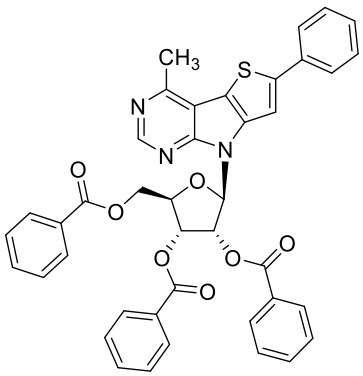
Following the general procedure for amination, protected nucleoside **22c** (250 mg, 0.34 mmol) gave product **24c** (101 mg, 74%) as a white solid. mp 224–228 °C. $[\alpha]_D$ = –34.2 (*c* = 0.292 g/100 mL in DMSO). IR (ATR): *v* = 3350, 3236, 2912, 1650, 1602, 1434, 1387, 1052, 1018, 751 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.67 (bdt, 1H, J_{gem} = 12.0 Hz, $J_{5'a,OH}$ = $J_{5'a,4'}$ = 4.0 Hz, H-5′a); 3.72 (bdt, 1H, J_{gem} = 12.0 Hz, $J_{5'b,OH}$ = $J_{5'b,4'}$ = 3.4 Hz, H-5′b); 3.97 (td, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.2 Hz, $J_{4',3'}$ = 2.5 Hz, H-4′); 4.19 (dd, 1H, $J_{3',2'}$ = 5.5 Hz, $J_{3',4'}$ = 2.4 Hz, H-3′); 4.65 (bt, 1H, $J_{2',3'}$ = $J_{2',1'}$ = 6.4 Hz, H-2′); 5.20 (bs, 1H, OH-3′); 5.27 (bs, 1H, OH-2′); 5.41 (bt, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 4.9 Hz, OH-5′); 6.29 (dd, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.19 (bs, 2H, NH_2); 7.33 (m, 1H, H-*p*-Ph); 7.43–7.48 (m, 2H, H-*m*-Ph); 7.68–7.73 (m, 2H, H-*o*-Ph); 8.08 (s, 1H, H-7); 8.17 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO-d_6): 62.0 (CH_2-5′); 70.7 (CH-3′); 72.1 (CH-2′); 85.6 (CH-4′); 87.0 (CH-1′); 97.5 (C-4a); 110.2 (CH-7); 113.4 (C-4b); 125.1 (CH-*o*-Ph); 127.8 (CH-*p*-Ph); 129.5 (CH-*m*-Ph); 134.9 (C-*i*-Ph); 139.4 (C-7a); 143.9 (C-6); 152.5 (CH-2); 155.0 (C-8a); 155.9 (C-4). MS (ESI): *m/z* (rel. %): 399 (100) [M

+ H]$^+$. HRMS (ESI): *m/z* [M + H]$^+$ Calcd for $C_{19}H_{19}O_4N_4S$ 399.11215; Found 399.11195.



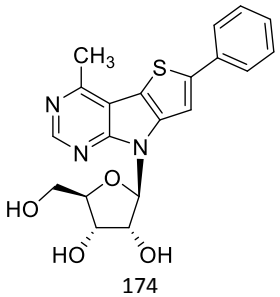
4-Methoxy-6-phenyl-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*] pyrimidine (**25c**)

Following the general procedure for methoxylation, protected nucleoside **22c** (250 mg, 0.34 mmol) gave product **25c** (130 mg, 92%) as a white solid. mp 282–284 °C. $[\alpha]_D$ = –42.9 (*c* = 0.296 g/100 mL in DMSO). IR (ATR): *v* = 3386, 2927, 1604, 1481, 1430, 1360, 1338, 1126, 1019, 899 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66–3.76 (m, 2H, H-5′); 4.01 (q, 1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'}$ = 3.1 Hz, H-4′); 4.16 (s, 3H, CH_3O); 4.21 (td, 1H, $J_{3',2'} = J_{3',OH}$ = 5.1 Hz, $J_{3',4'}$ = 2.3 Hz, H-3′); 4.66 (td, 1H, $J_{2',3'} = J_{2',1'}$ = 7.1 Hz, $J_{2',OH'}$ = 5.5 Hz, H-2′); 5.21 (d, 1H, $J_{OH,3'}$ = 4.4 Hz, OH-3′); 5.28–5.38 (m, 2H, OH-2′, OH-5′); 6.39 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.32–7.40 (m, 1H, H-*p*-Ph); 7.42–7.51 (m, 2H, H-*m*-Ph); 7.72–7.80 (m, 2H, H-*o*-Ph); 8.22 (s, 1H, H-7); 8.54 (s, 1H, H-2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 54.1 (CH_3O); 61.7 (CH_2-5′); 70.4 (CH-3′); 72.1 (CH-2′); 85.6 (CH-4′); 86.8 (CH-1′); 99.8 (C-4a); 110.6 (CH-7); 112.1 (C-4b); 125.3 (CH-*o*-Ph); 128.2 (CH-*p*-Ph); 129.3 (CH-*m*-Ph); 134.2 (C-*i*-Ph); 140.7 (C-7a); 145.8 (C-6); 151.4 (CH-2); 156.0 (C-8a); 160.9 (C-4). MS (ESI): *m/z* (rel. %): 414 (100) [M + H]$^+$. HRMS (ESI): *m/z* [M + H]$^+$ Calcd for $C_{20}H_{20}O_5N_3S$ 414.11182; Found 414.11161.



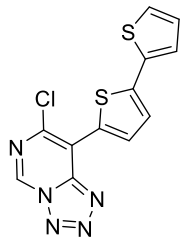
4-Methyl-6-phenyl-8-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (**26c**)

Following the general procedure for methylation, protected nucleoside **22c** (220 mg, 0.30 mmol) gave compound **26c** (170 mg, 80%) as a yellow solid. mp 92–97 °C. IR (ATR): v = 2923, 1720, 1450, 1428, 1260, 1089, 1067 cm^{-1}. ^1H NMR (401 MHz, $CDCl_3$): δ 2.83 (s, 3H, CH_3); 4.79 (dd, 1H, J_{gem} = 11.7 Hz, $J_{5'a,4'}$ = 3.7 Hz, H-5′a); 4.82–4.85 (m, 1H, H-4′); 4.88 (dd, 1H, J_{gem} = 11.7 Hz, $J_{5'b,4'}$ = 2.3 Hz, H-5′b); 6.28 (dd, 1H, $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 4.4 Hz, H-3′); 6.45 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.1 Hz, H-2′); 6.97 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1′); 7.26–7.30 (m, 3H, H-*m,p*-Ph); 7.32–7.43 (m, 6H, H-*m*-Bz); 7.43–7.48 (m, 2H, H-*o*-Ph); 7.48–7.62 (m, 3H, H-*p*-Bz); 7.56 (s, 1H, H-7); 7.90–7.98 (m, 2H, H-*o*-Bz); 7.98–8.05 (m, 2H, H-*o*-Bz); 8.08–8.15 (m, 2H, H-*o*-Bz); 8.81 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 22.6 (CH_3); 64.1 (CH_2-5′); 71.2 (CH-3′); 72.9 (CH-2′); 80.2 (CH-4′); 86.0 (CH-1′); 108.1 (CH-7); 114.1 (C-4a); 114.9 (C-4b); 126.0 (CH-*o*-Ph); 128.3 (CH-*p*-Ph); 128.60 and 128.66 (CH-*m*-Bz); 128.74 (C-*i*-Bz); 128.77 (CH-*m*-Bz); 129.0 (C-*i*-Bz); 129.1 (CH-*m*-Ph); 129.5 (C-*i*-Bz); 129.87, 129.97 and 129.99 (CH-*o*-Bz); 133.55, 133.77 and 133.80 (CH-*p*-Bz); 134.4 (C-*i*-Ph); 141.4 (C-7a); 149.3 (C-6); 152.0 (CH-2); 155.2 (C-8a); 157.0 (C-4); 165.4, 165.7 and 166.4 (CO). MS (ESI): *m/z* (rel. %): 710 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{41}H_{32}O_7N_3S$ 710.19555; Found 710.19500.



4-Methyl-6-phenyl-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*] pyrimidine (27c)

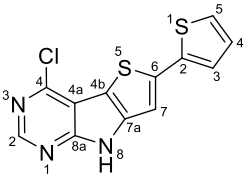
Following the general procedure for deprotection, protected nucleoside **26c** (218 mg, 0.31 mmol) gave final product **27c** (80 mg, 66%) as a white solid. mp 285–286 °C. $[\alpha]_D$ = –53.9 (*c* = 0.319 g/100 mL in DMSO). IR (ATR): *v* = 3362, 3261, 2924, 1590, 1426, 1408, 1091, 1024, 899 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.78 (s, 3H, CH_3); 3.68–3.78 (m, 2H, H-5′); 4.02 (q, 1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'}$ = 3.1 Hz, H-4′); 4.22 (td, 1H, $J_{3',2'} = J_{3',OH}$ = 5.3 Hz, $J_{3',4'}$ = 2.4 Hz, H-3′); 4.61–4.70 (m, 1H, H-2′); 5.22 (d, 1H, $J_{OH,3'}$ = 4.4 Hz, OH-3′); 5.27–5.38 (m, 2H, OH-2′, OH-5′); 6.42 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.34–7.41 (m, 1H, H-*p*-Ph); 7.48 (m, 2H, H-*m*-Ph); 7.74–7.82 (m, 2H, H-*o*-Ph); 8.28 (s, 1H, H-7); 8.75 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 22.1 (CH_3); 61.7 (CH_2-5′); 70.4 (CH-3′); 72.0 (CH-2′); 85.6 (CH-4′); 86.5 (CH-1′); 110.8 (CH-7); 112.6 (C-4a); 113.0 (C-4b); 125.4 (CH-*o*-Ph); 128.4 (CH-*p*-Ph); 129.4 (CH-*m*-Ph); 134.0 (C-*i*-Ph); 141.7 (C-7a); 147.1 (C-6); 151.5 (CH-2); 154.7 (C-8a); 156.2 (C-4). MS (ESI): *m/z* (rel. %): 398 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{20}H_{20}O_4N_3S$ 398.11690; Found 398.11675.



8-([2,2'-Bithiophen]-5-yl)-7-chlorotetrazolo[1,5-*c*]pyrimidine (19d)

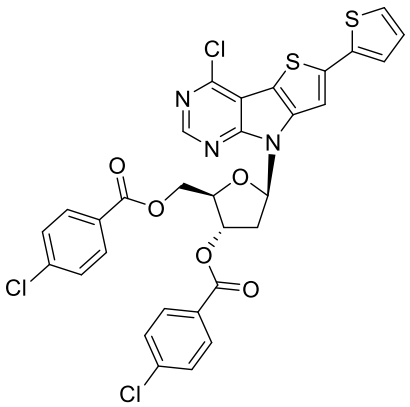
Following the general procedure for azidation, compound **18d** (2.39 g, 7.6 mmol) gave crude product **19d** (3.20 g) as a yellow solid. IR (ATR): *v* = 3377, 3086, 2135, 1633, 1577, 1434, 1353, 939, 811 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.18 (dd, 1H, $J_{4',5'}$ = 5.1 Hz, $J_{4',3'}$ = 3.6 Hz, H-4′-thienyl); 7.54 (dd, 1H, $J_{3',4'}$ = 3.6 Hz, $J_{3',5'}$ =1.1 Hz, H-3′-thienyl); 7.59 (d, 1H, $J_{3,4}$ = 4.1 Hz, H-3-thienyl); 7.66 (dd, 1H, $J_{5',4'}$ = 5.1 Hz, $J_{5',3'}$ = 1.1 Hz, H-5′-thienyl); 8.55 (d, 1H, $J_{4,3}$ = 4.1 Hz, H-4-thienyl); 10.14 (s, 1H, H-5). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 116.0 (C-8); 124.4 (CH-3-thienyl); 125.6 (CH-3′-thienyl); 127.2 (CH-5′-thienyl); 128.6 (C-5-thienyl); 128.8

(CH-4′-thienyl); 135.0 (CH-4-thienyl); 135.2 (C-2′-thienyl); 136.8 (CH-5); 141.0 (C-2-thienyl); 141.7 (C-7/C-8a); 149.9 (C-7/C-8a). MS (APCI): *m/z* (rel. %): 292 (100) $[M - N_2 + H]^+$. HRMS (APCI): *m/z* $[M]^+$ Calcd for $C_{12}H_6N_5ClS_2$ 318.97477; Found 318.97485.



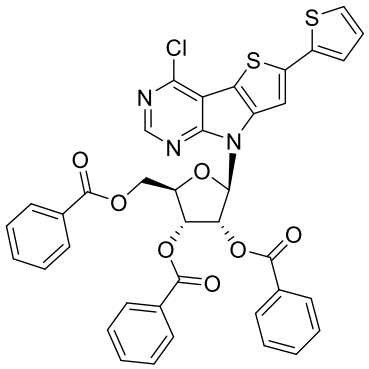
4-Chloro-6-(thiophen-2-yl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (20d)

Following the general procedure for thermal cyclization, tetrazole **19d** (3.2 g, crude product) gave nucleobase **20d** (1.5 g, 67% over 2 steps) as a brown solid. mp 271 °C (decomp.). IR (ATR): ν = 2848, 1602, 1561, 1417, 1229, 1068, 786 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 7.16 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.55 (dd, 1H, $J_{3,4}$ = 3.7 Hz, $J_{3,5'}$ = 1.3 Hz, H-3-thienyl); 7.56 (s, 1H, H-7); 7.62 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 8.64 (s, 1H, H-2), 13.08 (s, 1H, NH). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 108.9 (CH-7); 110.1 (C-4b); 111.7 (C-4a); 125.3 (CH-3-thienyl); 126.8 (CH-5-thienyl); 128.6 (CH-4-thienyl); 136.5 (C-2-thienyl); 141.7 (C-6); 143.3 (C-7a); 147.4 (C-8a); 150.9 (CH-2); 155.8 (C-4). MS (ESI): *m/z* (rel. %): 292 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{12}H_7N_3ClS_2$ 291.97644; Found 291.97636.



4-Chloro-6-(thiophen-2-yl)-8-(3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (21d)

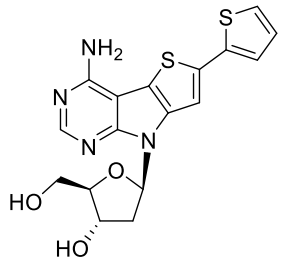
Following the general procedure for glycosylation to deoxyribonucleoside, nucleobase **20d** (400 mg, 1.4 mmol) gave product **21d** (580 mg, 62%) as a light yellow solid. mp 137–139 °C. IR (ATR): v = 2923, 1717, 1592, 1487, 1263, 1087, 1013, 847, 756 cm^{-1}. ^{1}H NMR (500.2 MHz, DMSO-d_6): 2.78 (ddd, 1H, J_{gem} = 14.4 Hz, $J_{2'a,1'}$ = 6.2 Hz, $J_{2'a,3'}$ = 2.7 Hz, H-2′a); 3.40 (m, 1H, H-2′b); 4.65 (m, 1H, H-4′); 4.65 (dd, 1H, J_{gem} = 13.7 Hz, $J_{5'a,4'}$ = 5.5 Hz, H-5′a); 4.75 (dd, 1H, J_{gem} = 13.7 Hz, $J_{5'b,4'}$ = 5.8 Hz, H-5′b); 5.89 (dt, 1H, $J_{3',2'b}$ = 7.1 Hz, $J_{3',2'a}$ = $J_{3',4'}$ = 2.8 Hz, H-3′); 6.92 (dd, 1H, $J_{1',2'b}$ = 8.3 Hz, $J_{1',2'a}$ = 6.2 Hz, H-1′); 7.12 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.42 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5}$ = 1.2 Hz, H-3-thienyl); 7.45–7.50 (m, 2H, H-*m*-C_6H_4Cl); 7.61 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 7.62–7.67 (m, 2H, H-*m*-C_6H_4Cl); 7.79 (s, 1H, H-7); 7.86–7.91 (m, 2H, H-*o*-C_6H_4Cl); 8.06–8.11 (m, 2H, H-*o*-C_6H_4Cl); 8.68 (s, 1H, H-2). ^{13}C{^{1}H} NMR (125.8 MHz, DMSO-d_6): 35.5 (CH_2-2′); 64.5 (CH_2-5′); 75.0 (CH-3′); 81.2 (CH-4′); 84.4 (CH-1′); 109.6 (CH-7); 111.3 (C-4b); 112.6 (C-4a); 125.7 (CH-3-thienyl); 127.3 (CH-5-thienyl); 128.2 and 128.3 (C-*i*-C_6H_4Cl); 128.7 (CH-4-thienyl); 129.0 and 129.1 (CH-*m*-C_6H_4Cl); 131.1 and 131.5 (CH-*o*-C_6H_4Cl); 136.2 (C-2-thienyl); 138.6 and 138.8 (C-*p*-C_6H_4Cl); 142.4 (C-6); 142.8 (C-7a); 148.1 (C-4); 151.1 (CH-2); 154.6 (C-8a); 164.8 and 164.9 (CO). MS (ESI): *m/z* (rel. %): 684 (92) [M + H]$^+$, 706 (4) [M + Na]$^+$. HRMS (ESI): *m/z* [M + H]$^+$ Calcd for $C_{31}H_{21}O_5N_3Cl_3S_2$ 683.99827; Found 683.99767.



4-Chloro-6-(thiophen-2-yl)-8-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (22d)

Following the general procedure for glycosylation to ribonucleoside, nucleobase **20d** (200 mg, 0.69 mmol) gave product **22d** (330 mg, 65%) as a light yellow solid. mp

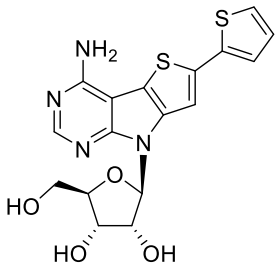
208–209 °C. IR (ATR): v = 1720, 1441, 1255, 1087, 1067 cm^{-1}. ^{1}H NMR (500.2 MHz, $CDCl_3$): 4.77 (dd, 1H, J_{gem} = 12.2 Hz, $J_{5'a,4'}$ = 3.8 Hz, H-5′a); 4.84 (m, 1H, H-4′); 4.89 (dd, 1H, J_{gem} = 12.2 Hz, $J_{5'b,4'}$ = 2.7 Hz, H-5′b); 6.26 (dd, 1H, $J_{3',2'}$ = 5.9 Hz, $J_{3',4'}$ = 4.7 Hz, H-3′); 6.45 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 5.9 Hz, H-2′); 6.86 (d, 1H, $J_{1',2'}$ = 5.9 Hz, H-1′); 6.96 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.07 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5}$ = 1.1 Hz, H-3-thienyl); 7.22 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.1 Hz, H-5-thienyl); 7.33 –7.44 (m, 6H, H-*m*-Bz); 7.40 (s, 1H, H-7); 7.51–7.56 (m, 2H, H-*p*-Bz); 7.58 (m, 1H, H-*p*-Bz); 7.91–7.95 (m, 2H, H-*o*-Bz); 8.00–8.04 (m, 2H, H-*o*-Bz); 8.05–8.09 (m, 2H, H-*o*-Bz); 8.63 (s, 1H, H-2). ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): 63.7 (CH_2-5′); 71.0 (CH-3′); 72.9 (CH-2′); 80.3 (CH-4′); 86.6 (CH-1′); 107.9 (CH-7); 113.2 (C-4b); 113.7 (C-4a); 124.9 (CH-3-thienyl); 125.9 (CH-5-thienyl); 128.1 (CH-4-thienyl); 128.50, 128.54 and 128.60 (CH-*m*-Bz); 128.8 and 129.3 (C-*i*-Bz); 129.74; 129.82 and 129.83 (CH-*o*-Bz); 133.44, 133.70 and 133.72 (CH-*p*-Bz); 136.8 (C-2-thienyl); 142.3 (C-7a); 143.7 (C-6); 149.2 (C-4); 150.9 (CH-2); 155.2 (C-8a); 165.2; 165.5 and 166.2 (CO). MS (ESI): *m/z* (rel. %): 736 (100) $[M + H]^+$, 758 (10) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{38}H_{27}O_7N_3ClS_2$ 736.09735; Found 736.09759.



4-Amino-6-(thiophen-2-yl)-8-(2′-deoxy-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5] pyrrolo[2,3-*d*]pyrimidine (23d)

Following the general procedure for amination, protected nucleoside **21d** (340 mg, 0.50 mmol) gave product **23d** (112 mg, 58%) as a white solid. mp 202–203 °C. $[\alpha]_D$ = +12.7 (c = 0.275 g/100 mL in DMSO). IR (ATR): v = 3440, 3303, 3097, 2911, 1639, 1590, 1543, 1442, 1379, 1051, 784 cm^{-1}. ^{1}H NMR (500.2 MHz, DMSO-d_6): 2.12 (ddd, 1H, J_{gem} = 13.1 Hz, $J_{2'a,1'}$ = 5.8 Hz, $J_{2'a,3'}$ = 2.1 Hz, H-2′a); 2.67 (ddd, 1H, J_{gem} = 13.1 Hz, $J_{2'b,1'}$ = 9.1 Hz, $J_{2'b,3'}$ = 6.2 Hz, H-2′b); 3.64 (dt, 1H, J_{gem} = 11.8 Hz, $J_{5'a,OH} = J_{5'a,4'}$ = 4.5 Hz, H-5′a); 3.69 (bdt, 1H, J_{gem} = 11.8 Hz, $J_{5'b,OH} = J_{5'b,4'}$ = 4.1 Hz, H-5′b); 3.89 (td,

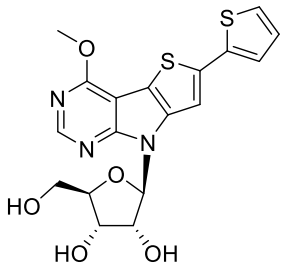
1H, $J_{4',5'a} = J_{4',5'b}$ = 3.8 Hz, $J_{4',3'}$ = 2.6 Hz, H-4′); 4.45 (m, 1H, H-3′); 5.21 (bt, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.2 Hz, OH-5′); 5.31 (bd, 1H, $J_{OH,3'}$ = 3.7 Hz, OH-3′); 6.72 (dd, 1H, $J_{1',2'b}$ = 9.1 Hz, $J_{1',2'a}$ = 5.8 Hz, H-1′); 7.13 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.19 (bs, 2H, NH_2); 7.34 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5'}$ = 1.2 Hz, H-3-thienyl); 7.53 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 7.84 (s, 1H, H-7); 8.16 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO-d_6): 38.9 (CH_2-2′); 61.9 (CH_2-5′); 71.0 (CH-3′); 83.4 (CH-1′); 87.4 (CH-4′); 97.2 (C-4a); 110.2 (CH-7); 113.0 (C-4b); 123.8 (CH-3-thienyl); 125.6 (CH-5-thienyl); 128.6 (CH-4-thienyl); 137.0 (C-6); 137.8 (C-2-thienyl); 138.5 (C-7a); 152.5 (CH-2); 154.3 (C-8a); 155.8 (C-4). MS (ESI): *m/z* (rel. %): 389 (6) $[M + H]^+$, 411 (100) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{17}H_{17}O_3N_4S_2$ 389.07366; Found 389.07325.



4-Amino-6-(thiophen-2-yl)-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (24d)

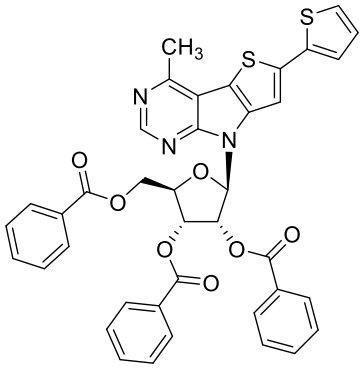
Following the general procedure for amination, protected nucleoside **22d** (250 mg, 0.34 mmol) gave product **24d** (93 mg, 68%) as a light yellow solid. mp 243–247 °C. $[\alpha]_D$ = –33.0 (*c* = 0.348 g/100 mL in DMSO). IR (ATR): *v* = 3351, 3234, 3119, 2914, 1649, 1604, 1388, 1361, 1054, 1020, 785 cm^{-1}. ^1H NMR (500.2 MHz, DMSO-d_6): 3.65 (ddd, 1H, J_{gem} = 12.0 Hz, $J_{5'a,OH}$ = 5.7 Hz, $J_{5'a,4'}$ = 3.4 Hz, H-5′a); 3.69 (ddd, 1H, J_{gem} = 12.0 Hz, $J_{5'b,OH}$ = 4.8 Hz, $J_{5'b,4'}$ = 3.3 Hz, H-5′b); 3.97 (btd, 1H, $J_{4',5'a} = J_{4',5'b}$ = 3.3 Hz, $J_{4',3'}$ = 2.5 Hz, H-4′); 4.17 (m, 1H, H-3′); 4.62 (q, 1H, $J_{2',1'} = J_{2',3'} = J_{2',OH}$ = 6.4 Hz, H-2′); 5.17 (d, 1H, $J_{OH,3'}$ = 4.3 Hz, OH-3′); 5.25 (d, 1H, $J_{OH,2'}$ = 6.7 Hz, OH-2′); 5.39 (bt, 1H, $J_{OH,5'a} = J_{OH,5'b}$ = 5.3 Hz, OH-5′); 6.27 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.13 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.20 (bs, 2H, NH_2); 7.34 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5'}$ = 1.2 Hz, H-3-thienyl); 7.53 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 7.87 (s, 1H, H-7); 8.16 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (125.8 MHz,

DMSO-d_6): 62.0 (CH_2-5′); 70.7 (CH-3′); 72.1 (CH-2′); 85.6 (CH-4′); 87.0 (CH-1′); 97.4 (C-4a); 110.3 (CH-7); 112.9 (C-4b); 123.8 (CH-3-thienyl); 125.6 (CH-5-thienyl); 128.6 (CH-4-thienyl); 137.0 (C-6); 137.8 (C-2-thienyl); 138.9 (C-7a); 152.5 (CH-2); 154.9 (C-8a); 155.8 (C-4). MS (ESI): *m/z* (rel. %): 405 (100) $[M + H]^+$, 427 (71) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{17}H_{17}O_4N_4S_2$ 405.06857; Found 405.06802.



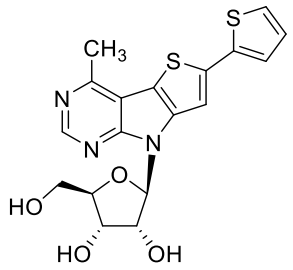
4-Methoxy-6-(thiophen-2-yl)-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (25d)

Following the general procedure for methoxylation, protected nucleoside **22d** (250 mg, 0.34 mmol) gave product **25d** (126 mg, 88%) as a light yellow solid. mp 259–262 °C. $[\alpha]_D$ = –58.3 (*c* = 0.307 g/100 mL in DMSO). IR (ATR): *v* = 3344, 3110, 2929, 1596, 1430, 1418, 1357, 1126, 1018 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 3.70 (bd, 2H, $J_{5'a,4'}$ = 2.5 Hz, H-5′); 4.00 (q, 1H, $J_{4',5'a} = J_{4',5'b} = J_{4',3'}$ = 3.0 Hz, H-4′); 4.15 (s, 3H, CH_3O); 4.20 (dd, 1H, $J_{3',2'}$= 5.5 Hz, $J_{3',4'}$= 2,3 Hz, H-3′); 4.61 (dd, 1H, $J_{2',1'}$ = 7.5 Hz, $J_{2',3'}$ = 5.4 Hz, H-2′); 5.29 (s, 3H, OH-3′, OH-2′, OH-5′); 6.37 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.14 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.42 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5'}$ = 1.2 Hz, H-3-thienyl); 7.59 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 8.01 (s, 1H, H-7), 8.53 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 54.1 (CH_3O); 61.6 (CH_2-5′); 70.4 (CH-3′); 72.1 (CH-2′); 85.6 (CH-4′); 86.8 (CH-1′); 99.7 (C-4a); 110.8 (CH-7); 111.5 (C-4b); 124.5 (CH-3-thienyl); 126.2 (CH-5-thienyl); 128.5 (CH-4-thienyl); 137.0 (C-2-thienyl); 138.9 (C-6); 140.2 (C-7a); 151.4 (CH-2); 156.0 (C-8a); 160.8 (C-4). MS (ESI): *m/z* (rel. %): 420 (100) $[M + H]^+$, 442 (2) $[M + Na]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{18}H_{18}O_5N_3S_2$ 420.06824; Found 420.06793.



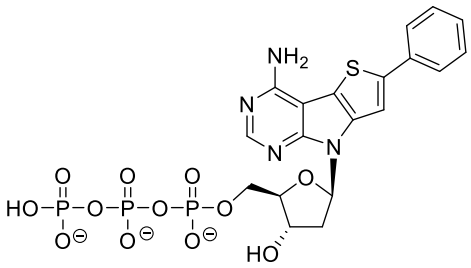
4-Methyl-6-(thiophen-2-yl)-8-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo[2,3-*d*]pyrimidine (26d)

Following the general procedure for methylation, protected nucleoside **22d** (365 mg, 0.50 mmol) gave compound **26d** (261 mg, 74%) as a yellow solid. mp 180–181 °C. IR (ATR): ν = 2923, 1720, 1447, 1257, 1241, 1088, 1068, 703 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.80 (s, 3H, CH_3); 4.77 (dd, 1H, J_{gem} = 11.9 Hz, $J_{5'a,4'}$ = 3.8 Hz, H-5′a); 4.80–4.84 (m, 1H, H-4′); 4.88 (dd, 1H, J_{gem} = 11.8 Hz, $J_{5'b,4'}$ = 2.5 Hz, H-5′b); 6.28 (dd, 1H, $J_{3',2'}$ = 6.1 Hz, $J_{3',4'}$ = 4.6 Hz, H-3′); 6.46 (t, 1H, $J_{2',1'} = J_{2',3'}$ = 6.0 Hz, H-2′); 6.90 (d, 1H, $J_{1',2'}$ = 5.9 Hz, H-1′); 6.95 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.03 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5}$ = 1.2 Hz, H-3-thienyl); 7.19 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.1 Hz, H-5-thienyl); 7.32 - 7.43 (m, 6H, H-*m*-Bz); 7.41 (s, 1H, H-7); 7.49–7.60 (m, 3H, H-*p*-Bz); 7.91–7.97 (m, 2H, H-*o*-Bz); 7.99–8.04 (m, 2H, H-*o*-Bz); 8.06–8.12 (m, 2H, H-*o*-Bz); 8.79 (s, 1H, H-2). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$): δ 22.6 (CH_3); 64.1 (CH_2-5′); 71.2 (CH-3′); 73.0 (CH-2′); 80.2 (CH-4′); 86.3 (CH-1′); 108.4 (CH-7); 114.0 and 114.3 (C-4b, C-4a); 124.6 (CH-3-thienyl); 125.6 (CH-5-thienyl); 128.1 (CH-4-thienyl); 128.61, 128.66 and 128.72 (CH-*m*-Bz); 128.8, 129.0 and 129.6 (C-*i*-Bz); 129.94, 129.97 and 129.99 (CH-*o*-Bz); 133.5 and 133.8 (CH-*p*-Bz); 137.3 (C-2-thienyl); 141.2 (C-7a); 142.0 (C-6); 152.0 (CH-2); 155.1 (C-8a); 156.9 (C-4); 165.4, 165.6 and 166.4 (CO). MS (ESI): *m/z* (rel. %): 716 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{39}H_{30}O_7N_3S_2$ 716.15197; Found 716.15137.



4-Methyl-6-(thiophen-2-yl)-8-(*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo [2,3-*d*]pyrimidine (**27d**)

Following the general procedure for deprotection, protected nucleoside **26d** (230 mg, 0.32 mmol) gave final product **27d** (112 mg, 86%) as a light yellow solid. mp 258–260 °C. $[\alpha]_D$ = –61.2 (*c* = 0.325 g/100 mL in DMSO). IR (ATR): *v* = 3354, 3277, 3109, 2928, 1589, 1440, 1416, 1120, 1093, 1024 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 2.75 (s, 3H, CH_3); 3.71 (d, 2H, $J_{5',4'}$ = 3.8 Hz, H-5′); 4.01 (q, 1H, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',3'}$ = 3.0 Hz, H-4′); 4.20 (dd, 1H, $J_{3',2'}$ = 5.6 Hz, $J_{3',4'}$ = 2.3 Hz, H-3′); 4.61 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 6.4 Hz, H-2′); 5.26 (bs, 1H, OH-3′); 5.32 (m, 2H, OH-2′, OH-5′); 6.39 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′); 7.16 (dd, 1H, $J_{4,5}$ = 5.1 Hz, $J_{4,3}$ = 3.6 Hz, H-4-thienyl); 7.46 (dd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{3,5'}$ = 1.2 Hz, H-3-thienyl); 7.60 (dd, 1H, $J_{5,4}$ = 5.1 Hz, $J_{5,3}$ = 1.2 Hz, H-5-thienyl); 8.07 (s, 1H, H-7); 8.73 (s, 1H, H-2). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 22.1 (CH_3); 61.7 (CH_2-5′); 70.5 (CH-3′); 72.1 (CH-2′); 85.7 (CH-4′); 86.5 (CH-1′); 110.9 (CH-7); 112.4, 112.5 (C-4a, C-4b); 124.8 (CH-3-thienyl); 126.5 (CH-5-thienyl); 128.7 (CH-4-thienyl); 136.9 (C-2-thienyl); 140.2 (C-6); 141.4 (C-7a); 151.5 (CH-2); 154.7 (C-8a); 156.3 (C-4). MS (ESI): *m/z* (rel. %): 404 (100) $[M + H]^+$. HRMS (ESI): *m/z* $[M + H]^+$ Calcd for $C_{18}H_{18}O_4N_3S_2$ 404.07332; Found 404.07303.



4-Amino-6-phenyl-8-(2′-deoxy-*β*-D-ribofuranosyl)-8*H*-thieno[2',3':4,5]pyrrolo [2,3-*d*]pyrimidine-5′-*O*-triphosphate triethylammonium salt (**23cTP**)

Nucleoside **23c** (30 mg, 0.078 mmol) was dissolved in trimethyl phosphate (0.4 mL), cooled to 0 °C, and treated with freshly distilled $POCl_3$ (11.2 µL, 0.118 mmol). The mixture was stirred at 0 °C for 3 h. A solution of $(NHBu_3)_2H_2P_2O_7$ (216 mg, 0.393 mmol) and Bu_3N (112 µL, 0.472 mmol) in anhydrous DMF (0.5 mL), which was prestirred at 0 °C for 5 min, was added to the former mixture and stirred at 0 °C for 2 h. Then, the mixture was treated with aqueous TEAB (2 M, 2 mL). The mixture was evaporated under reduced pressure and co-evaporated four times with water. The crude product was purified by HPLC (C-18, 10−100% MeOH in 0.1 M TEAB) and co-evaporated four times with water. And the residue was freeze-dried to obtain triphosphate **23cTP** (30 mg, 41%) as a white solid. ^{1}H NMR (600.1 MHz, D_2O): 2.45 (ddd, 1H, J_{gem} = 14.0 Hz, $J_{2'a,1'}$ = 6.1 Hz, $J_{2'a,3'}$ = 2.5 Hz, H-2′a); 2.87 (ddd, 1H, J_{gem} = 14.0 Hz, $J_{2'b,1'}$ = 9.1 Hz, $J_{2'b,3'}$ = 7.1 Hz, H-2′b); 4.28–4.36 (m, 3H, H-4′,5′); 4.38 (m, 1H, H-3′); 6.65 (dd, 1H, $J_{1',2'b}$ = 9.1 Hz, $J_{1',2'a}$ = 6.1 Hz, H-1′); 7.30 (m, 1H, H-*p*-Ph); 7.35–7.40 (m, 2H, H-*m*-Ph); 7.50 (s, 1H, H-7); 7.51–7.55 (m, 2H, H-*o*-Ph); 7.77 (s, 1H, H-2). ^{13}C{^{1}H} NMR (150.9 MHz, D_2O): 39.8 (CH_2-2′); 67.6 (d, $J_{C,P}$ = 4.8 Hz, CH_2-5′); 72.7 (CH-3′); 85.2 (CH-1′); 86.8 (d, $J_{C,P}$ = 8.7 Hz, CH-4′); 99.6 (C-4a); 109.9 (CH-7); 115.8 (C-4b); 126.8 (CH-*o*-Ph); 130.0 (CH-*p*-Ph); 131.1 (CH-*m*-Ph); 135.3 (C-*i*-Ph); 140.4 (C-7a); 147.9 (C-6); 152.0 (CH-2); 154.6 (C-8a); 155.7 (C-4). ^{31}P NMR (202.4 MHz, D_2O): –22.2 (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma}$ = 19.9 Hz, P_β); –10.5 (d, 1P, $J_{\alpha,\beta}$ = 19.8 Hz, P_α); –8.3 (d, 1P, $J_{\gamma,\beta}$ = 20.0 Hz, P_γ). MS (ESI): *m/z* (rel. %): 621 (100) $[M–3C_6H_{16}N + 2H]^-$, 643 (4) $[M–3C_6H_{16}N + H + Na]^-$. HRMS (ESI): *m/z* $[M–3C_6H_{16}N + 2H]^-$ Calcd for $C_{19}H_{20}N_4O_{12}P_3S$ 621.00168; Found 621.00111.

5.3.3 UV and fluorescence spectra

Materials and methods

Chemicals and spectroscopic grade solvents were purchased from Sigma-Aldrich and Lach-Ner and used without further purification. UV-VIS spectra were measured on a Cary 100 UV-VIS spectrometer (Agilent Technologies). Fluorescence spectra were measured on a Fluoromax 4 spectrofluorimeter (HORIBA Scientific).

Determination of absorption coefficients

Absorption coefficients were measured using 1 mL quartz cuvettes on a Cary 100 UV-VIS spectrometer (Agilent Technologies). The absorption coefficients were calculated according to the following equation

$$A = c * l * \varepsilon$$

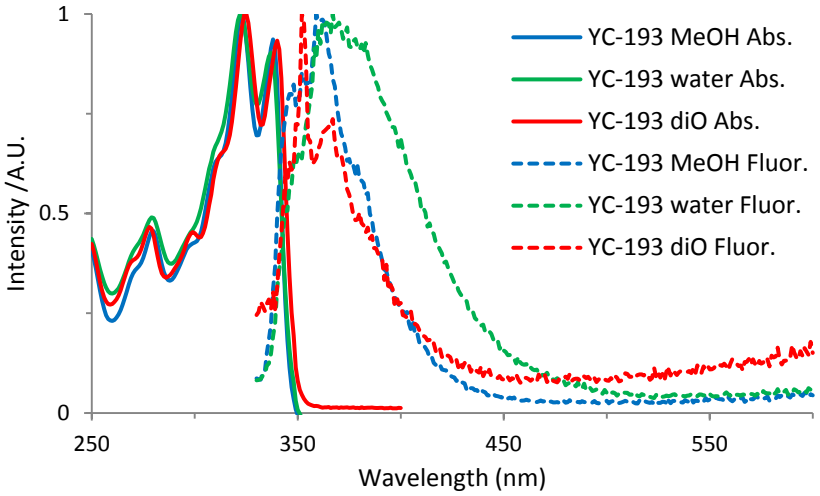
where ε is the absorption coefficient, c is the exact concentration of the sample in the cuvette, l is the length of the path that the light travels through the cuvette and A is the absorbance of the sample. Measurements were triplicated and the uncertainty of measured values of absorption coefficients did not exceed ± 0.2 × 10^4 M^{-1} cm^{-1}.

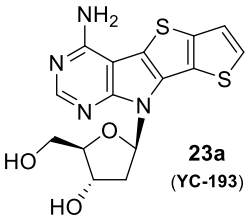
Determination of fluorescence quantum yields

Determination of the fluorescence quantum yields (Φ_f) was performed using anthracene in EtOH (Φ_f = 0.27 at 25 °C) as a standard.[139] Measurements were performed in 1 mL quartz fluorescence cuvettes (Hellma Analytics) on a Fluoromax 4 spectrofluorimeter equipped with a thermostated cuvette holder set to 25 °C. The solvents (MeOH, H_2O, 1,4-Dioxane) used were of spectroscopy or HPLC grade. The excitation wavelength was 320 nm and the emission spectra were recorded in the range of 330–600 nm for all compounds. The absorption of the solutions of samples was kept below 0.08 to prevent inner filter effect. The quantum yields were calculated using following equation:

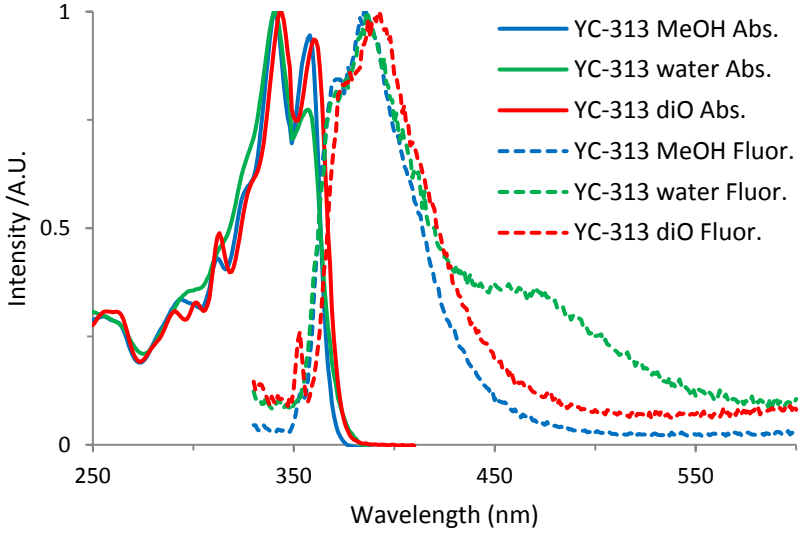
$$\Phi_{f,x} = \Phi_{f,st} \frac{F_x}{F_{st}} \frac{1 - 10^{-Abs_{st}}}{1 - 10^{-Abs_x}} \frac{n_x^2}{n_{st}^2}$$

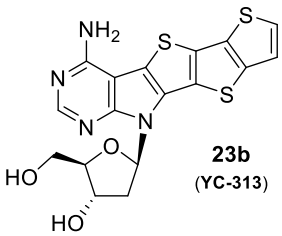
where Φ_f is the quantum yield, F is the integrated fluorescence intensity, Abs is the absorbance of solution at the excitation wavelength, n is the refractive index of the solvent. The subscripts x and st stand for the sample and standard, respectively. Measurements were triplicated.





23a
(YC-193)





23b
(YC-313)

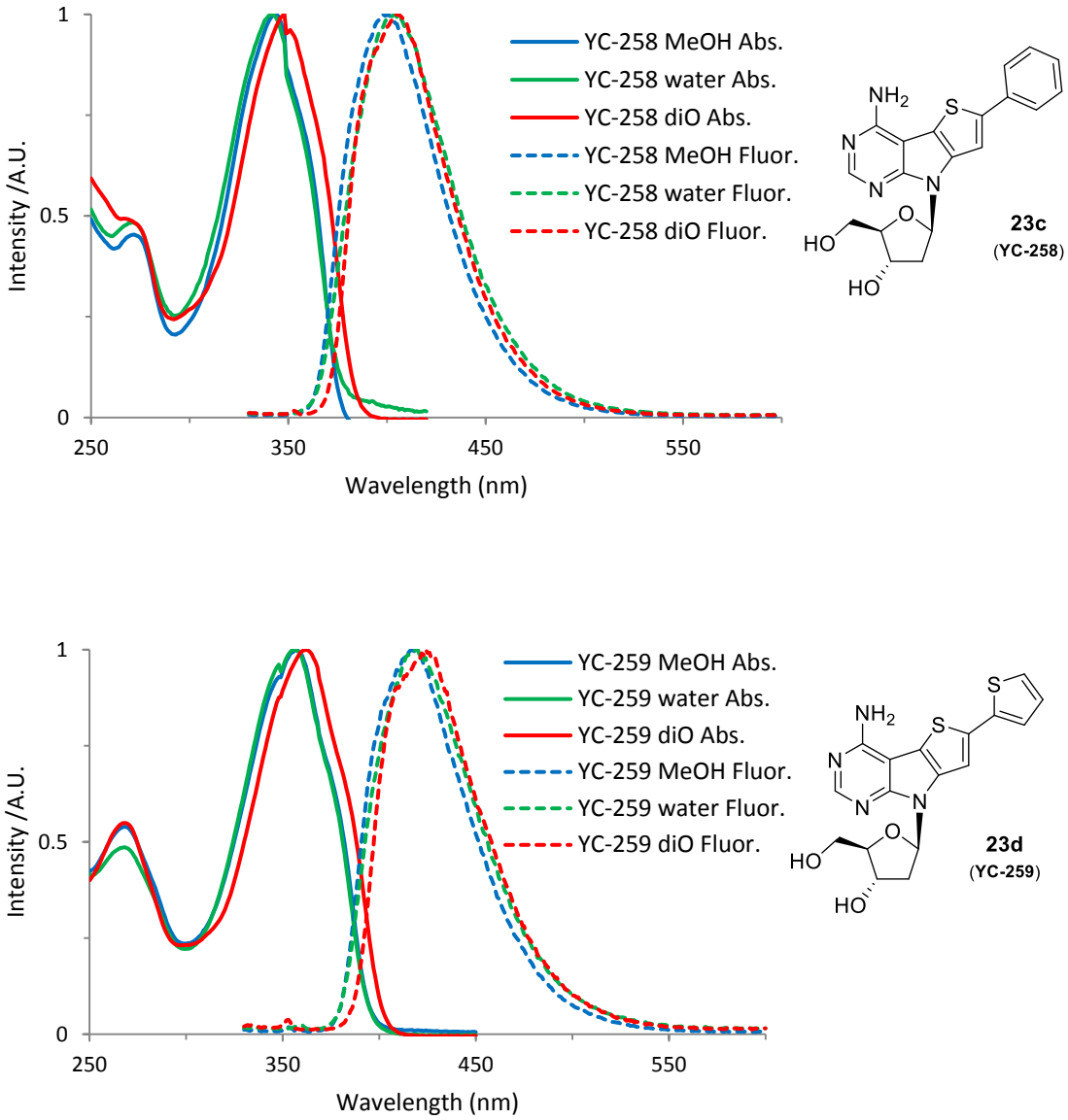


Figure 19. Normalized UV and fluorescence spectra of nucleosides **23a–23d**

5.3.4 Biochemistry of phenyl-thieno-fused 7-deazapurine nucleotide

5.3.4.1 Primer extension experiments (PEX)

The primer extension experiments were performed as described previously.[75] The reaction mixture (20 μL) contained FAM-labeled primer *Prim*^{FAM} (0.15 μM), template *Temp1A* or *Temp4A* (0.225 μM each), KOD XL DNA polymerase (0.0625 U) and KOD XL DNA polymerase buffer (2 μL) supplied by the manufacturer. For template *Temp1A*: reaction mixtures contained also dGTP (15 μM) and either dATP, 7-deaza-dATP or modified **dA^{PT}TP** (30 μM). Reactions were incubated at 60 °C for 15 min or 25 min. For template *Temp4A*: reaction mixtures contained also dGTP (30 μM), dCTP (30 μM), dTTP (30 μM) and either dATP, 7-deaza-dATP or modified **dA^{PT}TP** (120 μM). Reactions were incubated at 60 °C for 30, 45, 60 or 120 min. Reactions were stopped by addition of PAGE stop solution (20 μL) and heated at 95 °C for 5 min. Aliquots (4 μL) were separated by 12.5% denaturing PAGE and visualized by fluorescence imaging (Figure 13).

5.3.4.2 PEX experiments for comparison of reactivity of dA^{PT}TP

Comparison of the primer extension experiments was performed with *Temp4A* at two different concentrations of **23cTP** (**dA^{PT}TP**). (A) High concentration of the reaction mixture: Reaction mixture (20 μL) contained *Prim*^{FAM} (3.2 μM); *Temp4A* (3.2 μM), KOD XL DNA polymerase (1 U) and KOD XL DNA polymerase buffer (2 μL); dGTP (300 μM), dCTP (300 μM), dTTP (300 μM) and **dA^{PT}TP** (1.2 mM); (B) Low concentration of the reaction mixture: Reaction mixture (100 μL) contained *Prim*^{FAM} (0.15 μM); *Temp4A*^{bio} (0.225 μM), KOD XL DNA polymerase (0.0625 U) and KOD XL DNA polymerase buffer (10 μL); dGTP (30 μM), dCTP (30 μM), dTTP (30 μM) and **dA^{PT}TP** (120 μM). Reactions were incubated at 60 °C for 120 min, stopped by addition of PAGE stop solution (20 μL) and heated at 95 °C for 5 min. Aliquots (4 μL) were separated by 12.5% denaturing PAGE and visualized by fluorescence imaging

(Figure 14). Mixture containing **dA^{PT}TP** was also used for MALDI-TOF measurements (see more in section 5).

5.3.4.3 The rate of incorporation with template *Temp^{termA}*

1) Study of the rate of incorporation with template *Temp^{termA}*

The rate of incorporation was studied by preparation of samples with modified **dA^{PT}TP** in various time periods. PEX reaction mixture (20 μL) contained a FAM-labeled primer *Prim^{FAM}* (0.15 μM,), template Temp^{termA} (0.225 μM), **dA^{PT}TP** (30 μM) with KOD XL DNA polymerase (0.0625 U) in the enzyme reaction buffer (2 μL) as supplied by the manufacturer, reactions were incubated at 60 °C for various time periods (0.5, 1, 2, 3 h), followed by stopping of the reaction using the PAGE stop solution (20 μL) and immediately heated for 2 min at 95 °C. Samples were analyzed with a 12.5% denaturing PAGE (1h, 50°C) and visualized by fluorescence imaging (Figure 15).

2) Comparison of the rate of incorporation with template *Temp^{termA}*

Since the rate of **dA^{PT}TP** incorporation was slow, high concentrations of DNA polymerase and dNTPs were used for comparison of the rate of incorporation.

The rate of incorporation was compared by preparation of samples with natural dATP, 7-deaza-dATP, and modified **dA^{PT}TP** with various time periods. PEX reaction mixture (20 μL) contained a FAM-labeled primer *Prim^{FAM}* (0.15 μM,), template temp^{termA} (0.225 μM), either dATP, 7-deaza-dATP or **dA^{PT}TP** (200 μM each) with KOD XL DNA polymerase (0.125 U) in the enzyme reaction buffer (2 μL) as supplied by the manufacturer, and reactions were incubated at 60 °C for various time periods (0.1, 0.5, 1, 2, 5, 10, 15, 20, 30, 60, 90, 120 min), followed by stopping of the reaction using the PAGE stop solution (20 μL) and immediately heated for 2 min at 95 °C. Samples were analyzed with a 12.5% denaturing PAGE (1h 50°C) and visualized by fluorescence imaging (Figure 16A).

5.3.4.4 Comparison of the rate of incorporation with template *Temp1A*

The rate of incorporation was compared by preparation of samples with natural dATP, 7-deaza-dATP, and modified **dA^{PT}TP** with various time periods. PEX reaction mixtures (20 μL) contained a FAM-labeled primer *Prim^{FAM}* (0.15 μM,), template *Temp1A* (0.225 μM), either dATP, 7-deaza-dATP or **dA^{PT}TP** (200 μM each) with KOD XL DNA polymerase (0.125 U) in the enzyme reaction buffer (2 μL) as supplied by the manufacturer, another mixture with **dA^{PT}TP** also contained dGTP (100 μM), reactions were incubated at 60 °C for various time periods (0.1, 0.5, 1, 2, 5, 10, 15, 30 min), followed by stopping of the reaction using the PAGE stop solution (20 μL) and immediately heated for 2 min at 95 °C. Samples were analyzed with a 12.5% denaturing PAGE (1h, 50°C) and visualized by fluorescence imaging (Figures 16B).

5.3.4.5 Preparation of ONs for MALDI-TOF Analysis:

1) Primer extension experiments (PEX)

a) The ONs were prepared as described previously.[75] The reaction mixture (100 μL) contained primer *Prim* (3.2 μM), biotinylated template *Temp1A^{bio}* or *Temp4A^{bio}* or *Temp$^{termA\ bio}$* (3.2 μM), KOD XL DNA polymerase buffer (10 μL) supplied by the manufacturer. For template *Temp1A^{bio}*: reaction mixtures contained also KOD XL DNA polymerase (2.5 U), dGTP (300 μM) and modified **dA^{PT}TP** (600 μM). Reaction mixtures were incubated at 60 °C for 40 min. For template *Temp4A^{bio}*: reaction mixtures contained also KOD XL DNA polymerase (5 U), dGTP (300 μM), dCTP (300 μM), dTTP (300 μM) and modified **dA^{PT}TP** (1.2 mM). Reaction mixtures were incubated at 60 °C for 2 h. For template *Temp$^{termA\ bio}$*: reaction mixtures contained also KOD XL DNA polymerase (2.5 U), and modified **dA^{PT}TP** (600 μM). Reaction mixtures were incubated at 60 °C for 2 h.

b) For template *Temp4A^{bio}*, the reaction mixture was also prepared at low concentration. The reaction mixture (100 μL) contained *Prim^{FAM}* (0.15 μM);

Temp4A^{bio} (0.225 μM), KOD XL DNA polymerase (0.0625 U) and KOD XL DNA polymerase buffer (10 μL); dGTP (30 μM), dCTP (30 μM), dTTP (30 μM) and **dA^{PT}TP** (120 μM). Reaction mixtures were incubated at 60 °C for 2 h. This mixture was also used for PEX analysis (Figure 14B). The reaction was conducted in parallel 12 batches. Then all mixtures were collected together and evaporated at 50 °C to 100 μL.

c) For template *Temp1A* ^{bio}, the reaction mixture was also prepared with only **dA^{PT}TP**. The reaction mixture (100 μL) contained *Prim* (3.2 μM); *Temp1A*^{bio} (3.2 μM), KOD XL DNA polymerase (2.5 U) and KOD XL DNA polymerase buffer (10 μL); and **dA^{PT}TP** (600 μM). Reaction mixtures were incubated at 60 °C for 40 min.

2) Magnetoseparation procedure

The PEX products prepared in section 5.1 were applied for magnetoseparation on magnetic beads and was carried out under following conditions: A stock solution of streptavidin magnetic particles (Roche, 50 μL) was washed with buffer TEN_{100} (3× 200 μL; 10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5). The PEX solution and buffer TEN_{100} (50 μL) were added. The mixture was vigorously shaken at 15 °C for 30 min at 1400 rpm. The magnetic particles were collected on a magnet and washed with buffer TEN_{500} (3× 200 μL; 10 mM Tris, 1 mM EDTA, 500 mM NaCl, pH 7.5) and water (5× 200 μL). Water (50 μL) was added and the suspension was heated to 75 °C for 2 min at 900 rpm. The magnetic particles were then collected on a magnet and the supernatant was quickly transferred into a clean vial. The concentration of the ON product was determined by Nanodrop1000 spectrophotometer and analyzed by MALDI-TOF mass spectrometry. An overview of measured data is in Table 15 and copies of mass-spectra in Part 7, Figures 20 – 24.

5.3.4.6 Fluorescence Measurements with Oligonucleotides:

Fluorescence emission spectra of ONs were measured as described previously.[75] ONs (prepared in section 6.1 a; 1 μM or 1 μM of each strand for DNA duplexes) were

mixed in medium salt buffer (100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0) and heated to 95 °C for 5 min and cooled slowly to 25 °C over a period of 60 min. Fluorescence emission spectra were recorded by using the excitation wavelength of 320 nm and the integration range of 330–600 nm. Measurements were performed at 25 °C. Background spectra of the buffer solutions were recorded and subtracted from the spectra.

5.3.4.7 Copies of MALDI-TOF spectra

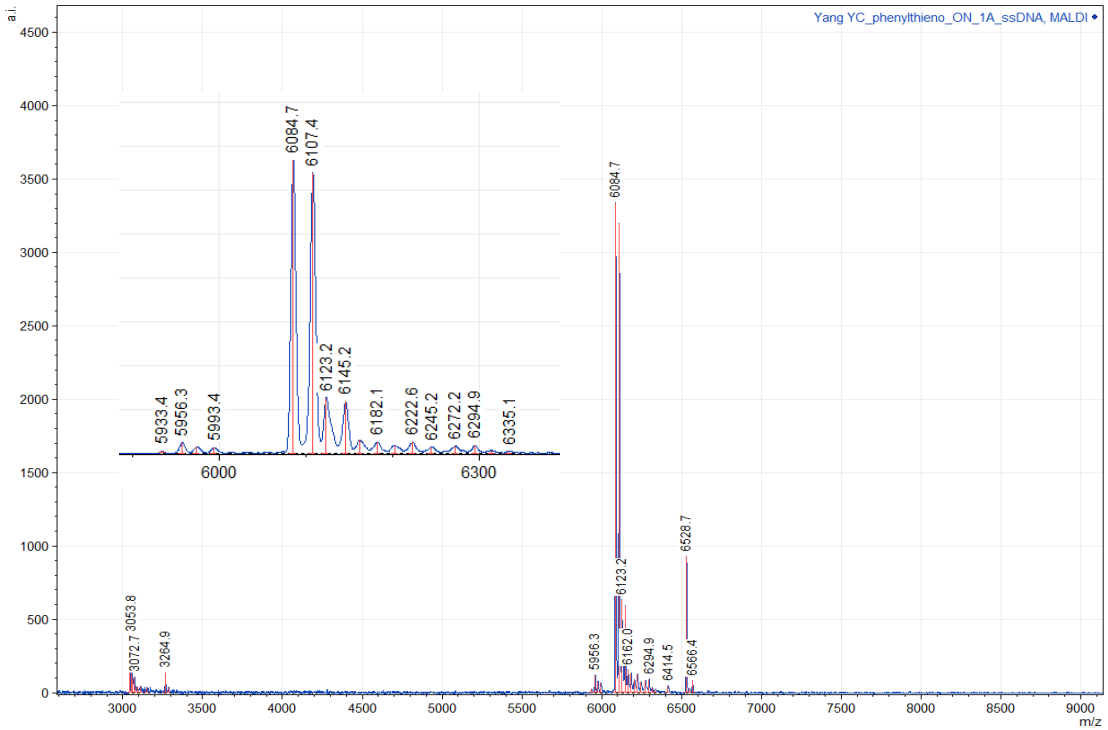


Figure 20. MALDI TOF spectrum of **19ON_1A^{PT}**: calculated: 6105.9 Da; found: 6107.4 Da; Δ = 1.5 Da.

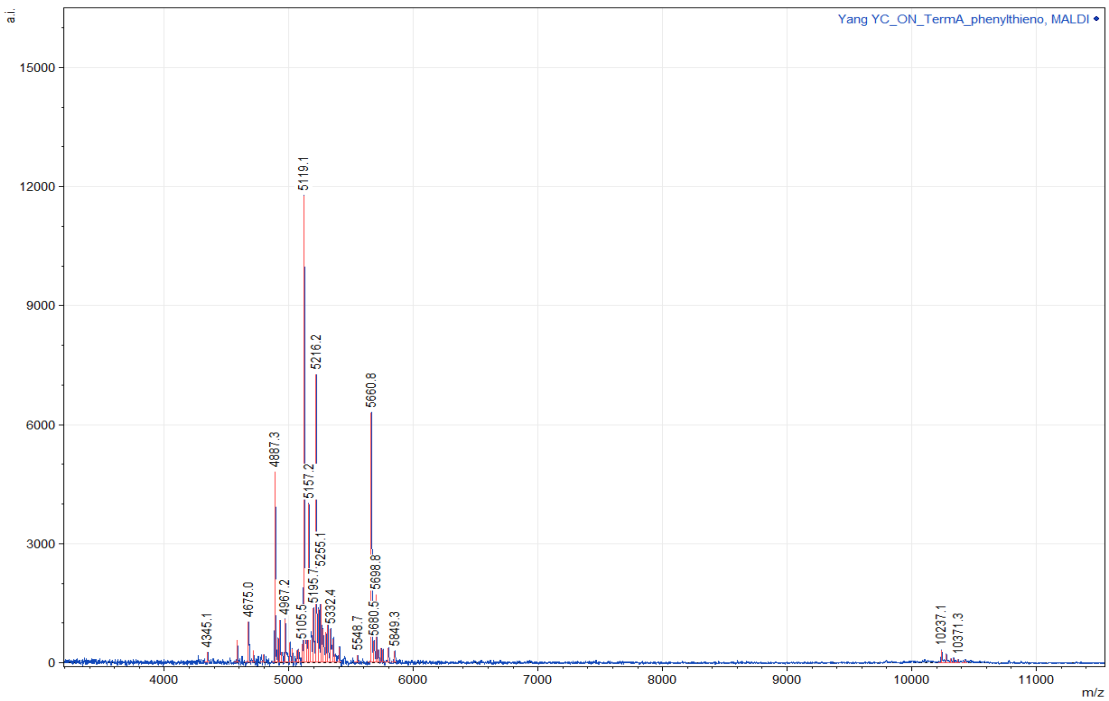


Figure 21. MALDI TOF spectrum of **16ON_1A^{PT}**: calculated: 5118.2 Da; found: 5119.1 Da; Δ = 0.9 Da.

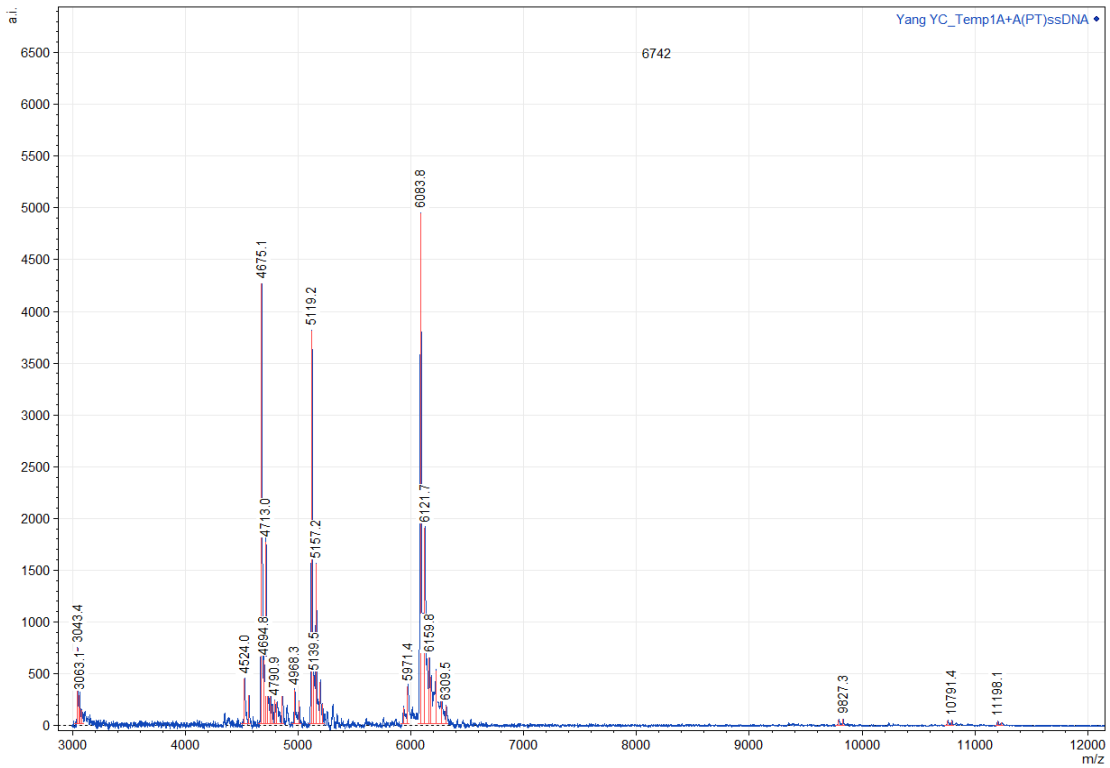


Figure 22. MALDI TOF spectrum of **16ON_1A^{PT}** (prepared with *Temp1A*^{bio} and only **dA^{PT}TP)**: calculated: 5118.2 Da; found: 5119.2 Da; Δ = 1.0 Da.

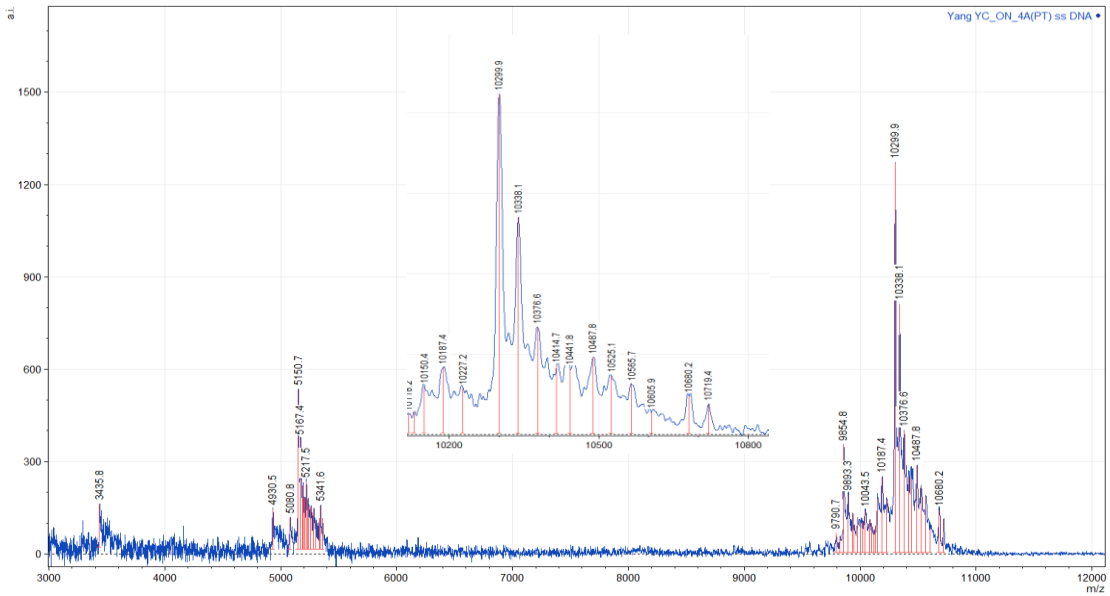


Figure 23. MALDI TOF spectrum of **31ON_4A^{PT}** (low concentration of PEX mixture; 5`-(6-FAM)-labelled): calculated: 10678.2 Da; found: 10680.2 Da; Δ = 2.0 Da.

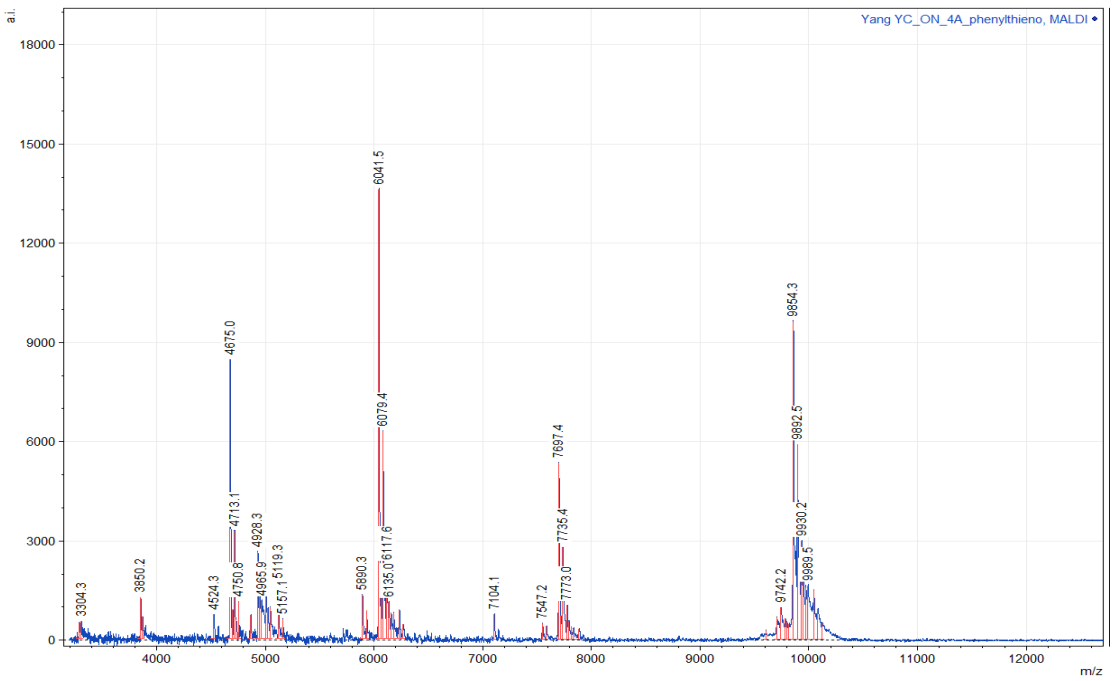


Figure 24. MALDI TOF spectrum of **31ON_4A^{PT}** (high concentration of PEX mixture): calculated: 10141.2 Da; found:
6041.5 Da (5′-CATGGGCGGCATGGGA^{PT}CTG, calcd 6040.8),
7697.4 Da (5′-CATGGGCGGCATGGGA^{PT}CTGA^{PT}GCTC calcd 7696.8).

5.3.5 HPLC purity of final products

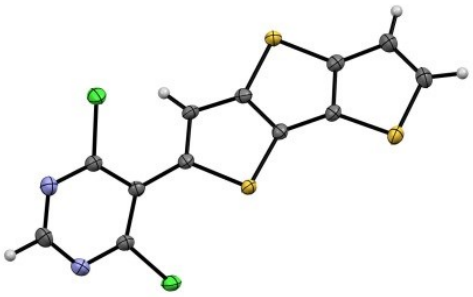
compd	method	t_r [min]	purity [%]	method	t_r [min]	purity [%]
23a	A	16.74	98.78	B	10.26	98.90
23b	A	19.52	98.77	B	12.78	98.91
23b*α*	A	18.82	98.00	B	12.43	97.64
23c	A	18.85	98.98	B	12.55	99.25
23d	A	18.28	97.82	B	12.14	98.51
24a	A	15.94	97.58	B	8.89	97.01
24b	A	18.89	97.22	B	12.46	97.83
24c	A	18.11	97.27	B	11.97	97.04
24d	A	17.60	98.04	B	11.53	98.38
25a	A	19.95	99.34	B	13.70	99.35
25b	A	22.42	99.02	B	16.55	98.96
25c	A	21.80	99.00	B	15.74	99.15
25d	A	21.45	99.26	B	15.29	99.41
27a	A	18.51	97.70	B	11.72	98.50
27b	A	21.26	98.54	B	14.27	98.58
27c	A	20.87	99.51	B	13.85	99.41
27d	A	20.33	98.38	B	13.38	98.59

Methods: A = 20% MeOH in H_2O to 100% MeOH in 15 min, 100% MeOH in 15 min, UV 280 nm; B = 20% MeCN in H_2O to 100% MeCN in 15 min, 100% MeCN in 15 min, UV 280 nm.

5.3.6 X-ray crystallography

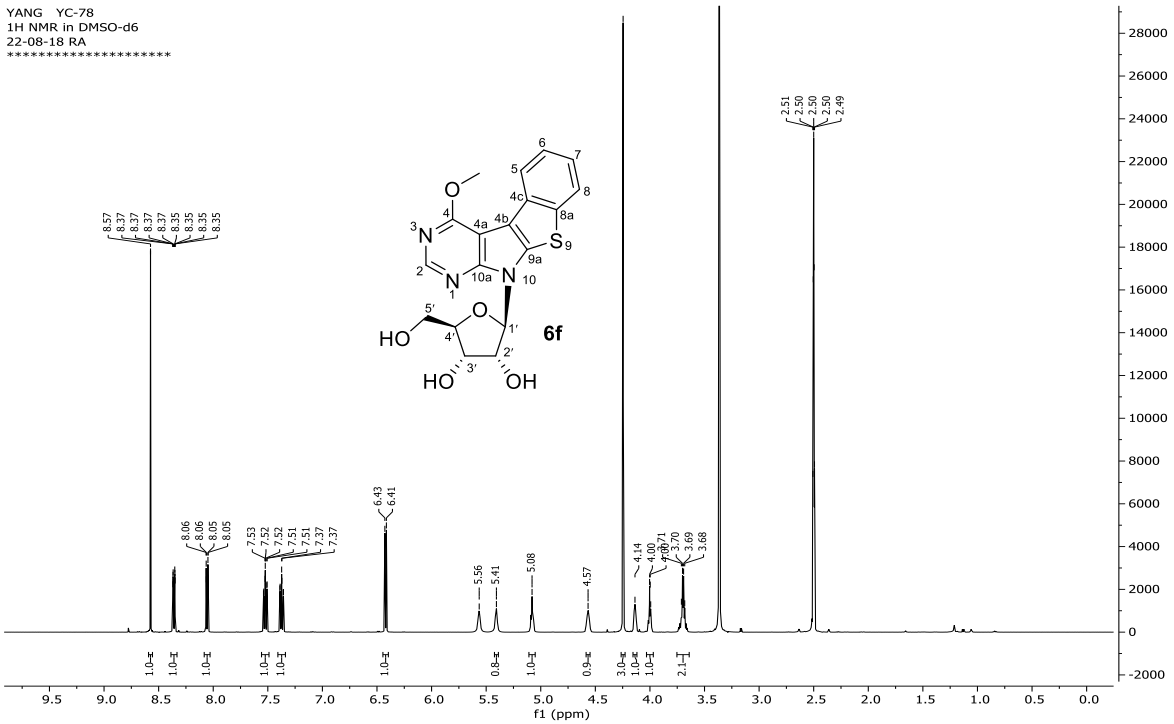
Single-crystal diffraction data of **18b** were collected using Bruker D8 VENTURE system equipped with a Photon 100 CMOS detector, a multilayer monochromator, and a CuKα Incoatec microfocus sealed tube (λ = 1.54178 Å) at 180 K. The frames were integrated with the with Bruker SAINT[140] software package. The structure was solved by direct methods with SIR92[141] and refined by full-matrix least-squares on F with CRYSTALS.[142] The positional and anisotropic thermal parameters of all non-hydrogen atoms were refined. All hydrogen atoms were located in a difference Fourier map and then they were repositioned geometrically. They were initially refined with soft restraints on the bond lengths and angles to regularize their geometry, then their positions were refined with riding constraints.

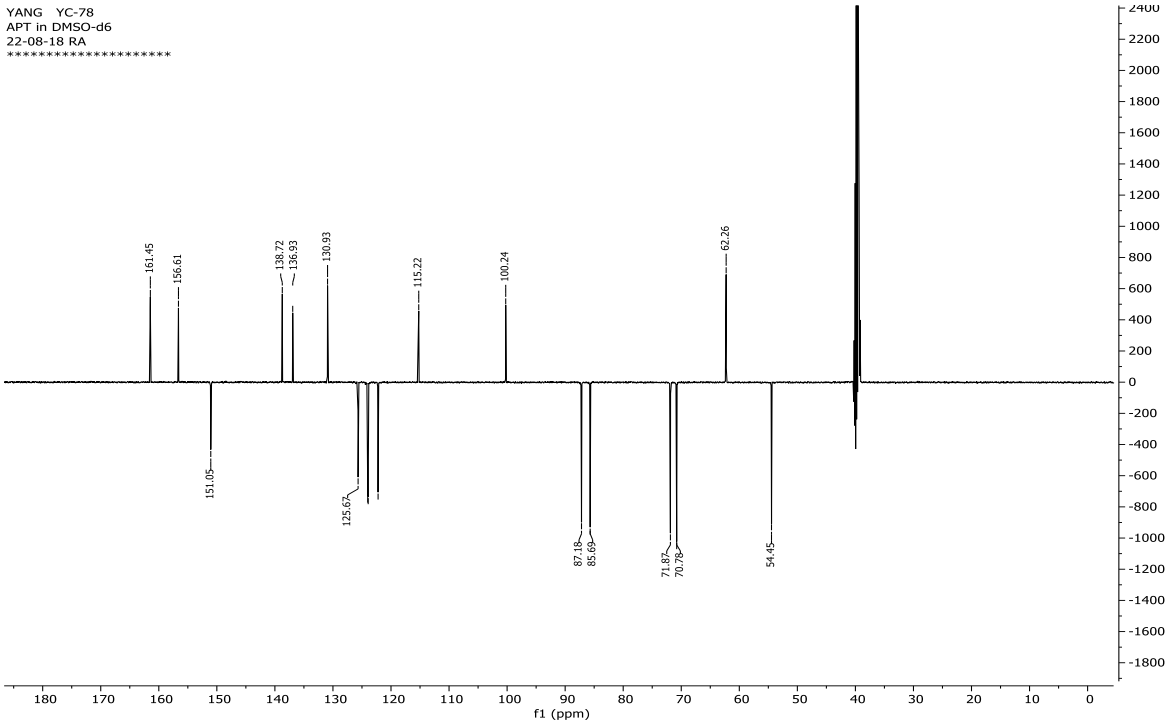
Crystal data for 18b (light green, 0.099 x 0.121 x 0.810 mm):
$C_{12}H_4Cl_2N_2S_3$, monoclinic, space group $P2_1/n$, a = 17.7569(4) Å, b = 3.88630(9) Å, c = 18.2519(4) Å, β = 96.1837(6)°, V = 1252.21(5) Å^3, Z = 4, M = 343.28, 24556 reflections measured, 2300 independent reflections. Final R = 0.026, wR = 0.028, GoF = 1.079 for 2293 reflections with $I > 2\sigma(I)$ and 172 parameters. CCDC2183422.

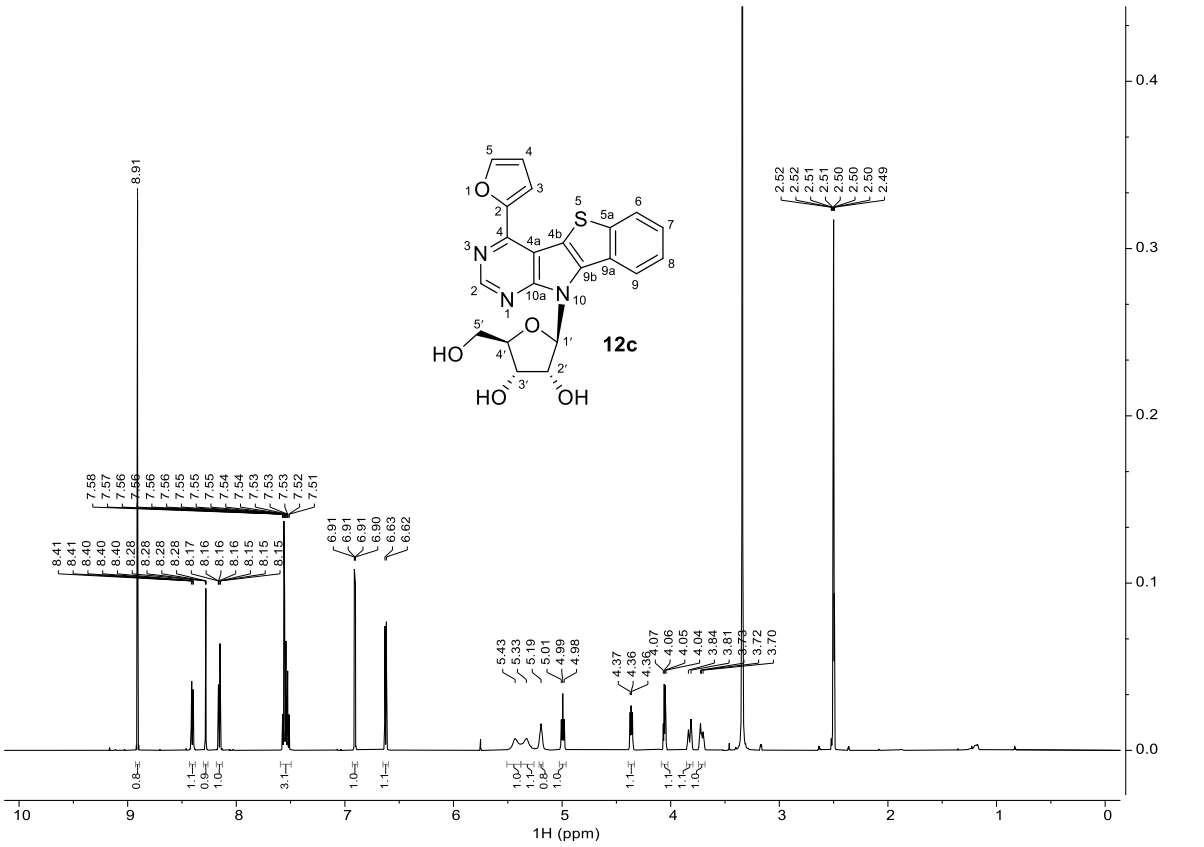


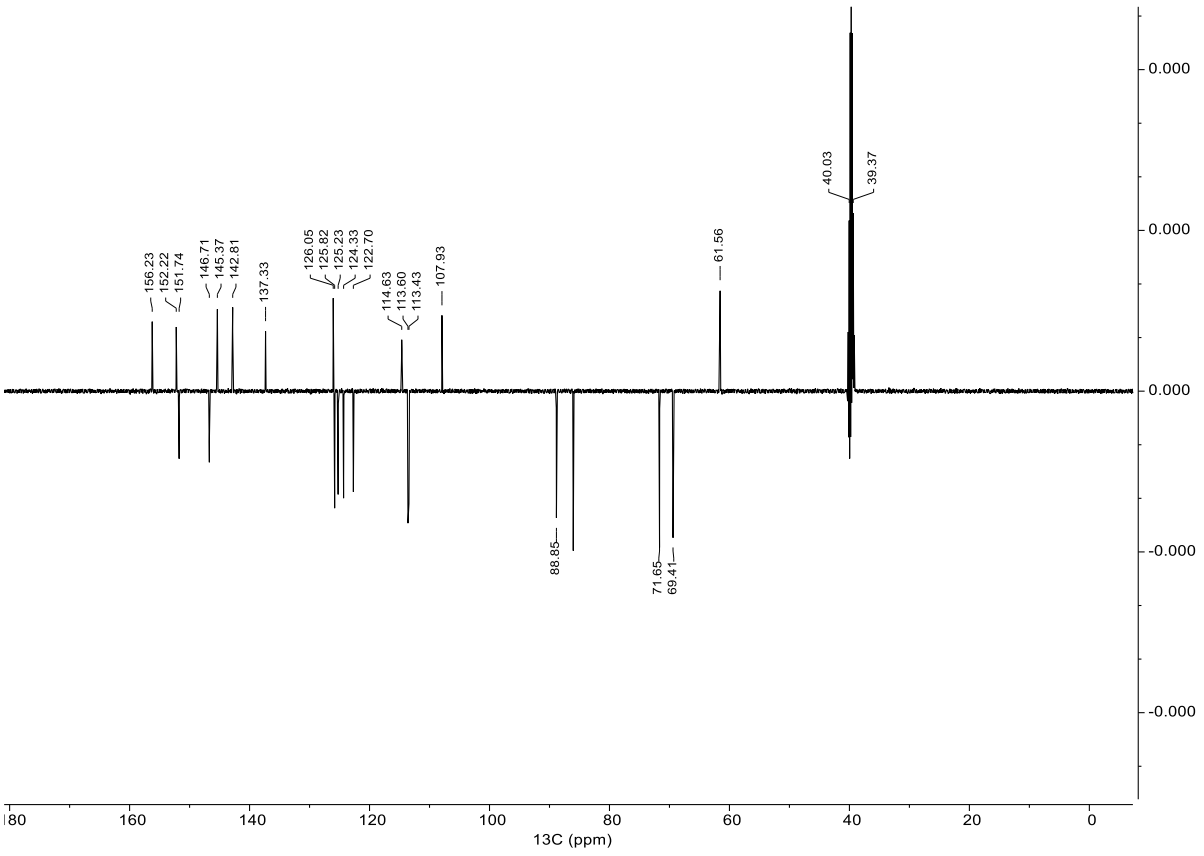
6 Appendix: Selected NMR Spectra

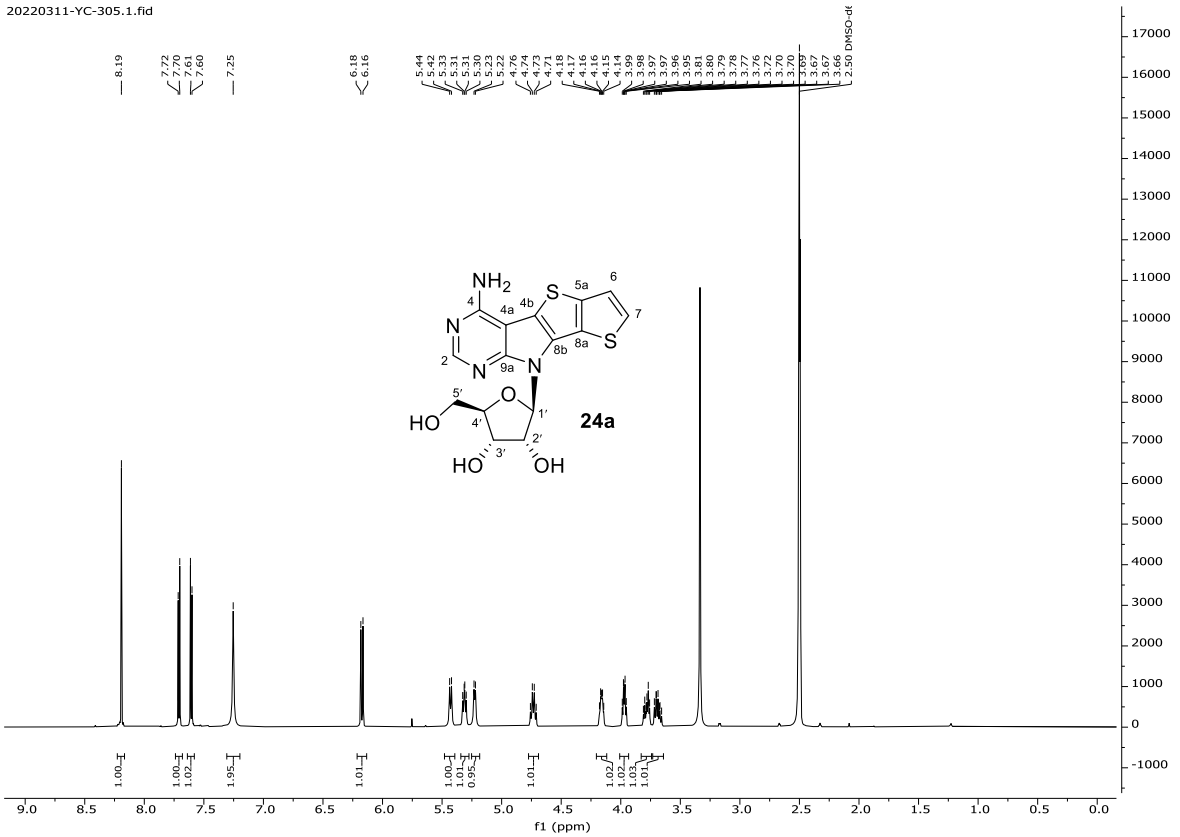
One NMR spectrum of final free nucleoside from each series was selected to show the atom numbering and characteristic peaks.






12c
1H (ppm)


13C (ppm)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7.70
7.61
7.60
7.25
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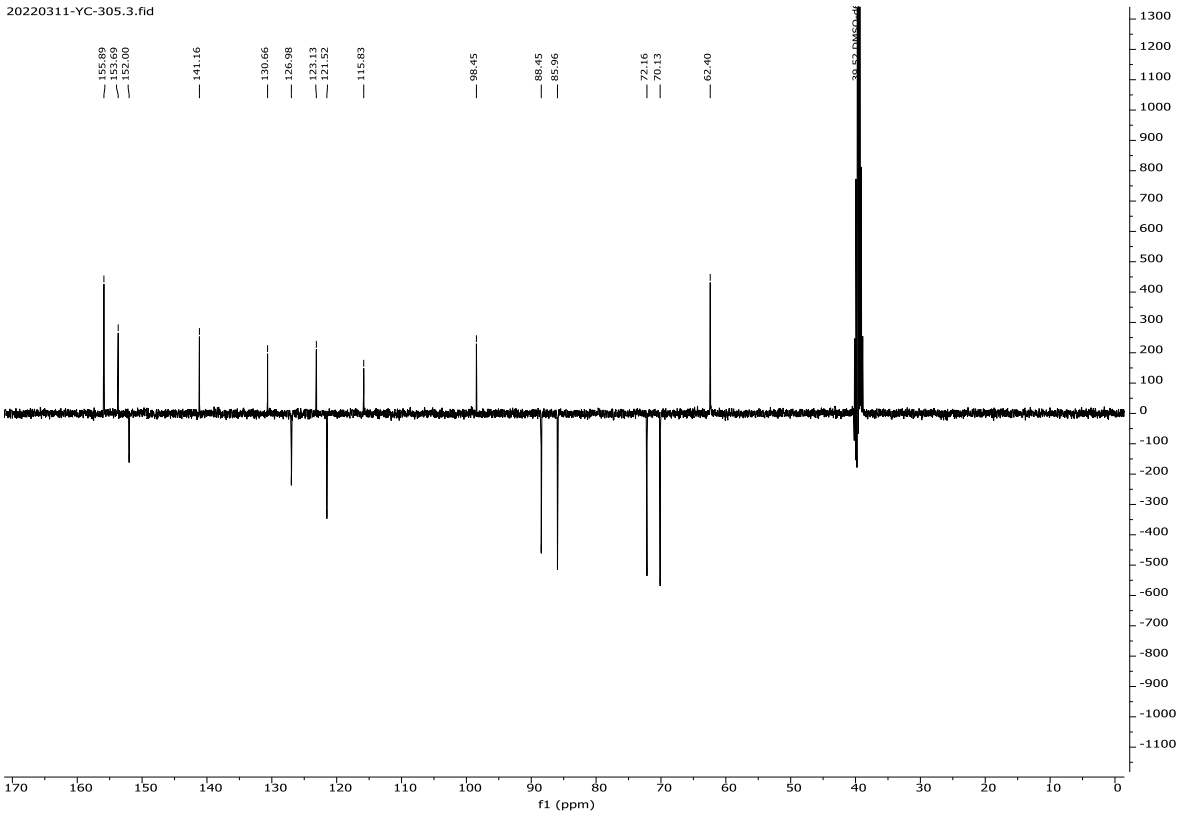
5.30
5.23
5.22
4.76
4.74
4.73
4.71
4.18
4.17
4.16
4.16
4.15
4.14
3.99
3.98
3.97
3.97
3.96
3.95
3.81
3.80
3.79
3.78
3.77
3.76
3.72
3.70
3.70
3.69
3.67
3.67
3.66
2.50 DMSO-d6
NH2
4
4a
4b
5a
6
7
S
N
2
N
9a
N
8b
8a
S
5′
O
1′
HO
4′
2′
3′
HO
OH
24a
1.00
1.00
1.02
1.95
1.01
1.00
1.01
0.95
1.01
1.02
1.02
1.01
1.01
f1 (ppm)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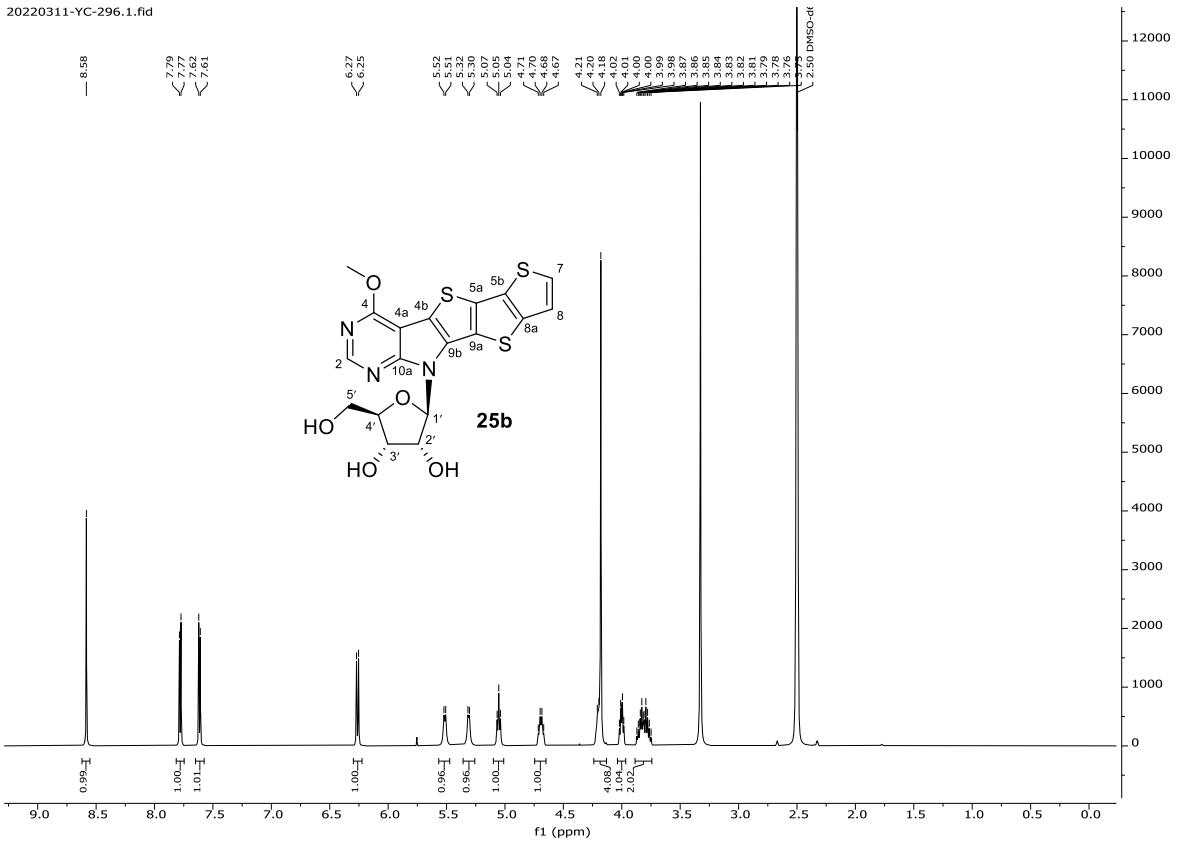
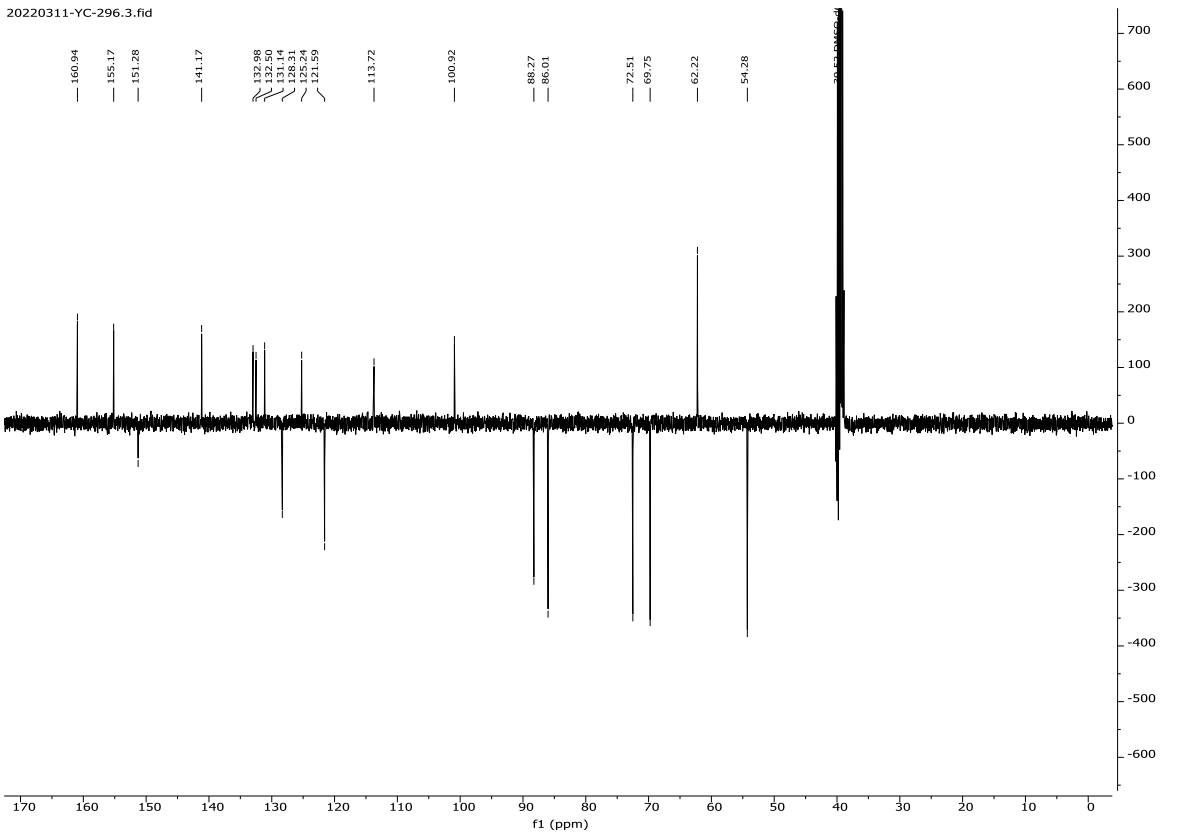
155.89
153.69
152.00
141.16
130.66
126.98
123.13
121.52
115.83
98.45
88.45
85.96
72.16
70.13
62.40
39.52 DMSO-d6
f1 (ppm)


20220311-YC-296.1.fid
8.58
7.79
7.77
7.62
7.61
6.27
6.25
5.52
5.51
5.32
5.30
5.07
5.05
5.04
4.71
4.70
4.68
4.67
4.21
4.20
4.18
4.02
4.01
4.00
4.00
3.99
3.98
3.87
3.86
3.85
3.84
3.83
3.82
3.81
3.79
3.78
3.76
3.74
2.50 DMSO-d6
0.99
1.00
1.01
1.00
0.96
0.96
1.00
1.00
4.08
1.04
2.02
25b
f1 (ppm)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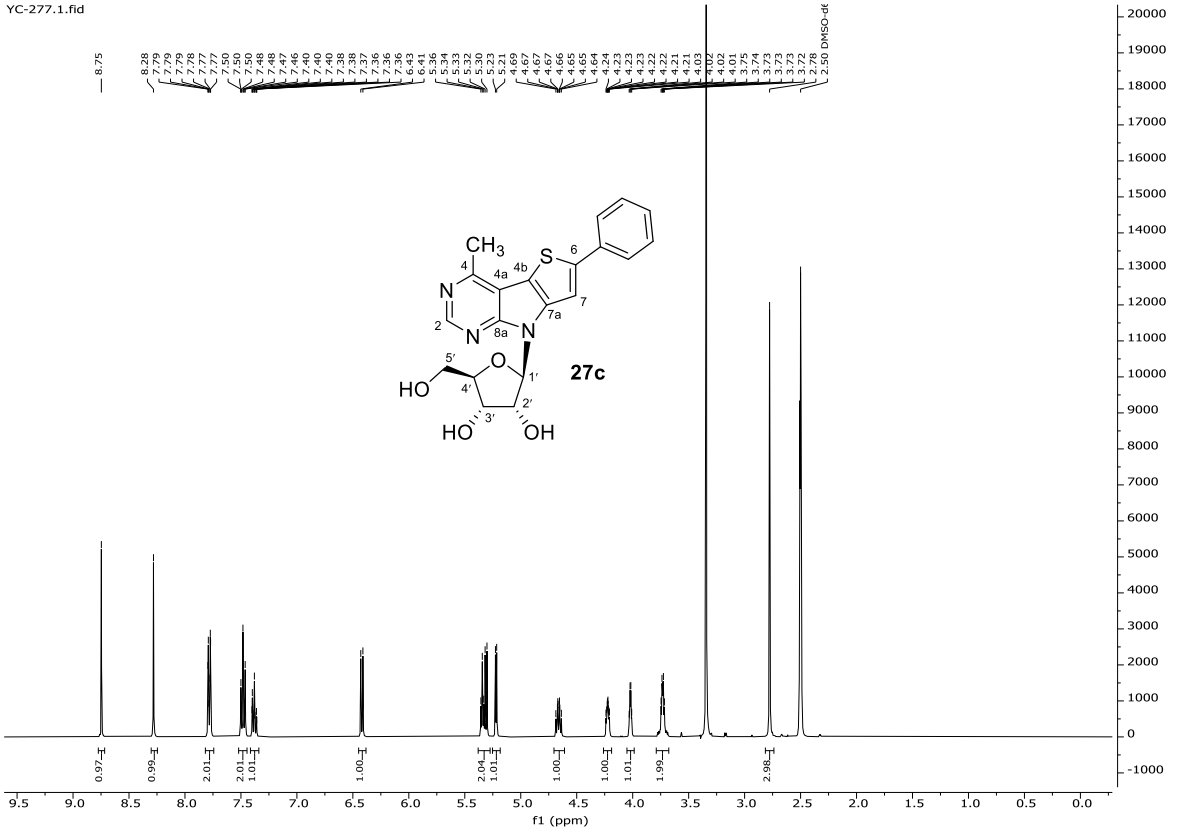
155.17
151.28
141.17
132.98
132.50
131.14
128.31
125.24
121.59
113.72
100.92
88.27
86.01
72.51
69.75
62.22
54.28
39.51 DMSO-d6
f1 (ppm)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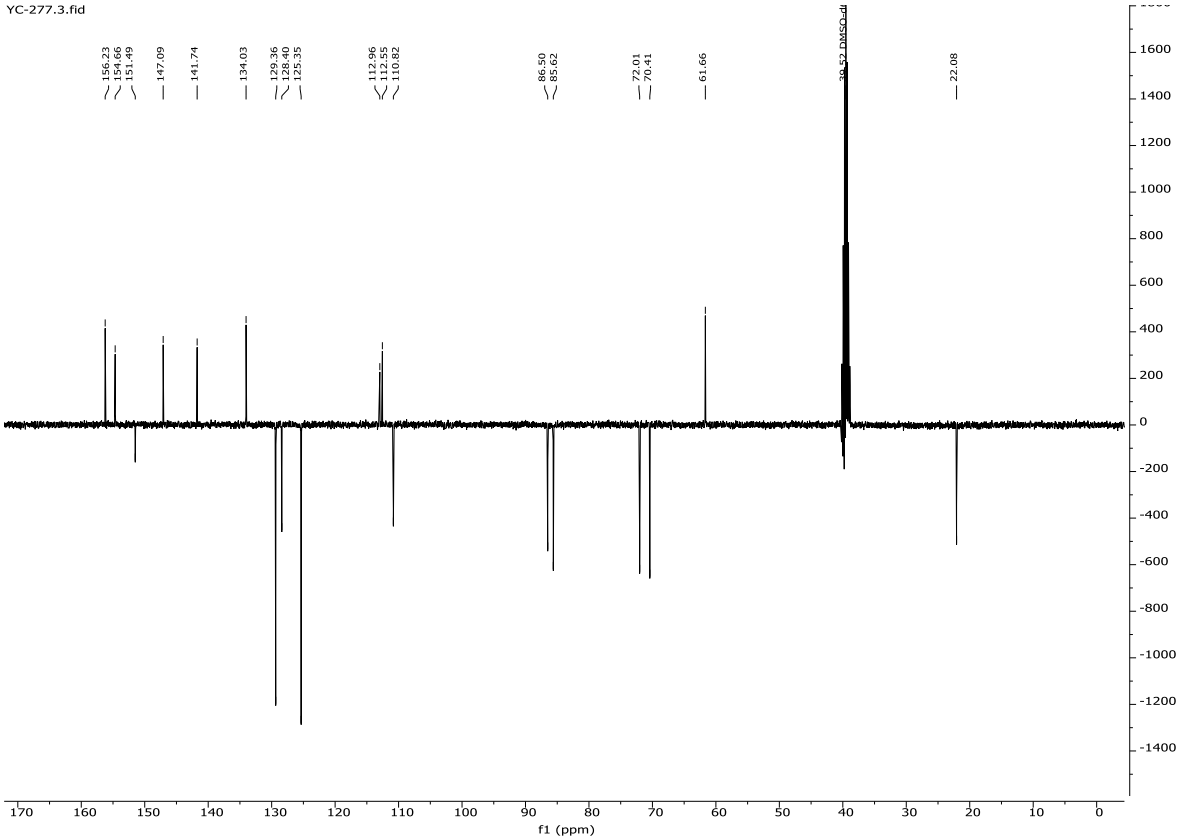
27c
f1 (ppm)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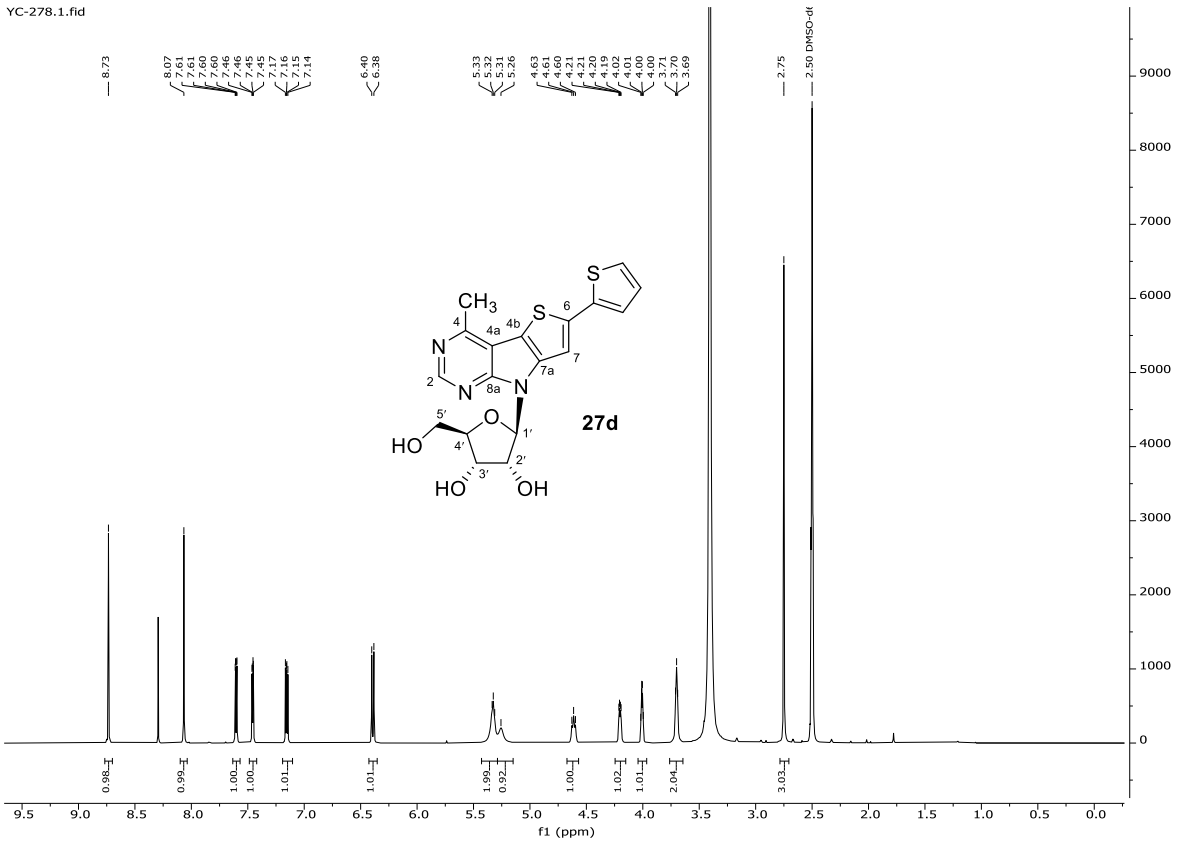
f1 (ppm)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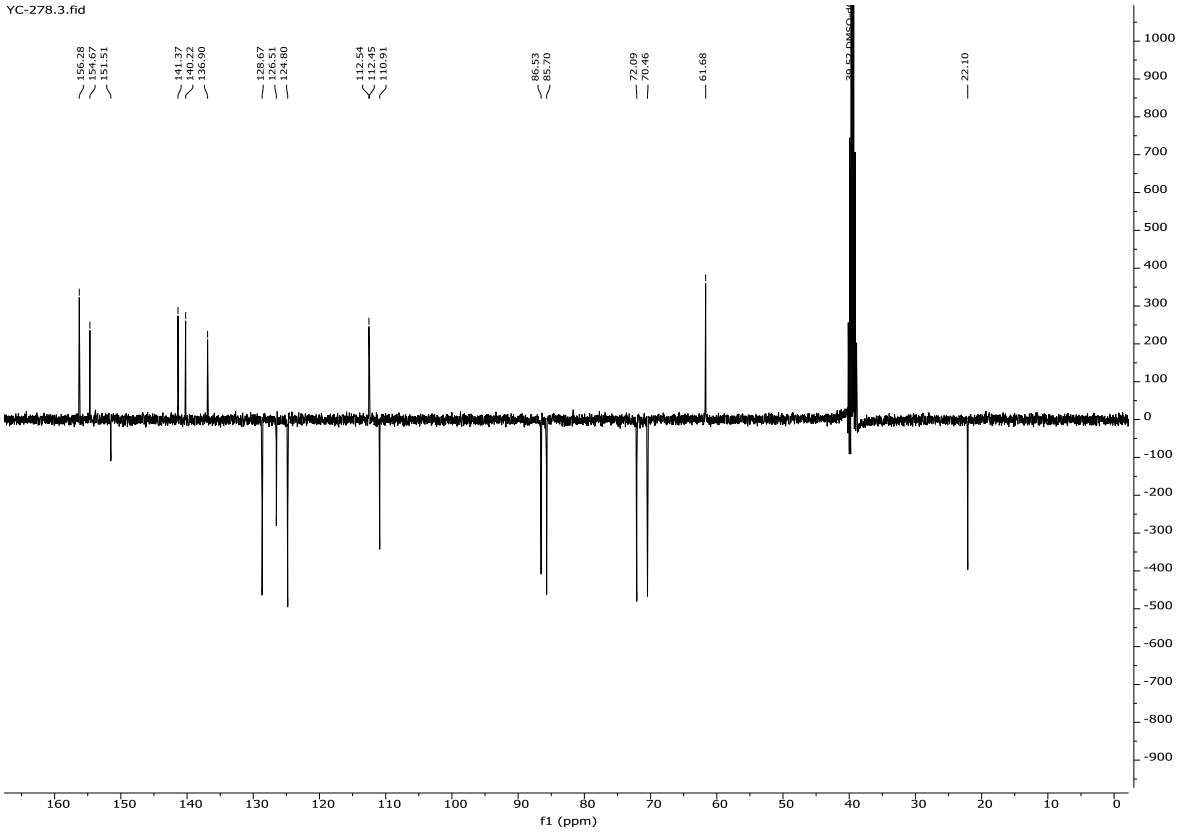
7.61
7.61
7.60
7.60
7.46
7.46
7.45
7.45
7.17
7.16
7.15
7.14
6.40
6.38
5.33
5.32
5.31
5.26
4.63
4.61
4.60
4.21
4.21
4.20
4.19
4.02
4.01
4.00
4.00
3.71
3.70
3.69
2.75
2.50 DMSO-d6
CH3
S
S
N
N
N
O
HO
HO
OH
27d
0.98
0.99
1.00
1.00
1.01
1.01
1.99
0.92
1.00
1.02
1.01
2.04
3.03
f1 (ppm)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151.51
141.37
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136.90
128.67
126.51
124.80
112.54
112.45
110.91
86.53
85.70
72.09
70.46
61.68
39.53 DMSO-d6
22.10
f1 (ppm)

7 References

1. Löffler, M.; Fairbanks, L. D.; Zameitat, E.; Marinaki, A. M.; Simmonds, H. A. *Trends Mol. Med.* **2005,** *11*, 430-437.
2. Legraverend, M.; Grierson, D. S. *Biorg. Med. Chem.* **2006,** *14*, 3987-4006.
3. KAWAI, S.; MURATA, K. *Biosci., Biotechnol., Biochem.* **2008,** *72*, 919-930.
4. Srivatsan, A.; Wang, J. D. *Curr. Opin. Microbiol.* **2008,** *11*, 100-105.
5. Mewies, M.; McIntire, W. S.; Scrutton, N. S. *Protein Sci.* **1998,** *7*, 7-20.
6. Rasmussen, H. *Science* **1970,** *170*, 404-412.
7. Zenchenko, A. A.; Drenichev, M. S.; Il'icheva, I. A.; Mikhailov, S. N. *Mol Biol* **2021,** *55*, 786-812.
8. Seley-Radtke, K. L.; Yates, M. K. *Antiviral Res.* **2018,** *154*, 66-86.
9. Yates, M. K.; Seley-Radtke, K. L. *Antiviral Res.* **2019,** *162*, 5-21.
10. Lin, X.; Liang, C.; Zou, L.; Yin, Y.; Wang, J.; Chen, D.; Lan, W. *Eur. J. Med. Chem.* **2021,** *214*, 113233.
11. Ashton, H.; Frenk, E.; Stevenson, C. J. *Br. J. Dermatol.* **1971,** *84*, 496-500.
12. Carmine, A. A.; Brogden, R. N.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1982,** *23*, 329-353.
13. Rabasseda, X. *Drugs Today (Barc)* **2003,** *39*, 359-371.
14. Power, D. G.; Kemeny, N. E. *Mol. Cancer Ther.* **2009,** *8*, 1015-1025.
15. Longley, D. B.; Harkin, D. P.; Johnston, P. G. *Nat. Rev. Cancer* **2003,** *3*, 330-338.
16. Giovannoni, G. *Neurotherapeutics* **2017,** *14*, 874-887.
17. Von Hoff, D. D.; Slavik, M., 5-Azacytidine—A New Anticancer Drug with Significant Activity in Acute Myeloblastic Leukemia. In *Advances in Pharmacology*, Garattini, S.; Goldin, A.; Hawking, F.; Kopin, I. J., Eds. Academic Press: 1977; Vol. 14, pp 285-326.
18. Čihák, A. *Oncology* **1974,** *30*, 405-422.
19. Mian, A. M.; Khwaja, T. A. *J. Med. Chem.* **1983,** *26*, 286-291.
20. Acs, G.; Reich, E.; Mori, M. *Proc. Natl. Acad. Sci.* **1964,** *52*, 493-501.
21. Štambaský, J.; Hocek, M.; Kočovský, P. *Chem. Rev.* **2009,** *109*, 6729-6764.
22. Du, Y.-X.; Chen, X.-P. *Clin. Pharmacol. Ther.* **2020,** *108*, 242-247.
23. Eggleston, M. *Infect. Control* **1987,** *8*, 215-218.
24. De Clercq, E. *J. Med. Chem.* **2016,** *59*, 2301-2311.
25. Roy-Burman, S.; Roy-Burman, P.; Visser, D. W. *Cancer Res.* **1968,** *28*, 1605-1610.
26. Whitley, R.; Alford, C.; Hess, F.; Buchanan, R. *Drugs* **1980,** *20*, 267-282.

27. Guchelaar, H. J.; Richel, D. J.; van Knapen, A. *Cancer Treat. Rev.* **1996,** *22*, 15-31.

28. Mini, E.; Nobili, S.; Caciagli, B.; Landini, I.; Mazzei, T. *Ann. Oncol.* **2006,** *17*, v7-v12.

29. Hirsch, M. S. *J. Infect. Dis.* **1988,** *157*, 427-431.

30. De Clercq, E.; Neyts, J., Antiviral Agents Acting as DNA or RNA Chain Terminators. In *Antiviral Strategies*, Kräusslich, H.-G.; Bartenschlager, R., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 2009; pp 53-84.

31. Whittington, R.; Brogden, R. N. *Drugs* **1992,** *44*, 656-683.

32. Riddler, S. A.; Anderson, R. E.; Mellors, J. W. *Antiviral Res.* **1995,** *27*, 189-203.

33. Chang, J. *Acc. Chem. Res.* **2022,** *55*, 565-578.

34. Haraguchi, K.; Takeda, S.; Tanaka, H.; Nitanda, T.; Baba, M.; Dutschman, G. E.; Cheng, Y.-C. *Bioorg. Med. Chem. Lett.* **2003,** *13*, 3775-3777.

35. Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* **1986,** *6*, 1-40.

36. Honkoop, P.; de Man, R. A. *Expert Opin. Investig. Drugs* **2003,** *12*, 683-688.

37. Han, S.-H. B. *Expert Opin. Investig. Drugs* **2005,** *14*, 511-519.

38. Perry, C. M.; Faulds, D. *Drugs* **1997,** *53*, 657-680.

39. Nishiyama, S.; Yamamura, S.; Kato, K.; Takita, T. *Tetrahedron Lett.* **1988,** *29*, 4743-4746.

40. Wagstaff, A. J.; Faulds, D.; Goa, K. L. *Drugs* **1994,** *47*, 153-205.

41. Jacobson, M. A. *J. Med. Virol.* **1993,** *41*, 150-153.

42. Reusser, P. *Expert Opin. Investig. Drugs* **2001,** *10*, 1745-1753.

43. Fan-Havard, P.; Nahata, M. C.; Brady, M. T. *J. Clin. Pharm. Ther.* **1989,** *14*, 329-340.

44. Schmid-Wendtner, M. H.; Korting, H. C. *Skin Pharmacol. Physiol.* **2004,** *17*, 214-218.

45. Bang, L. M.; Scott, L. J. *Drugs* **2003,** *63*, 2413-2424.

46. Saravolatz, L. D.; Saag, M. S. *Clin. Infect. Dis.* **2006,** *42*, 126-131.

47. Michailidis, E.; Huber, A. D.; Ryan, E. M.; Ong, Y. T.; Leslie, M. D.; Matzek, K. B.; Singh, K.; Marchand, B.; Hagedorn, A. N.; Kirby, K. A.; Rohan, L. C.; Kodama, E. N.; Mitsuya, H.; Parniak, M. A.; Sarafianos, S. G. *J. Biol. Chem.* **2014,** *289*, 24533-24548.

48. Faulds, D.; Brogden, R. N. *Drugs* **1992,** *44*, 94-116.

49. Hervey, P. S.; Perry, C. M. *Drugs* **2000,** *60*, 447-479.

50. Chen, Y.-L.; Yin, Z.; Duraiswamy, J.; Schul, W.; Lim, C. C.; Liu, B.; Xu, H. Y.; Qing, M.; Yip, A.; Wang, G.; Chan, W. L.; Tan, H. P.; Lo, M.; Liung, S.; Kondreddi, R. R.; Rao, R.; Gu, H.; He, H.; Keller, T. H.; Shi, P.-Y. *Antimicrob. Agents Chemother.* **2010,** *54*, 2932-2939.

51. Perry, C. M.; Wagstaff, A. J. *Drugs* **1995,** *50*, 396-415.

52. Lewis, D. J.; Duvic, M. *Expert Opin. Investig. Drugs* **2017,** *26*, 771-775.

53. Calabresi, F.; Repetto, M. *J. Surg. Oncol.* **1991,** *48*, 124-128.

54. Alanazi, A. S.; Miccoli, A.; Mehellou, Y. *J. Med. Chem.* **2021,** *64*, 16703-16710.

55. Zhang, Y.; Gao, Y.; Wen, X.; Ma, H. *Asian J. Pharm. Sci.* **2014,** *9*, 65-74.

56. Dando, T. M.; Plosker, G. L. *Drugs* **2003,** *63*, 2215-2234.

57. De Clercq, E. *Biochem. Pharmacol.* **2018,** *153*, 2-11.

58. Fung, H. B.; Stone, E. A.; Piacenti, F. J. *Clin. Ther.* **2002,** *24*, 1515-1548.

59. Antoniou, T.; Park-Wyllie, L. Y.; Tseng, A. L. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* **2003,** *23*, 29-43.

60. Herbst, D. A.; Reddy, K. R. *Expert Opin. Investig. Drugs* **2013,** *22*, 527-536.

61. Florini, J. R., Nucleocidin. In *Antibiotics: Volume I Mechanism of Action*, Gottlieb, D.; Shaw, P. D., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 1967; pp 427-433.

62. Eastman, R. T.; Roth, J. S.; Brimacombe, K. R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M. D. *ACS Cent. Sci.* **2020,** *6*, 672-683.

63. Pardo, J.; Shukla, A. M.; Chamarthi, G.; Gupte, A. *Drugs Context* **2020,** *9*.

64. Gordon, C. J.; Tchesnokov, E. P.; Woolner, E.; Perry, J. K.; Feng, J. Y.; Porter, D. P.; Götte, M. *J. Biol. Chem.* **2020,** *295*, 6785-6797.

65. Mehellou, Y. *ChemMedChem* **2010,** *5*, 1841-1842.

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

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

68. Bourderioux, A.; Naus, P.; Perlikova, P.; Pohl, R.; Pichova, I.; Votruba, I.; Dzubak, P.; Konecny, P.; Hajduch, M.; Stray, K. M.; Wang, T.; Ray, A. S.; Feng, J. Y.; Birkus, G.; Cihlar, T.; Hocek, M. *J. Med. Chem.* **2011,** *54*, 5498-507.

69. Naus, P.; Caletkova, O.; Konecny, P.; Dzubak, P.; Bogdanova, K.; Kolar, M.; Vrbkova, J.; Slavetinska, L.; Tloust'ova, E.; Perlikova, P.; Hajduch, M.; Hocek, M. *J. Med. Chem.* **2014,** *57*, 1097-110.

70. Tichy, M.; Pohl, R.; Xu, H. Y.; Chen, Y. L.; Yokokawa, F.; Shi, P. Y.; Hocek, M. *Bioorg. Med. Chem.* **2012,** *20*, 6123-33.

71. Tichý, M.; Smoleň, S.; Tloušťová, E.; Pohl, R.; Oždian, T.; Hejtmánková, K.; Lišková, B.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. *J. Med. Chem.* **2017,** *60*, 2411-2424.

72. Tokarenko, A.; Lišková, B.; Smoleň, S.; Táborská, N.; Tichý, M.; Gurská, S.; Perlíková, P.; Frydrych, I.; Tloušťová, E.; Znojek, P.; Mertlíková-Kaiserová, H.; Poštová Slavětínská, L.; Pohl, R.; Klepetářová, B.; Khalid, N.-U.-A.; Wenren, Y.; Laposa, R. R.; Džubák, P.; Hajdúch, M.; Hocek, M. *J. Med. Chem.* **2018,** *61*, 9347-9359.

73. Fleuti, M.; Bártová, K.; Slavětínská, L. P.; Tloušťová, E.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. *J. Org. Chem.* **2020,** *85*, 10539-10551.

74. Veselovská, L.; Kudlová, N.; Gurská, S.; Lišková, B.; Medvedíková, M.; Hodek, O.; Tloušťová, E.; Milisavljevic, N.; Tichý, M.; Perlíková, P.; Mertlíková-Kaiserová, H.; Trylčová, J.; Pohl, R.; Klepetářová, B.; Džubák, P.; Hajdúch, M.; Hocek, M. *Chem. Eur. J.* **2020,** *26*, 13002-13015.

75. Ghosh, K.; Perlíková, P.; Havlíček, V.; Yang, C.; Pohl, R.; Tloušťová, E.; Hodek, J.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. *Eur. J. Org. Chem.* **2018,** *2018*, 5092-5108.

76. Balzarini, J.; McGuigan, C. *J. Antimicrob. Chemother.* **2002,** *50*, 5-9.

77. Yang, L.; Dan, H. C.; Sun, M.; Liu, Q.; Sun, X.-m.; Feldman, R. I.; Hamilton, A. D.; Polokoff, M.; Nicosia, S. V.; Herlyn, M.; Sebti, S. M.; Cheng, J. Q. *Cancer Res.* **2004,** *64*, 4394-4399.

78. Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975,** *40*, 356-363.

79. Seley-Radtke, K. L.; Zhang, Z.; Wauchope, O. R.; Zimmermann, S. C.; Ivanov, A.; Korba, B. *Nucleic Acids Symp. Ser.* **2008,** *52*, 635-636.

80. Wauchope, O. R.; Johnson, C.; Krishnamoorthy, P.; Andrei, G.; Snoeck, R.; Balzarini, J.; Seley-Radtke, K. L. *Biorg. Med. Chem.* **2012,** *20*, 3009-3015.

81. Clercq, E. D.; Li, G. *Clin. Microbiol. Rev.* **2016,** *29*, 695-747.

82. Eyer, L.; Nencka, R.; de Clercq, E.; Seley-Radtke, K.; Růžek, D. *Antiviral Chem. Chemother.* **2018,** *26*, 1-28.

83. Parker, W. B. *Chem. Rev.* **2009,** *109*, 2880-2893.

84. Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. *Nat. Rev. Drug Discov.* **2013,** *12*, 447-464.

85. Szakács, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. *Nat. Rev. Drug Discov.* **2006,** *5*, 219-234.

86. Tichy, M.; Pohl, R.; Tloust'ova, E.; Weber, J.; Bahador, G.; Lee, Y. J.; Hocek, M. *Bioorg. Med. Chem.* **2013,** *21*, 5362-72.

87. Zhang, Y.-M.; Razler, T.; Jackson, P. F. *Tetrahedron Lett.* **2002,** *43*, 8235-8239.

88. Li, Z.; Wang, X.; Eksterowicz, J.; Gribble, M. W., Jr.; Alba, G. Q.; Ayres, M.; Carlson, T. J.; Chen, A.; Chen, X.; Cho, R.; Connors, R. V.; DeGraffenreid, M.; Deignan, J. T.; Duquette, J.; Fan, P.; Fisher, B.; Fu, J.; Huard, J. N.; Kaizerman, J.; Keegan, K. S.; Li, C.; Li, K.; Li, Y.; Liang, L.; Liu, W.; Lively, S. E.; Lo, M. C.; Ma, J.; McMinn, D. L.; Mihalic, J. T.; Modi, K.; Ngo, R.; Pattabiraman, K.; Piper, D. E.; Queva, C.; Ragains, M. L.; Suchomel, J.; Thibault, S.; Walker, N.; Wang, X.; Wang, Z.; Wanska, M.; Wehn, P. M.; Weidner, M. F.; Zhang, A. J.; Zhao, X.; Kamb, A.; Wickramasinghe, D.; Dai, K.; McGee, L. R.; Medina, J. C. *J. Med. Chem.* **2014,** *57*, 3430-49.

89. Mizar, P.; Myrboh, B. *Tetrahedron Lett.* **2008,** *49*, 5283-5285.

90. Srogl, J.; Allred, G. D.; Liebeskind, L. S. *J. Am. Chem. Soc.* **1997,** *119*, 12376-12377.

91. Kaiser, D.; Klose, I.; Oost, R.; Neuhaus, J.; Maulide, N. *Chem. Rev.* **2019,** *119*, 8701-8780.

92. Berger, F.; Plutschack, M. B.; Riegger, J.; Yu, W.; Speicher, S.; Ho, M.; Frank, N.; Ritter, T. *Nature* **2019,** *567*, 223-228.

93. Li, J.; Chen, J.; Sang, R.; Ham, W.-S.; Plutschack, M. B.; Berger, F.; Chabbra, S.; Schnegg, A.; Genicot, C.; Ritter, T. *Nat. Chem.* **2020,** *12*, 56-62.

94. Engl, P. S.; Häring, A. P.; Berger, F.; Berger, G.; Pérez-Bitrián, A.; Ritter, T. *J. Am. Chem. Soc.* **2019,** *141*, 13346-13351.

95. Berger, F.; Alvarez, E. M.; Frank, N.; Bohdan, K.; Kondratiuk, M.; Torkowski, L.; Engl, P. S.; Barletta, J.; Ritter, T. *Org. Lett.* **2020,** *22*, 5671-5674.

96. Kafuta, K.; Korzun, A.; Böhm, M.; Golz, C.; Alcarazo, M. *Angew. Chem. Int. Ed.* **2020,** *59*, 1950-1955.

97. Yorimitsu, H. *Chem. Rec.* **2021,** *21*, 3356-3369.

98. Vasu, D.; Yorimitsu, H.; Osuka, A. *Synthesis* **2015,** *47*, 3286-3291.

99. Tian, Z.-Y.; Wang, S.-M.; Jia, S.-J.; Song, H.-X.; Zhang, C.-P. *Org. Lett.* **2017,** *19*, 5454-5457.

100. Kozhushkov, S. I.; Alcarazo, M. *Eur. J. Inorg. Chem.* **2020,** *2020*, 2486-2500.

101. Lu, Y.; Liu, Q.; Wang, Z.-X.; Chen, X.-Y. *Angew. Chem. Int. Ed.* **2022,** *61*, e202116071.

102. Wang, D. J.; Targos, K.; Wickens, Z. K. *J. Am. Chem. Soc.* **2021,** *143*, 21503-21510.

103. Tian, Z.-Y.; Lin, Z.-H.; Zhang, C.-P. *Org. Lett.* **2021,** *23*, 4400-4405.

104. Minami, H.; Otsuka, S.; Nogi, K.; Yorimitsu, H. *ACS Catal.* **2018,** *8*, 579-583.

105. Xie, C.; Han, D.; Liu, J.; Xie, T. *Synlett* **2009,** *2009*, 3155-3158.

106. McGarrigle, E. M.; Fritz, S. P.; Favereau, L.; Yar, M.; Aggarwal, V. K. *Org. Lett.* **2011,** *13*, 3060-3063.

107. Mosrin, M.; Knochel, P. *Chem. Eur. J.* **2009,** *15*, 1468-1477.

108. Haas, D.; Hammann, J. M.; Greiner, R.; Knochel, P. *ACS Catal.* **2016,** *6*, 1540-1552.

109.Stokes, B. J.; Jovanović, B.; Dong, H.; Richert, K. J.; Riell, R. D.; Driver, T. G. *J. Org. Chem.* **2009,** *74*, 3225-3228.

110. Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *J. Am. Chem. Soc.* **2003,** *125*, 9296-9297.

111. Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. *ChemBioChem* **2004,** *5*, 958-963.

112.Yang, H.; Robinson, M.; Corsa, A. C.; Peng, B.; Cheng, G.; Tian, Y.; Wang, Y.; Pakdaman, R.; Shen, M.; Qi, X.; Mo, H.; Tay, C.; Krawczyk, S.; Sheng, X. C.; Kim, C. U.; Yang, C.; Delaney, W. E. *Antimicrob. Agents Chemother.* **2014,** *58*, 647-653.

113. Nosková, V.; Džubák, P.; Kuzmina, G.; Ludkova, A.; Stehlik, D.; Trojanec, R.; Janostakova, A.; Korinkova, G.; Mihal, V.; Hajduch, M. *Neoplasma* **2002,** *49*, 418-425.

114. Riss, T. L.; Moravec, R. A.; Niles, A. L.; Duellman, S.; Benink, H. A.; Worzella, T. J.; Minor, L., Cell Viability Assays. In *Assay Guidance Manual*, **2013**.

https://www.ncbi.nlm.nih.gov/books/NBK144065/

115. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988,** *48*, 4827-4833.

116. Xu, P.; Zhao, D.; Berger, F.; Hamad, A.; Rickmeier, J.; Petzold, R.; Kondratiuk, M.; Bohdan, K.; Ritter, T. *Angew. Chem. Int. Ed.* **2020,** *59*, 1956-1960.

117. Alvarez, E. M.; Karl, T.; Berger, F.; Torkowski, L.; Ritter, T. *Angew. Chem. Int. Ed.* **2021,** *60*, 13609-13613.

118. Juliá, F.; Shao, Q.; Duan, M.; Plutschack, M. B.; Berger, F.; Mateos, J.; Lu, C.; Xue, X.-S.; Houk, K. N.; Ritter, T. *J. Am. Chem. Soc.* **2021,** *143*, 16041-16054.

119. Berger, F.; Ritter, T. *Synlett* **2021,** *33*, 339-345.

120. Chen, X.-Y.; Wu, Y.; Wang, P. *Synthesis* **2022,** *54*, 3928-3940.

121. McCann, L. C.; Organ, M. G. *Angew. Chem. Int. Ed.* **2014,** *53*, 4386-4389.

122. Yang, C.; Pohl, R.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. *J. Org. Chem.* **2020,** *85*, 8085-8101.

123. Bosáková, A.; Perlíková, P.; Tichý, M.; Pohl, R.; Hocek, M. *Biorg. Med. Chem.* **2016,** *24*, 4528-4535.

124. Dziuba, D. *Methods Appl. Fluoresc.* **2022,** *10*, 044001.

125. Hulpia, F.; Van Hecke, K.; França da Silva, C.; da Gama Jaen Batista, D.; Maes, L.; Caljon, G.; de Nazaré C. Soeiro, M.; Van Calenbergh, S. *J. Med. Chem.* **2018,** *61*, 9287-9300.

126. Hulpia, F.; Mabille, D.; Campagnaro, G. D.; Schumann, G.; Maes, L.; Roditi, I.; Hofer, A.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. *Nat. Commun.* **2019,** *10*, 5564.

127. Hulpia, F.; Campagnaro, G. D.; Scortichini, M.; Van Hecke, K.; Maes, L.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. *Eur. J. Med. Chem.* **2019,** *164*, 689-705.

128. Bouton, J.; Furquim d'Almeida, A.; Maes, L.; Caljon, G.; Van Calenbergh, S.; Hulpia, F. *Eur. J. Med. Chem.* **2021,** *216*, 113290.

129. De Clercq, E.; Balzarini, J.; Madej, D.; Hansske, F.; Robins, M. J. *J. Med. Chem.* **1987,** *30*, 481-486.

130. Seela, F.; Zulauf, M.; Chen, S.-F. *Nucleosides, Nucleotides & Nucleic Acids* **2000,** *19*, 237-251.

131. Bourderioux, A.; Nauš, P.; Perlíková, P.; Pohl, R.; Pichová, I.; Votruba, I.; Džubák, P.; Konečný, P.; Hajdúch, M.; Stray, K. M.; Wang, T.; Ray, A. S.; Feng, J. Y.; Birkus, G.; Cihlar, T.; Hocek, M. *J. Med. Chem.* **2011,** *54*, 5498-5507.

132. Milisavljevic, N.; Konkolová, E.; Kozák, J.; Hodek, J.; Veselovská, L.; Sýkorová, V.; Čížek, K.; Pohl, R.; Eyer, L.; Svoboda, P.; Růžek, D.; Weber, J.; Nencka, R.; Bouřa, E.; Hocek, M. *ACS Infect. Dis.* **2021,** *7*, 471-478.

133. Krueger, A. T.; Lu, H.; Lee, A. H. F.; Kool, E. T. *Acc. Chem. Res.* **2007,** *40*, 141-150.

134. Krueger, A. T.; Kool, E. T. *J. Am. Chem. Soc.* **2008,** *130*, 3989-3999.

135. Luecke, L. Stereoselective Synthesis of Heterocyclic Steroids - a Novel Entry Through the Dubble Heck Reaction. Ph.D. Thesis, The Georg-August University of Goettingen, **2001**.

136. Sabat, N.; Nauš, P.; Matyašovský, J.; Dziuba, D.; Slavětínská, L. P.; Hocek, M. *Synthesis* **2016,** *48*, 1029-1045.

137. Rolland, V.; Kotera, M.; Lhomme, J. *Synth. Commun.* **1997,** *27*, 3505-3511.

138. Bolli, M. H.; Boss, C.; Binkert, C.; Buchmann, S.; Bur, D.; Hess, P.; Iglarz, M.; Meyer, S.; Rein, J.; Rey, M.; Treiber, A.; Clozel, M.; Fischli, W.; Weller, T. *J. Med. Chem.* **2012,** *55*, 7849-7861.

139. Brouwer, A. M. *Pure Appl. Chem.* **2011,** *83*, 2213-2228.

140. SAINT. Bruker AXS Inc., Madison, Wisconsin, USA, **2015**.

141. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.* **1994,** *27*, 435.

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