Univerzita Karlova, Přírodovědecká fakulta
Charles University, Faculty of Science
Organická Chemie / Organic Chemistry

Synthesis of novel hetero-fused 7-Deazapurine Nucleosides and Nucleotides with potential biological Activity or for the Modifications of DNA and RNA

-

Syntéza nových heteroanelovaných 7-Deazapurinových Nukleosidů a Nukleotidů s potenciální biologickou Aktivitou a pro Modifikace DNA a RNA

Marianne H. Fleuti, MSc ETH

Doctoral thesis

under the Supervision of Prof. Michal Hocek

Prague 2024

Declaration of Originality

I declare that I prepared the final thesis independently and that I have listed all the information sources and literature used. This thesis, or any substantial part of it, has not been submitted for another or the same academic degree.

Prohlášení

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

Prague / V Praze, 31st March 2024

Marianne Heidi Fleuti, MSc ETH

Acknowledgment

First and foremost, I would like to thank my supervisor, Prof. Michal Hocek, for allowing me to work on such interesting and synthetic challenging projects, as well as for your trust and guidance.

Furthermore, I would like to thank all current and former group members for their support. Especially, I would like to thank Dr. Zuzana Vaníková and Dr. Pedro Güixens Gallardo for helping me get started in Prague and feel welcome in the group.

As my work was part of a larger project on fused 7-deazapurine ribonucleosides in general, and I would like to thank everyone involved: Dr. Michal Tichý, Dr. Anna Tokarenko, Dr. Lucia Sirotová (née Veselovská), Dr. Sabina Pisarek (née Smoleń), Dr. Ketaki Ghosh and Dr. Chao Yang. All your suggestions and the transparent discussion of your synthetic struggles helped me advance in my endeavors. Aside from Anna and Chao, I also shared my lab space with Dr. Nazarii Sabat, Tomáš Vyskocil, and Dr. Pratap Jagtap: Thank you all for the interesting discussions and the support needed. My thanks are also going to Katarína Markušová and Jason Archdeacon for being the best students possible. I will always remember fondly the discussions that I had especially with Ema Chaloupecká, Dr. Iveta Chena, Dr. Petr Kasal, and Dr. Dana Schwarz from neighboring labs.

Although I did all the synthetic work by myself, I had some help: I would like to thank Dr. Jiří Rybáček from the research group under Dr. I. Starý at IOCB Prague for introducing me to the world of flow chemistry. I would like to thank my co-worker Tania Sanchez Quirante for introducing me to the world of biochemistry and *in vitro* transcription. I will always cherish our discussions. For the characterization of my compounds, I would like to especially thank Kateřina Bártová and Dr. Lenka Poštová Slavětínská at IOCB Prague for their help with the interpretation of the NMR measurements and their patience in answering all my questions. Furthermore, I would like to thank Bohunka Šperlichová at Charles University (melting points), Lucie Holasová from the Analytical Laboratory at IOCB Prague (optical rotation and precise weighing for biological testing) and the service group under Dr. J. Cvačka (mass spectroscopy) for their routine measurements.

The biological screening of the final nucleosides was performed by our collaborating partners in the research group under Prof. M. Hajdúch at the *Institute of Molecular and Translational Medicine* of the Palacký University in Olomouc, Czech Republic; in the *Biochemical Pharmacology* group under Dr. H. Mertlíková-Kaiserová as well as in the *Virology* group under Dr. J. Weber at IOCB Prague. The anti-HCV screening was performed by Drs. Joy Feng and Gina Bahador at *Gilead Sciences, Inc*.

Science is not possible without administrative support: Most importantly, I would like to thank Kristina Jarolímová of the Department of Organic Chemistry at Charles University Prague for her continuous help with the administrative side of running the laboratory as well as for answering all my questions and for all the translation for better understanding life in Czech Republic – especially during those trying times of ever-changing COVID restrictions. I would also like to thank Šárka Kněžická and her team at IOCB Prague for their help and the ladies from Euraxess Prague for taking care of the immigration paperwork in Czech.

Furthermore, I would like to thank my friends and family for their mental support and their patience with me: Pedro, Dr. Nicole Hauser, Denicija Miladinoska-Stojanovska, and Dr. Maya Schneebeli you are the best! I would also like to thank my truck-driving friends from the GMMB-BM and the SFiA ladies for keeping me sane. Last but not least, I would like to thank my family for letting me go abroad and for their understanding when I had to miss family events because of work. A special thanks goes to my grandparents Marie-Lisette and

Georges Fleuti-Bardet for always believing in me and supporting me. And Grossmami, thank you for letting me keep my promise… There is one person without whom this thesis would not have been possible: Dr. Martin T. Peschel, thank you for everything – the interesting discussions about chemistry and the never-ending support.

This work is supported by the Czech Science Foundation (19-08124S), the Czech Ministry of Education, Youth and Sports (CZOPENSCREEN: LM2018130, EATRIS-CZ: LM2018133), the European Regional Development Fund; OP RDE; Project: "ChemBioDrug" (No. CZ.02.1.01/0.0/0.0/16_019/0000729), and by the National Institute for Cancer Research (EXCELES program, ID Project No. LX22NPO5102, funded by the European Union – Next Generation EU).

Summary

In medicinal chemistry, 7-deazapurine ribonucleosides are important analogues to purine nucleosides and represent a privileged scaffold in the research for new treatments against cancer, leukemia, and diseases caused by viruses. This thesis discusses the synthesis of new heteroaryl-fused 7-deazapurine ribonucleosides and reports on the biological activity of the target nucleosides against different cancer cell lines and viruses *in vitro*.

In the first part of the thesis, the focus lies on the comparison of strategies for the synthesis of different methylpyrazolo-fused 7-deazapurine ribonucleosides. The tricyclic nucleobase was furnished by (i) a six-step classical heterocyclization approach starting from 5-chloro-1-methyl-4-nitropyrazole, and by (ii) a three-step cross-coupling and cyclization approach starting from the zincated 4,6-dichloropyrimidine and 5-iodo-1-methylpyrazole. Both strategies resulted in comparable yields overall. Then, three different glycosylation methods were explored to give the *β*-anomeric nucleoside intermediate. Only the Vorbrüggen glycosylation yielded the intermediate as a pure *β*-anomer. Derivatization and deprotection gave a series of eight different pyrazolo-fused deazapurine ribonucleosides, some of which were weakly fluorescent. Methyl, amino, and methylsulfanyl derivatives exerted submicromolar cytotoxic effects *in vitro* against a panel of cancer and leukemia cell lines as well as antiviral effects against the Hepatitis C virus in the replicon assay.

In the second part of the thesis, the focus lies on the systematic study of the C-H functionalization of 5-membered heterocycles through the formation of different sulfonium salts and their use as an alternative substrate to heteroaryl iodides in the Negishi cross-coupling reaction with zincated 4,6-dichloropyrimidine. Twelve different 5-membered heterocycles bearing one or two heteroatoms were subjected to thianthrenation and dibenzothiophenation with thianthrene *S*-oxide (TT=O) and dibenzothiophene *S*-oxide (DBT=O), respectively. For both sulfoxides, only the corresponding aryl sulfonium salts with pyrrole, 1-methylpyrrole, thiophene, and 1-methylpyrazole were formed. The Negishi cross-coupling between the methylpyrazol-4-yl-thianthrenium salt and zincated 4,6-dichloro-pyrimidine did not yield the desired pyrazolyl pyrimidine cross-coupling product. The same reaction was performed also with the dibenzothiophenium salts but only the corresponding thiophene derivative gave the desired cross-coupling product.

In the third part of the thesis, the focus lies on the synthesis of different quinolino-fused 7-deazapurine ribonucleosides and their application in biochemistry. The synthesis of the tetracyclic nucleobase was based on the cross-coupling and cyclization approach. Different cyclization methods were explored and compared. The Vorbrüggen glycosylation gave the desired nucleoside intermediate as pure *β*-anomer. Derivatization and deprotection gave a series of seven different quinolino-fused ribonucleosides which showed moderate to weak cytotoxic activity against cancer cell lines and fluorescent properties. The amino derivative was then triphosphorylated and, as an ATP analogue, successfully incorporated into RNA using *in vitro* transcription with T7 RNA polymerase.

Souhrn

V medicinální chemii jsou 7-deazapurinové ribonukleosidy důležitými analogy purinových nukleosidů a představují výsadní strukturu ve výzkumu nových léčebných přípravků na rakovinu, leukémii a virová onemocnění. Tato práce diskutuje syntézu nových heteroaryl-fúzovaných 7-deazapurinových ribonukleosidů a uvádí biologickou aktivitu cílových nukleosidů proti různým liniím rakovinných buněk a virům *in vitro*.

První část práce se zaměřuje na porovnání strategií pro syntézu různých methylpyrazolo-fúzovaných 7-deazapurinových ribonukleosidů. Tricyklická nukleobáze byla připravena (i) šestikrokovým klasickým heterocyklizačním přístupem vycházejícím z 5-chlor-1-methyl-4-nitropyrazolu a (ii) tříkrokovým přístupem s využitím cross-couplingu a cyklizace vycházejícím ze zinkovaného 4,6-dichloropyrimidinu a 5-jod-1-methylpyrazolu. Obě strategie vedly celkově ke srovnatelným výtěžkům. Poté byly prozkoumány tři různé metody glykosylace pro získání *β*-anomerního nukleosidového intermediátu. Pouze glykosylace podle Vorbrüggena poskytla intermediát jako čistý *β*-anomer. Derivatizace a deprotekce poskytly sérii osmi různých pyrazolo-fúzovaných deazapurinových ribonukleosidů, z nichž některé byly slabě fluorescenční. Methyl-, amin- a methylsulfanylderiváty prokázaly submikromolární cytotoxické účinky in vitro proti řadě linií buněk rakoviny a leukémie, stejně jako antivirové účinky proti viru hepatitidy C.

Druhá část práce se zaměřuje na systematické studium funkčního nahrazení C-H u pětičlenných heterocyklů formováním různých sulfoniových solí a jejich využitím jako alternativního substrátu k heteroaryl jodidům v Negishiho cross-couplingu se zinkovaným 4,6-dichloropyrimidinem. Dvanáct různých pětičlenných heterocyklů nesoucích jeden nebo dva heteroatomy bylo podrobeno thianthrenaci a dibenzothiofenaci s thianthren *S*-oxidem (TT=O) a dibenzothiofen *S*-oxidem (DBT=O), v tomto pořadí. Pro oba sulfoxidy byly vytvořeny pouze odpovídající arylsulfoniové soli s pyrrolem, 1-methylpyrrolem, thiofenem a 1-methylpyrazolem. Negishiho cross-coupling mezi methylpyrazol-4-yl-thianthreniovou solí a zinkovaným 4,6-dichloropyrimidinem neposkytl kýžený pyrazolyl pyrimidin produkt. Tato reakce byla provedena také s dibenzothiofeniovými solemi, ale pouze odpovídající derivát thiofenu poskytl požadovaný produkt.

Třetí část práce se zaměřuje na syntézu různých chinolin-fúzovaných 7-deazapurinových ribonukleosidů a jejich aplikaci v biochemii. Syntéza tetracyklické nukleobáze byla založena na přístupu kombinujícím cross-coupling a cyklizaci. Byly prozkoumány a porovnány různé metody cyklizace. Glykosylace podle Vorbrüggena poskytla kýžený nukleosidový intermediát jako čistý *β*-anomer. Derivatizace a deprotekce poskytly sérii sedmi různých chinolin-fúzovaných ribonukleosidů, které vykazovaly mírnou až slabou cytotoxickou aktivitu proti liniím rakovinných buněk a fluorescenční vlastnosti. Aminový derivát byl poté trifosforylován a jako analog ATP úspěšně začleněn do RNA pomocí in vitro transkripce s T7 RNA polymerázou.

List of Publications relevant for this Dissertation

Fleuti, M.; Bártová, K.; Poštová Slavětínská, L.; Tloušťová, E.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Biological Profiling of Pyrazolo-Fused 7‑Deazapurine Nucleosides. *J. Org. Chem.* **2020**, *85*, 10539−10551. DOI: 10.1021/acs.joc.0c00928

Yang, C.; Poštová Slavětínská, L.; **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. DOI: 10.1021/jacs.2c07517

Fleuti, M.; Sanchez-Quirante, T.; Poštová Slavětínská, L.; Tloušťová, E.; Tichý, M.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Biological Profiling of Quinolino-Fused 7-Deazapurine Nucleosides. *ACS Omega*, **2024**, *manuscript under revision.*

List of Abbreviations

AcOH	acetic acid
ADDP	1,1'-(azodicarbonyl)dipiperidine
ADK	adenosine-dependent kinase
APT	attached proton test
ATR	attenuated total reflection
aq.	aqueous
b.p.	boiling point
BSA	bis(trimethylsilyl)-acetamide
BTEA-Cl	benzyltriethylammonium chloride
Bz	benzoyl
calcd	calculated
CDK	cyclin-dependent kinase
cHex	cyclohexane
compd	compound
concd	concentrated
d	days
Da	Dalton
DBT	dibenzothiophene
DBT=O	dibenzothiophene *S*-oxide
DCM	dichloromethane
decomp.	decomposition
DEPC	diethyl dicarbonate
DMAc	*N*,*N*-dimethylacetamide
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated DMSO
DNA	deoxyribonucleic acid
DQF-COSY	double-quantum filter correlation spectroscopy
ds	double stranded
dtbbpy	4,4’-di-tert-butyl-2,2’-dipyridyl
ED-XRF	energy dispersive X-ray fluorescence
EI	electron ionization
EMA	European Medicines Agency
ESI	electrospray ionization
EtOAc	ethyl acetate
equiv.	equivalent
FC	flash chromatography
FDA	Food and Drug Administration
h	hour

$HBF_4 \cdot OEt_2$	tetrafluoroboric acid diethyl ether complex
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HFIP	Hexafluoropropan-2-ol
HMBC	hetero multiple-bond coherence spectroscopy
HMPT	tris(dimethylamino)phosphine
HR MS	high-resolution mass spectroscopy
HPLC-MS	high-pressure liquid chromatography mass spectroscopy
HPFC	high-performance flash chromatography
HSQC	hetero single-quantum coherence spectroscopy
Hz	Hertz
IC_{50}	half maximal inhibitory concentration
IR	infrared spectroscopy
IVT	*in vitro* transcription
J	coupling constant
LC	liquid chromatography
LiHMDS	lithium bis(trimethylsilyl) amide
MHz	Mega Hertz
m.p.	melting point
m/*z*	mass per charge
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic spectroscopy
PAGE	polyacrylamide gel electrophoresis
ppm	parts per million
ppy	2-phenylpyridine
R_f	retention factor
RNA	ribonucleic acid
ROESY	rotational nuclear Overhauser effect spectroscopy
rotavap	rotary evaporator
rxn	reaction
satd	saturated
SLM	sangivamycin-like molecule
ss	single stranded
TBDMS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
TDA-1	tris[2-(2-methoxyethoxy)ethyl]amine
TEAB	triethylammonium bicarbonate
temp.	temperature
TFA	trifluoroacetic acid
TFA^-	trifluoroacetate
TFAA	trifluoroacetanhydride
TFT	2,3,7,8-tetrafluoro-thianthrene
TFT=O	2,3,7,8-tetrafluoro-thianthrene *S*-oxide

THF	tetrahydrofuran
TLC	thin-layer chromatography
TMP	2,2,6,6-tetramethylpiperidyl
TMS	trimethylsilyl
TMSOTf	trimethylsilyl triflate
Tr	trityl, triphenylmethyl
TT	thianthrene
TT=O	thianthrene *S*-oxide
WHO	World Health Organization
wt%	weight percent

Table Of Content

1. Introduction

1.1. Nucleosides and Nucleotides in Nature

Nucleosides and Nucleotides are essential biomolecules and are involved in all aspects of cellular life. They are the building blocks of nucleic acids that store and carry the genetic information for all biochemical processes. They regulate the cell metabolism and are involved in many biochemical pathways.

Chemically speaking, nucleosides consist of a heteroaromatic nucleobase and a sugar moiety (ribose). They are linked via an *N*-glycosidic bond resulting in a *β*-anomeric center at the 1'-C of the sugar. The 2'-position of the sugar either bears a hydroxyl group (in RNA) or is deoxygenated (in DNA). Nucleotides are phosphorylated at the 5'-hydroxyl group of the sugar. One differentiates between mono-, di- and triphosphates depending on the number of attached phosphate groups. Nucleoside triphosphates are used for the biosynthesis of DNA and RNA. In these polymers, a phosphate group connects the primary 5'-hydroxyl group of a nucleoside and the secondary 3'-hydroxyl group of the next monomer. (**Figure 1**)

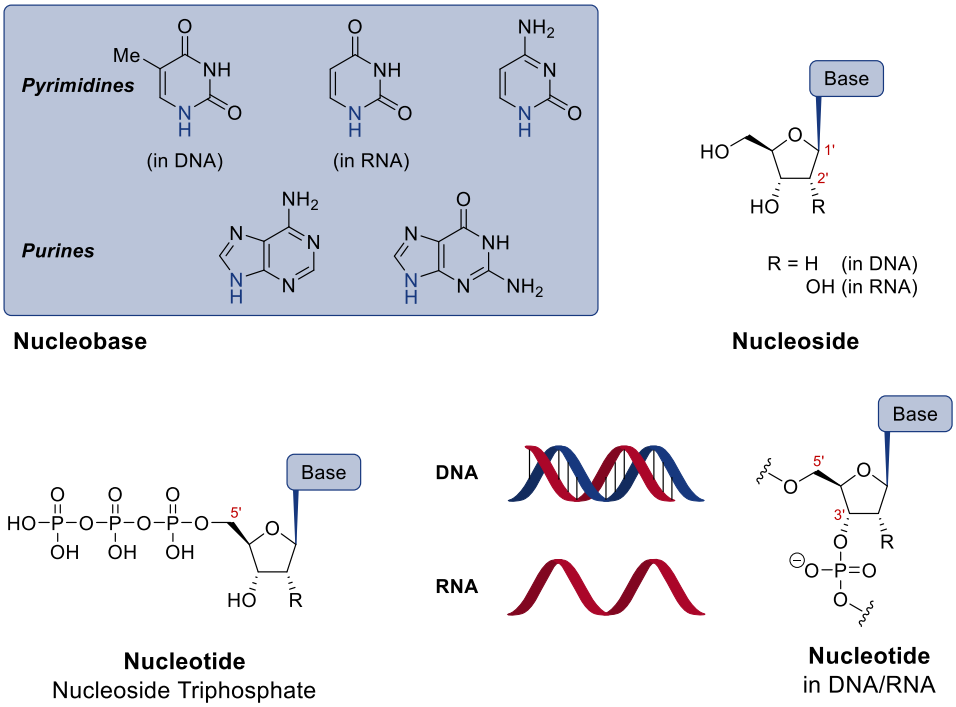


Figure 1. Nucleosides and nucleotides as building blocks of DNA and RNA.

The nucleobases are divided into two groups: pyrimidines and purines. The pyrimidines cytosine, thymine (in DNA), and uracil (in RNA), consist of a 6-membered heterocyclic structure similar to pyridine. The scaffold of the purine nucleobases, adenine and guanine, consists of a pyrimidine ring fused to an imidazole ring. The nucleosides and nucleoside analogues described and discussed in this thesis are based on adenosine and modifications to its scaffold.

Adenosine is one of the four canonical nucleosides which make up the nucleic acids in the cell. Its function goes beyond that of a simple building block. Its triphosphate, adenosine triphosphate (ATP) is the energy provider for all cellular processes.[1] The cyclic adenosine

monophosphate (cAMP) is essential for intracellular signal transduction.[2] Coenzyme A is involved in several metabolic pathways and its acetylated counterpart is a key intermediate in Krebs' citric acid cycle.[3] Nicotinamide adenine diphosphate (NAD^+/NADH) is a redox cofactor and can be found in all living cells.[4] (**Figure 2**)

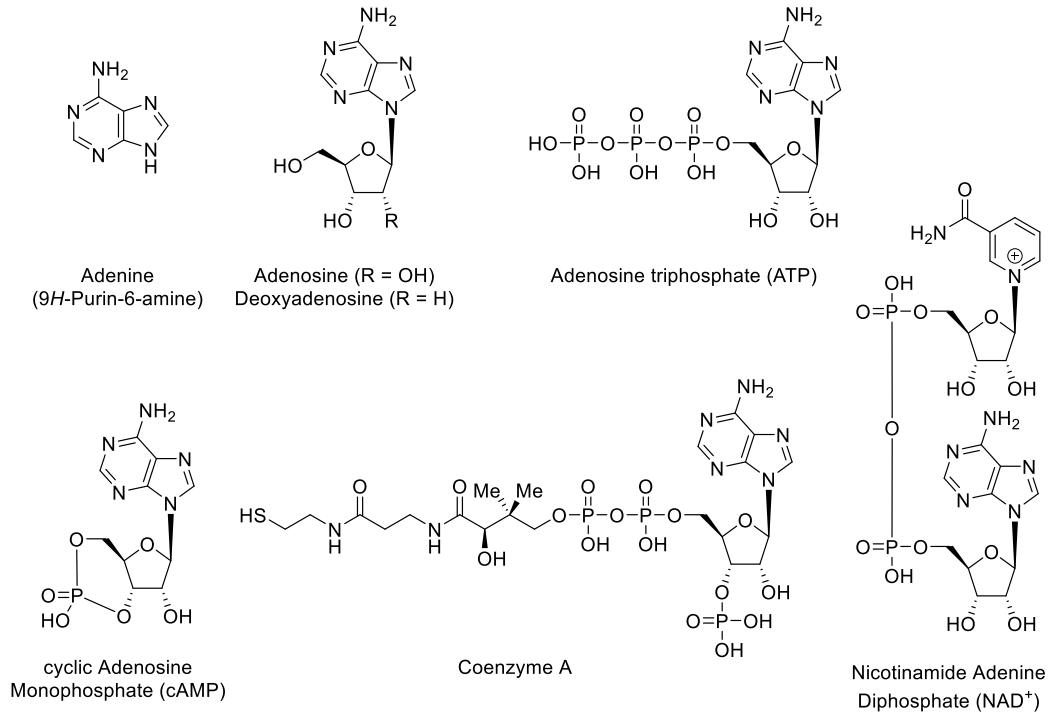


Figure 2. Adenine, adenosine and its derivatives are essential for cellular life.

Beyond the four canonical nucleosides that make up the majority of RNA building blocks in nature, more than 100 modified RNA nucleosides alone are found throughout all domains of life. The modifications can be small, such as methylations, hydroxylations, or thiolations; or can be more complex such as glycosylations or acylations. Almost all modifications are introduced by an enzyme, only a few are the result of oxidative stress or nonenzymatic processes. In general, these modifications are necessary for the cell's survival. Repair and control mechanisms in every cell make sure that the cellular processes are not affected by any unwanted modifications.[5]

1.2. Nucleosides and Nucleotides in Medicinal Chemistry

Nature is an excellent resource; and the naturally occurring nucleosides and nucleotides are an outstanding starting point for medicinal chemists in drug design. Through modifications of their structures, large libraries of potential target compounds can be synthesized, tested, and adapted. Ideally, these newly developed nucleoside analogues mimic the structure of their natural precursors in such a way that they are recognized by their target enzymes solely to disrupt or terminate the cellular process altogether.[6]

When considering any potential modifications to the nucleoside scaffold, one needs to take into account which molecular feature is necessary for the recognition by and the interaction with the target enzyme and which features can be altered. Modifications can be made by simply adding a substituent to the scaffold, exchanging atoms in the scaffold, or via an extension of the scaffold. These changes to the nucleoside structure can be categorized into: (1) phosphate modifications, (2) sugar modifications, and (3) nucleobase modifications. Most nucleoside analogues bear modifications in more than one moiety.[7] (**Figure 3**)

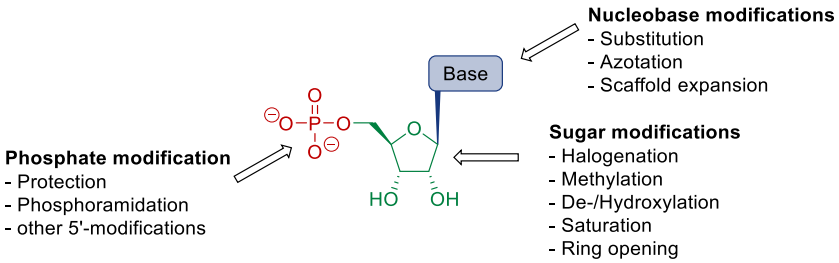


Figure 3. Nucleoside modifications in drug design.

Each modification introduced can develop its influence in a different cellular process that originally involved the unmodified nucleoside. Hence, a broad range of potential modes of action are available. In general, nucleoside analogues can be used as cytotoxic agents when they induce irreversible cell damage or apoptosis; as cytostatic agents when they inhibit cell growth and proliferation; or as antivirals when they specifically inhibit the replication of a virus. The efficacy of this influence can be quantified by the IC_{50} value determined during biological testing.

Any modification, big or small, to the adenosine scaffold may influence different aspects of the cell's biochemistry. Accordingly, the impact of any such change to the structure cannot be predicted. Thus, each derivative has to be prepared and tested separately for its suitability as an active pharmaceutical ingredient. Some of the success stories are depicted in **Figure 4** (in alphabetical order):

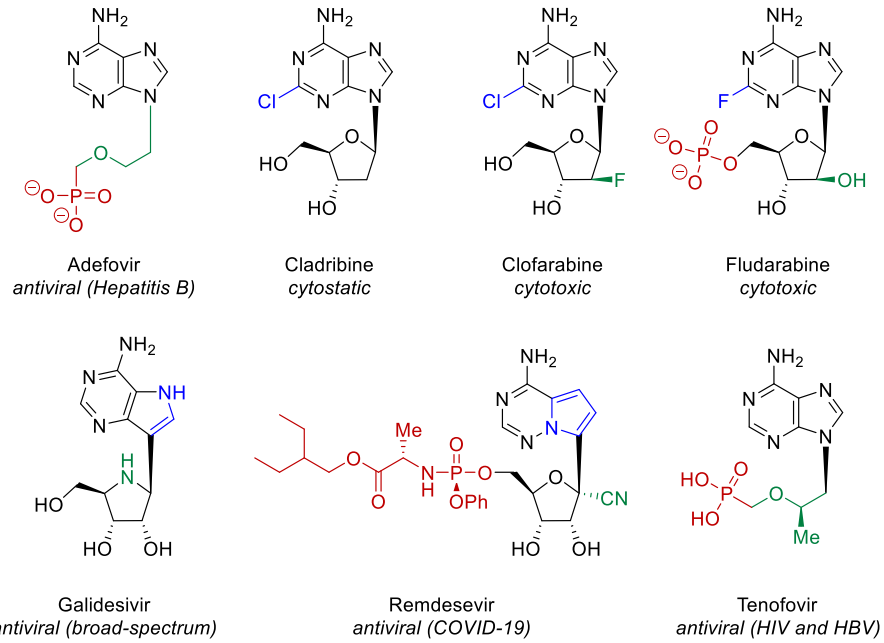


Figure 4. A selection of success stories of adenosine analogues as active pharmaceutical ingredients. Changes to the adenosine scaffold are color-coded.

Adefovir is an acyclic nucleotide analogue based on adenosine. While the nucleobase structure remains unchanged, the sugar moiety has been altered significantly. Furthermore, Adefovir contains a phosphonate group instead of a phosphate group. The molecule was first synthesized in 1987 by the Holý group at IOCB Prague and was initially developed as an anti-HIV drug by *Gilead Sciences*. Unfortunately, the required dose for the treatment resulted in liver damage. Its prodrug *Adefovir dipivoxil* is effective at much lower concentrations and was approved by the FDA in 2002 as an antiviral drug against chronic Hepatitis B (HBV).[8]

Cladribine and *Clofarabine*, both mimic deoxyadenosine while bearing an isosteric chloride in the pyrimidine ring (position C2). In fast proliferating cells, *Cladribine* is converted to its triphosphate and competes with ATP in DNA synthesis where it interferes with DNA repair.[9] In 1993, it was approved by the FDA for the treatment of hairy cell leukemia, chronic lymphocytic leukemia, and low-grade non-Hodgkin's lymphoma.[10] In 2002, the EMA approved the use of Cladribine for the treatment of relapsing–remitting multiple sclerosis.[11] *Clofarabine* has a fluorinated position in the sugar moiety derived from arabinose and interferes with the DNA synthesis and repair using a different pathway. It was approved by the FDA in 2004 for the treatment of pediatric acute lymphoblastic leukemia if two prior treatments have failed.[12]

Fludarabine is chemically similar to *Cladribine* and *Clofarabine*. Instead of the chloro modification in the nucleobase, the same position is fluorinated. The sugar moiety is not derived from ribose but from arabinose and the 5'-hydroxyl group is phosphorylated. In 1991, it was approved by the FDA for the treatment of different forms of leukemia and lymphoma, such as chronic lymphocytic leukemia, non-Hodgkin's lymphoma, acute myeloid leukemia, and acute lymphocytic leukemia.[13] In 2015, it was added to the list of essential medicines by the WHO.[14]

Galidesivir has modifications in both, the sugar moiety and the nucleobase. After activation, it inhibits the viral RNA-dependent RNA polymerase. *In vitro* studies showed broad-spectrum activity against more than 20 RNA viruses in nine different families. In the animal model, *Galidesivir* exerts antiviral effects against Ebola, Marburg and Zika viruses among others. Clinical studies are ongoing.[15]

Remdesivir is a prodrug and has modifications to the adenosine scaffold in all three domains mentioned above. After activation, its triphosphate inhibits the viral RNA-dependent RNA polymerase in the cell. Although originally developed for the treatment of Hepatitis C followed by the investigation of efficacy against Ebola and Marburg infections, it was approved by the FDA for the treatment of COVID-19 and became well known also to the general public.[16]

Tenofovir is an acyclic phosphonate analogue of adenosine monophosphate (AMP). After activation of its prodrug, *Tenofovir* is phosphorylated to its diphosphate and inhibits the viral reverse transcriptase of retroviruses in infected cells. The molecule was developed by Antonín Holý at IOCB Prague and Erik DeClerq at KU Leuven (Belgium) together with John C. Martin from Gilead Sciences. Tenofovir is on the WHO's list of essential medicines for the treatment of HIV and chronic Hepatitis B.[14,17]

1.3. Evolution of 7-Deazapurines and their Ribonucleosides in Drug Discovery

A simple but important structural change to the purine scaffold opens a lot of possibilities for further modifications: By exchanging the N7 nitrogen with a carbon atom, the newly formed 7-deazapurine (7*H*-pyrrolo[2,3-*d*]pyrimidine) moiety is susceptible to further modifications at C7 and C8.[18] (**Figure 5**)

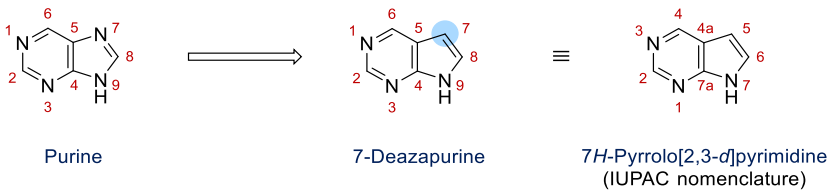


Figure 5. Purine nomenclature and numbering used throughout the introduction.

Over the years, some correlations between modifications of the (deaza)purine scaffold and the biological activity of the corresponding nucleosides were discovered. [19] Structural changes to the pyrimidine ring in the (deaza)purine scaffold, such as replacing the N1 or the N3 nitrogen, as well as large substitution in the C2 position, lead to a significant drop in biological activity or the complete loss thereof.[20] (**Figure 6**)

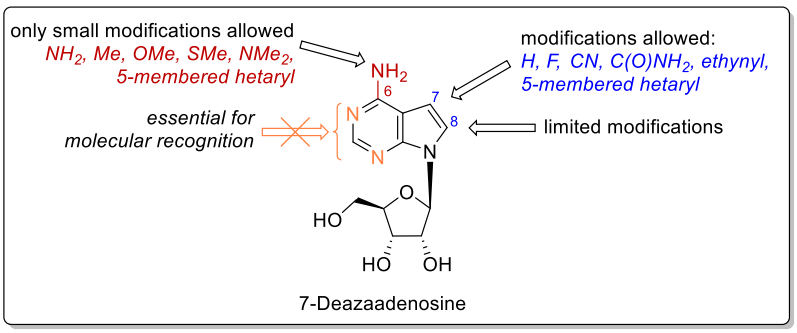


Figure 6. Structure-activity relationship (SAR study) of 7-deazapurine nucleosides.

1.3.1. Natural 7-Deazapurine Nucleosides with intriguing Biological Activities

Several of the 7-deazapurine nucleosides occur in nature and show interesting biological activities. Tubercidin (7-dazaadenosine) was first isolated in the 1950s from bacterial *Streptomyces tubercidicus* cultures. Its biological activity ranges from antibiotic properties against tuberculosis to antitumor properties against animal fibroblasts and KB cells[21] as well as antiviral properties against vaccinia viruses (DNA virus), mengovirus (ssRNA virus), and reoviruses (dsRNA virus).[22] Its derivative toyocamycin bearing a cyano group in position 7 was isolated from a different *Streptomyces* strain and has similar antibiotic and antitumor properties but is too cytotoxic for clinical use.[23] Sangivamycin bearing a carboxamido group in position 7 is active against cancer cells with low toxicity in human cells.[24] Additionally, Sangivamycin can also be used as a competitive inhibitor for protein kinases (competition with ATP).[25] (**Figure 7**)

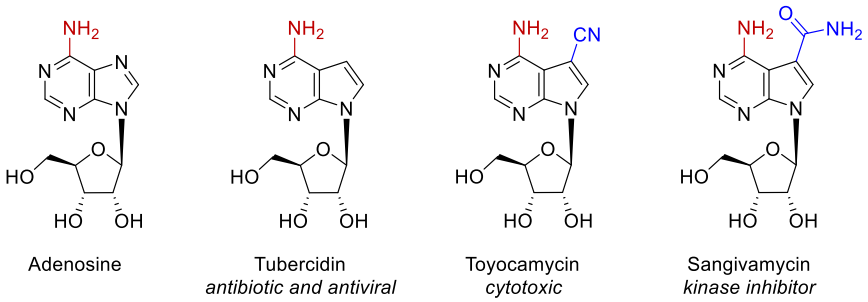


Figure 7. Adenosine and naturally occurring 7-deazapurine nucleosides with interesting biological activities.

These 7-dezapurine nucleosides strongly resemble adenosine and their biological activities suggest that this class of derivatives is recognized by their biological targets where it can disrupt the nucleic-acid metabolism and kinase-related signaling pathways.[26]

1.3.2. 6-Substituted 7-Deazapurine Nucleosides

Exchanging the amino group in position 6 with the non-polar methyl group leads to 6-methyl-7-deazapurine nucleoside which is a highly potent antiviral agent against polio and *Dengue* as its cytotoxic profile is improved compared to tubercidin.[27] The isosteric 7-dezapurine nucleoside bearing a CF_3 group in position 6 shows similar cytotoxicity against cancer cell lines.[28] Even though less toxic than tubercidin, the 6-methyl and the 6-trifluoromethyl-7-deazapurine nucleosides are still too cytotoxic for clinical use as antiviral agents. (**Figure 8**)

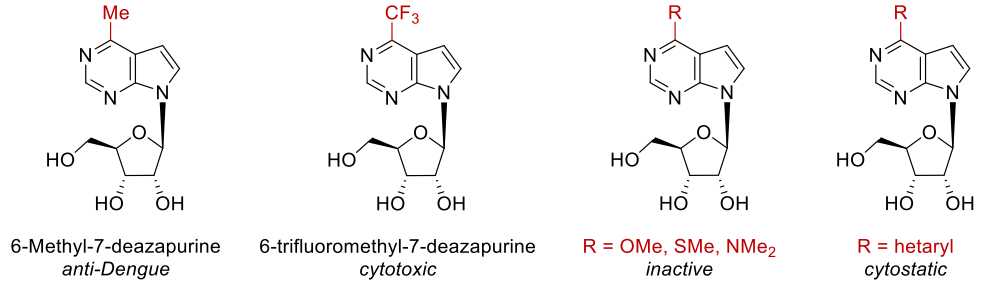


Figure 8. 6-Substituted 7-deazapurine nucleosides with biological activities.

The amino group (H-bond donor) can also be replaced by H-bond acceptors such as methoxy, methylsulfanyl, dimethylamino groups. 7-Deazapurines with only this single modification in position 6 are not biologically active by themselves.[29] However, the substituent in this position can finetune the activity introduced by modifications in other positions of the heterocyclic nucleobase. Introducing a heteroaryl substituent in position 6 leads to a group of cytostatic derivatives with submicromolar activity against several cancer cell lines.[30] As a

prodrug with a *cyclo*Sal-phosphate group at the 5'-hydroxyl group of the sugar moiety, the cytostatic activity is similar. 5-membered heteroaryl group are well accepted whereas larger substituents render the derivative inactive.[31]

1.3.3. 7-Substituted 7-Deazapurine Nucleosides

Switching from the purine framework to the 7-deazapurine framework opens up the possibility for modifications in position 7. After introducing a methyl group, 7-methyltubercidin is no longer cytotoxic but shows promising antitrypanosomal effects instead. Further derivatization in position 6 leads to different 6-alkoxy-7-methyl-7-deazapurine nucleosides with submicromolar activities giving promising lead structures for the development of treatments against sleeping sickness.[32] Modifications bearing aryl groups in position 7 lead to cytotoxic derivatives which inhibit RNA synthesis in treated cells, inducing apoptosis. The corresponding heteroaryl derivatives are cytostatic even at low nanomolar concentrations.[33] Its most prominent derivative (AB-61) bears a thienyl group in position 7 and exerts subnanomolar cytotoxic activity against human lymphoblastic leukemia (CCRF-CEM).[34] Even larger substituents are tolerated, and the dibenzofuranyl derivative selectively inhibits the mycobacterial adenosine kinase while not showing any cytotoxicity.[35] (**Figure 9**)

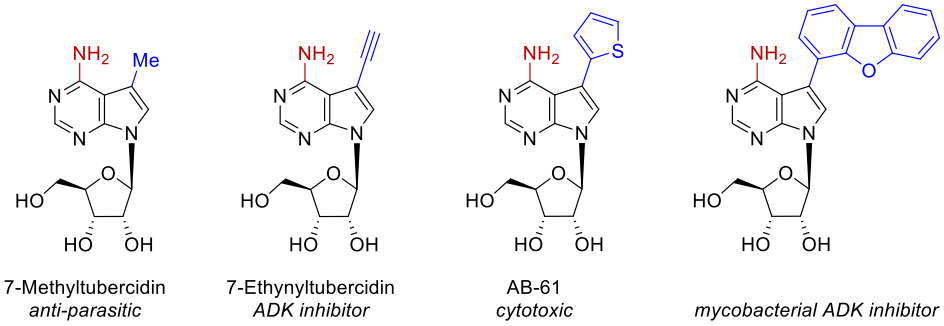


Figure 9. 7-Substituted 7-deazapurine nucleosides with biological activities.

This wide range of 7-(het)aryl-7-deazaadenosine derivatives (over 30 examples) proved that there is enough room in position 7 for future modifications without sterically compromising the interaction with the target.

1.3.4. 8-Substituted 7-Deazapurine Nucleosides

With sangivamycin as a parent structure (**Figure 7**), the carboxamide substituent can be replaced by other carboxylic-like substituents or an additional substituent can be introduced in position 8. One of these sangivamycin-like molecules (SLM) is SLM6 bearing a carbohydrazonamide group in position 7. SLM6 inhibits the cyclin-dependent kinase 9 (CDK9) which leads to cytotoxicity in multiple myeloma cells (MM), a hematologic form of cancer.[36] 8-Hydrazinosangivamycin (ARC) exerts cytotoxic activities against several cancer cell lines.[37] Xylocydine is a 8-bromosangivamycin analogue with a modified α-anomeric sugar moiety. It selectively inhibits several cyclin-dependent kinases (CDK1, CDK2, CDK7, and CDK9) which leads to apoptosis in hepatocellular carcinoma.[38] However, derivatives bearing larger groups in position 8 such as *p*-tolyl, *m*- or *p*-methoxyphenyl, and *m*- or *p*-bromophenyl groups are inactive and no longer inhibit CDK1 or CDK2. Most probably, this loss in biological activity correlates with a change in the conformational structure of the sugar moiety as 8-unsubstituted nucleosides prefer an *anti*-conformation of the nucleoside, whereas large substituents in position 8 lead to the *syn*-conformer.[39] (**Figure 10**)

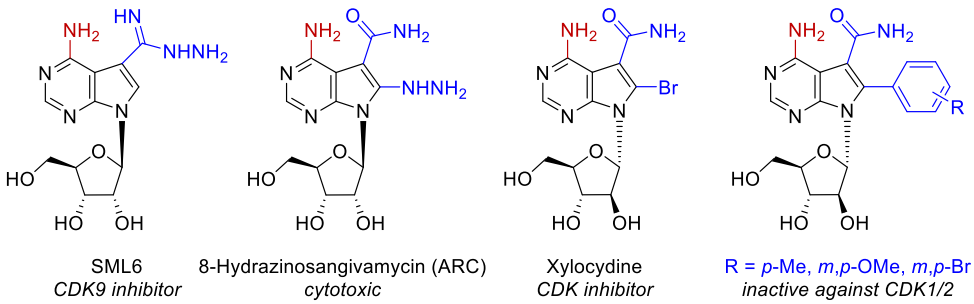


Figure 10. 8-Substituted 7-deazapurine nucleosides based on sangivamycin with biological activities.

1.3.5. Fused 7-Deazapurine Nucleosides

After the modification of the 7-deazapurine nucleoside scaffold with diverse substituents, the heterocyclic nucleobase can be further extended by annulation of (het)aryl rings leading to fused nucleosides. As one of the first tricyclic nucleosides, triciribine was synthesized in 1971 and selectively inhibits AKT kinases overexpressed in tumor cells. Its more soluble 5'-monophosphate showed cytotoxic tendencies at higher concentrations. With the introduction of phosphoramidite prodrugs, the corresponding triciribine derivative showed much better bioavailability and is now in clinical testing against breast and ovarian cancer.[40] In 1975, the Leonard group synthesized three different benzoadenosines where the annulation of the benzene ring is positioned in between the pyrimidine and the imidazole ring of the purine moiety. With the extension of the aromatic system, the polarizability of the tricyclic nucleobase was increased compared to purine. This increased π-π stacking ability was used to study enzyme binding sites.[41] The thieno-expanded adenosine exerted antiviral activity against HCV.[42] A further class of tricyclic nucleosides are pyrimido-fused 7-deazapurines. These Janus-type nucleosides can present as either adenosine or as guanosine during Watson-Crick base-pairing and therefore can form stable base pairs with two different canonical nucleobases. They show only moderate cytotoxicity but are active against HCV.[43] (**Figure 11**)

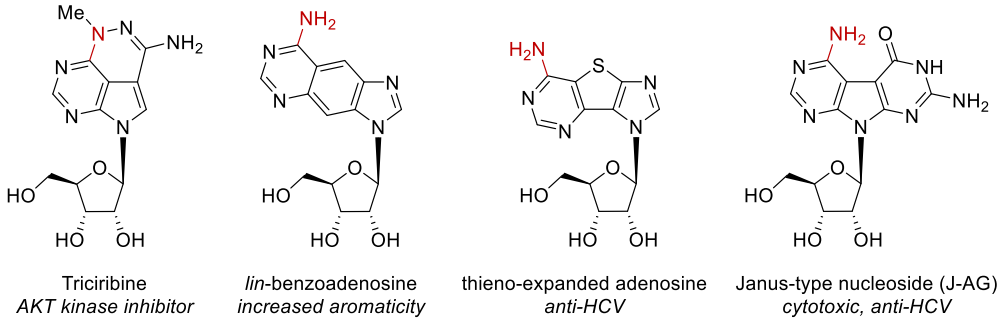


Figure 11. Tricyclic nucleosides with biological activities.

After the successful introduction of differently sized substituents in position 7, it was clear that there is sufficient room for the annulation of (het)aryl rings onto the C7/C8 bond of 7-deazapurine nucleosides. This led to a large library with several series of different (het)aryl-fused 7-deazapurine nucleosides. (**Figure 12** and **Figure 13**)

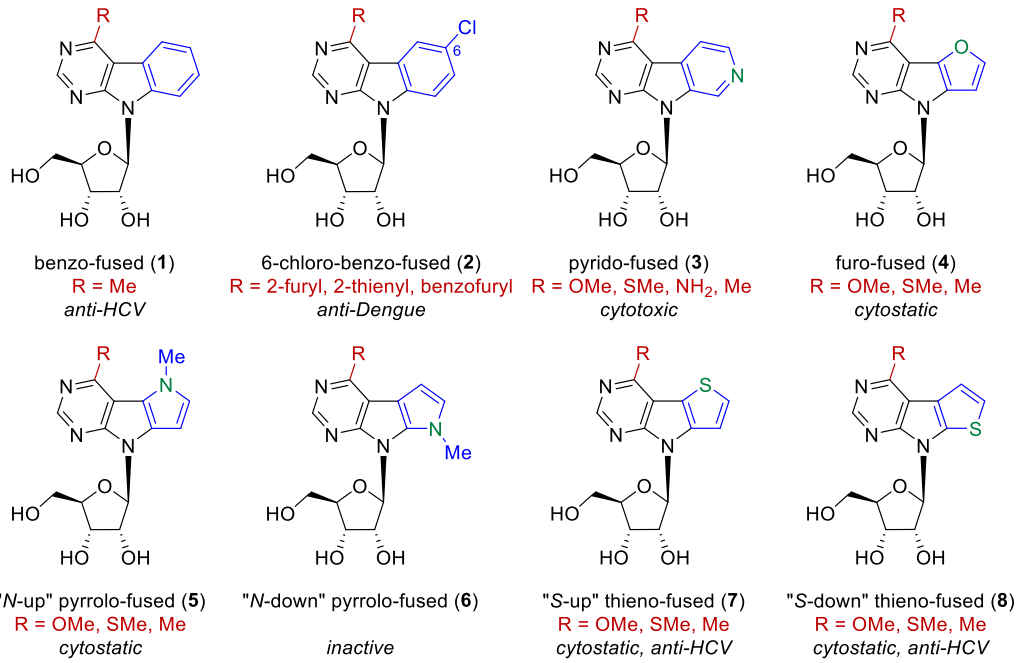


Figure 12. Fused 7-deazapurine nucleosides bearing a tricyclic nucleobase.

First, the benzo-fused 7-deazapurine nucleoside series (**1**) was synthesized in our group. Unfortunately, none of the derivatives exerted any cytotoxicity. Its methyl derivative showed antiviral activity against Hepatitis C (HCV).[44] In the chloro-substituted benzo-fused series (**2**), the 2-furyl, 2-thienyl, and 2-benzofuryl derivatives displayed antiviral activity against Dengue virus.[45] Then, all four isomeric pyrido-fused 7-deazapurine nucleosides (**3**) were synthesized which are derived from the benzo-fused nucleosides by formal introduction of nitrogen into the fused 6-membered ring. The methoxy, methylsulfanyl, amino, and methyl derivatives (**3**, isomer shown in **Figure 12**) exerted submicromolar cytotoxic activity against a broad panel of cancer cells with good selectivity.[46] A comparison between the biological activities of the benzo-fused 7-deazapurines (**1**) and of the pyrido-fused 7-deazapurines (**3**) revealed that the strategic placement of nitrogen into the fused ring can result in a strong increase in biological activity. Then, by annulation of 5-membered rings, the furo-fused 7-deazapurine nucleosides **4** were prepared. Their methoxy, methylsulfanyl and methyl derivatives all showed submicromolar cytostatic activity against the same panel of cancer cells. The "*N*-up" (**5**) and "*N*-down" methylpyrrolo-fused 7-deazapurine nucleosides (**6**) were prepared as well. The methoxy, methylsulfanyl and methyl derivatives in the "*N*-up" series **5** showed similar biological activities as the corresponding derivatives in the furo-fused series **4**. However, the "*N*-down" series **6** proved to be biologically inactive, probably due to the steric influence of the *N*-methyl group on the syn/anti-conformational equilibrium of the nucleoside.[47] The derivatives in both thieno-fused 7-deazapurine series **7** and **8** showed cytostatic properties against a broad panel of cancer cells as well as antiviral properties against HCV. Furthermore, the methyl derivatives of **3** and **4** both efficiently underwent intracellular phosphorylation by ADK in BJ fibroblasts and in HCT116 cancer cells.[48] (**Figure 12**) This library of tricyclic (het)aryl-fused 7-deazapurine nucleosides can be extended with the annulation of 5-membered rings bearing two heteroatoms.[49]

The library was further extended to tetracyclic and polycyclic 7-deazapurine nucleosides. The "angular" naptho-fused series (**9**) proved to be too bulky and none of its derivatives were biologically active. The 2-furyl and 2-benzofuryl derivatives in the "linear" series **10** showed moderate cytostatic activity.[50] Next, two benzothieno-fused series **11** and **12** were prepared. Only the benzofuryl derivative in series **12** exerted antiviral properties against HCV. All the other derivatives in both series **11** and **12** were inactive.[51] In the benzofuro-fused series **13**, the sequence of annulation is different: the 7-deazapurine scaffold was first extended by a

benzene ring before the furan moiety is fused onto it. Only the amino derivative of this series was cytotoxic.[52] As the thieno-fused series **7** and **8** showed cytotoxic and antiviral properties, the scaffold was extended by annulation of additional thiophene ring(s). The bithieno-fused series (**14**) was inactive. However, the amino derivative of the corresponding bithieno-fused 7-deazapurine deoxynucleoside **d14** was cytotoxic against T-lymphoblastic leukemia (CCRF-CEM). The same was observed with the polythieno-fused series **15**: while the amino derivative of **15** was inactive, the one of the deoxyseries **d15** was cytotoxic.[53] (**Figure 13**) This library of tetra- and polycyclic nucleosides can be further extended by annulation of heteroaryl-fused ring systems bearing a nitrogen to the 7-deazapurine scaffold.[54]

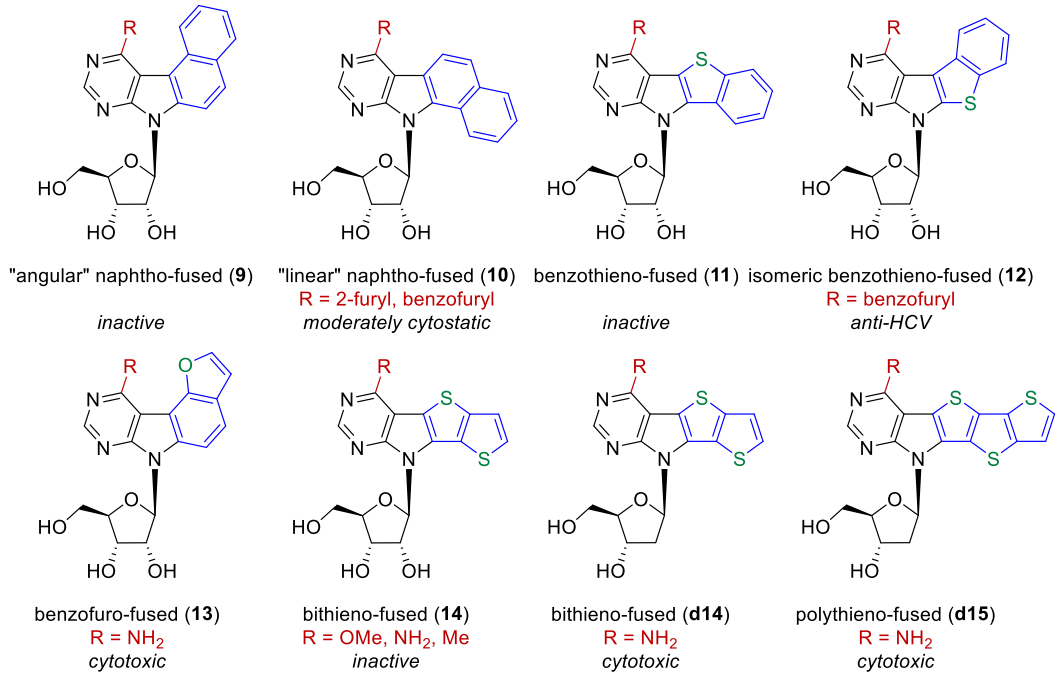


Figure 13. Fused 7-deazapurine nucleosides bearing a tetra- or polycyclic nucleobase.

1.4. Synthetic Strategy

The synthesis of (het)aryl-fused 7-deazapurine nucleosides can be divided into three parts: (1) Formation of the heterocyclic nucleobase, (2) glycosylation of the key nucleoside intermediate and (3) derivatization and deprotection furnishing a series of free nucleosides. For the application in biochemistry, the nucleosides can be phosphorylated to their nucleotide triphosphates.

1.4.1. Heteroaryl-fused 7-Deazapurine Nucleobases

The annulation of (het)aryl rings to the parent 7-deazapurine scaffold forms tricyclic heterocycles. They consist of three parts: the "western" pyrimidine is responsible for the molecular recognition, the "central" pyrrole ring connects the nucleobase to the sugar moiety and the "eastern" ring system represents the heterocycle fused to the C7/C8 bond of the 7-deazapurine scaffold. (**Figure 14**)

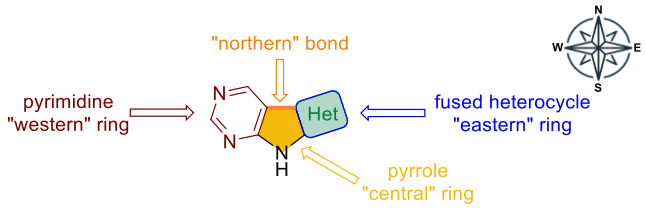
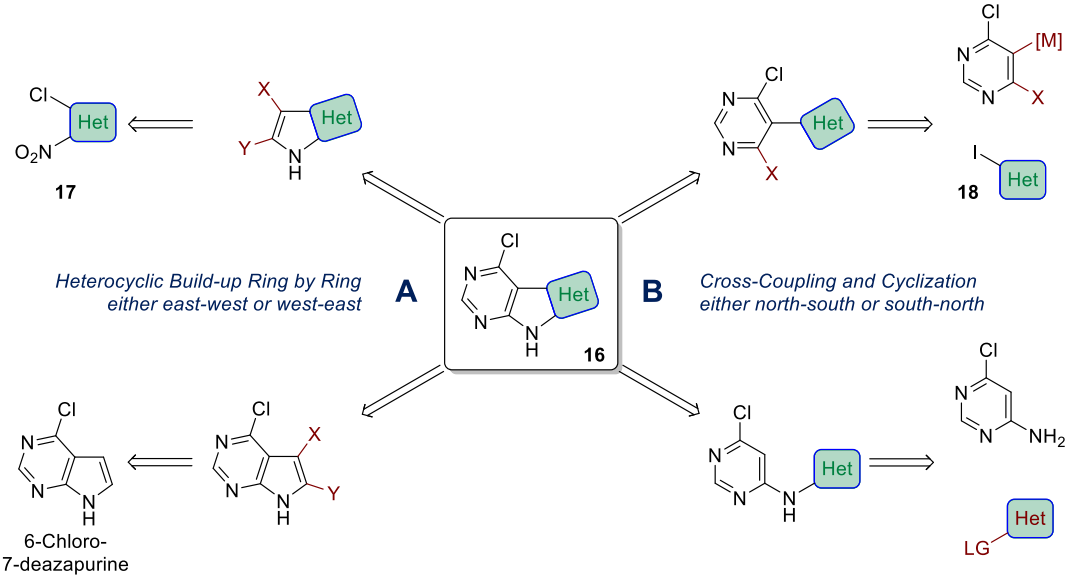


Figure 14. Schematic of the (het)aryl-fused 7-deazapurine scaffold.

When looking at the (het)aryl-fused 7-deazapurine scaffold (**16**) from a retrosynthetic point of view, these tricyclic nucleobases can be built in four different ways: (A) A horizontal strategy involving a heterocyclic build-up ring by ring either in the east-west direction starting from the chloro-nitro bifunctionalized (het)arenes or in the west-east direction starting from 6-chloro-7-deazapurine. (B) A vertical strategy starting by connecting the outer rings either by a cross-coupling reaction forming the northern bond or by amination forming the southern connection followed by cyclization and formation of the "central" pyrrole ring. (**Scheme 1**)



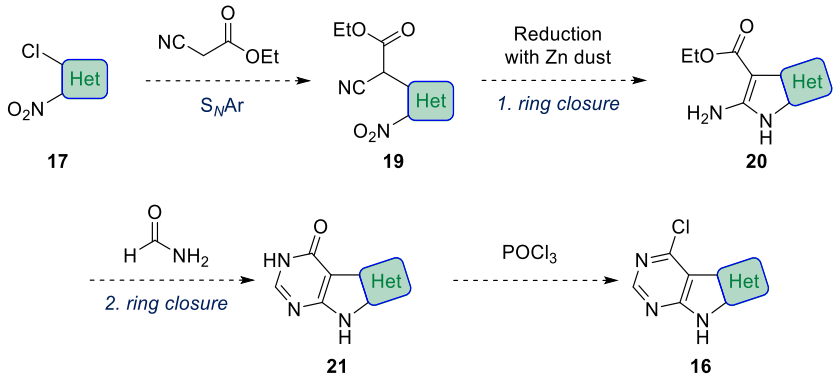
Scheme 1. Retrosynthetic analysis of (het)aryl-fused 7-deazapurine nucleobases.

In our group, two approaches are established: Approach A (direction east-west) relies on a multistep reaction cascade starting from the "eastern" (het)aromatic ring system with a

chloro-nitro bifunctionality (**17**). The nucleobase is built up ring by ring: first, the "central" pyrrole ring is formed; then, the "western" pyrimidine is established.[55] Approach B (direction north-south) starts with the cross-coupling reaction between the "western" pyrimidine ring and the "eastern" (het)aromatic iodide (**18**) forming the "northern" bond. Then, the "central" pyrrole ring is closed.[47,48,50–52]

1.4.1.1. Classical Heterocyclization (Approach A)

Most chloro-nitro bifunctionalized (het)arenes (**17**) are either commercially available or can be established in one or two steps. In the first step of the reaction cascade, the chloro position of the (het)arene **17** undergoes nucleophilic aromatic substitution with the anion of ethyl cyanoacetate furnishing **19**. In the second step, the nitro group is reduced to the amine with zinc dust in hot acetic acid followed by spontaneous cyclization during the work-up with satd $NaHCO_3$ yielding **20** with the newly formed "central" pyrrole ring.[56] For establishing the "western" pyrimidine ring and the formation of the (het)aryl-fused 7-deazahypoxanthine **21**, the intermediate **20** undergoes Niementowski condensation with formamide which requires elevated temperatures.[57] Different formamide sources are known and will be discussed in the main part of this thesis. In the last step of the cascade, **21** is transformed to its 6-chloro-7-deazapurine analogue **16** with freshly distilled $POCl_3$, *N*,*N*-dimethylaniline and benzyl-triethylammonium chloride (BTEA-Cl).[58] (**Scheme 2**)

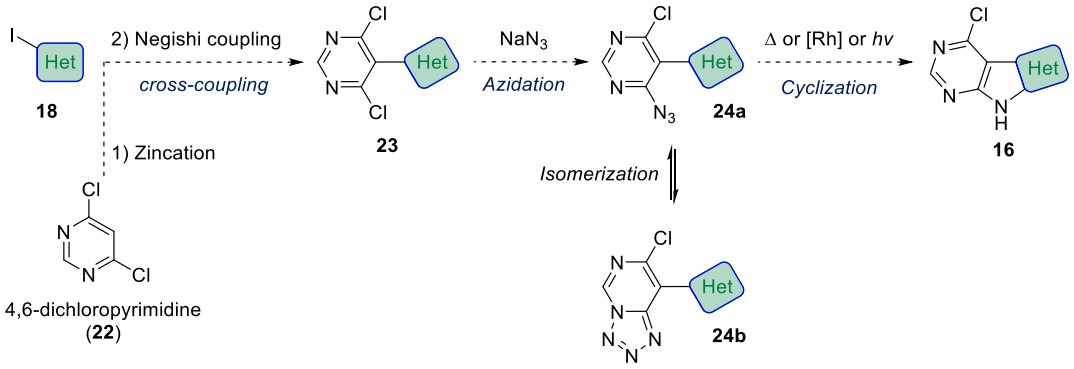


Scheme 2. Heterocyclization approach starting from the chloro-nitro bifunctionalized (het)arene **17** to the desired tricyclic (het)aryl-fused 7-deazapurine **16**.

This heterocyclization approach was employed to furnish the benzo-fused (**1**,**2**)[44,45] as well as the pyrido-fused 7-deazapurines (**3**)[46] previously reported by our group. I used this approach for the synthesis of the pyrazolo-fused 7-deazapurine.[49]

1.4.1.2. Cross-Coupling & Cyclization (Approach B)

Alternatively, (het)arenes can be accessed through their iodo derivatives (**18**). A Negishi cross-coupling reaction between the zincated 4,6-dichloropyrimidine **22** and the (het)aryl iodide **18** furnishes the intermediate **23**. Azidation of **23** with NaN_3 and LiCl results in **24**. The equilibrium between its azide form **24a** and its tetrazole form **25b** highly depends on the environment.[59] To form the desired (het)aryl-fused 7-deazapurine **16**, the azide **24a** undergoes a cyclization reaction which resembles the carbazole formation from *o*-azidobiaryls.[60] To accomplish this transformation, N_2 can be cleaved from the azide using different techniques. The nitrene intermediate then undergoes an electrocyclic ring-closure (forming the "central" pyrrole ring) followed by an intramolecular hydrogen shift furnishing the desired nucleobase **16**. (**Scheme 3**)



Scheme 3. Cross-coupling and cyclization approach starting from the (het)aryl iodide **18** to the desired tricyclic (het)aryl-fused 7-deazapurine **16**.

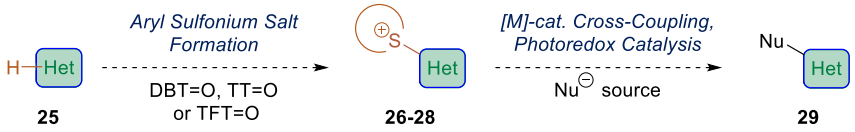
Three different principles have been employed for the cyclization: (1) Thermal cyclization of **24** in 1,4-dibromobenzene, a high-boiling solvent;[61] (2) rhodium-catalyzed cyclization using Rh_2esp_2,[62] Rhodium octanoate dimer ($Rh_2(O_2CC_7H_{15})_4$) or Rhodium heptafluorobutyrate dimer ($Rh_2(O_2CC_3F_7)_4$)[63] as a catalyst to form the nitrene intermediate; and (3) photocyclization in trifluoroacetic acid (TFA), a solvent that shifts the **24a**/**24b** equilibrium to the azide by protonation of the N1-nitrogen in the pyrimidine ring.[64]

This cross-coupling and cyclization approach was used for the synthesis of the (het)aryl-fused 7-deazapurines in the preparation of the corresponding nucleosides **4**–**13**.[47–52] I used this approach for the synthesis of the pyrazolo-fused and the (iso)quinoline-fused 7-deazapurine.[49,54]

1.4.1.3. Alternatives to Heteroaryl Iodides for Approach B

Not all the (het)aryl iodides **18** are commercially available. Some can be prepared by lithiation/iodination of their (het)aryl bromides or by direct lithiation/iodination of their unsubstituted (het)arenes.[47,48] For other (het)arenes, this option is not available.

Recently, the Ritter group published a new C–H functionalization approach by using aryl sulfonium salts as an alternative to the inaccessible (het)aryl halides.[65–67] First, the (het)arene **25** undergoes site-selective substitution by an aryl sulfoxide forming the sulfonium salts **26-28**. Then, the intermediates **26-28** can be used as a substrate in a variety of palladium-catalyzed cross-coupling reactions such as Heck, Sonogashira, Negishi or Suzuki. Also, the exchange of the sulfonium salt by another functionality via photoredox catalysis is possible furnishing the borylated, phosphorylated, cyanated or halogenated product **29**.[65] (**Scheme 4**)



Scheme 4. C–H functionalization through the formation of the aryl sulfonium salt as intermediates.

Depending on the nature of the (het)arene, one of three aryl sulfoxides were used in the formation of the aryl sulfonium salt (**26-28**). The thianthrenation of the (het)arenes with thianthrene *S*-oxide (TT=O) in the presence of trifluoroacetic anhydride (TFAA) is the most reliable (**26**).[66] In some cases, its fluorinated analogue 2,3,7,8-tetrafluorothianthrene *S*-oxide (TFT=O) furnished **27** more successfully.[65] A third option is the use of dibenzothiophene *S*-oxide (DBT=O) in the presence of either TFAA or triflic anhydride (Tf_2O) for the formation of the sulfonium salt **28**.[67] (**Figure 15**)

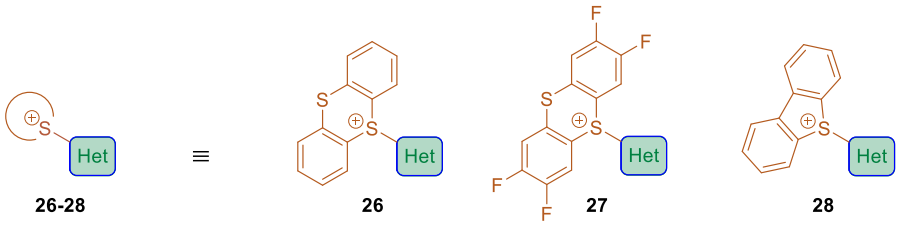
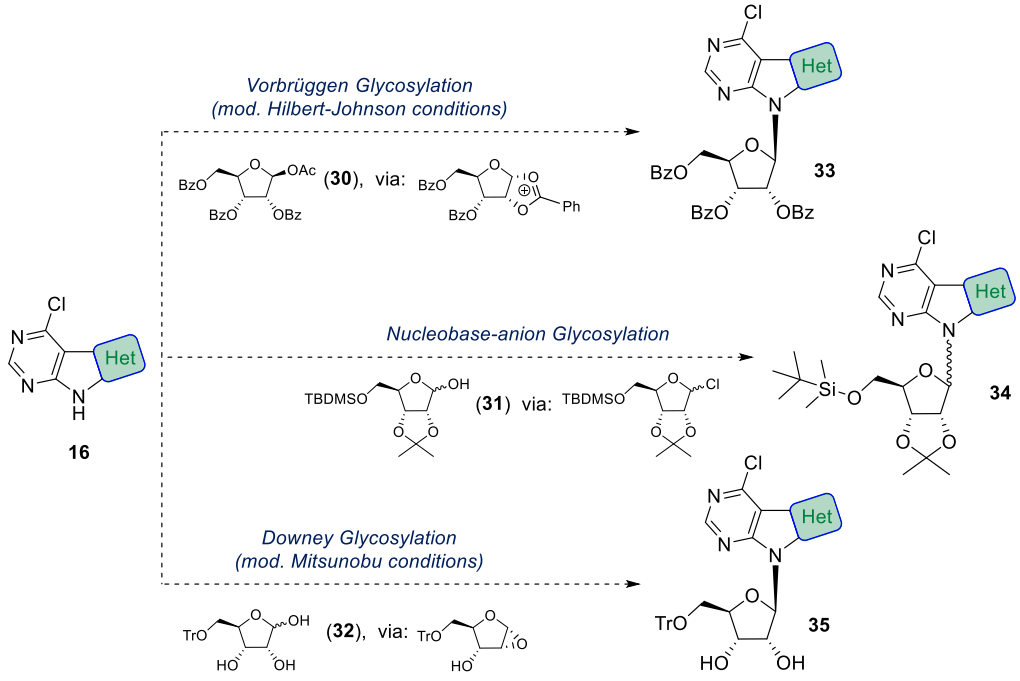


Figure 15. Aryl sulfonium salts are formed from the (het)arene with TT=O (**26**), with TFT=O (**27**), or with DBT=O (**28**),

When studying the published examples of aryl sulfonium salts (**26-28**),[65–67] there is no specific trend or correlation visible between the electronic nature of (het)arene **25** and the successfully prepared sulfonium salts **26-28**.

1.4.2. Glycosylation

With the desired nucleobase **16** in hand, the *N*-glycosylation can be performed to connect the base to the sugar moiety. Different glycosylation options are available, each with its own scope, and its own advantages and disadvantages:[68] (1) The Vorbrüggen glycosylation is a modified Silyl-Hilbert-Johnson reaction and very reliable in the preparation of nucleosides.[69] (2) A great alternative is the nucleobase-anion glycosylation even though the reaction is not stereo selective at the anomeric center.[70] (3) Recently, my former co-worker A. Michael Downey developed a glycosylation procedure based on the Mitsunobu reaction.[71] All of the *N*-glycosylation reactions have in common that the used sugars **30-32** are activated first. Then, the glycosyl intermediates are subjected to the nucleophilic attack by nucleobase **16** furnishing the corresponding nucleosides **33-35**. (**Scheme 5**)



Scheme 5. Glycosylation of nucleobase **16** to form the nucleosides **33-35** as a key intermediate.

For the Vorbrüggen glycosylation, the nucleobase **16** is first silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in the amine position N9. The newly installed N–Si bond enhances the nucleophilic character of the base.[72] Then, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**30**) reacts with trimethylsilyl triflate (TMSOTf), a Lewis acid, in the glycosyl position forming a 1,3-dioxolane cation with the neighboring benzoyl protecting group *in*

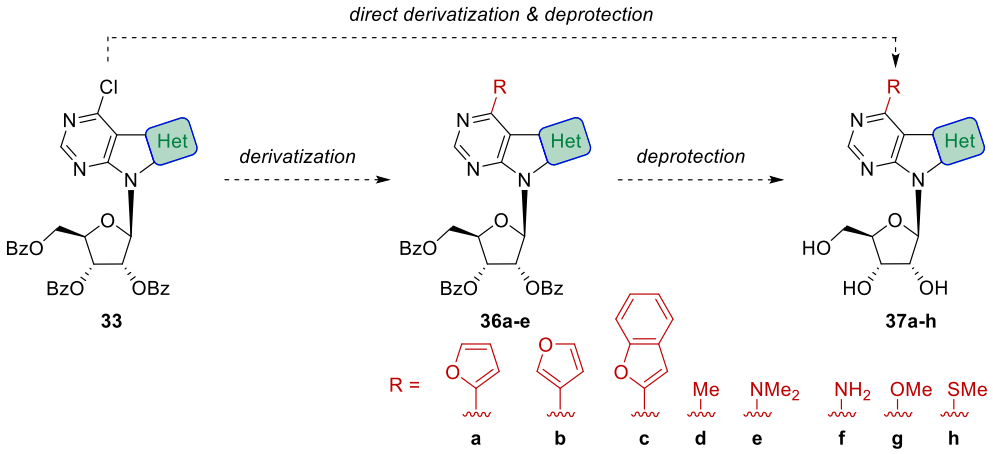
situ.[73] Finally, the stabilized cation reacts with the previously silylated nucleobase giving the desired ribonucleoside **33** as a pure *β*-anomer.

For the nucleobase-anion glycosylation,[70] the nucleobase **16** is first deprotonated by KOH. To improve the solubility of the formed potassium salt, the phase-transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1) is used. Separately, the halosugar is prepared from its hydroxy precursor (**31**)[74] by treatment with CCl_4 and tris(dimethylamino)phosphine (HMPT). Then, the mixtures are combined and the newly installed chloride acts as a leaving group during the nucleophilic attack of the nucleobase anion on the sugar. As the chloride cannot be installed stereoselectively, the resulting nucleoside **34** is obtained as an anomeric mixture.

The Downey glycosylation[71] uses a 5-monotritylated ribose[75] (**32**) treated with 1,1'-(azodicarbonyl)dipiperidine (ADDP) and tributylphosphine to form the reactive epoxide intermediate. The previously deprotonated nucleobase **16** undergoes a nucleophilic attack on the sugar and opens the epoxide furnishing the desired ribonucleoside **35** as a pure *β*-anomer.

1.4.3. Derivatization and Deprotection

The protected nucleoside **33** is used as a key building block in the preparation of free nucleosides bearing different substituents in position 6 (**37**). Based on prior biological testing with 6-substituted 7-deazapurine ribonucleosides,[27–30] a selection of 8 different modifications were chosen for the library of (het)aryl-fused 7-deazapurine ribonucleosides **37a-e**: The 2-furyl (a) and 3-furyl (b) are 5-membered heteroaryl substituents, the 2-benzofuryl (c) represents a bulky modification, the methyl group (d) is a small nonpolar substituent, the amino group (f) is a H bond donor while the *N*,*N*-dimethylamino (e), the methoxy (g) and the methylsulfanyl (h) are considered H bond acceptors. (**Scheme 6**) The library can be extended with further modifications in position 6 if the initial testing reveals any interesting biological activity.[46]



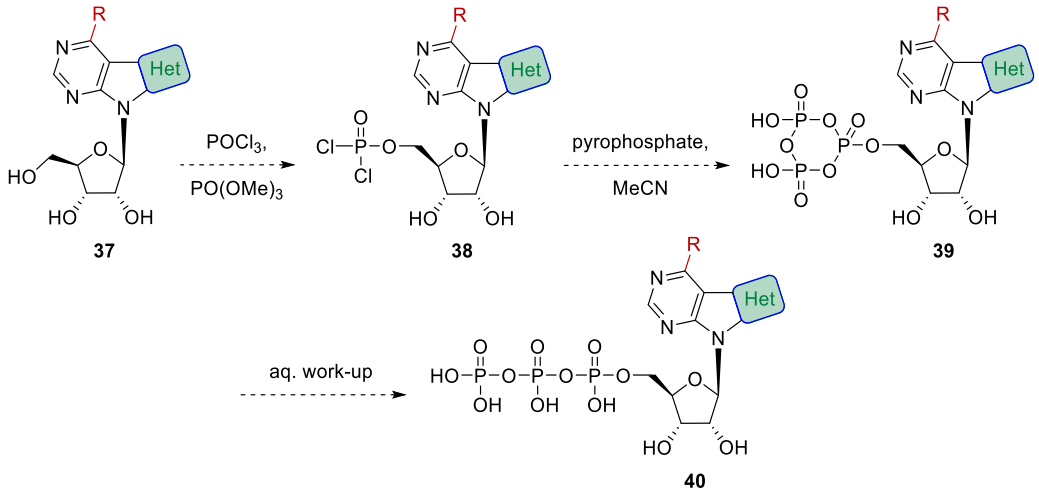
Scheme 6. Preparation of a library with the ribonucleosides **37a-h** either by stepwise derivatization and deprotection via **36a-e** or by simultaneous derivatization and deprotection directly from **33** (for **37f-h**).

The nucleosides **37a-e** are prepared in two steps, first derivatization, then deprotection: The 2-furyl derivative **36a** can be prepared via cross-coupling of **33** with 2-(tributylstannyl)furan. Both, the 3-furyl and the 2-benzofuryl derivatives **36b** and **36c** can be synthesized from **33** and their corresponding boronic acids. The methyl derivative **36d** is the result of cross-coupling reaction between **33** and $AlMe_3$. The *N*,*N*-dimethylamino derivative **36e** is obtained by nucleophilic substitution of **33** with dimethylamine. The protected nucleoside derivatives **36a-e** are then subjected to the Zemplén deprotection of the sugar moiety with NaOMe in MeOH giving the free ribonucleosides **37a-e**.[76]

The derivatives **37f-h** are prepared from **33** directly by nucleophilic substitution in position 6 and the simultaneous deprotection of the sugar moiety with ammonia, NaOMe, or with NaSMe, respectively.

1.4.4. Triphosphorylation

The biological testing is performed with the free ribonucleosides (**37**). For experiments in biochemistry, the corresponding triphosphates (**40**) are required. One of the most well-known methods is the Yoshikawa protocol which makes the selective 5'-phosphorylation of the unprotected sugar moiety possible.[77]



Scheme 7. Triphosphorylation of the free ribonucleoside **37** furnishing the corresponding tiphosphate **40**.

The unprotected ribonucleoside **37** is treated with $POCl_3$ to furnish the highly reactive 5'-monophosphorodichlorate intermediate **38**. Aqueous work-up after this step would give the monophosphate. Instead, **38** then reacts *in situ* with pyrophosphate resulting in the formation of the cyclic triphosphate **39**. During aqueous work-up, **39** is hydrolyzed to the desired triphosphate **40**.

2. Aim of Thesis

1. Synthesis of a library of pyrazolo-fused 7-deazapurine ribonucleosides and comparison of different synthetic strategies
2. Systematic study of C-H functionalization of five-membered heterocycles by dibenzothiophenation and thianthrenation
3. Synthesis of (iso)quinolino-fused 7-deazapurine ribonucleosides and their application as triphosphates in biochemistry.

Rationale of the Specific Aims

As discussed in the previous section, nucleoside analogues are an important class of compounds for the development of new treatments against cancers and viral diseases.[78] The canonical nucleoside adenosine is involved in nearly every cellular process and its triphosphate is the cell's energy currency. Changes to its scaffold, even small ones, can influence biochemical reactions heavily but the effects of each possible modification can not be predicted. Thus, each target structure has to be synthesized and tested individually. 7-Deazapurine nucleosides are a new class of adenosine analogues and allow modifications in position 6 and 7 as well as the annulation of (het)aryl rings to the C7/C8 bond.[19]

After the success of 7-(2-thienyl)-7-deazaadenosine (AB-61) during biological screening,[79] (het)aryl-fused 7-deazapurine nucleosides were prepared and tested against a broad panel of cancer cell lines and against several viruses. While the benzo-fused deazapurines[44,45] proved only active against Hepatitis B or Dengue, the thieno-fused 7-deazapurines also showed broad anticancer properties.[48] Furo- and methylpyrrolo-fused 7-deazapurines were biologically active as well.[47] My goal was to extend the library of 5-membered heteroaryl-fused 7-deazapurine nucleosides with the annulation of 5-membered rings bearing two heteroatoms. During the synthesis of the pyrazolo-fused 7-deazapurine nucleosides, I also compared the synthetic strategies for the preparation of the nucleobase as well as for the glycosylation established in our group.

As not all 5-membered heterocycles can be accessed through established strategies, an alternative strategy for the annulation of these heterocycles to the 7-deazapurine scaffold is needed. My goal was to apply sulfonium chemistry to unsubstituted 5-membered heteroaromatic rings and use the prepared aryl sulfonium salts as substrates for the Negishi cross-coupling in the preparation of additional (het)aryl-fused nucleosides.

Unfortunately, the "angular" naphtho-fused 7-deazapurine was completely inactive during the biological screening.[50] Previously, with the introduction of a nitrogen to the benzo-fused system giving the pyrido-fused 7-deazapurine nucleosides, the biological activity was recovered.[46] My goal was to introduce a nitrogen to the naphtho-fused system, resulting in the (iso)quinolino-fused system, to determine whether the biological activity can be recovered as well. Furthermore, the naphtho-fused nucleosides were already too bulky for the incorporation into DNA. Hence, the incorporation of quinolino-fused nucleosides was studied as well.

3. Results and Discussion

The structure of this chapter can be divided into three parts. In the first part, I discuss the synthesis of all target compounds. I start with the pyrazolo-fused 7-dazapurine nucleosides where I compare synthetic strategies for the formation of the nucleobase as well as for the glycosylation towards the key intermediate nucleoside.[49] Then, I have a look at other 5-membered heterocycles and their availability as a substrate for the Negishi cross-coupling and discuss the use of aryl sulfonium salts as a possible alternative to the iodo derivatives.[53] Finally, I shift my focus towards nitrogen-containing tetracyclic heteroaryl-fused 7-deazapurines and their ribonucleosides. During the preparation of the quinolino-fused nucleobase, a more detailed discussion of the cyclization step will be provided.[54] Throughout the discussion, I am using the systematic numbering. (**Figure 16**)

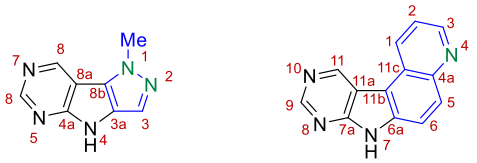


Figure 16. Systematic numbering used throughout this chapter.

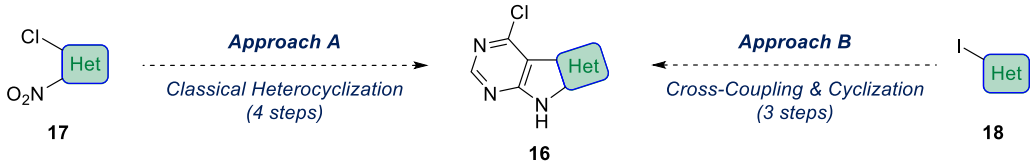
After the synthetic part, I evaluate these ribonucleoside libraries for their photophysical properties as well as their biological activities. The biological testing for this evaluation was performed by the research group under Prof. M. Hajdúch at the *Institute of Molecular and Translational Medicine* of the Palacký University in Olomouc, Czech Republic as well as the research-service groups under Dr. H. Mertlíková-Kaiserová, and under Dr. J. Weber at IOCB Prague.[49,54]

In the last part, I will discuss a possible application of the amino-substituted quinolino-fused 7-deazapurine ribonucleoside triphosphate as an ATP analogue for transcription together with the photophysical properties of the products. This work was done in cooperation with Tania Sánchez Quirante from our research group.[54]

3.1. Pyrazolo-Fused 7-Deazapurine Ribonucleosides

3.1.1. Synthesis of the Pyrazolo-fused 7-Deazapurine Nucleobase

The fused 7-deazapurine nucleobase (**16**) can be synthesized using two different approaches: Approach A starts from the chloro-nitro derivative **17** of the "eastern" ring and builds each ring up step by step resulting in the desired tricyclic nucleobase. Approach B starts from the iodo derivative **18** of the "eastern" ring undergoing a cross-coupling reaction with the "western" pyrimidine ring followed by azidation and ends with cyclization of the "middle" ring resulting in the desired tricyclic nucleobase. (**Scheme 8**)



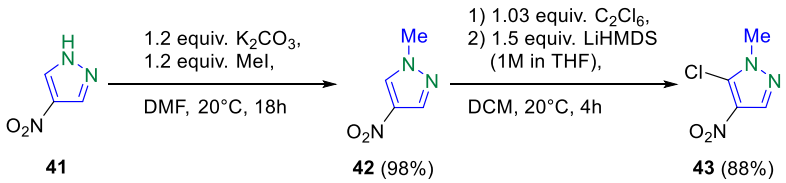
Scheme 8. Synthetic strategy for the formation of the nucleobase: Approach A involves a classical heterocyclization with a stepwise heterocycle built-up (4 steps). Approach B starts with a cross-coupling reaction and results in a cyclization step (3 steps).

Both approaches have been used in the synthesis of tricyclic nucleobases. The classical heterocyclization approach (Approach A) was used in the synthesis of benzo-[44,45] and pyrido-fused nucleobases.[46] Whereas the cross-coupling approach (Approach B) was used in the synthesis of the thieno-[48], pyrrolo- and furano-fused nucleobases.[47] Furthermore, the tetra-[50–52] and polycyclic nucleobases[53] were synthesized in the same manner.

For the synthesis of the pyrazolo-fused nucleobase, both approaches were utilized and then compared.

3.1.1.1. Classical Heterocyclization (Approach A)

One of the key conditions for the classical heterocyclization leading to the nucleobase is the presence of the *ortho*-chloro-nitro bifunctionality. In the case of the pyrazolo-fused 7-deazapurine, this was achieved in two steps from the commercially available 4-nitropyrazole (**41**). (**Scheme 9**) In the first step, the 4-nitropyrazole (**41**) was methylated at one of the nitrogen positions using methyl iodide and K_2CO_3 in DMF resulting in the 1-methyl-4-nitropyrazole (**42**) in nearly quantitative yield (98%).[80] Scale-up is non-problematic and the reaction can be performed even with 20.0 g of starting material.

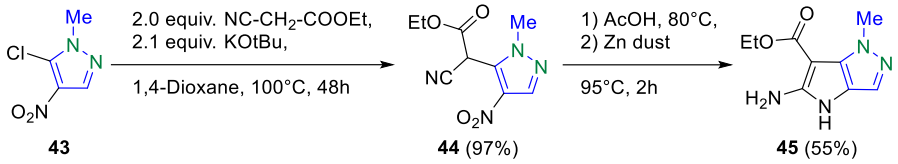


Scheme 9. Preparation of the starting material with the chloro-nitro bifunctionality.

In the second step, **42** was selectively chlorinated in position 5 with hexachloroethane and LiHMDS in DCM giving the desired 5-chloro-4-nitropyrazole **43** in 88% yield.[81] The slow addition of LiHMDS was crucial for the success of the reaction especially for larger batch sizes (e.g. 22.0 g of **42**). The deprotonation in position 5 is regioselective as this is the most acidic position in the pyrazole ring while position 3 is the least reactive.[82]

With the chloro-nitro-bifunctionality installed, the pyrazole **43** underwent heterocyclization to build up the neighboring rings towards the tricyclic nucleobase. First, the pyrrole moiety is

built, then the pyrimidine ring. The formation of the fused pyrrole ring was achieved in two steps.[55] (**Scheme 10**)

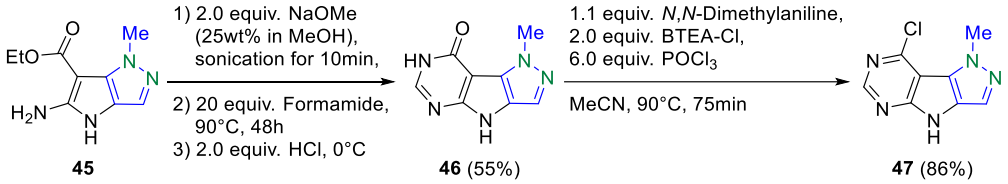


Scheme 10. Formation of the fused pyrrolo[3,2-*c*]pyrazole ring system.

First, the nucleophilic aromatic substitution in the chloro position of **43** with an excess of ethyl cyanoacetate and KO*t*Bu in 1,4-dioxane gave **44** in 97% yield. The reaction worked best with 2.0 equiv. ethyl cyanoacetate; when using only 1.5 equiv. of ethyl cyanoacetate, a significant drop in yield (76%) was observed. Unfortunately, the required excess proved problematic during the purification process. Flash chromatography (FC) worked nicely with small batch sizes (e.g. 1.0 g of **43**); however, it was impractical at larger scales since ethyl cyanoacetate is not UV active and was present in nearly all fractions. Instead, for larger batches, the excess of ethyl cyanoacetate was removed by vacuum distillation (lit[83]: b.p. = 208–210 °C at 1 bar; 98 °C at 22 mbar) prior to solidification in the freezer and washing with ice-cold DCM.

In the next step, the nitro group in intermediate **44** was reduced by zinc dust in AcOH to the amine which underwent spontaneous cyclization during the work-up with satd $NaHCO_3$ forming the desired pyrrole ring. Filtration and washing with cold water gave the desired product **45** (43%). The volume of the filtrate was reduced *in vacuo* and was neutralized with diluted AcOH resulting in further precipitation **45** (+12%). Small traces of zinc were confirmed by ED-XRF[84] but they did not interfere in the next step. I also tried to use activated zinc dust[85] for this reaction but it did not improve the yield. For the work-up, I also tried to dilute the crude oil with EtOAc instead and extract it with satd $NaHCO_3$. However, the subsequent HPFC of the organic layers gave the product **45** in low purity as everything was washed out within the first 5 min at 5% EtOAc in cHex.

The formation of the pyrimidine moiety resulting in the 7-deazahypoxanthine **46** and the chlorination in the pyrimidine position 4 gave the pyrazolo-fused 7-deazapurine nucleobase **47**. (**Scheme 11**)



Scheme 11. Formation of the tricyclic 7-deazahypoxanthine **46** and transformation towards the pyrazolo-fused 7-deazapurine **47**.

The commonly used procedure for the formation of the fused 7-deazahypoxanthine **46** involves an excess of formamide, elevated temperature (150–190 °C) and stirring under reflux conditions overnight.[86] Unfortunately, this resulted in decomposition for my pyrazolo-fused ring system. Hence, extensive testing of different conditions was warranted. (**Table 1**)

Table 1. Conditions for the condensation reaction with a formamide source furnishing **46**. A sample from the reaction mixture was diluted in MeOH and the ratio between the starting material **45** and the product **46** was determined by HPLC-MS.

Entry	Conditions	Temp.	Time	ratio **45**:**46**	Ref.
1	formamide (45 equiv.)	150 °C	22 h	- (decomp.)	[86]
2	formamide (45 equiv.)	100 °C	66 h	4:1	
3	ammonium formate (1.2 equiv.), formamide	170 °C	19 h	- (decomp.)	[87]
4	ammonium formate (1.2 equiv.), formamide	80 °C	5 d	17:3	
5	molecular sieves (3 Å), formamide	80 °C	10 d	2:3	
6	$HN{=}CHNH_2{\cdot}AcOH$ (1.5 equiv.), ethanol	77 °C	4.5 d	n/a [a]	[88]
7	formamide/formic acid/DMF (4:1:2)	80 °C	10 d	43:7	[89]
8	NaOMe, 25 wt% in MeOH (2.0 equiv.) formamide (20 equiv.)	90 °C	40 h	product **46** only	[90]

[a] precipitation during the reaction, the ratio was not determined by HPLC-MS.

Reducing the temperature from 150 °C to 100° C resulted in the formation of **46** without decomposition. After 66 hours, a substantial amount of **45** remained. (**Table 1**, entry 2) When using ammonium formate as an additive in formamide at 170 °C, decomposition was observed after 19 hours. The same conditions at 80 °C resulted in **46** with poor conversion. These four conditions (**Table 1**, entries 1–4) demonstrated that reduced heating had to be employed to avoid decomposition. The reaction in formamide with molecular sieves (3 Å) to trap the formed ethanol gave the desired **46** after 10 days at 80 °C with moderate conversion.[57] (**Table 1**, entry 5) The reaction with formamidinium acetate ($HN{=}CHNH_2{\cdot}AcOH$) in ethanol at 77 °C resulted in precipitation. Filtration and analysis by ^{1}H NMR revealed that the precipitate was not the desired product. (**Table 1**, entry 6) When using formamide, formic acid and DMF with the ratio 4:1:2, only a small amount of product **46** was formed after 10 days. (**Table 1**, entry 7) Finally, the reaction in formamide with NaOMe as an additive resulted in the full consumption of **45** after 40 hours at 90 °C. (**Table 1**, entry 8) During my literature search, I also came across some conditions that asked for triethyl orthoformate.[91] I did not try these after obtaining the results depicted in **Table 1**.

To obtain an optimal yield, the powdered **45** was treated with NaOMe (25 wt% in MeOH) and sonicated for 10 min to improve solubility. Formamide was added and the mixture was stirred at 90 °C for 48 hours. After neutralization and precipitation, the product **46** was obtained in only 46% yield despite full consumption of the starting material (confirmed by HPLC-MS). After the filtration step, the mother liquor was analyzed by HPLC-MS and only small amounts of product were detected. Thus, it is likely that the lower yield can be explained by the formation of a side product which I was not able to identify.

The last step towards the desired nucleobase **47** is the chlorination of **46** in position 8.[92] (**Scheme 11**) Accordingly, **46** was treated with an excess of $POCl_3$ in the presence of BTEA-Cl and *N*,*N*-dimethylaniline in acetonitrile giving the nucleobase **47** in 86% yield. It was essential that the $POCl_3$ was freshly distilled. During work-up, the mixture was slowly treated with diluted ammonia (1 M in water) until pH 4–5 to avoid accidental amination of **47** in position 8.

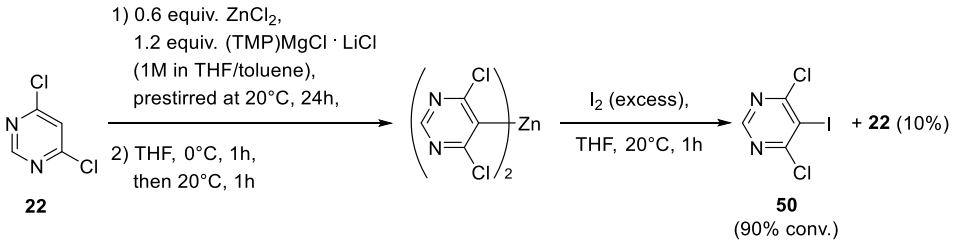
In short, the starting material **43** required for the classical heterocyclization (Approach A) was prepared in two steps from the commercially available 4-nitropyrazole **41** in 81% yield. (**Scheme 9**) Then, the nucleobase **47** was prepared with 21% yield over four steps. (**Scheme 10** and **Scheme 11**)

3.1.1.2. Cross-Coupling & Cyclization (Approach B)

The second approach to the nucleobase **47** consists of three steps: (1) Negishi cross-coupling of the zincated "western" pyrimidine ring with the iodo derivative of the "eastern" pyrazole ring forming the "northern" bond of the future pyrrole ring, (2) azidation and (3) cyclization of the "central" pyrrole ring.

Under standard conditions, the Negishi cross-coupling between the commercially available 5-iodo-1-methyl-pyrazole (**48**) and the zincated 4,6-dichloropyrimidine **22** in the presence of 5 mol% $Pd(PPh_3)_4$ gave the cross-coupling product **49** with low conversion (24%). Hence, an optimization of the reaction conditions was pivotal:

First, I confirmed that the zincation of **22** with $(TMP)_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$, a Turbo-Hauser base, was indeed successful. The base itself was prepared *in situ* from $ZnCl_2$ and a solution of (TMP)MgCl·LiCl. Usually after stirring the mixture for 12 hours, the $ZnCl_2$ was completely dissolved. If this was not the case, I sonicated the mixture for 5 min to improve the solubility of $ZnCl_2$. In the end, the mixture was stirred for at least 8–10 hours without any visible solids present before adding **22**. As the zincated pyrimidine cannot be isolated in this form, I treated a sample of the zincated pyrimidine with large excess of I_2 in THF, followed by stirring for 1 hour at 20 °C. Then, I performed the same work-up as published by Prof. P. Knochel and his team.[93] ^{1}H NMR of the crude mixture in $CDCl_3$ agreed well with the published data when looking at the H-2 signal of the pyrimidine ring: 90% of the zincated pyrimidine was converted to the 4,6-dichloro-5-iodopyrimidine **50** ($\delta_{H\text{-}2}$ = 8.65 ppm) and 10% did not react resulting in the starting **22** after the work-up with water ($\delta_{H\text{-}2}$ = 8.81 ppm). This corresponds well with the published yield of 91% for 4,6-dichloro-5-iodopyrimidine **50**.[93] (**Scheme 12**)



Scheme 12. Confirmation of the zincation step by trapping the zincated pyrimidine with excess of iodine.

Then, a detailed reaction screening was performed. I first looked at different catalytic systems (**Table 2**, entries 1–6), then at different catalyst loadings of $Pd(PPh_3)_4$ (**Table 3**, entries 2–4). All reactions were performed in THF at 65 °C for 19 hours. After extraction with EtOAc and concentration *in vacuo*, the conversion of the reaction mixture was determined by ^{1}H NMR in $CDCl_3$ by comparing H-2 in the pyrimidine moiety as these signals are very distinct. The signal of H-2 in the starting material **22** is at $\delta_{H\text{-}2}$ = 8.81 ppm. The signal of H-2 in the cross-coupling product **49** is shifted downfield ($\delta_{H\text{-}2}$ = 8.88 ppm).

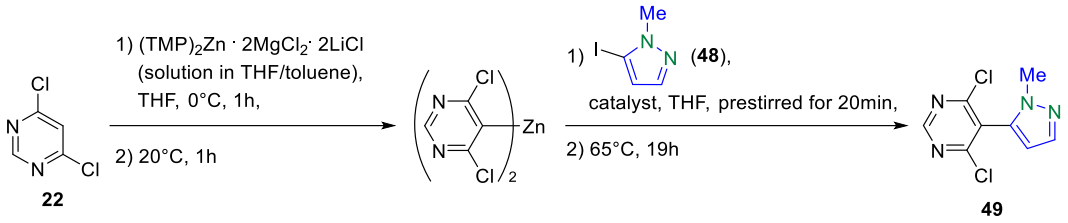


Table 2. Screening of the Negishi cross-coupling reaction between **48** and zincated **22** with the corresponding catalyst system in THF at 65 °C for 19 hours towards **49**. The conversion from **22** to **49** was determined by ^{1}H NMR in $CDCl_3$.

Entry	**22** [equiv.]	**48** [equiv.]	Catalytic system	**49** (conv.)	unreacted **22**
1	1.0	1.1	5 mol%. $Pd(PPh_3)_4$	24%	42%
2	1.0	1.1	5 mol% $Pd_2(dba)_3$, 10 mol% JohnPhos	3%	61%
3	1.0	1.1	5 mol% $Pd_2(dba)_3$, 10 mol% XPhos	4%	28%
4	1.0	1.1	5 mol% $Pd_2(dba)_3$, 10 mol% P(*t*Bu)$_3$ · HBF_4	4%	75%
5	1.0	1.1	5 mol% PEPPSI-IPr	0%	71%
6	1.0	1.1	5 mol% Pd(dppf)Cl_2, 10 mol% DIBAL-H	0%	62%

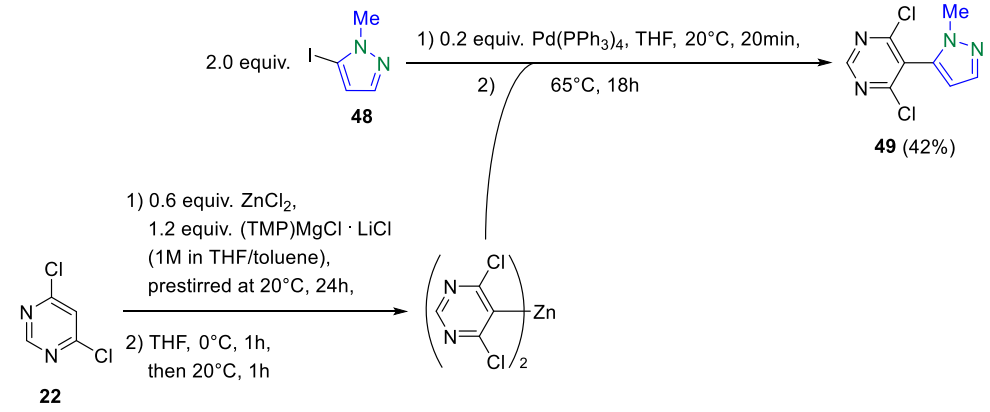
As mentioned above, the Negishi coupling between **48** and zincated **22** with the initial catalytic system (5 mol% $Pd(PPh_3)_4$) resulted in **49** with only 24% conversion. (**Table 2**, entry 1) Next, I tried $Pd_2(dba)_3$ as a palladium(0) source with 3 different phosphine ligands giving **49** with marginal conversion of 3–4%. (**Table 2**, entries 2–4) Additionally, I also tried PEPPSI-IPr and Pd(dppf)Cl_2 (reduced by DIBAL-H) as catalysts, but these reactions did not proceed at all. (**Table 2**, entries 5 and 6)

Finally, I decided to use the pyrazole **48** in excess (2.0 equiv.) and increase the catalyst loading from 5 mol% to 20 mol% of $Pd(PPh_3)_4$ resulting in 45% of **49** after purification. (**Table 3**)

Table 3. Screening of the Negishi cross-coupling reaction between **48** and zincated **22** with catalyst loading in THF at 65 °C for 19 hours towards **49**.

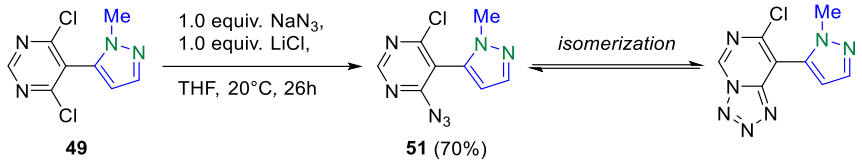
Entry	**22** [equiv.]	**48** [equiv.]	Catalytic system	**49** (yield)
1	1.0	1.1	5 mol%. $Pd(PPh_3)_4$	10%
2	1.0	2.0	5 mol% $Pd(PPh_3)_4$	14%[a]
3	1.0	2.0	10 mol% $Pd(PPh_3)_4$	34%[a]
4	1.0	2.0	20 mol% $Pd(PPh_3)_4$	45%[a]

With these results in hand, the optimized cross-coupling reaction between **48** and **22** was performed at a larger scale. (**Scheme 13**) First, $ZnCl_2$ was treated with a solution of (TMP)MgCl·LiCl resulting in the Turbo-Hauser base $(TMP)_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$ which was used to zincate 4,6-dichloropyrimidine **22**. Then, the *in situ* formed zincated pyrimidine underwent Negishi coupling with 5-iodo-1-methylpyrazole (**48**) in presence of $Pd(PPh_3)_4$ at 65 °C for 18 hours giving the coupling product **49** in 20–50% yield depending on the reaction scale.



Scheme 13. Optimized Negishi cross-coupling towards **49**.

The second step in the reaction cascade towards the nucleobase **47** was the nucleophilic substitution: The Negishi cross-coupling product **49** was treated with NaN_3 and LiCl in THF giving **51** in 70% yield. (**Scheme 14**)



Scheme 14. Azidation of **10** with NaN_3 and LiCl in THF resulting in **51**.

Working with inorganic azides (e.g. NaN_3) can be dangerous. So, I took the following precautions: (1) I used a plastic spatula instead of a metallic one, (2) I used a fresh resp. designated stirring bar for this reaction as any metal residues from a previous reactions could form highly explosive, shock-, friction- and static-sensitive azide salts and (3) I did not work with anything in parallel involving halogenated solvents to avoid any accidental contact as e.g. in the presence of DCM, inorganic azides can easily form diazidomethane which is highly explosive and shock-sensitive.[94] Additionally, exact weighing is required as even a small excess of NaN_3 can lead to double azidation due to the simila reactivity of the chloro-positions in **49** and in **51**. To avoid any other side reactions or decomposition of the product **51**, the reaction vessel was wrapped in aluminium foil and the reaction was run under light seclusion. Even product **51** had to be handled with care as with a molecular formula of $C_8H_5N_7Cl$ its characteristic C/N ratio is close to 1 which is an indicator for potentially explosive materials that should not be concentrated.[95]

The product **51** can be present in its azide **51a** or its tetrazole form **51b**, the equilibrium between these isomers is highly dependent on the environment.[59] This can be easily observed during ^{1}H NMR measurements, where the azide **51a** occurs exclusively in $CDCl_3$; but in DMSO-d_6, the tetrazole **51b** is the major form. This can be best observed by looking at the shift of H-2 in the pyrimidine moiety. (**Table 4**)

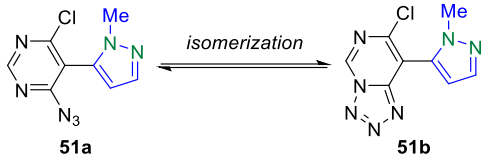


Table 4. ^{1}H NMR measurements to determine the azide/tetrazole equilibrium of **51**.

Entry	Measurement in	azide **51a** ($\delta_{H\text{-}2}$ [ppm])	tetrazole **51b** ($\delta_{H\text{-}2}$ [ppm])	ratio **51a** : **51b**
1	$CDCl_3$	8.75[a]	-	100 : 0
2	DMSO-d_6	8.94	10.35	23 : 77

[a] The presence of the azide **51a** in $CDCl_3$ was confirmed by IR measurement ($\tilde{\nu}$ = 2145 cm^{-1}).

The third and final step of Approach B is the cyclization and formation of the "central" pyrrole ring. Three different cyclization procedures were used in our group: (1) thermal cyclization in 1,4-dibromobenzene,[61] (2) rhodium-catalyzed cyclization,[62,63] and (3) photocyclization in TFA.[61b,64] (**Table 5**)

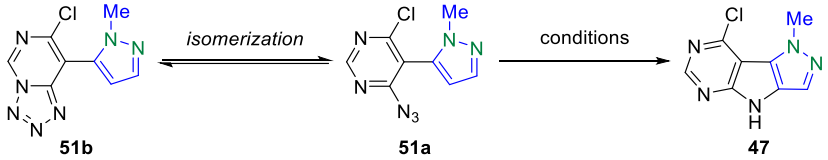


Table 5. Photo-, thermal and Rh^{II}-catalyzed cyclization of **51** towards the tricyclic nucleobase **47**.

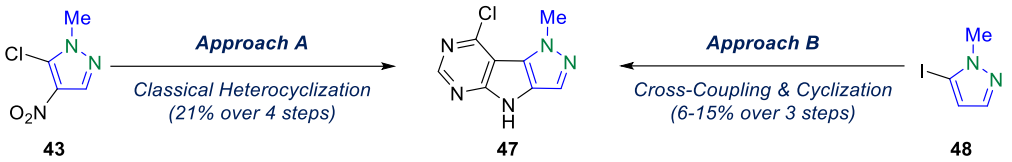
Entry	Conditions	Temp.	Time	Yield
1	1,4-dibromobenzene (500 w%)	170 °C	0.5 h	44% (30% of **51** recovered)
2	10 mol% Rh_2esp_2, molecular sieves (4 Å), toluene/TFA (1:1)	90 °C	24 h	- (no reaction)
3	UV (254 nm, 4 W), TFA	20 °C	48 h	- (decomposition)

The thermal cyclization of **51** in 1,4-dibromobenzene at 170 °C for 30 min gave **47** with 44% yield (60% pure). The purification by HPFC was tedious due to the poor solubility of **47**. (**Table 5**, entry 1) The catalytic cyclization with Rh_2esp_2 in toluene/TFA (1:1) did not proceed at all while the photocyclization with UV light (254 nm, 4 W) in TFA resulted in decomposition. (**Table 5**, entry 2 and 3)

In short, using the cross-coupling and cyclization approach (Approach B) gave nucleobase **47** with 6–15% yield over three steps. (**Scheme 13**, **Scheme 14** and **Table 5**)

3.1.1.3. Summary

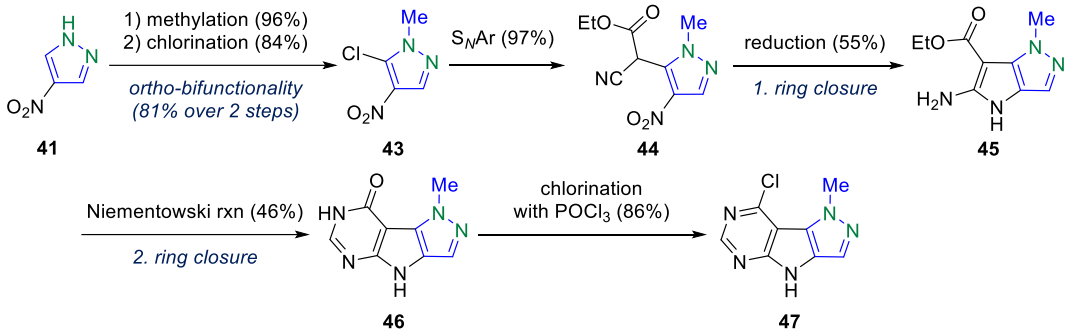
After a detailed discussion of both synthetic procedures (the classical heterocyclization approach A in **Chapter 3.1.1.1** and the cross-coupling/cyclization approach B in **Chapter 3.1.1.2**), both strategies are summarized, and the main advantages and disadvantages of each strategy are discussed. (**Scheme 15**)



Scheme 15. Synthetic strategy for the formation of the nucleobase **47**: Approach A requires four steps, approach B only three steps.

Approach A

The starting material **3** with the required chloro-nitro-bifunctionality is not commercially available and has to be prepared (81% over 2 steps). Both, the methylation and the chlorination with C_2Cl_6 have been performed at a multi-gram scale (20 g of **41** resp. 22 g of **42**) and only require a short chromatographic purification each. (**Scheme 16**)

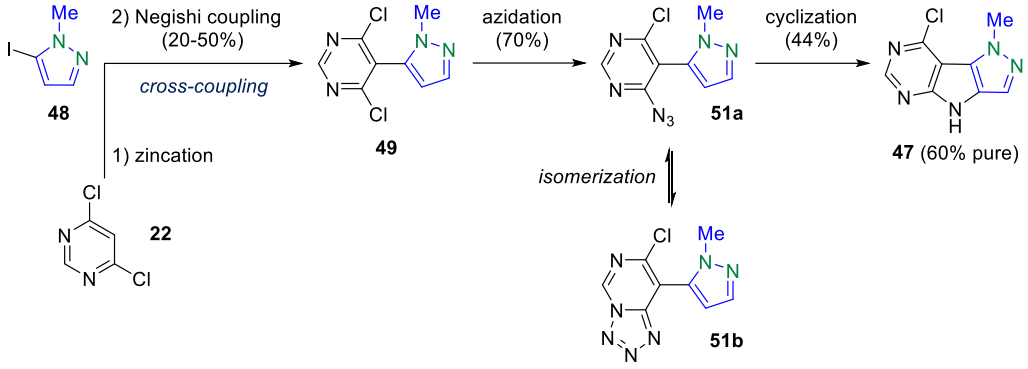


Scheme 16. Approach A – Synthesis of the nucleobase **7** from **3** in 4 steps (21% overall) or from the commercially available **1** in 6 steps (18% overall).

The nucleophilic aromatic substitution of **43** was performed at a multi-gram scale as well (10 g of **43**) and required vacuum distillation and solidification in the freezer followed by a simple filtration. The reduction of the nitro group followed by spontaneous cyclization during work-up was performed with 4 g of **44** and required a simple filtration after precipitation in the fridge overnight. Non-precipitated product during the filtration was recovered together with the filtrate, reduced in volume *in vacuo*, neutralized, and subjected to precipitation again. Both, the Niementowski reaction resulting in the second ring closure and the chlorination with $POCl_3$ were performed at a smaller scale (< 1 g each) and only required filtration after precipitation in the fridge overnight. The yield for this classical heterocyclization approach from the chloro-nitro bifunctionalized pyrazole **43** to the tricyclic nucleobase **47** was 21% over 4 steps. The overall yield for this reaction cascade from the commercially available 4-nitropyrazole **41** was 18% over 6 steps. (**Scheme 16**)

Approach B

The required iodo heteroaryl derivative **48** for the Negishi cross-coupling reaction is commercially available and the zincated species of **22** was prepared *in situ*. The reaction itself requires a large excess of **8** (2 equiv.) and high catalyst loading (20 mol% $Pd(PPh_3)_4$) and the purification is tedious. A large batch with 4 g of **48** and 1.6 g of **22** gave **49** with 42% yield. (**Scheme 17**)



Scheme 17. Approach B – Synthesis of the nucleobase **47** from the commercially available **48** in three steps (6–15% overall).

The azidation of **51** requires equimolar amounts of NaN_3: Any excess of NaN_3 could lead to undesired azidation of both chloro positions. For safety reasons, the reaction was performed in smaller batches and multi-gram reactions are not advisable. Several cyclization options are available and only the thermal cyclization of **51** gave the nucleobase **47** successfully. The purification was difficult and the resulting product **47** was only 60% pure. The yield for this cross-coupling and cyclization approach from **48** to the tricyclic nucleobase **47** was 6–15% yield over 3 steps. (**Scheme 17**)

Comparison: Approach A vs. Approach B

Even though approach B is shorter (3 steps) than approach A (4 resp. 6 steps), the overall yield of approach A is higher (21% resp. 18%) than approach B (6–15%). Additionally, the large-scale synthesis of nucleobase **47** using approach A is far less problematic as no chromatography is required and the synthesis does not involve a nitrogen-rich intermediate for the potentially hazardous thermal cyclization (Approach B, step 3). (**Table 6**)

Table 6. Comparison of both synthetic approaches towards the tricyclic nucleobase **47**.

	Approach A	Approach B
Strategy (overall yield)	Classical heterocyclization (21% over 4 steps or 18% over 6 steps)	Cross-coupling and cyclization (6–15% yield over 3 steps)
Key steps	1) Aromatic substitution 2) Reduction and 1. ring closure 3) Niementowski rxn (2. ring closure) 4) Chlorination with $POCl_3$	1) Negishi coupling 2) Azidation 3) Thermal cyclization
Advantages:	+ large-scale synthesis unproblematic + no chromatography required, only precipitation and filtration	+ shorter reaction cascade (3 steps) + milder reaction conditions
Disadvantages:	– starting material not commercially available (synthesis: 81% yield over 2 steps) – longer reaction cascade (4 resp. 6 steps)	– tedious chromatography after each step – Negishi coupling requires high catalyst loading and large excess of **48** – nitrogen-rich intermediate **51** (C/N = 1.14!) – nucleobase **47** is not pure

rxn = reaction

In approach A, there are two key elements in the synthesis of the nucleobase that strongly depend on the used heterocycle: (1) installation of the chloro-nitro *ortho*-bifunctionality and (2) the Niementowski reaction resulting in the 2. ring closure and formation of the heteroaryl-fused 7-deazahypoxanthine. As the pyrazole-moiety is already a highly deactivated ring system, other heterocyclic systems might pose fewer problems in this step.

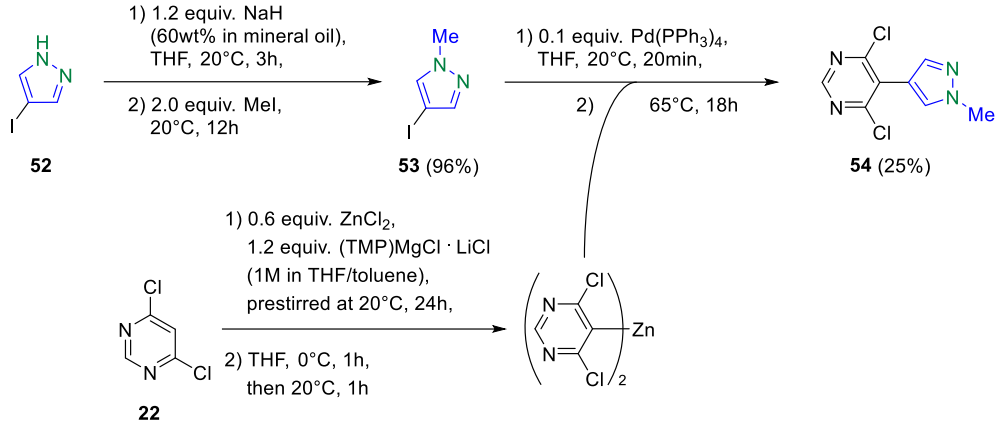
In approach B, the Negishi cross-coupling and the cyclization reactions are both crucial for the success of the strategy. Especially for small heterocycles, the nitrogen-rich azide might pose a safety risk and has to be handled with care by an experienced chemist. For this pyrazolo ring system, thermal cyclization was the only option otherwise any other cyclization option is definitively to be preferred.

In conclusion, there are two different synthetic strategies to obtain the heteroaryl-fused 7-deazapurine nucleobase. (**Scheme 15**) For the tricyclic pyrazolo-fused nucleobase **47**, the classical heterocyclization (Approach A) is longer but results in better yield overall with less tedious purification. However, this comparison for the synthesis of pyrazolo-fused 7-deazapurine **47** cannot be generalized for all heterocycles and their synthesis towards their respective heteroaryl-fused 7-deazapurines.

3.1.1.4. Isomeric pyrazolo-fused Nucleobase

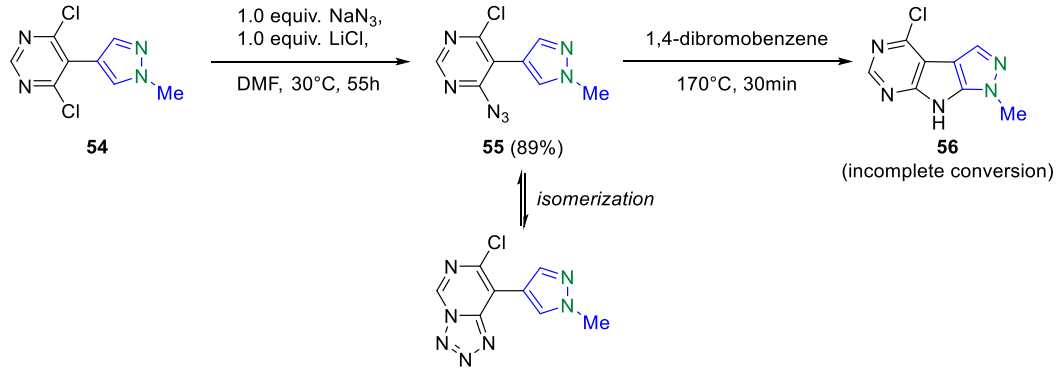
Next, I also attempted to prepare the isomeric pyrazolo-fused 7-deazapurine using the cross-coupling and cyclization approach B.

First, 4-iodopyrazole (**52**) was methylated with iodomethane in the presence of sodium hydride (60% dispersion in mineral oil) in THF furnishing **53** in 96% yield.[96] Then, the Negishi cross-coupling between **53** and the zincated pyrimidine **22** furnished **54** in 25% yield. (**Scheme 18**) I also tried the same reaction conditions and CuI (0.05 equiv.) but it didn't improve the outcome.[97]



Scheme 18. Methylation of **52** and subsequent Negishi cross-coupling of **53** with zincated **22** gave **54** in 25% yield.

After the azidation of **54** with equimolar amounts of sodium azide and LiCl in DMF at 30 °C for 55 hours, the reaction mixture was treated with water. Precipitation and filtration gave the desired azide **55** in 89% yield. (**Scheme 19**)



Scheme 19. Azidation of **54** and cyclization of **55** furnished the desired nucleobase **56**.

The initial test reactions for the cyclization of **55** were performed with the same conditions as for **47** (**Table 7**).

Table 7. Photo-, thermal and Rh^{II}-catalyzed cyclization of **55** towards the tricyclic nucleobase **56**.

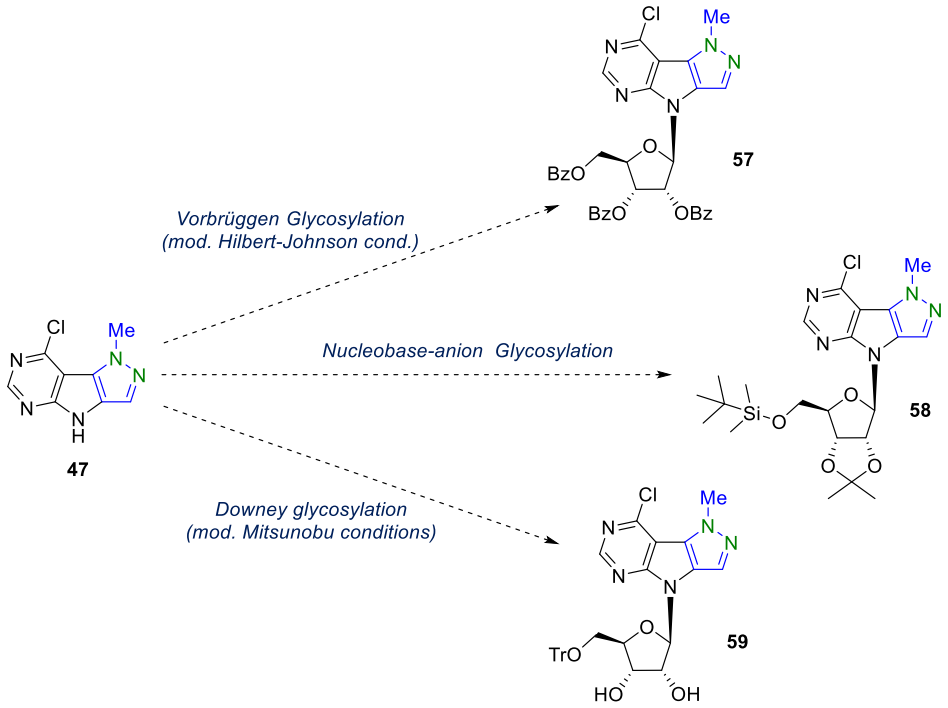
Entry	Conditions	Temp.	Time	Outcome
1	1,4-dibromobenzene (500 w%)	170 °C	0.5 h	incomplete reaction (ratio **55**:**56** = 7:3)
2	10 mol% Rh_2esp_2, molecular sieves (4 Å), toluene/TFA (1:1)	90 °C	24 h	- (no reaction)
3	UV (254 nm, 4 W), TFA	20 °C	48 h	- (decomposition)

Only the thermal cyclization of **55** in 1,4-dibromobenzene at 170 °C gave **56**. ^{1}H NMR analysis of the reaction mixture in DMSO-d_6 after 30 min revealed the reaction was

incomplete and the ratio of **55**:**56** was 7:3. A common side reaction is the complex polymerization of **55**. Its characteristic is the formation of dark insoluble mass.[98] Hence, longer reaction times do not necessarily lead to higher amounts of **56**. (**Table 7**, entry 1) The reaction of **55** with Rh_2esp_2 in toluene/TFA (1:1) did not proceed and the photocyclization of **55** in TFA resulted in decomposition. (**Table 7**, entries 2 and 3)

3.1.2. Glycosylation towards the protected Ribonucleoside as a key Intermediate

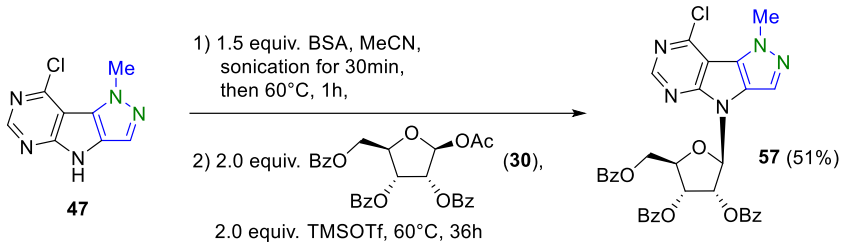
With the pyrazolo-fused 7-deazapurine **47** in hand, three different glycosylation procedures were tested: (1) The Vorbrüggen glycosylation is a modified Hilbert-Johnson reaction where the silylated nucleobase reacts with a benzoyl-stabilized sugar cation. (2) The Nucleobase-anion glycosylation uses the deprotonated nucleobase for an S_N2 reaction with an *in situ*-formed halosugar with a chloride acting as a leaving group. (3) The recently developed Downey glycosylation uses modified Mitsunobu conditions for the S_N2 attack of the deprotonated nucleobase on an epoxide formed *in situ* from a tritylated sugar. (**Scheme 20**)



Scheme 20. Glycosylation strategies to obtain the pyrazolo-fused 7-deazapurine *β*-ribonucleosides **57-59**.

3.1.2.1. Vorbrüggen glycosylation (modified Hilbert-Johnson reaction)

The Vorbrüggen procedure for *N*-glycosylation of the nucleobase **47** can be used, resulting in the desired ribonucleoside **57** as a pure *β*-anomer. This method is based on the Hilbert-Johnson reaction and gives very reliable results.[99] (**Scheme 21**) It has been used successfully with all heteroaryl-fused 7-deazapurines prepared in our group.[44–48,50–53]



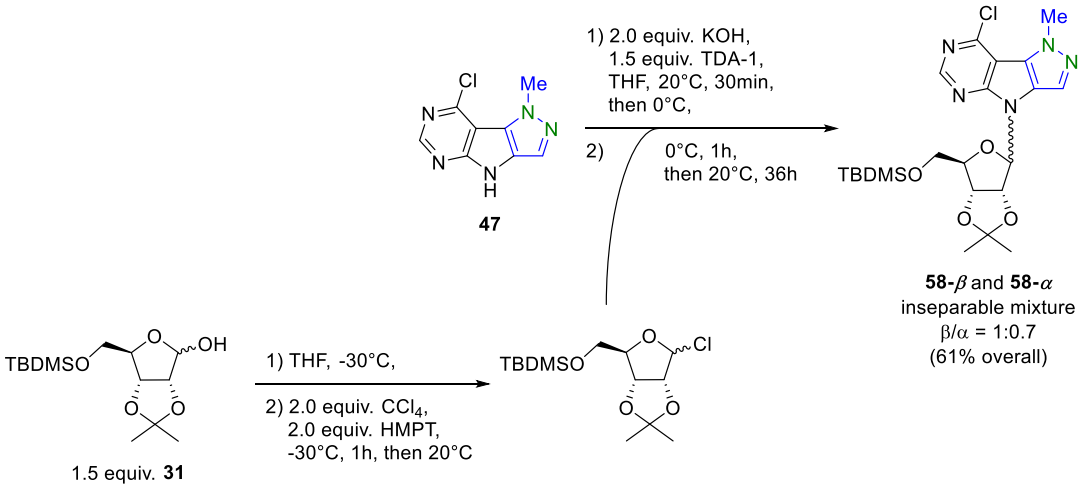
Scheme 21. Vorbrüggen glycosylation of nucleobase **47** towards the pyrazolo-fused 7-deazapurine ribonucleoside **57** as a pure *β*-anomer.

The nucleobase **47** was silylated in position 4 with BSA and reacted with the protected sugar **30** in the presence of TMSOTf furnishing the desired ribonucleoside **57** as a pure *β*-anomer in 51% yield. The complete silylation of the nucleobase **57** with BSA is essential for the success of the glycosylation reaction and is normally done at r.t. but it can be challenging. I tried several options: Longer silylation times at r.t. did not improve the outcome. A larger excess of BSA (2 equiv. instead of 1.5 equiv.) led to unwanted silylation of nitrogen in the pyrimidine ring. In the end, I prepared the silylation mixture, sonicated the suspension for 30 min and stirred it at 60 °C for 1 hour resulting in a homogenous solution before continuing with the addition of the protected sugar **30**.

The stereochemistry of **57** was confirmed by H,H-ROESY using the relations between H-3 of the nucleobase and H-2’ and H-3’ as well as between H-1’ and H-4’ of the sugar moiety.

3.1.2.2. Nucleobase-anion glycosylation

In one previous case, the nucleobase-anion glycosylation was performed for fused 7-deazapurines.[100] Unfortunately, this procedure is not stereoselective as the halosugar formed *in situ* is not anomerically pure. (**Scheme 22**)



Scheme 22. Nucleobase-anion glycosylation of **47** furnishing the pyrazolo-fused 7-deazapurine *β*-ribonucleoside **58-*β*** in an inseparable mixture with the *α*-anomer **58-*α***.

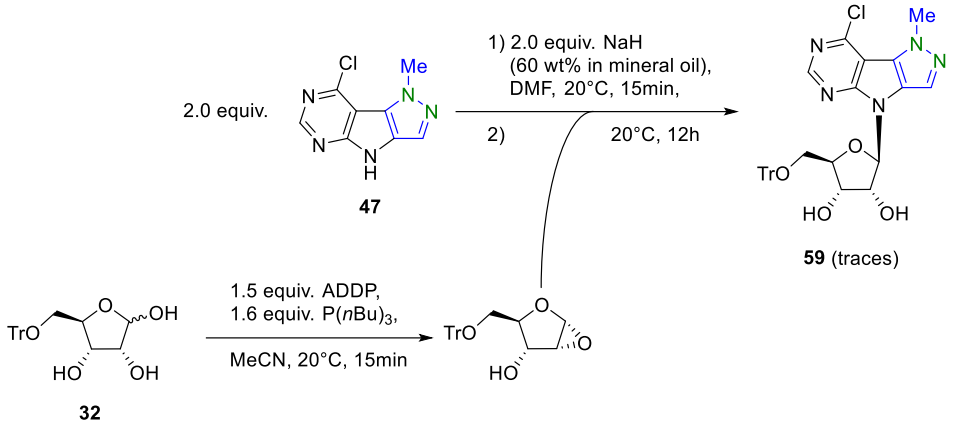
Nucleobase **47** was deprotonated with KOH in the presence of TDA-1. Separately, the halosugar precursor **31** was dissolved in THF and treated with CCl_4 and HMPT at –30 °C for 1 hour before the addition of the deprotonated nucleobase solution. After purification by

HPFC, the desired ribonucleoside **58-*β*** was obtained in an inseparable mixture with its corresponding *α*-anomer **58-*α*** with 61% overall yield (*β*/*α* = 1:0.7). I was not successful in separating the anomers, but I was able to collect a few early fractions for the characterization of the desired *β*-anomer **58-*β***.

The stereochemistry of **58-*β*** was confirmed by H,H-ROESY using the same relations between H-3 of the nucleobase and H-2' and H-3' as well as between H-1' and H-4' of the sugar moiety as above.

3.1.2.3. Downey glycosylation (modified Mitsunobu conditions)

A previous co-worker in our group, A. Michael Downey, developed a novel glycosylation method for pyrimidine, purine and 7-deazapurine bases with 5-*O*-protected ribose (**32**). So, I attempted these modified Mitsunobu conditions with the nucleobase **47** to see whether the substrate scope could be extended to tricyclic nucleobases. (**Scheme 23**)

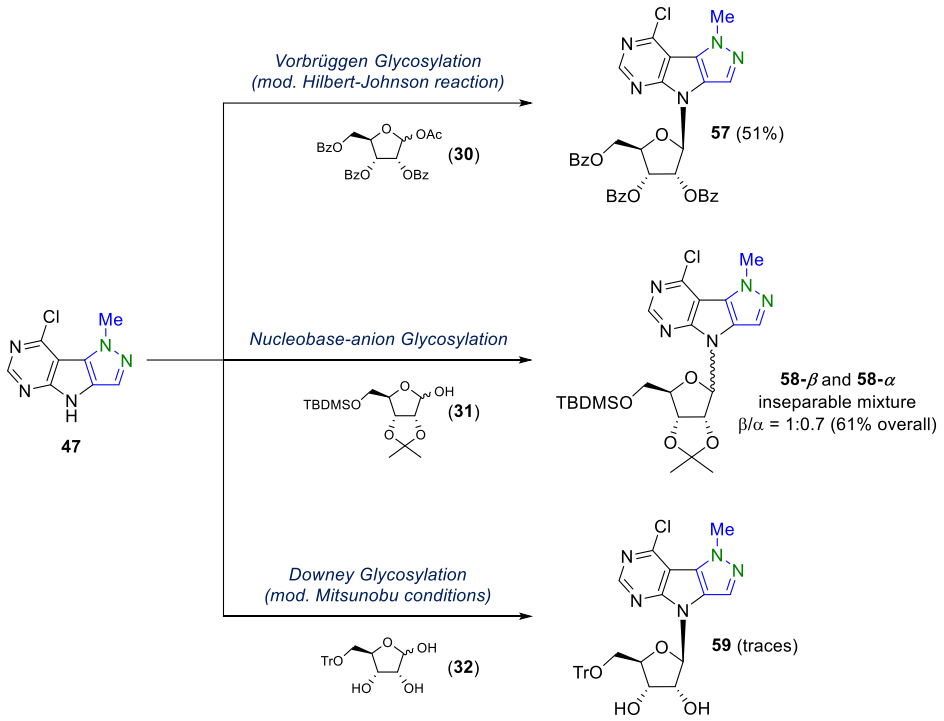


Scheme 23. Strategy for the glycosylation of **47** was developed by A. M. Downey based on modified Mitsunobu conditions.

5-*O*-trityl ribose (**32**) was treated with ADDP and tributylphosphine in MeCN forming the epoxide intermediate which then reacted with the previously deprotonated nucleobase **47**. After purification, only a small fraction contained traces of the desired ribonucleoside **59**. Unfortunately, these results were not reproducible.[101]

3.1.2.3. Summary

The pyrazolo-fused and sugar-protected β-ribonucleosides **57-59** were prepared from nucleobase **47** via different *N*-glycosylation methods. The Vorbrüggen procedure yielded the desired nucleoside **57** as a pure β-anomer in 51% yield. The nucleobase-anion glycosylation gave the desired nucleoside **58-*β*** together with its α-anomer **58-*α*** in an inseparable mixture in the ratio of β/α = 1:0.7 with 61% yield overall. The Downey glycosylation resulted in traces of **59** only. (**Scheme 24**)



Scheme 24. Overview of the tested glycosylation options.

The protected sugar **30** for the Vorbrüggen glycosylation is commercially available. The halosugar precursor **31** used in the nucleobase-anion glycosylation can be prepared from D-ribose in two steps (multi-gram scale).[74] The chromatographic purification after each step has to be done by hand as the product is not UV active. The tritylated sugar **32** used for the Downey glycosylation can be prepared from D-ribose in one step at a multi-gram scale.[75]

In conclusion, the Vorbrüggen glycosylation was the only method which furnished the ribonucleoside as a pure β-anomer with acceptable yield. Additionally, the required sugar **30** is commercially available and does not need to be prepared from D-ribose.

3.1.3. Derivatization and Deprotection

The key nucleoside intermediate **18** was used for the derivatization in position 8 and deprotection to give a series of different 8-substituted free ribonucleosides **25a-h**. (**Table 8**)

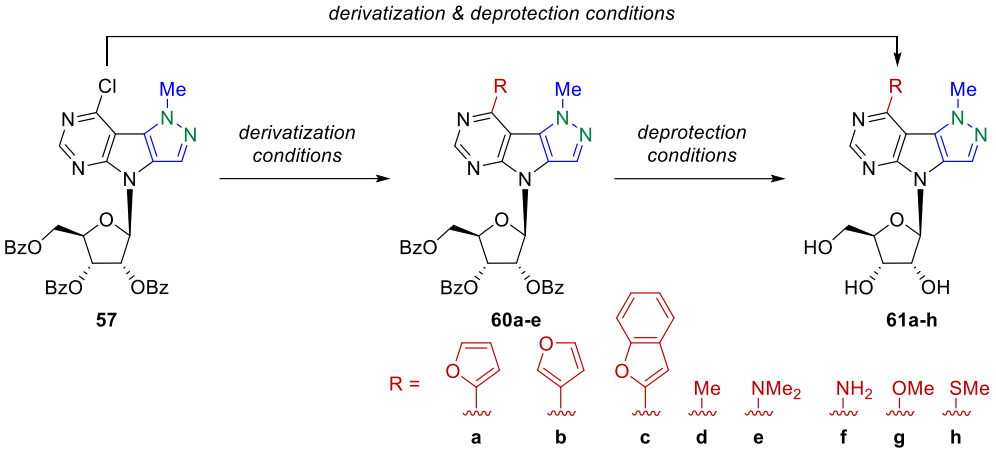


Table 8. Preparation of a library with the ribonucleosides **61a-h** either by stepwise derivatization and deprotection via **60a-e** or by simultaneous derivatization and deprotection directly from **57** (for **61f-h**).

Entry	R =	Conditions	Temp.	Time	Product	Yield
1	2-furyl	1.2 equiv. 2-(tributylstannyl)furan, 0.1 equiv. $PdCl_2(PPh_3)_2$, DMF	100 °C	10 h	**60a**	69%
2	3-furyl	1.5 equiv. 3-furylboronic acid, 2.0 equiv. K_2CO_3, 0.1 equiv. $Pd(PPh_3)_4$, toluene	100 °C	48 h	**60b**	32%
3	2-benzofuryl	1.5 equiv. 2-benzofurylboronic acid, 2.0 equiv. K_2CO_3, 0.1 equiv. $Pd(PPh_3)_4$, toluene	100 °C	48 h	**60c**	38%
4	methyl	2.0 equiv. $AlMe_3$, 0.05 equiv. $Pd(PPh_3)_4$, THF	65 °C	3 h	**60d**	64%
5	dimethylamino	1.5 equiv. Me_2NH, 2-propanol	60 °C	24 h	**60e**	47%
6	2-furyl	0.3 equiv. NaOMe (25 wt% in MeOH), MeOH	60 °C	6 h	**61a**	87%
7	3-furyl			6 h	**61b**	72%
8	2-benzofuryl			6 h	**61c**	98%
9	methyl			4 h	**61d**	73%
10	dimethylamino			6 h	**61e**	98%
11	amino	aq. ammonia/1,4-dioxane (5:2)	120 °C	24 h	**61f**	81%
12	methoxy	2.2 equiv. NaOMe (25 wt% in MeOH), MeOH	25 °C	76 h	**61g**	80%
13	methylsulfanyl	6.0 equiv. NaSMe, 1,4-dioxane	60 °C	14 h	**61h**	51%

The 2-furyl derivative **60a** was prepared in 69% yield via Stille cross-coupling of **57** with 2-(tributylstannyl)furan in the presence of $PdCl_2(PPh_3)_2$ in DMF at 100 °C. (**Table 8**, entry 1) During the purification by HPFC, the organotin residue is usually removed from the column by flushing with immense amounts of cHex prior to running the gradient. Instead, I attempted to remove the Sn species by precipitation first. Based on the publications by the Mascaretti group[102] and Leibner/Jacobus,[103] a solution containing Bu_3SnCl can be treated with KF or with CsF/CsOH resulting in the formation of the insoluble organotin fluoride which then can be removed by simple filtration. Thus, I concentrated the reaction mixture *in vacuo*, dissolved

it in EtOAc and treated it with 1.2 equiv. of KF in MeOH. Unfortunately, the expected precipitation was not observed. I then continued with the purification by HPFC (SiO_2; cHex/EtOAc, gradient 0→100% EtOAc) unfortunately with the initial cHex washing step.

The Suzuki-Miyaura cross-coupling of **57** with 3-furyl- or with 2-benzofurylboronic acid in the presence of K_2CO_3 and $Pd(PPh_3)_4$ in toluene at 100 °C for 48 h gave **60b** and **60c** in moderate yields (32% and 38%, respectively). (**Table 8**, entries 2 and 3) The methyl derivative **60d** was prepared by cross-coupling reaction of **57** with $AlMe_3$ and $Pd(PPh_3)_4$ in THF at 65 °C for 3 h in good 64% yield. (**Table 8**, entry 4) The *N*,*N*-dimethylamino derivative **60e** was obtained by nucleophilic substitution of **57** with dimethylamine in 2-propanol at 60 °C for 24 h in 47% yield. (**Table 8**, entry 5)

The protected nucleoside derivatives **60a-e** underwent the Zemplén deprotection of the sugar moiety with NaOMe in methanol at 60 °C for 4–6 h and gave the free nucleosides **61a-e** in very good to excellent yields (72–98%). (**Table 8**, entries 6–10)

The preparation of **61f-h** included the nucleophilic substitution at position 8 directly with simultaneous deprotection. The amino derivative **61f** was obtained in 81% yield through treatment of **57** with aqueous ammonia/1,4-dioxane (5:2) at 120 °C for 24 h in a screw-cap pressure tube. (**Table 8**, entry 11) The reaction of **57** with NaOMe in methanol at 25 °C after 76 h gave **61g** in 80% yield. (**Table 8**, entry 12) The methylsulfanyl derivative **61h** was obtained from **57** using NaSMe in 1,4-dioxane at 60 °C for 14 h in 51% yield. (**Table 8**, entry 13) I first performed this reaction in MeOH but I obtained **61h** together with **61g** as an inseparable mixture.

3.2. Aryl Sulfonium salts as an Alternative Substrate for Negishi Coupling

Most tricyclic nucleobases containing one heteroatom in the "eastern" 5-membered ring have been synthesized using the corresponding iodo derivatives as a starting material for the cross-coupling and cyclization approach (Approach B, discussed in **Chapter 3.1.1.2**). (**Figure 17**)

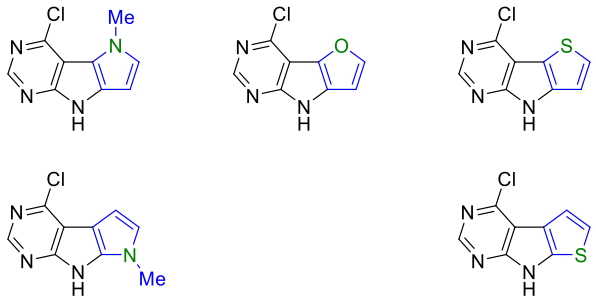


Figure 17. Previously prepared 5-membered heteroaryl-fused 7-deazapurines with the "eastern" ring containing only one heteroatom.

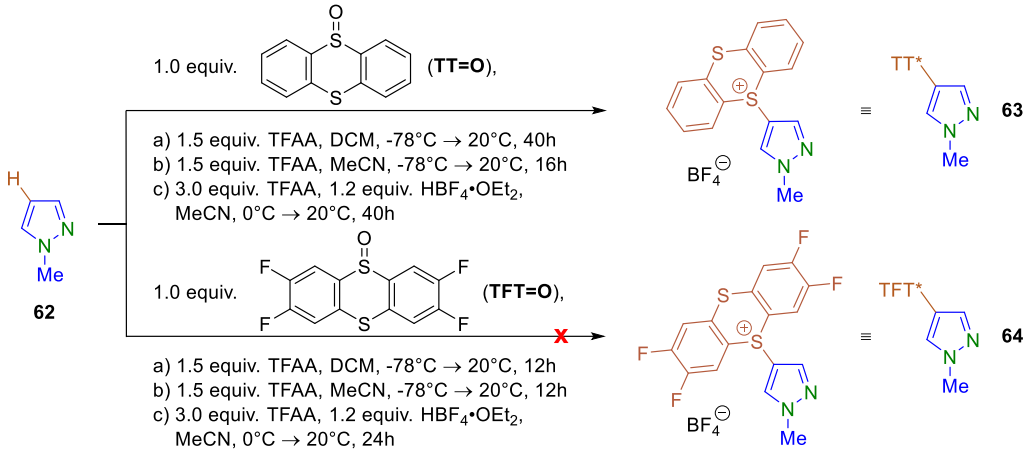
To expand the library of fused-deazapurine nucleosides further, a second heteroatom is introduced in the "eastern" ring of the tricyclic nucleobase. The formation of the pyrazolo-fused nucleobase has already been discussed in detail in the previous chapter. One of the main problems is the availability and accessibility of the iodo-substituted 5-membered heterocycles required for the Negishi cross-coupling reaction. (see **Table A1**in the Appendix)

Recently, the Ritter group published several papers on the C-H functionalization of (het)arenes through the formation of sulfonium salts and their application as a potential alternative to the corresponding iodo-substituted substrates in palladium-catalyzed cross-coupling reactions.[65–67] The published substrate scope for these sulfonium salts contained a large selection of aryl-substituted 5- and 6-membered (het)aryl rings as well as fused ring systems – but none of the published compounds have been prepared from simple 5-membered heterocycles so far. While my co-worker Chao Yang was working with mostly published examples of aryl sulfonium salts and focused on their application in the Negishi cross-coupling reaction with the zincated pyrimidine **22** and the subsequent preparation of further fused 7-deazapurines; I was tasked to apply the published concepts to prepare the sulfonium salts of simple 5-membered heterocycles.

3.2.1. Thianthrenium Salts from 5-membered Heterocycles

The Ritter group published the formation of sulfonium salts with different sulfoxide species: The thianthrenation of (het)arenes with thianthrene *S*-oxide (TT=O) was the most reliable one. In some cases, the fluorinated analogue 2,3,7,8-tetrafluorothianthrene *S*-oxide (TFT=O) was the better alternative. The required sulfoxides for the reaction screening were prepared according to literature.[65]

For the thianthrenation of nitrogen-containing heterocycles, most published examples were prepared using the following conditions: TT=O or TFT=O either with (a) TFAA in DCM, with (b) TFAA in MeCN, or with (c) TFAA and tetrafluoroboric acid ($HBF_4 \cdot OEt_2$) in MeCN. I applied all six conditions to the thianthrenation of 1-methylpyrazole (**62**). After the reaction, the mixtures were neutralized with satd $NaHCO_3$ and extracted with DCM and washed with aqueous $NaBF_4$ (10 wt% in water). The organic layers were concentrated *in vacuo* and the conversion was determined by ^1H NMR in $CDCl_3$. (**Scheme 25**)



Scheme 25. Preparation of aryl sulfonium salts from 1-methylpyrazole (**62**).

The reaction of **62** with TT=O in the presence of TFAA in DCM resulted in 5% conversion towards the desired sulfonium salt **63**. Thus, the reaction mixture was not purified. The same reaction in MeCN resulted in 66% of **63** after 16 hours. Using a larger excess of TFAA (3 equiv.) as well as tetrafluoroboric acid ($HBF_4 \cdot OEt_2$) in MeCN resulted in full conversion after 40 hours and 86% yield of **63**. The substitution in position 4 was selective and no other isomers were detected. The same reaction of **62** with TFT=O did not proceed to the desired sulfonium salt **64** with any of the conditions used above.

With the successful preparation of **62** in hand, I applied the same conditions to the thianthrenation of other 5-membered heterocycles bearing one or two heteroatoms. After 24 hours, the same work-up was performed as before and then, the crude mixtures were analyzed by ^1H NMR in $CDCl_3$. (**Table 9**)

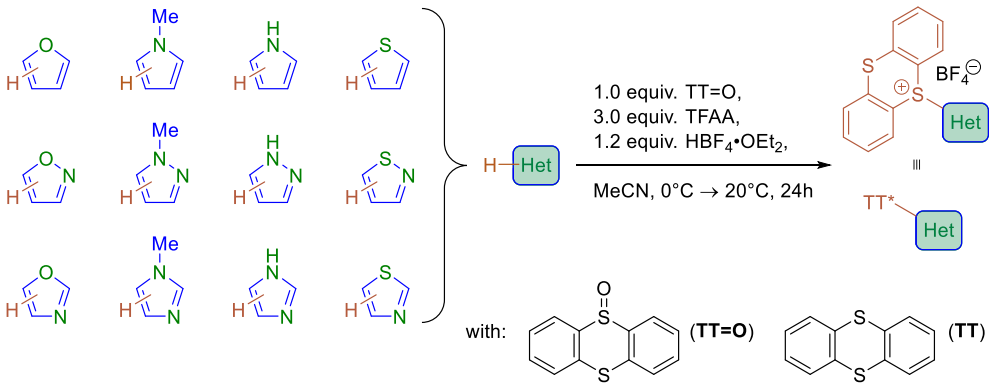


Table 9. Reaction screening with 5-membered heterocycles and TT=O towards their thianthrenium salts. After work-up, the crude mixtures were analyzed by ^{1}H NMR in $CDCl_3$.

Entry	Heterocycle	reaction outcome determined by NMR[a]
1	furan	TT
2	1-methylpyrrole	**65** and TT (4:7)
3	pyrrole	*inconclusive NMR* after HPFC: inseparable mixture of **66** and TT (4:1)
4	thiophene	**67** and TT (10:3)
5	isoxazole	TT=O and TT (1 : 2.45)
6	1-methylpyrazole	*full conversion after 40 hours* after HPFC: **63** (86% at larger scale)
7	pyrazole	TT=O and TT (2 : 0.9)
8	isothiazole	TT=O and TT (1 : 3.5)
9	oxazole	TT
10	1-methylimidazole	TT=O and TT (1 : 14.5)
11	imidazole	TT=O and TT (1 : 0.95)
12	thiazole	TT

[a] NMR presence of the starting heterocycle omitted for clarity, most starting heterocycle are volatile and were removed during the concentration *in vacuo* prior to the NMR measurement.

The reaction with furan resulted in deoxygenation of TT=O giving thianthrene (TT). (**Table 9**, entry 1) The reaction with 1-methylpyrrole resulted in 5-(1-methyl-1*H*-pyrrol-3-yl)-5*H*-thianthren-5-ium tetrafluoroborate (**65**) and TT with a 4:7 ratio.(**Table 9**, entry 2) After the reaction with pyrrole, the ^{1}H NMR of the crude mixture was inconclusive and purification by HPFC gave the 5-(1*H*-pyrrol-3-yl)-5*H*-thianthren-5-ium tetrafluoroborate (**66**) together with thianthrene (TT) as an inseparable mixture (ratio 4:1). (**Table 9**, entry 3) The reaction with thiophene gave 5-(thiophene-2-yl)-5*H*-thianthren-5-ium tetrafluoroborate (**67**) and TT (ratio 10:3). (**Table 9**, entry 4) As these 5-membered heterocycles containing one heteroatom can be accessed through their iodo derivatives for the Negishi coupling, further purification or characterization was not performed.

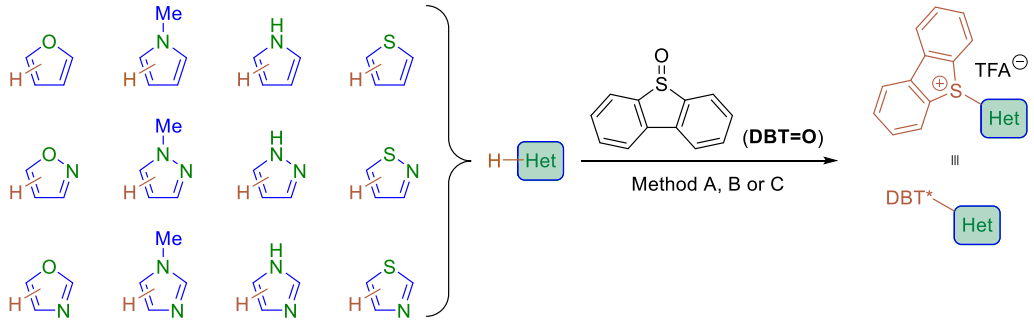
During the reactions involving 5-membered rings with two heteroatoms, (**Table 9**, entries 5–12), no product was formed, instead partial deoxygenation of TT=O towards TT was observed. The only exception was the reaction with 1-methylpyrazole which resulted in full conversion and the isolation of 5-(1-methylpyrazol-4-yl)-5*H*-thianthren-5-ium tetrafluoroborate (**63**) in 86% yield.

In summary, 5-membered heterocycles bearing one heteroatom (furan), two neighboring heteroatoms (isoxazole, pyrazole, isothiazole), or two non-neighboring heteroatoms (oxazole, 1-methylimidazole, imidazole, thiazole) did not react with TT=O in the presence of TFAA and $HBF_4 \cdot OEt_2$. Instead TT=O was fully or partially deoxygenated to TT. However, the thianthrenium salts (**63**, **65-67**) were successfully formed from 1-methylpyrrole, pyrrole, thiophene and 1-methylpyrazole. All the substitutions were regioselective in the most nucleophilic position of the ring. This is in agreement with the published results by the Ritter group.[65,66]

3.2.2. Dibenzothiophenium Salts from 5-membered Heterocycles

Another source for sulfonium salts is dibenzothiophene *S*-oxide (DBT=O). My co-worker Chao Yang successfully employed DBT=O to form the sulfonium salts of aryl-substituted and fused thiophenes. Thus, I speculated that the dibenzothiophenation of other 5-membered heterocycles might be more successful than their thianthrenation.

The initial reaction of 1-methylpyrazole (**62**) with DBT=O (1.0 equiv.), large excess of TFAA (3.0 equiv.) in MeCN, direct warming up from –78 °C to 20 °C and stirring for 24 hours overall, did not give the desired dibenzothiophenium salt **68**. Hence, the reaction screening with the 5-membered heterocycles tested above, was performed using the following three methods: *Method A* used equimolar amounts of DBT=O, a smaller excess of TFAA (1.5 equiv.) and was stirred at –78 °C for 2 h prior to warming up. Compared to method A, *method B* employed the heterocycle using a small excess (1.05 equiv.) and stopped the reaction after stirring for 1 hour at 20 °C. *Method C* used equimolar amounts of triflic anhydride (Tf_2O) instead of TFAA, the reaction times were kept the same as in method B. (**Scheme 26**)



Scheme 26. Formation of dibenzothiophenium salts with DBT=O using three different methods. *Method A*: with DBT=O and TFAA in MeCN at –78 °C for 2 h, then slowly warmed to 20 °C within 2 h and stirred for 48 h; *Method B*: with DBT=O and TFAA in MeCN at –78 °C for 1 h, –78 °C to 20 °C within 2 h, then 20 °C for 1 h; or *Method C*: with DBT=O and Tf_2O in MeCN at –78 °C for 1 h, –78 °C to 20 °C within 2 h, then 20 °C for 1 h.

I started with the same selection of twelve different 5-membered heterocycles bearing one or two heteroatoms and subjected them to dibenzothiophenation with DBT=O and TFAA in MeCN using method A. (**Table 10**)

Table 10. Reaction screening with 5-membered heterocycles and DBT=O towards their dibenzothiophenium salts using *method A*: Heterocycle (1.0 equiv.), DBT=O (1.0 equiv.), TFAA (1.5 equiv.) in MeCN at –78 °C for 2 h, then slowly warmed to 20 °C within 2 h and stirred for 48 h.

Entry	Heterocycle	reaction outcome
1	furan	- [a] (DBT only)
2	1-methylpyrrole	inseparable mixture of **69** and **70** (3:5, 54%) and DBT
3	pyrrole	**71** (7%) and DBT
4	thiophene	**72** (91%) and DBT
5	isoxazole	- [a] (DBT=O only)
6	1-methylpyrazole	**68** (82%) and DBT
7	pyrazole	- [a] (DBT=O only)
8	isothiazole	dimer (no product isolated)
9	oxazole	- [a] (DBT only)
10	1-methylimidazole	- [a] (dimer only)
11	imidazole	- [a] (DBT and dimer, 5:1)
12	thiazole	- [a] (DBT and dimer)

[a] NMR analysis of crude mixture, no HPFC performed

The reaction with furan resulted in deoxygenation of DBT=O yielding dibenzothiophene (DBT). (**Table 10**, entry 1) The reaction with 1-methylpyrrole gave an inseparable mixture of two different regioisomers: the dibenzothiophenium substitution in position 2 of the pyrrole ring (**69**) as well as the substitution in position 3 (**70**) in 3:5 ratio (54% yield overall). (**Table 10**, entry 2) The reaction with pyrrole furnished 5-(1*H*-pyrrole-2-yl)dibenzothiophenium trifluoroacetate (**71**) in 7% yield. A major side product was DBT. (**Table 10**, entry 3) The reaction with thiophene led to the formation of 5-(thiophen-2-yl)dibenzothiophenium trifluoroacetate (**72**) and partial deoxygenation of DBT=O. (**Table 10**, entry 4)

The reactions with isoxazole and with 1*H*-pyrazole did not proceed at all and DBT=O was recovered. (**Table 10**, entries 5 and 7) The reaction with 1-methylpyrazole yielded 5-(1-methylpyrazol-4-yl)dibenzothiophenium trifluoroacetate (**68**, 82%). (**Table 10**, entry 6) The reaction with isothiazole resulted in the dimerization of DBT=O. (**Table 10**, entry 8) The reactions with oxazole, 1-methylimidazole, imidazole and thiazole led to deoxygenation of DBT=O and/or to dimerization of DBT=O. (**Table 10**, entries 10–12)

The deoxygenation of DBT=O as well as the dimerization and trimerization of DBT=O are known side reactions and have previously been described by the Alcarazo group. (**Figure 18**)

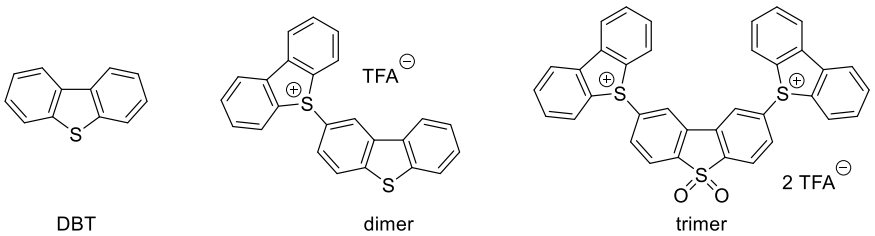


Figure 18. Known side products observed during the formation of dibenzothiophenium salts. DBT = deoxygenation of DBT=O, dimer = dimerization of DBT, trimer = symmetrical polymerization of DBT=O.

As the dibenzothiophenation using method A was successful with the same 5-membered heterocycles as the thianthrenation (chapter 3.2.1), method B was employed with only the most intriguing heterocycles bearing two heteroatoms. (**Table 11**)

Table 11. Reaction screening with 5-membered heterocycles and DBT=O towards their dibenzothiophenium salts using *method B*: Heterocycle (1.05 equiv.), DBT=O (1.0 equiv.), TFAA (1.5 equiv.) in MeCN at –78 °C for 1 h, –78 °C to 20 °C within 2 h, then 20 °C for 1 h.

Entry	Heterocycle	reaction outcome
1	isoxazole	DBT=O and dimer (no product isolated)
2	isothiazole	DBT=O and dimer (no product isolated)
3	oxazole	DBT=O and DBT (no product isolated)
4	thiazole	dimer (no product isolated)
5	thiophene	**72** (84%)[104]
6	1-methylpyrazole	**68** (93%)[104]

Unfortunately, the reactions with isoxazole, isothiazole, oxazole and thiazole did not proceed to any of the possible dibenzothiophenium salts. Instead, deoxygenation and dimerization of DBT=O occurred. (**Table 11**, entries 1–4) Larger scale reactions using method B with thiophene and with 1-methylpyrazole gave **72** (84%) and **68** (93%), respectively.

Looking at the examples prepared by Chao Yang, sometimes using Tf_2O instead of TFAA worked better. So, the four heterocycles, isoxazole, isothiazole, oxazole and thiazole, were subjected to method C as well. (**Table 12**)

Table 12. Reaction screening with 5-membered heterocycles and DBT=O towards their dibenzothiophenium salts using *method C*: Heterocycle (1.05 equiv.), DBT=O (1.0 equiv.), Tf_2O (1.0 equiv.) in MeCN at –78 °C for 1 h, –78 °C to 20 °C within 2 h, then 20 °C for 1 h.

Entry	Heterocycle	reaction outcome
1	isoxazole	- (no product isolated)
2	isothiazole	trimer (no product isolated)
3	oxazole	trimer (no product isolated)
4	thiazole	trimer (no product isolated)

Unfortunately, none of the reactions gave the desired sulfonium salts. Instead, trimerization occurred. (**Table 12**, entries 1–4)

In summary, I was not able to access more 5-membered heterocycles by dibenzothiophenation than the ones already accessed by thianthrenation. 1-methylpyrrole gave an inseparable mixture of two regioisomers **69** and **70** (3:5, 54% overall) using method A. Under the same conditions dibenzothiophenium salt **71** was prepared from pyrrole in 7% yield. Methods A and B, both successfully yielded the sulfonium salt **72** from thiophene in similar yield (91% vs. 84%). Both methods also furnished pyrazole derivative **68** in 82% and 91%, respectively. All other reactions with any of the heterocycles resulted in the formation of side products even though methods B and C were successfully used for aryl-substituted and fused thiophenes.[53]

3.2.3. Sulfonium Salts as Substrates for the Negishi Cross-coupling

With the prepared thianthrenium salts (**Chapter 3.2.1**) and dibenzothiophenium salts (**Chapter 3.2.2**) in hand, their use as substrate for the Negishi cross-coupling with the zincated pyrimidine **22** was explored.

Unfortunately, the initial test reaction with the sulfonium salt **63** derived from 1-methylpyrazole under standard conditions showed substantial solubility problems. When adding the solution of **63** and $Pd(PPh_3)_4$ to the zincated pyrimidine **22**, instant agglutination occurred. This solid rock-like clump persisted, and I was not able to break up the insoluble mass even by extensive sonication or by prolonged heating. Hence, an extensive solvent screening was warranted. (**Table 13**)

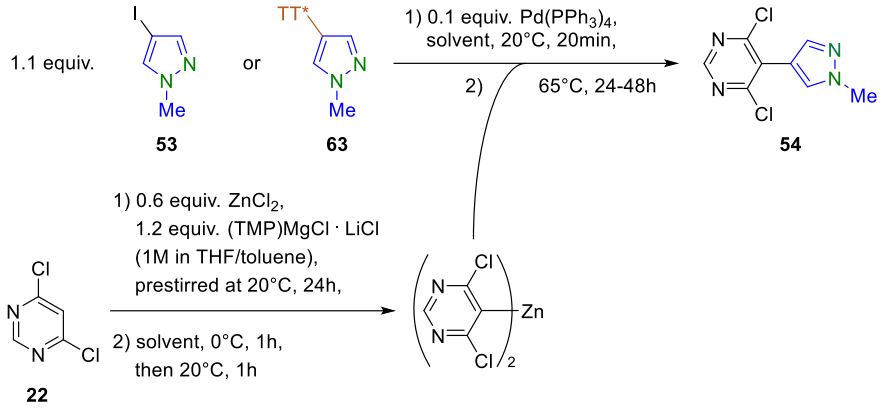


Table 13. Solvent screening for the Negishi coupling with **63** as an alternative for **53**.

Entry	**53** or **63** (1.1 equiv.)	Solvent	Time	Conversion[a]	Comment[b]
1	**53**	THF	48 h	n/a (25%[c])	
2	**63**	THF	48 h	0%	Agglutination after addition
3	**63**	THF (volume ×2)	48 h	0%	Agglutination after addition
4	**63**	1,4-dioxane	48 h	0%	Agglutination after addition, resolved after 4 h at 65 °C
5	**63**	DMF	48 h	0%	
6	**63**	DMAc	48 h	0%	
7	**63**	DMSO	24 h	0%	Slurry, solubility improved after 12 h at 65 °C
8	**63**	NMP	24 h	0%	
9	**63**	THF, then $CHCl_3$ (×2)	24 h	0%	Agglutination after addition, resolved after 1 h at 65 °C

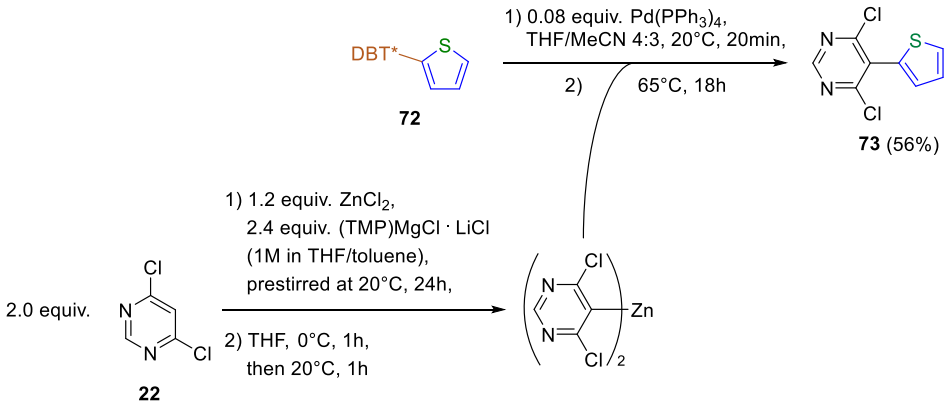
[a] Conversion was determined by ^1H NMR of the crude mixture in $CDCl_3$ by comparing H-2 in the pyrimidine moiety ($\delta_{H\text{-}2}$ (**22**) = 8.81 ppm, $\delta_{H\text{-}2}$ (**54**) = 8.88 ppm), additionally a shift of the methyl group signal in the pyrazole moiety was observed (δ_{NMe} (**53**) = 3.92 ppm, δ_{NMe} (**63**) = 3.91 ppm, δ_{NMe} (**54**) = 4.01 ppm).
[b] Observation after the addition of the **54** mixture to the solution with the zincated pyrimidine.
[c] isolated yield.

The $(TMP)_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$ solution for the zincation step was prepared in bulk and used for the 4,6-dichloropyrimidine (**22**) solutions in the solvents indicated in the table above. As a reference, the reaction with 1.1 equiv. of 4-iodo-1-methyl-pyrazole (**53**) and 0.1 equiv. $Pd(PPh_3)_4$ in THF gave the desired cross-coupling product **54** with 25% yield. (**Table 13**, entry 1)

The initial test reaction with 1.1 equiv. of the thianthrenium salt **63** and 0.1 equiv. $Pd(PPh_3)_4$ in THF resulted in instant agglutination after the addition of the solution containing **63** to the zincated pyrimidine. Neither sonication nor heating the reaction mixture to 65°C for several hours helped with solubility. (**Table 13**, entry 2) Increasing the amount of THF in the reaction mixture twofold did not help with the problem of agglutination either. (**Table 13**, entry 3) When changing the solvent to 1,4-dioxane, the agglutination still occurred after the addition of the **63** to the zincated pyrimidine in 1,4-dioxane but it resolved after several hours of stirring the resulting mixture at 65°C. But after 48 hours, the desired cross-coupling product **54** was still not formed. (**Table 13**, entry 4) While solubility was not an issue in DMF, DMAc, or in NMP, no product was formed nonetheless. (**Table 13**, entries 5, 6 and 8) For the reaction in DMSO, a slurry formed after the addition of the **63** and the solubility improved after continuous stirring for 12 hours at 65 °C. (**Table 13**, entry 7) In a last attempt to solve the solubility problems, I went back to the basics. As the sulfonium salt **63** was characterized in $CDCl_3$, I prepared the solution of **63** in chloroform and added it to the zincated pyrimidine **22** in THF. Unfortunately, I still observed agglutination after the addition and increasing the volume of chloroform twofold did not improve the solubility of the mixture. After stirring at 65 °C for 1 hour, the agglutination resolved on its own. Unfortunately, the reaction did not result in the formation of **54** after 24 hours. (**Table 13**, entry 9) I also tried toluene as a solvent, the reaction mixture turned gelatinous after the addition of **63**. This slime-like consistency made the stirring impossible – neither sonication nor heating were able to resolve this problem and the reaction was abandoned.

In conclusion, the Negishi cross-coupling between the sulfonium salt **63** and the zincated pyrimidine **22** was unsuccessful. Instead, solubility was an great issue. Changing the solvent system did not lead to the formation of the desired cross-coupling product **54**. The possible side reactions were not investigated further. However, some kind of polymerization in the reaction mixture might explain the change of consistency observed. Thus, thianthrenium salts of 5-membered heterocycles were deemed to be unsuitable as substrates for the Negishi cross-coupling with the zincated pyrimidine **22**.

Instead, dibenzothiophenium salts of biaryl and fused heterocycles had been successfully employed in Negishi cross-coupling reactions with the zincated pyrimidine **22** using a THF/MeCN solvent system. Thus, the same conditions were applied for the sulfonium salts **68** and **72**.[104]

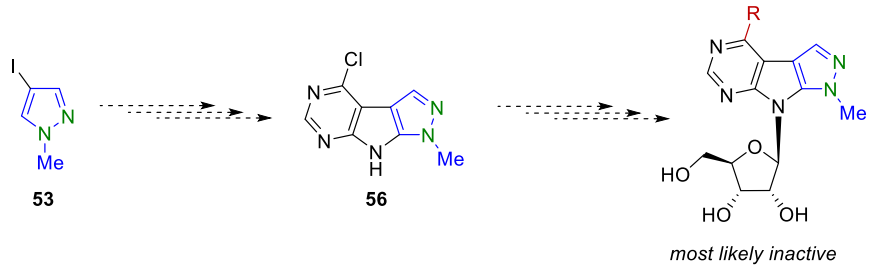


Scheme 27. Successful Negishi cross-coupling with sulfonium salt **72**.

Only the reaction with the thiophene derivative **72** was successful and furnished the cross-coupling product **73** in 56% yield. (**Scheme 27**) The reaction with the pyrazole derivative **68** led to the decomposition of the sulfonium salt and the cross-coupling product **54** was not formed.

In conclusion, sulfonium salts can be a feasible substitute for their corresponding iodo derivatives if they cannot be accessed themselves. Unfortunately, it requires larger than 5-membered ring systems such as biaryl or fused ring systems for this tool to work reliably. To make otherwise unsubstituted 5-membered heterocycles containing two heteroatoms (exception: 1-methylpyrazole) accessible as substrates for cross-coupling reactions, one must find an approach other than sulfonium salts or change the synthetic strategy altogether.

As the Negishi cross-coupling between 4-iodo-1-methylpyrazole (**53**) and the zincated pyrimidine **22** only gave the cross-coupling product **54** in 20–25% yield, the synthesis towards the corresponding nucleobase **56** is not feasible. (**Scheme 28**)



Scheme 28. Synthetic strategy towards the library of isomeric pyrazolo-fused 7-deazapurine ribonucleosides.

Additionally, biological testing revealed that the isosteric methylpyrrolo-fused 7-deazapurine ribonucleosides are inactive.[47] Most probably, the methyl group sterically hinders the interaction with the target as the corresponding thieno-fused series showed interesting activities *in vitro*.[48] Hence, the project was terminated and I shifted my focus towards (iso)quinoline fused 7-deazapurines.

3.3. Quinolino-Fused 7-Deazapurine Ribonucleosides

Pyrido-fused 7-deazapurine ribonucleosides **3** synthesized by my former co-worker Lucia Sirotová (née Veselovská) showed interesting biological activities and were easily incorporated into RNA and into DNA.[46] The naphtho-fused 7-deazapurine ribonucleosides **9** synthesized by co-workers Ketaki Ghosh and Chao Yang were no longer incorporated into DNA but showed interesting fluorescent properties.[50] So, I was tasked to combine the two structural motives and prepare a library with (iso)quinolino-fused deazapurine ribonucleosides **74**. (**Figure 19**)

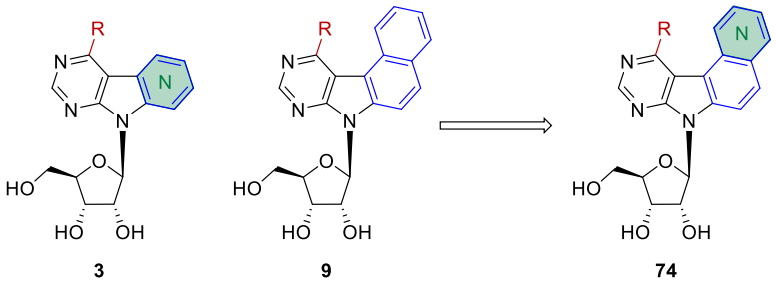
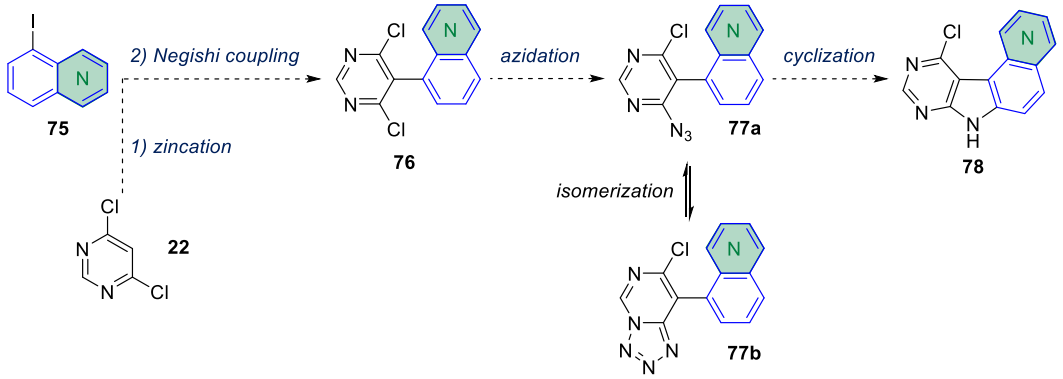


Figure 19. Previously prepared pyrido-fused and naphtho-fused 7-deazapurine ribonucleosides (**3** and **9**) led to the proposition of (iso)quinolino-fused 7-deazapurine nucleosides (**74**).

The isomeric pyrido-fused 7-deazapurines were prepared from their corresponding chloronitropyridines in 4–46% overall yield using the same classical heterocyclization strategy as discussed in **Chapter 3.1.1.1** (Approach A).[46] The tetracyclic naphtho-fused 7-deazapurine was prepared from 1-iodonaphthalene (12% overall) using the cross-coupling and cyclization strategy discussed in **Chapter 3.1.1.2**. (Approach B).[50]

As the iodo(iso)quinolines (**75**) are commercially available in small quantities, I intended to use the shorter approach B for the synthesis of the tetracyclic nucleobases (**78**). (**Scheme 29**)



Scheme 29. Synthetic Strategy towards the tetracyclic nucleobase **78**.

The first key step is the Negishi cross-coupling reaction between the zincated pyrimidine **22** and the iodo(iso)quinonline **75** and will be discussed in detail in **Chapter 3.3.1**. The second key step is the cyclization of **77** yielding the tetracyclic nucleobase **78** and will be discussed in **Chapter 3.3.2**. With the optimization of both key steps in place, the large-scale synthesis of the nucleobase is described in **Chapter 3.3.3** and **Chapter 3.3.4** followed by glycosylation and derivatization.

3.3.1. Key step 1: Negishi Cross-Coupling

The zincation of pyrimidine **22** is a two-step one-pot process: First, the Turbo-Hauser base $(TMP)_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$ is prepared *in situ* with (TMP)MgCl·LiCl and $ZnCl_2$. Then, 4,6-chloropyrimidine (**22**) is added to form the zincated species. The details are discussed in **Chapter 3.1.1.2** and the same procedure is used for the synthesis discussed here.

The initial Negishi cross-coupling between the zincated pyrimidine **22** and 1.1 equiv. of 5-iodoquinoline (**79**) in the presence of $Pd(PPh_3)_4$ in THF at 65 °C for 24 hours did not result in **83** with full conversion; instead, 32% of **43** was recovered. This called for adjustment of the reaction conditions. In the previous synthesis of the pyrazolo-fused 7-deazapurine (**47**), 5-iodo-1-methylpyrazole (**48**) was used in large excess (**Scheme 13**). As the iodo(iso)quinolines (**79-82**) are not cheap, conditions with the zincated pyrimidine **22** in excess were tried first. At the same time, I also tested the same conditions with the other three isomeric iodo(iso)quinolines: 5-iodoisoquinline (**80**), 8-iodoisoquinoline (**81**) and 8-iodoquinoline (**82**). (**Table 14**)

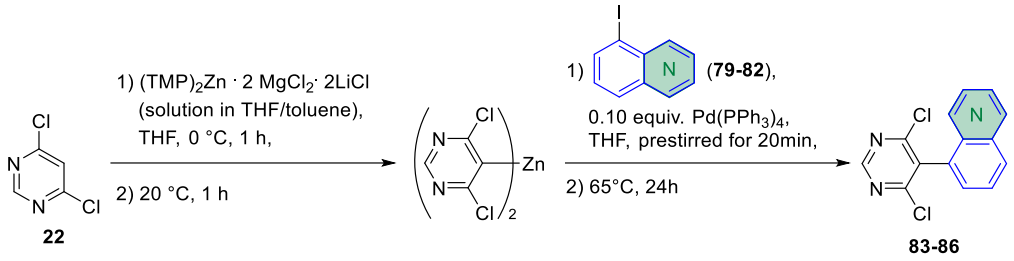
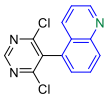
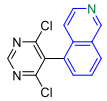
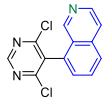
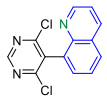


Table 14. Optimization of the Negishi cross-coupling between the zincated pyrimidine **22** and iodo(iso)quinoline **79-82** in THF at 65 °C for 24 hours forming **83-86**.

Entry	**22** [equiv.]	$(TMP)_2Zn$ [equiv.]	**79-82**	[equiv.]	**83-86**	Yield (conv.)	recovered **79-82**
1	1.0	0.6		1.1		66%	32%
2	2.0	1.2	**79**	1.0	**83**	83%	-
3	1.0	0.6		1.1		45%	26%
4	2.0	1.2	**80**	1.0	**84**	50%	-
5	1.0	0.6		1.1		27%	41%
6	2.0	1.2	**81**	1.0	**85**	44%	24%
7	1.0	0.6		1.1		0%	46%
8	2.0	1.2	**82**	1.0	**86**	-[a] (0%)	- *(ndt)*

[a] not isolated, conversion determined by ^1H NMR of the reaction mixture in $CDCl_3$,
ndt = not determined.

The reaction with 2.0 equiv. of zincated pyrimidine **22** and 1.0 equiv. of 5-iodoquinoline (**79**) resulted in full conversion and **83** was obtained in 83% yield which is an increase of 17% compared to the initial conditions with 1.0 equiv. of **22**. (**Table 14**, entries 1 and 2) The reaction with 1.0 equiv. of **22** and 1.1 equiv. of 5-iodoisoquinoline (**80**) gave 45% of **84**; additionally, 26% of **80** was recovered during purification by HPFC. Increasing the amount of zincated pyrimidine **22** (2.0 equiv.) resulted in full conversion and **84** was isolated in 50% yield (+5%). (**Table 14**, entries 3 and 4) An increase of zincated pyrimidine **22** in the reaction with 8-iodoisoquinoline (**81**) improved the yield of **85** by 17% (44%), and the amount of

recovered **81** dropped significantly (41 vs. 24%). (**Table 14**, entries 5 and 6) The reactions with 8-iodoquinoline (**82**) did not proceed to **86** at all. (**Table 14**, entries 7 and 8)

It seems that the position of the nitrogen in the quinoline ring system plays a significant role in the success of the Negishi cross-coupling reaction with the zincated pyrimidine **9**. The further apart the nitrogen position is from the iodo position, the better the outcome of the Negishi cross-coupling: the aryl halides **82** – **81** – **80** – **79** correspond to 0% – 44% – 50% – 83% yield. The reason for this correlation was not investigated further but a side reaction during the transmetalation step is possible.

3.3.2. Key step 2: Cyclization

In preparation for the cyclization of **87** towards the tetracyclic nucleobase **88**, the intermediate **87** with its two isomers was studied by ^{1}H NMR in different solvents. The equilibrium between the azide **87a** and the tetrazole form **87b** highly depends on the polarity of the solvent and can be easily observed when looking at the shift of H-2 in the pyrimidine moiety.[59] (**Table 15**)

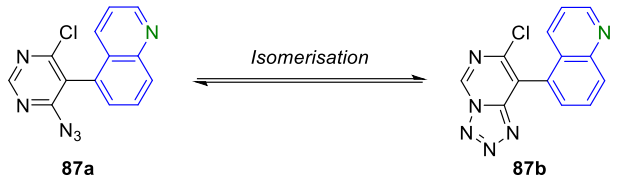


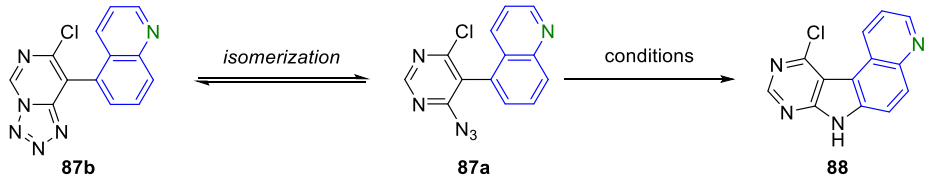
Table 15. ^{1}H NMR measurements to determine the azide/tetrazole equilibrium of intermediate **51**.

Entry	Measurement in	azide **87a** ($\delta_{H\text{-}2}$ [ppm])	tetrazole **87b** ($\delta_{H\text{-}2}$ [ppm])	ratio **87a** : **87b**
1	Benzene-d_6	8.43	-	100 : 0
2	$CDCl_3$	8.82[a]	-	100 : 0
3	THF-d_8	8.82	10.03	5 : 1
4	DMF-d_7	9.03	10.51	32 : 68
5	DMSO-d_6	8.99	10:40	23 : 77
6	TFA-d_1	9.08	-	100 : 0

[a] The presence of the azide **87a** in $CDCl_3$ was confirmed by IR measurement ($\tilde{\nu}$ = 2138 cm^{-1}).

The NMR study showed a preference for the azide **87a** in apolar solvents (benzene-d_6, $CDCl_3$) resp. in protonating solvents such as TFA (protonation of N-1 prevents tetrazole formation) which corresponds to the findings in **Chapter 3.1.1.2**. In THF-d_8, DMSO-d_6 and DMF-d_7 both forms, azide **87a** and tetrazole **87b**, are detected in various ratios.

With these findings in mind, I performed the initial cyclization screening with **87** using three different concepts: (1) thermal cyclization,[61] (2) rhodium-catalyzed cyclization,[62,63] and (3) photocyclization.[64] (**Table 16**)



87b **87a** **88**

Table 16. Photo-, thermal and Rh^{II}-catalyzed cyclization of **87** towards nucleobase **88**.

Entry	Conditions	Temp.	Time	Yield
1	6 equiv. 1,4-dibromobenzene	170 °C	0.5 h	11% (21% of **87** recovered)
2	10 mol% Rh_2esp_2, 100 wt% molecular sieves (4 Å), toluene/TFA (1:1)	90 °C	24 h	- (no reaction)
3	UV (254 nm, 4 W), TFA	20 °C	48 h	- (decomposition)

The thermal cyclization of **87** in 1,4-dibromobenzene at 170 °C for 30 min gave **88** with 11% yield. (**Table 16**, entry 1) The catalytic cyclization with Rh_2esp_2 in toluene/TFA (1:1) did not proceed at all while the photocyclization with UV light (254 nm, 4 W) in TFA resulted in decomposition. (**Table 16**, entries 2 and 3)

3.3.2.1. Thermal Cyclization

During the initial cyclization screening, the thermal cyclization in 1,4-dibromobenzene was the only one that furnished the desired nucleobase **88**. Thus, this strategy was pursued and I tried to improve its yield. (**Table 17**)

Table 17. Thermal cyclization of **87** in 1,4-dibromobenzene.

Entry	Conditions	Temp.	Time	Yield	recovered **87**
1	6 equiv. 1,4-dibromobenzene	170 °C	30 min	11%	21%
2	6 equiv. 1,4-dibromobenzene	170 °C	10 min	8%	59%
3	6 equiv. 1,4-dibromobenzene	170 °C	3×10 min	17%	31%

The initial reaction of **87** in 1,4-dibromobenzene at 170 °C for 30 min resulted in the formation of the desired nucleobase **88** (11%). Additionally, 21% of **87** recovered and a substantial amount of a dark insoluble mass was formed by polymerization, a known side reaction during the thermal cyclization of azides.[98] (**Table 17**, entry 1) Hence, the reaction time cannot be prolonged indefinitely. A shorter reaction time of only 10 min resulted in 8% of product **88** and 59% of **87** was recovered. (**Table 17**, entry 2) The thermal cyclization of **87** with three iterations of 10 min reaction time gave **88** in 17% yield. After each iteration, the product was removed from the reaction mixture by HPFC. By the third iteration, only a small amount of **88** was formed as the ratio between **87** and 1,4-dibromobenzene in the reaction mixture was no longer favorable and polymerization became predominant.[105] (**Table 17**, entry 3)

A different way to control short reaction times is the use of a flow reactor.[106] The starting material is dissolved in the reaction feed which leads to an HPLC pump. From there, the feed runs through a stainless steel capillary (Ø = 1.0 mm, length = 10 m, internal volume = 8 mL) coiled around a heating block. After cooling to r.t., the output feed passes through an automatic backpressure regulator and can then be collected. (**Figure 20**)

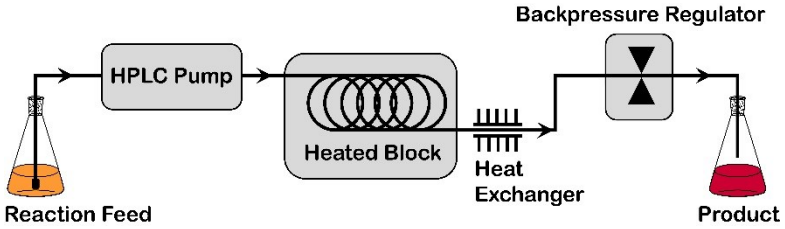


Figure 20. Schematic of a flow reactor, courtesy of Dr. Jiří Rybáček, Starý group at IOCB Prague.[107]

For this to work, two conditions have to be fulfilled: (1) All reaction partners such as starting material, reagents, products and any potential side products have to be soluble in the chosen solvent at room temperature. (2) Monomolecular reactions are preferred as their reaction rate is not concentration-dependent.

As the azide form of intermediate **87** is required, an apolar solvent such as benzene or chloroform is recommended. Unfortunately, the nucleobase **88** is only soluble in very polar solvents such as DMSO or MeOH. As a compromise, a polar solvent such as MeOH with 1% TFA was chosen, where the azide **87a** is protonated and the nucleobase **88** is soluble.

A reaction screening with different temperatures (130–170 °C) and various flow rates resulting in different reaction times ($t_{residence}$) was performed. The output feed was collected and analyzed by TLC (SiO_2; cHex/EtOAc 1:1, UV 254 nm) and/or by ^{1}H NMR in DMSO-d_6. (**Table 18**)

Table 18. Thermal cyclization of **87** using a flow reactor set-up. The reaction scale was 5 mg resp. 10 mg of azide and 2 mL of solvent. The post input feed was pure solvent without any TFA. The reaction output was analyzed by TLC and/or by ^{1}H NMR in DMSO-d_6.

Entry	Conditions	Temp.	Flow rate	$t_{residence}$	Outcome
1	MeOH with 1% TFA (0.035 M)	170 °C	1 mL/min	8 min	TLC: no **87** NMR: **89**, decomp.
2	MeOH with 1% TFA (0.035 M)	170 °C	2 mL/min	4 min	TLC: no **87** NMR: **89**, decomp.
3	MeOH with 1% TFA (0.035 M)	130 °C	2 mL/min	4 min	TLC: no **87** NMR: **89**, decomp.
4	MeOH with 1% TFA (0.035 M)	150 °C	2 mL/min	4 min	TLC: no **87** NMR: **89**, decomp.
5	MeOH with 1% TFA (0.035 M)	170 °C	3 mL/min	2.6 min	TLC: no **87** NMR: **89**, decomp.
6	NMP with 1% TFA (0.07 M)	170 °C	2 mL/min	4 min	TLC: - NMR: traces of **88**
7	TFA (0.07 M)	170 °C	2 mL/min	4 min	TLC: - NMR: decomp.

decomp. = decomposition

Unfortunately, the thermal cyclization of **87** in MeOH with 1% TFA using a flow reactor set-up did not give the desired product **88** but resulted in the amine side product **89** instead. Interestingly, the amine **89** has the same R_f value as the nucleobase **88**. (**Table 18**, entries 1–

5) The same reaction in NMP with 1% TFA gave only traces of product **88**. (**Table 18**, entry 6) The reaction in TFA led to decomposition. (**Table 18**, entries 7)

Next, I tried the thermal cyclization of **87** in toluene at 170 °C under microwave conditions. (**Table 19**)

Table 19. Thermal cyclization of **87** under m.w. conditions in toluene.

Entry	Conditions	Temp.	Time	Yield	recovered **87**
1	m.w., toluene (0.025 M)	170 °C	60 min	10%	-
2	m.w., toluene (0.05 M)	170 °C	60 min	14%	-

The initial reaction of **87** gave **88** with 10% yield. (**Table 19**, entry 1) Increasing the concentration of **87** in toluene twofold to 0.05 M gave **88** with 14% yield (+4%). (**Table 19**, entry 1) During both reactions, the formation of a dark insoluble mass was observed.[98] Further increasing the concentration of **87** increases the risk of more unwanted side products as seen in 1,4-dibromobenzene. Additionally, reactions at a larger scale could be potentially hazardous due to pressure build-up caused by the released nitrogen during the cyclization which can be problematic in a closed system such as a microwave reactor.

In conclusion, the thermal cyclization of **87** at 170 °C is less than ideal. The reaction in 1,4-dibromobenzene only gives up to 17% yield after three iterations. The cyclization in the flow reactor results in the undesired amine **89**. The reaction under microwave conditions gave **88** in 10–14%.

Before performing the thermal cyclization of **87** at a larger scale, I took another look at the Rh-catalyzed reactions as well as at the photocyclization.

3.3.2.2. Rhodium-catalyzed Cyclization

The initial test reaction with Rh_2esp_2 in toluene/TFA (1:1) was not successful (**Table 16**) but I wanted to extend the testing scope: (1) Previously in our group, the solvent system toluene/TFA (1:1) has been used to shift the azide/tetrazole equilibrium towards the azide for such reactions.[51] As the NMR study in benzene-d_6 showed the equilibrium of **87a**:**87b** to be 100:0 (**Table 15**), pure toluene as a solvent was tested as well. (2) Rh-catalyzed cyclizations of azides towards carbazoles have been published using three different catalysts: Rh_2esp_2,[62] Rhodium octanoate dimer ($Rh_2(O_2CC_7H_{15})_4$) and Rhodium heptafluorobutyrate dimer ($Rh_2(O_2CC_3F_7)_4$).[63] (3) Some of the published reaction conditions called for molecular sieves (4 Å) and some did not, so both conditions were tested.

After 24 hours, the reaction mixtures were concentrated *in vacuo*, treated with DMSO-d_6 and filtered through a syringe filter (nylon membrane, 0.45 µm pore size). The conversion was determined by ^{1}H NMR in DMSO-d_6 by comparing H-2 in the pyrimidine moiety. The signal of H-2 in tetrazole **87b** is $\delta_{H\text{-}2}$ = 10.40 ppm. The signal of H-2 in nucleobase **88** is shifted upfield ($\delta_{H\text{-}2}$ = 8.80 ppm). (**Table 20**)

Table 20. Reaction screening of Rh-catalyzed cyclization of **87** towards nucleobase **88**.

Entry	Conditions	Temp.	Time	Outcome
1	10 mol% $Rh_2(esp)_2$, toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
2	10 mol% $Rh_2(esp)_2$, 50 wt% molecular sieves (4 Å), toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
3	10 mol% $Rh_2(esp)_2$, toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition
4	10 mol% $Rh_2(esp)_2$, 50 wt% mol. sieves (4 Å), toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition
5	10 mol% $Rh_2(O_2CC_7H_{15})_4$, toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
6	10 mol% $Rh_2(O_2CC_7H_{15})_4$, 50 wt% mol. sieves (4 Å), toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
7	10 mol% $Rh_2(O_2CC_7H_{15})_4$, toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition
8	10 mol% $Rh_2(O_2CC_7H_{15})_4$, 50 wt% mol. sieves (4 Å), toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition
9	10 mol% $Rh_2(O_2CC_3F_7)_4$, toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
10	10 mol% $Rh_2(O_2CC_3F_7)_4$, 50 wt% mol. sieves (4 Å), toluene (0.2 M)	90 °C	24 h	no reaction (**87** only)
11	10 mol% $Rh_2(O_2CC_3F_7)_4$, toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition
12	10 mol% $Rh_2(O_2CC_3F_7)_4$, 50 wt% mol. sieves (4 Å), toluene/TFA 1:1 (0.2 M)	90 °C	24 h	decomposition

Independent of the catalyst, all the reactions in toluene did not proceed at all, only starting material was recovered. On the other hand, all the reactions in toluene/TFA led to the decomposition of intermediate **87**. No product **88** was observed in any rhodium-catalyzed cyclization reaction.

3.3.2.3. Photocyclization

The initial photocyclization of **87** under UV light (254 nm, 4 W) in TFA under ambient atmosphere resulted in decomposition (**Table 21**, entry 1). During the extended testing for conditions using thermal cyclization (**Table 18**, entry 7) as well as using rhodium catalysis (**Table 20**), all reactions involving TFA resulted in decomposition. Hence, TFA-free conditions were required. As the azide **87a** is soluble in DCM, I also determined its UV absorbance with the maxima at λ_{abs} = 232, 271 and 317 nm.

In photo-induced reactions, the starting azide can either be directly excited with a UV light source (λ_{max} = 225–300 nm), or a photosensitizer can be excited which then transfers this energy to the azide.[108] (**Figure 21**)

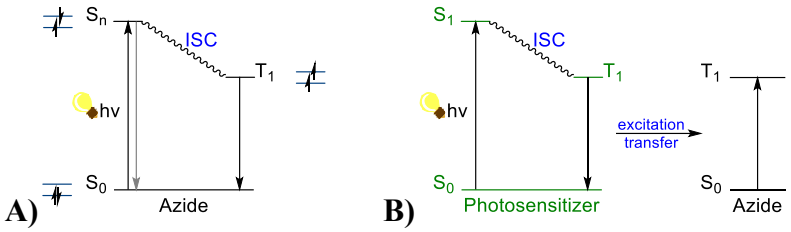


Figure 21. Jablonski diagram of photochemical pathways from the ground state to the triplet state:[109] **A)** Direct excitation of the azide and access of the excited triplet state T_1 via inter-system crossing (ISC) from a highly excited singlet state S_n; **B)** Photosensitization and access of T_1 via excitation transfer from the T_1-S_0 of the photosensitizer.

I tested different photocyclization conditions with various light sources and photosensitizers under Argon atmosphere. (**Table 21**)

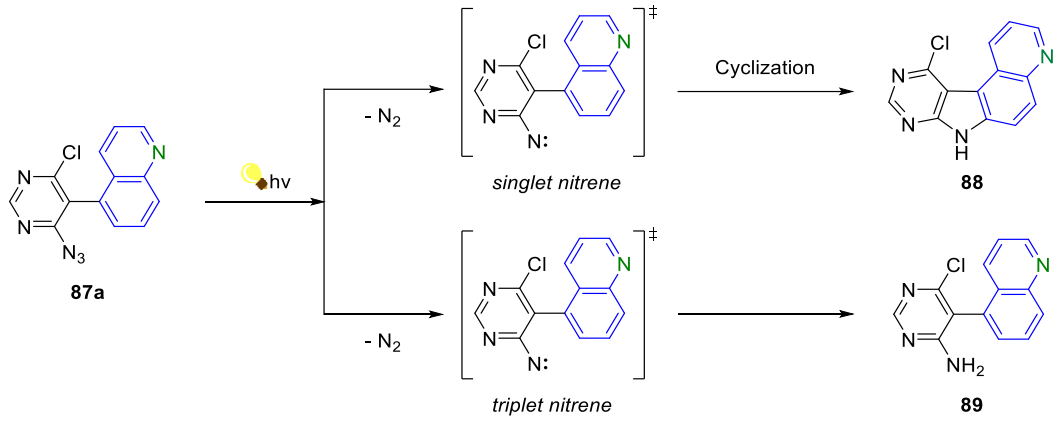
Table 21. Photocyclization of **87** towards the nucleobase **88**.

Entry	Conditions	Temp.	Time	Yield	recovered **87**
1	UV (λ_{max} = 254 nm), TFA (0.05 M)	20 °C	48 h	0% (decomposition)	-
2	UV (λ_{max} = 254 nm), DCM (0.025 M)	20 °C	96 h	39%, inseparable mixture of **88** and **89** (3:2)	13%
3	UV (λ_{max} = 275 nm), DCM (0.025 M)	20 °C	96 h	19%, inseparable mixture of **88** and **89** (3:2)	7%
4	5 mol% *fac*-Ir(ppy)$_3$, UV (λ_{max} = 400 nm), DCM (0.025 M)	20 °C	96 h	0% (31% of **89**)	-
5	5 mol% [Ir(ppy)$_2$(tbbpy)]PF$_6$, UV (λ_{max} = 400 nm), DCM (0.025 M)	20 °C	6 d	0%	-
6	UV (λ_{max} = 254 nm), THF (0.025 M)	–10 °C, then 20 °C	96 h, then 48 h	44%, inseparable mixture of **88** and **89** (4:1)	-
7	UV (λ_{max} = 254 nm), THF (0.025 M)	20 °C	6 days	19%	-
8	1.0 equiv. pyrene, UV (λ_{max} = 254 nm), THF (0.025 M)	20 °C	72 h	26%	-

The reactions of **87** in DCM under UV light with different wavelengths (λ_{max} = 254 and 275 nm) were still not complete after 96 hours. Both resulted in the formation of the desired nucleobase **88** as well as the formation of the unwanted amine **89**. (**Table 21**, entries 2 and 3) The reaction in the presence of *fac*-Ir(ppy)$_3$, a known triplet photosensitizer (lit[110]: ΔE_T = 58 kcal/mol) and UV light (λ_{max} = 400 nm) furnished the amine **89** exclusively (31%). (**Table 21**, entry 4) The reaction of **87** in the presence of [Ir(ppy)$_2$(tbbpy)]PF$_6$, a different photosensitizer (lit[110]: ΔE_T = 50 kcal/mol) and the same UV light source did not proceed at all. (**Table 21**, entry 5)

Additionally, my co-worker Chao Yang once performed the photocyclization in THF with reduced temperature for naphtho-fused 7-deazapurines.[50] So, I tried these as well: The reaction was still not complete after 4 days. Then, it was warmed to r.t. and stirred for a further 2 days until all the azide was consumed furnishing **88** and **89** as an inseparable mixture. (**Table 21**, entry 6)

With these experiments, I learned the following: (1) TFA-free conditions are crucial. (2) Direct excitation of the azide **87a** in DCM leads to a mixture of product **88** and amine **89**. As both, **88** and **89**, have the same R_f, they cannot be separated by HPFC. So, it is pivotal to find conditions where **88** is formed exclusively. (3) The amine is formed via the triplet state nitrene. (**Scheme 30**)



Scheme 30. Decomposition of the azide **87a** and formation of the singlet nitrene resulting in the cyclized product **88**. If triplet nitrene is formed, the reaction results in the formation of the amine **89**.

With this new information in mind, I performed two more experiments: The reaction of **87** in THF under UV light (λ_{max} = 254 nm) took 6 days to complete and resulted in 19% of **88**. (**Table 21**, entry 7) The same reaction with 1.0 equiv. of pyrene, a singlet photosensitizer and triplet quencher,[111] was complete after 3 days yielding 26% of nucleobase **88**. (**Table 21**, entry 8)

As a result, the Jablonski diagram in **Figure 21** can be updated as well. (**Figure 22**)

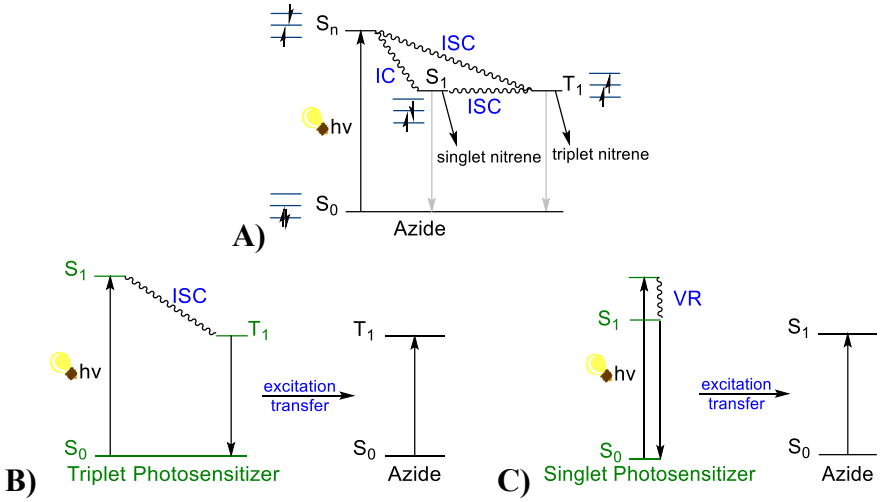
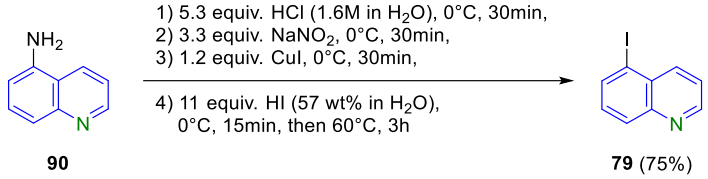


Figure 22. Updated Jablonski diagram of the photochemical pathways from the ground state of the azide **87a** to the nitrene formation: **A)** Direct excitation of azide **87a** yields a highly excited singlet state S_n and subsequent relaxation forms the lowest excited triplet state T_1 via inter-system crossing (ISC) or the lowest excited singlet state S_1 via internal conversion (IC). T_1 leads to the triplet nitrene and the amine **89** while S_1 leads to the singlet nitrene and the desired ring closure (**88**). **B)** Photosensitization and access to T_1 via excitation transfer from the T_1-S_0 of the triplet photosensitizer. **C)** Photosensitization and access to S_1 via vibrational relaxation (VR) and subsequent excitation transfer from the S_1-S_0 of the singlet photosensitizer.

3.3.3. From 5-Aminoquinoline to quinolino-fused 7-deazapurine nucleobase

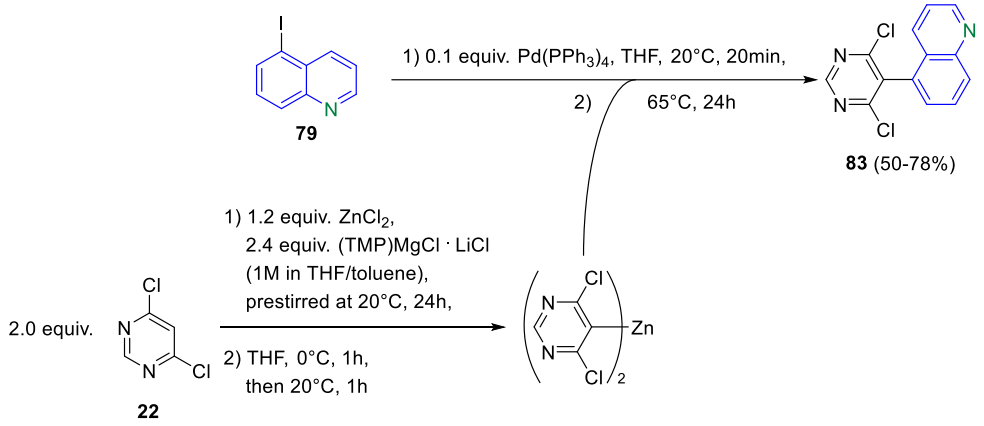
With both key steps, the Negishi cross-coupling (**Chapter 3.3.1**) and the cyclization (**Chapter 3.3.2**) optimized, I was able to prepare the quinolino-fused 7-deazapurine nucleobase **88** at a larger scale. First, I prepared the 5-iodoquinoline **79** from 5-aminoquinoline **90** via Staudinger reaction.[112] (**Scheme 31**)



Scheme 31. Staudinger reaction of **90** towards **79**.

In the first step, aminoquinoline **90** was protonated with HCl and treated with $NaNO_2$ forming the diazonium salt. Subsequently, CuI and HI were added leading to the formation of the desired 5-iodoquinoline (**79**). After neutralization with NaOH and extraction, the crude was purified by HPFC. Prior to the cHex/EtOAc gradient, a violet impurity (probably I_2) was washed out with pure cHex. The product **79** was obtained with 75% yield. Extensive gas formation during the addition of $NaNO_2$, or the addition of CuI led to a significant drop in yield. Not removing the violet discoloration at the beginning of the chromatographic purification led to problems in the next step.

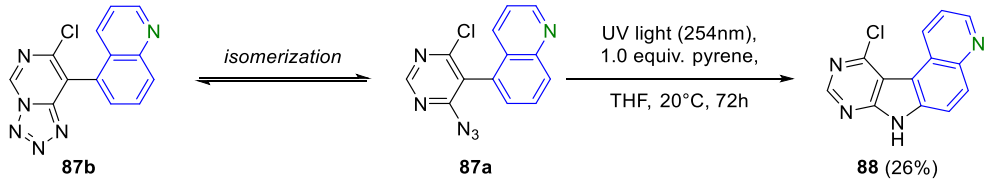
In the second step, the iodoquinoline **79** underwent Negishi cross-coupling with the *in situ* formed zincated pyrimidine **22** in the presence of $Pd(PPh_3)_4$ in THF at 65 °C for 24 hours furnishing the cross-coupling product **83** in 50–78% yield. (**Scheme 32**) The details of the reaction, including the zincation step have already been discussed in **Chapter 3.1.1.2**.



Scheme 32. Negishi cross-coupling between the zincated pyrimidine **22** and iodoquinoline **79**.

In the third step, the cross-coupling product **83** underwent aromatic nucleophilic substitution with sodium azide in the presence of LiCl in THF giving **87** in 88% yield. The equilibrium between azide **87a** and tetrazole **87b** is highly dependent on the polarity of the solvent and is discussed at the beginning of **Chapter 3.3.2** in preparation for the cyclization reactions.

The photocyclization of **87** was performed in THF with pyrene as a singlet sensitizer and triplet quencher under UV light (254 nm, 4 W) and furnished the nucleobase **88** in 26% yield. (**Scheme 33**)



Scheme 33. Photocyclization of intermediate **87** with pyrene in THF resulted in the formation of the desired nucleobase **88**.

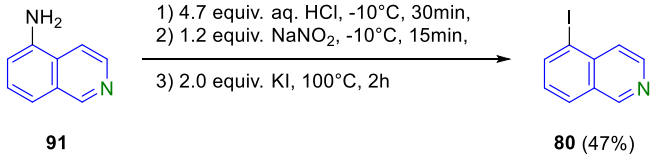
With the available UV lamp (λ_{max} = 254 nm, 4 W) in our group, the maximal batch size per cyclization was 300 mg of **87**. Every 12 hours, the reaction mixture was sonicated for 30 seconds to remove any precipitation from the light source. I usually ran two batches at the same time and combined them prior to purification.

The overall yield of this three-step reaction cascade from 5-iodoquinoline **79** towards the quinolino-fused 7-deazapurine **88** was 11–18%. The yield from 5-aminoquinoline **90** to the nucleobase **88** was 9–13% over four steps.

3.3.4. From 5-Aminoisoquinoline to isoquinolino-fused 7-deazapurine nucleobase

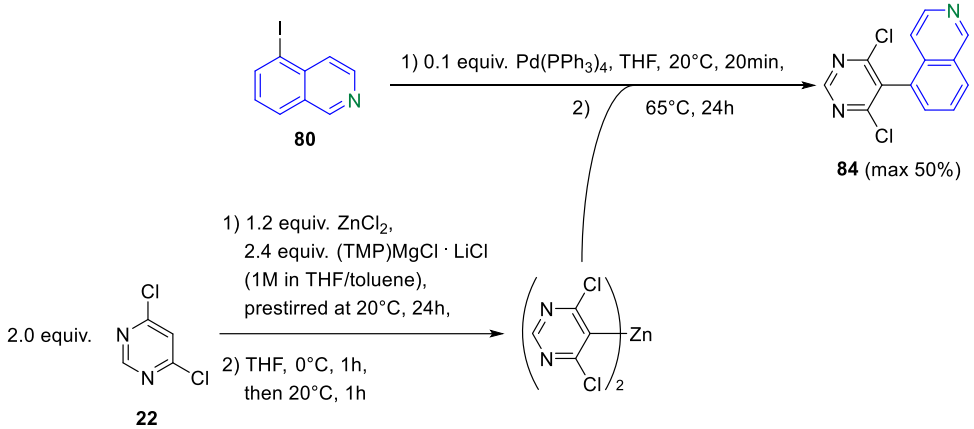
Analog to the formation of the quinolino-fused nucleobase in **Chapter 3.3.4**, the synthesis towards the isoquinolino-fused nucleobase was explored:

In the first step, the 5-aminoisoquinoline (**91**) underwent a Staudinger reaction in the presence of HCl and $NaNO_2$ to form the diazonium salt. Subsequent treatment with KI led to the formation of 5-iodoisoquinoline (**80**) in 47% yield.[113] (**Scheme 34**)



Scheme 34. Staudinger reaction of **91** towards **80**.

In the second step, the iodoquinoline **80** underwent Negishi cross-coupling with the *in situ* formed zincated pyrimidine **22** in the presence of $Pd(PPh_3)_4$ in THF at 65 °C for 24 hours furnishing the cross-coupling product **84** in with up to 50% yield. Any impurities from the previous step led to a significant drop in yield. (**Scheme 35**)



Scheme 35. Negishi coupling between the zincated pyrimidine **22** and 5-iodoisoquinoline **80**.

In the third step, the cross-coupling product **84** underwent aromatic nucleophilic substitution with sodium azide in the presence of LiCl in THF giving **92** in 64% yield. Then, I attempted

to perform the cyclization of **92** furnishing the isoquinolino-fused 7-deazapurine **93**. (**Table 22**)

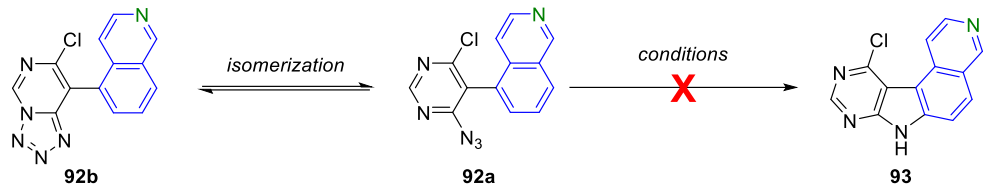


Table 22. Photo-, thermal and metal-catalyzed cyclization of **92** towards nucleobase **93**. For entries 3–7: After 36 h, the reaction mixtures were concentrated *in vacuo*, dissolved in DMSO-d_6 prior to analysis by ^{1}H NMR. Afterwards, the NMR samples were analyzed by MS.

Entry	Conditions	Temp.	Time	Outcome
1	1.0 equiv. pyrene, UV (λ_{max} = 254 nm), THF (0.025 M)	20 °C	5 d	- (decomposition)
2	6 equiv. 1,4-dibromobenzene	170 °C	0.5 h	- (decomposition)
3	5 mol% $Rh_2(esp)_2$, 100 wt% molecular sieves (4 Å), toluene (0.1 M)	100 °C	36 h	NMR: **92** and decomposition MS: ratio of **92**:**93** = 1:3
4	5 mol% $Rh_2(O_2CC_7H_{15})_4$, 100 wt% mol. sieves (4 Å), toluene/TFA 1:1 (0.1 M)	100 °C	36 h	NMR: **92** and decomposition MS: ratio of **92**:**93** = 2:3
5	5 mol% $Rh_2(O_2CC_3F_7)_4$, 100 wt% mol. sieves (4 Å), toluene (0.1 M)	100 °C	36 h	NMR: **92** and decomposition MS: ratio of **92**:**93** = 1:3
6	10 mol% $RuCl_3$, DME (0.1 M)	60 °C	36 h	NMR: **92** and decomposition MS: ratio of **92**:**93** = 8:1
7	10 mol% CuI 100 wt% mol. sieves (4 Å), toluene (0.1 M)	60 °C	36 h	NMR and MS: decomposition

First, I tried the conditions which were successful with **87**: The photocyclization of **92** with equimolar amounts of pyrene did not furnish the desired nucleobase **93**. (**Table 22**, entry 1) The thermal cyclization of **92** in 1,4-dibromobenzene at 170 °C for 30 min mainly resulted in decomposition. (**Table 22**, entry 2)

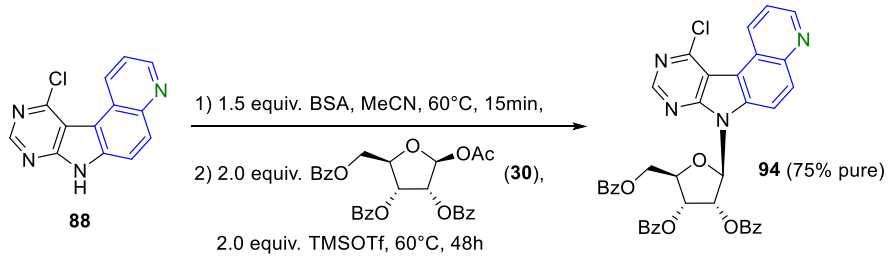
Next, I tried the Rh-catalyzed cyclizations using the same catalysts as in **Chapter 3.3.2.2**: Rh_2esp_2,[62] Rhodium octanoate dimer ($Rh_2(O_2CC_7H_{15})_4$) and Rhodium heptafluorobutyrate dimer ($Rh_2(O_2CC_3F_7)_4$).[63] Analysis of the NMR samples by ESI-MS showed a mass corresponding to **93**. (**Table 22**, entries 3–5) The analysis of the reaction of **92** with $RuCl_3$ in DME gave the same result.[114] (**Table 22**, entry 6) The reaction of **92** with CuI and molecular sieves in toluene resulted in decomposition. (**Table 22**, entry 7)

Then, I combined the crude mixtures from Rh- and Ru-catalyzed reactions (entries 3–6) and tried to isolate an analytical sample whose mass in the MS would correspond to nucleobase **93**. Unfortunately, I was unable to do so: I tried filtration with different solvents. I also tried Soxhlet extraction from a larger sample, but I was unable to isolate the desired product.

3.3.5. Glycosylation

With the quinolino-fused 5-deazapurine **88** in hand, I performed the Vorbrüggen glycosylation, (**Scheme 36**) and compared the results to the nucleobase anion glycosylation. (**Scheme 37**)

During the Vorbrüggen glycosylation, the nucleobase **88** was first silylated in position 7 with BSA and then underwent glycosylation with the protected sugar **30** in the presence of TMSOTf producing the key nucleoside **94** as pure *β*-anomer in 52% yield (**Scheme 36**).

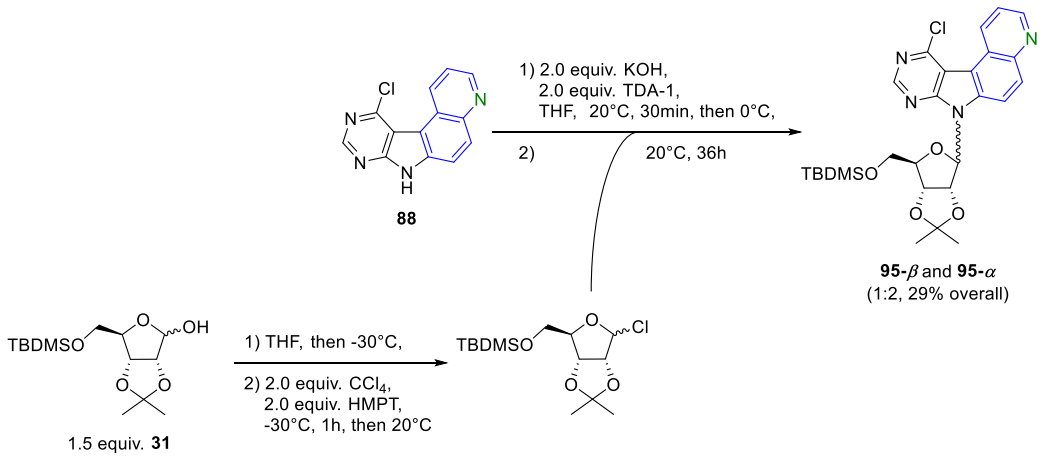


Scheme 36. Vorbrüggen glycosylation of nucleobase **88** towards the quinolino-fused 7-deazapurine ribonucleoside **94** as a pure *β*-anomer.

Details of this reaction were discussed in **Chapter 3.1.2**. Unfortunately, the purification at a larger scale proved to be difficult and I used the crude material (ca. 75% pure) directly for the derivatization. The impurity most likely stemmed from decomposition of the sugar. A small, repurified sample was used for characterization.

The stereochemistry of **94** was confirmed by H,H-ROESY using the relations between H-6 of the nucleobase and H-2' resp. H-3' as well as between H-1' and H-4' of the sugar moiety.

For the anion base glycosylation, the required halogenase was formed *in situ* from the protected sugar[74] **31** with CCl_4 and HMPT first. Then, it reacted with the deprotonated nucleobase **88** in the presence of KOH and TDA-1 resulting in a mixture of **95**-*β* and **95**-*α* (2:1) with 29% overall yield (**Scheme 37**).



Scheme 37. Nucleobase-anion glycosylation of **88** towards the quinolino-fused 7-deazapurine *β*-ribonucleoside **95**-*β* in a mixture with the *α*-anomer **95**-*α*.

The stereochemistry of the *β*-anomer **95**-*β* was also confirmed by H,H-ROESY using the same relations between H-6 of the nucleobase and H-2' resp. H-3' as well as between H-1' and H-4' of the sugar moiety. The *α*-anomer **95**-*α* was confirmed by using the relations between H-6 of the nucleobase, H-4' and the methyl in the isopropylidene protecting group as well as between H-1', H-2' and H-3' of the sugar moiety.

3.3.6. Derivatization and Deprotection

The crude nucleoside intermediate **94** (75% pure) was used in a series of reactions for the derivatization in position 11 and subsequent deprotection of the sugar moiety to give the desired 11-substituted quinolino-fused 7-deazapurine ribonucleosides **97a-h** (**Table 23**).

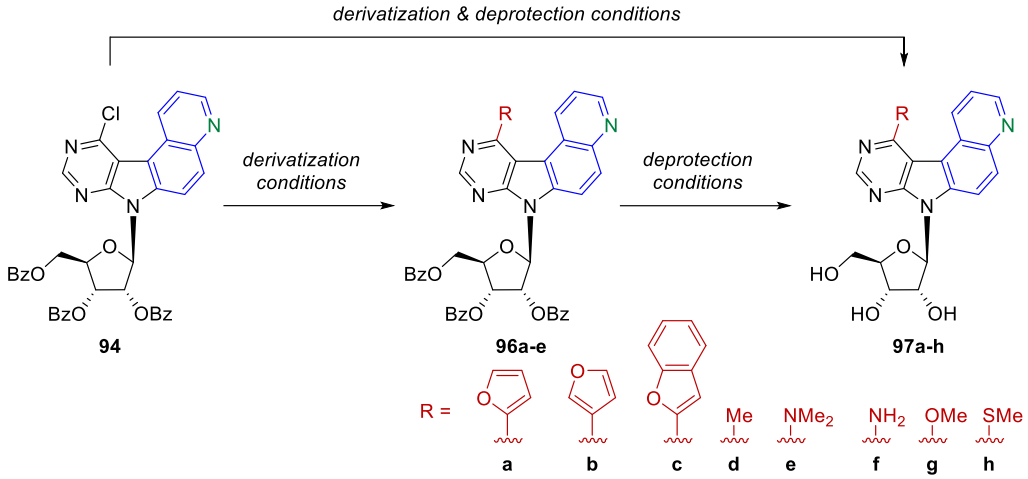


Table 23. Preparation of a library with the ribonucleosides **97a-h** either by stepwise derivatization and deprotection via **96a-e** or by simultaneous derivatization and deprotection directly from **94** (for **97f-h**).

Entry	R =	Conditions	Temp.	Time	Product	Yield
1	2-furyl	1.2 equiv. 2-(tributylstannyl)furan, 0.1 equiv. $PdCl_2(PPh_3)_2$, DMF	100 °C	24 h	**96a**	26%
2	3-furyl	2.0 equiv. 3-furylboronic acid, 2.5 or 3.0 equiv. K_2CO_3, 0.1 equiv. $Pd(PPh_3)_4$, toluene	100 °C	24 h	**96b**	*crude*
3	2-benzofuryl	2.0 equiv. 2-benzofurylboronic acid, 2.5 equiv. K_2CO_3, 0.1 equiv. $Pd(PPh_3)_4$, toluene	100 °C	24 h	**96c**	82%
4	methyl	3.0 equiv. $AlMe_3$, 0.05 equiv. $Pd(PPh_3)_4$, THF	65 °C	3 h	**96d**	42%
5	dimethylamino	2 equiv. Me_2NH, 2-propanol	60 °C	24 h	**96e**	64%
6	2-furyl	0.6 equiv. NaOMe (5.4 M in MeOH), MeOH	60 °C	24 h	**97a**	27%
7	3-furyl			24 h	**97b**	-
8	2-benzofuryl			18 h	**97c**	48%
9	methyl			18 h	**97d**	53%
10	dimethylamino			24 h	**97e**	64%
11	amino	aq. ammonia/1,4-dioxane (5:2)	120 °C	24 h	**97f**	52%
12	methoxy	6.0 equiv. NaOMe (5.4 M in MeOH), MeOH	60 °C	4 h	**97g**	22%
13	methylsulfanyl	6.0 equiv. NaSMe, THF	60 °C	18 h	**97h**	29%

The Stille cross-coupling of **94** with 2-(tributylstannyl)furan in the presence of $PdCl_2(PPh_3)_2$ in DMF at 100 °C for 24 h gave the 2-furyl derivative **96a** (26%). (**Table 23**, entry 1) The Suzuki-Miyaura cross-coupling of **94** with 3-furylboronic acid, K_2CO_3 and $Pd(PPh_3)_4$ gave **96b**. I performed this reaction twice (starting from 425 mg and from 200 mg of **94**); but both times, I was unable to purify the product. I used the crude **96b** in the Zemplén deprotection with NaOMe in MeOH. After purification by HPFC (C18; water/MeOH, gradient 0→100%

MeOH), I was left with less than 10 mg of impure **97b**. (**Table 23**, entries 2 and 7) The same Suzuki-Miyaura cross-coupling reaction of **94** with 2-benzofurylboronic acid gave the 2-benzofuryl derivative **96c** in high 82% yield. (**Table 23**, entry 3) The cross-coupling of **94** with $AlMe_3$ and $Pd(PPh_3)_4$ in THF at 65 °C for 3 h gave the methyl derivative **96d** with 42% yield. (**Table 23**, entry 4) The nucleophilic substitution of **94** with dimethylamine in 2-propanol at 60 °C for 24 h gave the *N*,*N*-dimethylamino derivative **96e** (64%). (**Table 23**, entry 5)

Then, the sugar moiety of the protected nucleosides **96a-e** underwent Zemplén deprotection with NaOMe in MeOH at 60 °C for 18–24 h providing the free nucleosides **97a-e** in 27–64% yield. (**Table 23**, entries 6 and 8–10)

The nucleosides **97f-h** were obtained from **94** in a single step as the derivatization in position 11 and the deprotection of the sugar moiety happened simultaneously. Treating **94** with aqueous ammonia/1,4-dioxane (5:2) in a screw-cap pressure vial at 120 °C for 18 hours resulted in the formation of the free amino derivative **97f** (52%). (**Table 23**, entry 11) The reaction of **94** with NaOMe in MeOH at 60 °C for 4 hours gave the free methoxy derivative **97g** in 22% yield. (**Table 23**, entry 12) The reaction with NaSMe in THF at 60 °C for 18 hours gave the free methylsulfanyl derivative **97h** (29%). (**Table 23**, entry 13)

3.4. Photophysical Properties of Heteroaryl-fused Nucleosides

Some of the previously synthesized heteroaryl-fused 7-deazapurine ribonucleosides showed interesting spectroscopic properties. Several derivatives in the thieno-,[48] furano-,[47] naphtho-[50] and pyrido-fused 7-deazapurine ribonucleoside[46] series exhibit strong fluorescence with quantum yields of 39–79%. Anisolo-fused 7-deazapurine 2'-deoxyribonucleosides have been used as nucleic acid probes.[115]

3.4.1. Pyrazolo-fused 7-Deazapurine Ribonucleosides

The absorption and emission spectra of the nucleosides **61a-h** were measured in MeOH and their quantum yields Φ_f. were determined using quinine sulfate in 0.1 M H_2SO_4 as a standard (Φ_f = 0.546 at 25 °C).[116] (**Table 24**) The exact procedure is described in Appendix 7.2.

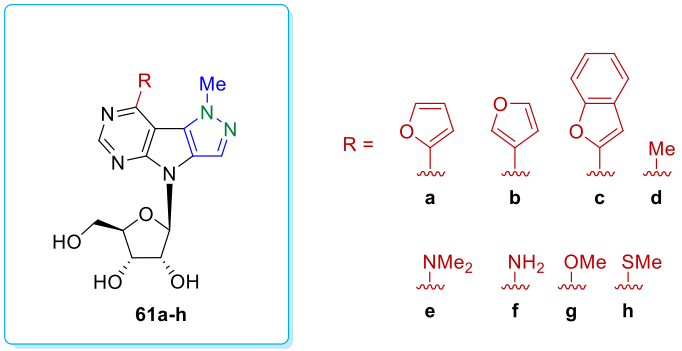


Table 24. UV and fluorescence maxima of nucleosides **61a-h** in MeOH.

compd	Absorption max. λ_{abs} (ε) [nm (M^{-1} cm^{-1})]	Emission max. λ_{em} [nm]	Quantum Yield Φ_f
61a	284 (10700), 323 (12000), *254* (29800)	506	0.077
61b	301 (4600), *250* (24200)	476	0.060
61c	336 (19800), *258* (20900)	534	0.014
61d	281 (6600), *246* (39300)	434	–
61e	*305* (7400)	417	0.045
61f	*281* (7000)	390	–
61g	*271* (9300)	398	–
61h	*303* (5000)	449	0.076

UV and fluorescence maxima were measured in MeOH. The used excitation wavelength for fluorescence are in *italics*. Fluorescence quantum yields Φ_f were determined using quinine sulfate in 0.1 M H_2SO_4 as a standard (Φ_f = 0.546 at 25 °C).[117]

The nucleosides **61a-c,e,h** exhibited fluorescence with moderate Φ_f 1.4–7.7%. The quantum yields Φ_f for nucleosides with maximal absorbance λ_{abs} below 300 nm were not determined (**61d**, **61f**, **61g**), as these values could no longer be compared with quinine sulfate as a standard.

3.4.1. Quinolino-fused 7-Deazapurine Ribonucleosides

The absorption and emission spectra of the nucleosides **97a-h** were measured in MeOH. I then determined their molar extinction coefficient ε as well as their quantum yields Φ_f using quinine sulfate in 0.1 M H_2SO_4 as a standard ($\Phi_f = 0.546$ at 25 °C).[116] (**Table 25**) The exact procedure is described in Appendix 7.2.

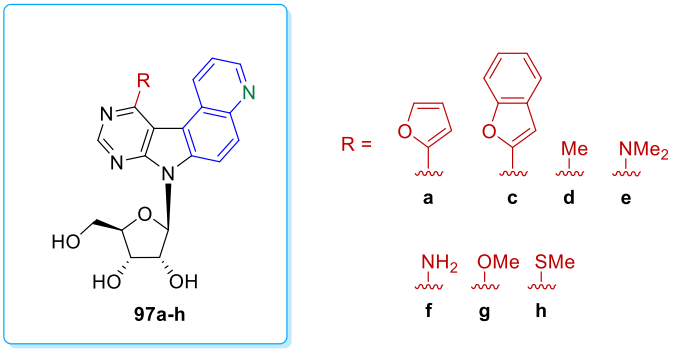


Table 25. UV and fluorescence maxima of nucleosides **97a-h** in MeOH.

compd	Absorption max. λ_{abs} (ε) [nm (M^{-1} cm^{-1})]	Emission max. λ_{em} [nm]	Quantum Yield Φ_f
97a	255 (27500), *330* (5400)	436	0.073
97c	259 (38200), *355* (13600)	466	0.510
97d	259 (50300), *319* (12900), 353 (4800)	383	0.402
97e	256 (29800), 298 (7700), *339* (7900), 359 (7100)	439, 610	0.136
97f	219 (13600), *330* (6800)	426	0.046
97g	255 (38000), 282 (16300), *315* (9900), 346 (5700)	375, 502	0.163
97h	252 (36600), *329* (10100), 359 (6600)	390	0.255

UV and fluorescence maxima were measured in MeOH. Used excitation wavelength for fluorescence are in *italics*. Fluorescence quantum yields Φ_f were determined using quinine sulfate in 0.1 M H_2SO_4 as a standard ($\Phi_f = 0.546$ at 25 °C).[117]

The nucleosides **97a** and **97f** exhibited fluorescence with moderate Φ_f of 4.6–7.3%. Intermediate quantum yields were observed with nucleosides **97e,g,h** (Φ_f = 14–26%). The methyl derivative **97d** exhibited fluorescence with very good Φ_f of 40%. The benzofuryl derivative **97c** even exhibited excellent fluorescence quantum yield Φ_f of 51%.

The quinolino-fused nucleoside **97c** exhibited higher fluorescence than the corresponding naphtho-fused derivative (Φ_f = 40%),[50] but not as high as the fluorescence of the corresponding pyrido-fused derivative ($\Phi_f = 62\%$).[46]

3.5. Biological Evaluation

The nucleosides **61a-h** and **97a-h** were tested for their cytotoxic activity. The MTS assay was performed by the research group under Prof. M. Hajdúch at the *Institute of Molecular and Translational Medicine* of the Palacký University in Olomouc, Czech Republic. The XTT and CellTiter-Glo assays were performed by the *Biochemical Pharmacology* group under Dr. H. Mertlíková-Kaiserová at IOCB Prague.

The nucleosides **61a-h** and **97a-h** were also evaluated for antiviral activity. The required testing was performed by the *Virology* group under Dr. J. Weber at IOCB Prague and the Anti-HCV screening for **61a-h** was performed at *Gilead Sciences, Inc*.

3.5.1. Pyrazolo-fused 7-Deazapurine Ribonucleosides

The following cancer cell lines were used for the study of *in vitro* cytotoxic activity: A549 (lung cancer), CCRF-CEM and CEM-DNR (acute T-lymphoblastic leukemia without and with drug resistance), HCT116 and HCT116p53$^-$ (colon carcinoma), K562 and K562-TAX (chronic myelogenous leukemia without or with multi-drug resistance), U2OS (bone osteosarcoma) in a MTS assay; as well as HeLaS3 (cervical cancer), HepG2 (hepatocellular liver carcinoma) and HL60 (acute promyelocytic leukemia) in an XTT assay.[118] For comparison, non-malignant fibroblast cell lines (BJ and MRC-5) were used in the MTS assay. The required concentrations of **61a-h** for the reduction of the respective cell viability by 50% (IC_{50}) were determined by colorimetric assays.[119] In the MTS assay,[120] after a 3-day treatment of the cell line with the respective nucleoside, the samples were stained with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) followed by detection at 492 nm. Initial testing is done with 50 μM concentration. In the XTT assay,[121] after incubation for 3 days the samples were treated with 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) prior to visualization. The initial testing concentration was 10 μM. (**Table 26**)

Table 26. Cytotoxic activities of nucleosides **61a–h**.

compd	MTS assay: IC_{50} [μM]										XTT assay: IC_{50} [μM]		
	BJ	MRC-5	A549	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53-	K562	K562-TAX	U2OS	HeLa	HepG2	HL60
61a	> 50	> 50	> 50	3.9	35	49	> 50	49.8	3.6	> 50	> 10	> 10	> 10
61b	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 10	> 10	> 10
61c	> 50	> 50	> 50	28	42	34	48	48	33	> 50	> 10	> 10	> 10
61d	27	> 50	-	0.46	2.0	1.8	4.9	1.9	2.2	2.9	> 10	3.8	1.4
61e	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 10	> 10	> 10
61f	47	> 50	3.6	0.54	1.1	0.83	2.0	1.4	0.58	2.3	> 10	1.15	1.11
61g	> 50	> 50	> 50	0.6	> 50	18	17	1.2	> 50	19	> 10	> 10	1.39
61h	3.2	> 50	37	0.51	1.3	0.32	0.43	0.49	0.9	2.0	> 10	> 10	1.2

Out of the tested compounds **61a-h**, the most cytotoxic were the 8-methyl derivative **61d**, 8-amino derivative **61f** and 8-methylsulfanyl derivative **61h** that exhibited low micromolar activity in most leukemia and cancer cell lines and submicromolar activity in CCRF-CEM leukemia cell line. Compounds **61d** and **61f** were less cytotoxic in non-cancerous BJ and MRC-5 fibroblasts, while **61h** showed micromolar cytotoxicity in BJ cells.

In general, the methylpyrazolo-fused deazapurine nucleosides **61a-h** were found to be less cytotoxic than the methylpyrrolo-fused nucleosides[47] but still, the compounds **61d** and **61e**

show an interesting potential therapeutic window between the cytotoxicity in the cancer cell lines and non-proliferating cells.

The antiviral activity testing of compounds **61a-h** was performed against influenza, hepatitis C virus (HCV), herpes simplex, Coxsackie, human immunodeficiency virus (HIV) and dengue viruses. All the nucleosides were inactive against most of these viruses.

Table 27. Antiviral activities of nucleosides **61a–h**.

	HCV replicon 1B		HCV replicon 2A	
compd	EC_{50} [μM]	CC_{50} [μM]	EC_{50} [μM]	CC_{50} [μM]
61a	13.9	>44.4	30.4	>44.4
61b	>44.4	>44.4	>44.4	>44.4
61c	34.9	>44.4	>44.4	>44.4
61d	0.6	>44.4	0.3	> 44.4
61e	>44.4	>44.4	>44.4	>44.4
61f	> 44.4	35.1	0.1	> 44.4
61g	0.5	> 44.4	0.2	> 44.4
61h	0.3	>44.4	0.08	>44.4

Only the anti-HCV screening[122] showed that the 8-methyl, 8-amino-, 8-methoxy- and 8-methylsulfanyl derivatives **61d,f,g,h** exerted submicromolar activity in 1B and 2A replicon assays (**Table 27**) with low toxicity against the Huh-7 cells. Unfortunately, the same compounds also exerted cytotoxicity at submicromolar concentrations against several leukemia and cancer cell lines so the selectivity is not sufficient for further development of these compounds as antivirals.

3.5.2. Quinolino-fused 7-Deazapurine Ribonucleosides

All the quinolino-fused nucleosides **97a-h** were tested for their *in vitro* cytotoxic activity. The following cancer cell lines were used for the study: A549 (lung cancer), CCRF-CEM (acute T-lymphoblastic leukemia), HCT116 and HCT116p53^{-} (colon carcinoma, parental and p53 deficient), K562 (chronic myelogenous leukemia), U2OS (bone osteosarcoma) using a colorimetric MTS assay.[118,120] Additionally, HeLa (cervical cancer), HepG2 (hepatocellular liver carcinoma) and HL60 (acute promyelocytic leukemia) cell lines were tested using the luminescent CellTiter-Glo assay. For comparison, non-malignant fibroblast cell lines (BJ and MRC-5) were included in the MTS assay, while non-cancerous human dermal fibroblasts (NHDF) were assessed with the CellTiter-Glo assay.[119] Initial screenings were done at 50 μM concentration for the MTS assay and 10 μM for the CellTiter-Glo assay. (**Table 28**)

Table 28. Cytotoxic activities of nucleosides **97a–h**.

compd	MTS assay: IC_{50} [μM]								CellTiter-Glo assay: IC_{50} [μM]			
	BJ	MRC-5	A549	CCRF-CEM	HCT116	HCT116 p53-	K562	U2OS	NHDF	HeLa	HepG2	HL60
97a	>50	>50	>50	38.5	>50	>50	>50	>50	>10	>10	>10	>10
97c	>50	>50	>50	18.6	36.0	43.2	>50	>50	>10	>10	2.8	>10
97d	>50	>50	26.4	6.5	17.0	17.0	11.9	13.1	>10	>10	>10	6.7
97e	>50	>50	>50	>50	>50	>50	>50	>50	>10	>10	>10	>10
97f	>50	>50	>50	8.5	18.9	17.3	10.0	17.1	>10	>10	8.6	5.2
97g	>50	>50	27.4	8.0	9.8	23.1	40.2	17.3	>10	>10	>10	5.2
97h	36.8	36.8	17.0	5.1	9.3	16.2	18.6	15.0	>10	>10	>10	9.3

Of all the title nucleosides, the dimethylamino derivative **97e** is the only one that did not show any cytotoxicity whatsoever, which is consistent with all other heteroaryl-fused nucleosides. Both 2-furyl and benzofuryl derivatives **97a** and **97c**, respectively, showed only weak activity against CCRF-CEM leukemia line, the methyl derivative **97d** also exhibited activity against both HCT116/HCT115p53- colon carcinoma lines and pronounced effect on the HepG2 hepatocellular carcinoma cell line, with an IC_{50} value of 2.8 μM indicating a specificity not observed in **97a**. The methylsulfanyl derivative **97h** displayed moderate cytotoxic activity against a spectrum of tested cell lines including non-malignant BJ and MRC-5, thus showing no significant selectivity toward cancerous cells.

The most promising nucleosides in this series are methyl **97d**, amino **97f**, and methoxy derivative **97g**, which all showed comparable activities against several cancer cell lines, with CCRF-CEM and HL60 being the most sensitive ones with single-digit micromolar IC_{50} values and no toxicity against non-malignant cell lines BJ, MRC-5 or NHDF. Although the nucleosides **97d**, **97f** and **97g** are more potent against CCRF-CEM line compared to their naphtho-fused analogs (6–8 μM vs 20–23 μM),[50] their activities are still two orders of magnitude lower than their tricyclic thieno-,[48] furo-, *N*-methylpyrrolo[47] and pyrido-fused[46] analogues.

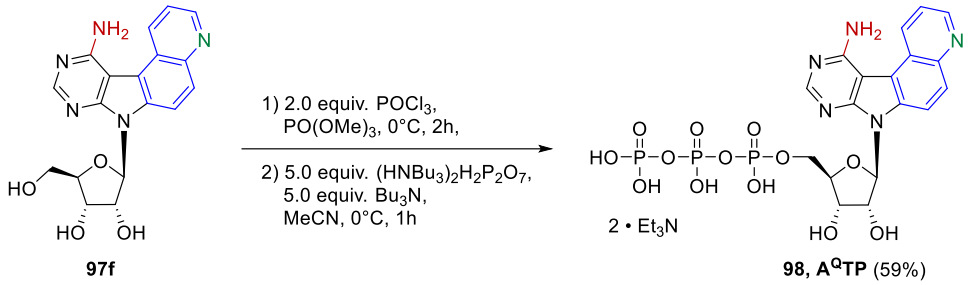
These findings together with the findings from the benzothieno-[51] and benzofuro-fused series[52] suggest that ribonucleosides with tetracyclic nucleobases might already be too bulky to be activated by phosphorylation and to interact with their biological target(s).

3.6. Biochemistry

The amino derivative **97f** was used as an adenosine analogue to study its incorporation by *in vitro* transcription and its fluorescent properties. This part of my thesis was done in cooperation with my colleague Tania Sánchez Quirante at IOCB Prague.

3.6.1. Triphosphorylation

The adenosine analogue **97f** was triphosphorylated at the 5'-OH according to standard procedures[123] resulting in the triphosphate **98** (**A^{Q}TP**) with good 59% yield. (**Scheme 38**)



Scheme 38. Triphosphorylation of the adenosine analogue **97f** furnishing the ATP analogue **98** (**A^{Q}TP**).

The formation of the monophosphate and cyclic triphosphate intermediates was indicated by a distinct color change and was monitored by TLC. The number of triethylammonium counterions was determined by ^{1}H NMR in D_2O.

3.6.2. *In vitro* Transcription[124]

The **A^{Q}TP** was then used as a substrate for the T7 RNA polymerase in the *in vitro* transcription (IVT) experiments.[125] We used **35DNA_A7** DNA template encoding for 35-mer RNA containing seven **A^{Q}** modifications (**Table 29**). We used the *T7 High Yield RNA Synthesis Kit* with a high concentration of NTPs (7.5 mM each) but without any further additives. The reaction time at 37 °C was 16 hours.

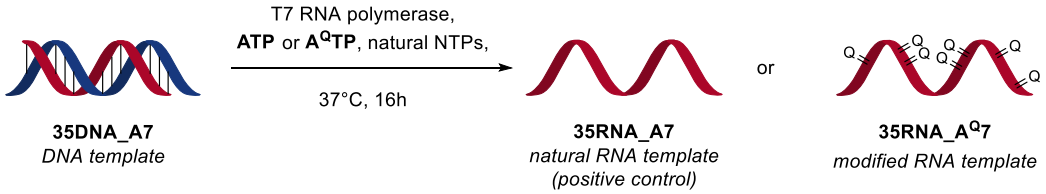


Table 29. Sequences of oligonucleotides and RNA products

Oligonucleotide	Sequence
35DNA_A7	5'-TAATACGACTCACTATAGGGCTTGCACGTGAATCGCTCTTAATGGATCGCGA-3' 3'-ATTATGCTGAGTGATATCCCGAACGTGCACTTAGCGAGAATTACCTAGCGC[mT]-5'
35RNA_A7	5'-pppGGGCUUGCACGUGAAUCGCUCUUAAUGGAUCGCGA
35RNA_A^{Q}7	5'-pppGGGCUUGCA^{Q}CGUGA^{Q}A^{Q}UCGCUCUUA^{Q}A^{Q}UGGA^{Q}UCGCGA^{Q}

The positive control experiment was performed with all four natural NTPs giving non-modified transcript **35RNA_A7** (lane 2). The negative control contained only the natural CTP, GTP, and UTP in the absence of ATP (lane 3). The real IVT experiment was performed with **A^{Q}TP** and three natural NTPs (lane 4). The transcription products were visualized by

denaturing 20% denaturating PAGE (**Figure 23A**) and characterized by LC-MS (**Figure 23B** and **Figure 23C**).

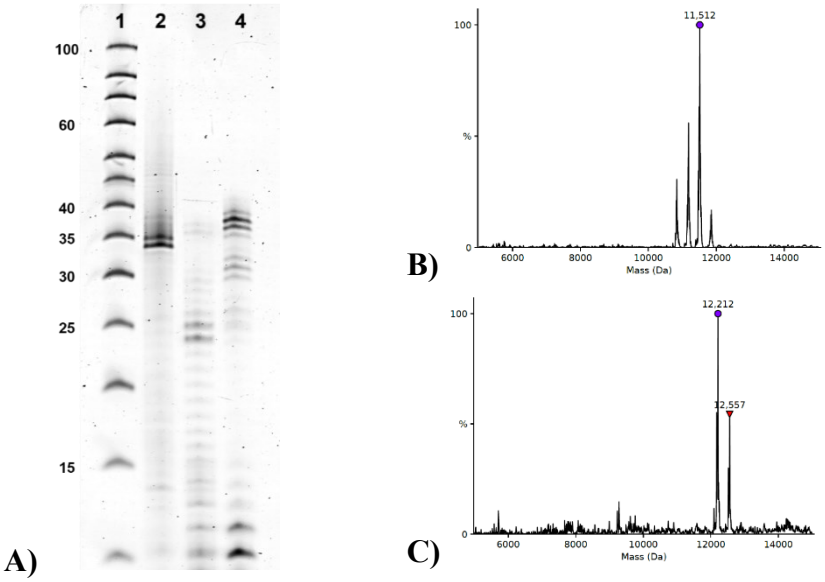


Figure 23. **A)** Denaturing PAGE analysis of the *in vitro* transcription reaction with T7 RNA polymerase and **35DNA_A7** template which provides 7 incorporations of the modified nucleotide. Lane 1: RNA ladder, lane 2: positive control (all natural NTPs), lane 3: negative control (CTP, GTP, UTP), lane 4: modification (**A^{Q}TP**, CTP, GTP, UTP). **B)** After IVT, the natural **35RNA_A7** (positive control) was analyzed by LC-ESI-MS. Deconvoluted mass spectrum of **35RNA_A7**: calcd 11471 Da, found 11512 Da [+K^+]. **C)** After IVT, the modified **35RNA_A^{Q}7** was analyzed by LC-ESI-MS. Deconvoluted mass spectrum of **35RNA_A^{Q}7**: calcd 12174 Da, found 12212 Da [+K^+] and 12557 Da [+G,+K^+].

We observed the formation of a full-length RNA resulting in the modified RNA **35RNA_A^{Q}7** containing seven **A^{Q}** modifications and partial formation of an n+1 product containing additional guanosine at the 3'-end of the RNA strand as a result of non-templated addition. Unfortunately, also some truncated products were observed indicating that the incorporation of this bulky modified nucleotide by the T7 RNA polymerase was less efficient compared to standard 7-substituted 7-deaza-ATP derivatives.

3.6.3. Spectroscopic Properties

With the IVT products in hand, I also studied the absorption and emission spectra of the triphosphate **98** (**$A^{Q}TP$**) and the oligonucleotide **35RNA_A^{Q}7** in water. (**Table 30**).

Table 30. UV and fluorescence maxima of nucleoside **97f**, triphosphate **98** (**$A^{Q}TP$**), natural ATP and all transcription products in water.

	Absorption max. λ_{abs} (ε) [nm (M^{-1} cm^{-1})]	Emission max. λ_{em} [nm]	Quantum Yield Φ_f
Nucleoside **97f**	290 (11200), 333 (5700)	420	0.02855
Triphosphate **98** (**$A^{Q}TP$**)	258 (21000), 290 (12600), 333 (6600)	579	0.00005
Natural ATP	256 (6300)	-	
Positive control (**35RNA_A7**)	256	-	
Negative control (IVT without T7 polymerase)	-	-	
Modification (**35RNA_A^{Q}7**)	256, 333	576	0.01120

UV and fluorescence maxima were measured in MilliQ water. The emission spectra were recorded after excitation at λ_{ex} = 333 nm. The molar extinction coefficient ε was not calculated for the transcription products as their absolute concentration cannot be determined by *Nanodrop*. Fluorescence quantum yields Φ_f were determined using quinine sulfate in 0.1 M H_2SO_4 as a standard (Φ_f = 0.546 at 25 °C).[116]

The real quantum yield of nucleoside **97f** in water might be masked by the presence of DMSO in the first solvation sphere of **97f** from the stock solution prior to the dilution in water. This would also explain the drop in fluorescence after triphosphorylation (stock solution in water). The fluorescence of the triphosphate **98** is very weak (Φ_f = 0.05‰). Once incorporated into RNA, the fluorescence increased more than 200-fold with only seven modifications in the 35mer (Φ_f = 11.2‰). This increase is significant but the quantum yield of the modified RNA template is still low. High concentration of RNA would be required for the detection. Thus, the triphosphate **98** might not be the best choice for fluorescent labelling of RNA *in vitro*.

4. Conclusion

Ribonucleosides with tri- and tetracyclic nucleobases (1-methylpyrazolo- and quinolino-fused 7-deazapurines) were prepared and the different synthetic strategies were evaluated. The final ribonucleoside derivatives **61a-h** and **97a-h** were tested for their biological activity against a broad panel of cancer cell lines as well as different viruses *in vitro*.

The tricyclic pyrazolo-fused 7-deazapurine nucleobase **47** was synthesized using two different approaches: (i) a six-step classical heterocyclization approach starting from 5-chloro-1-methyl-4-nitropyrazole **43** with 18% overall yield, and (ii) a three-step cross-coupling and cyclization approach starting from the zincated 4,6-dichloropyrimidine **22** and 5-iodo-1-methylpyrazole **48** with 6–15% overall yield. Neither one of these approaches was superior and the choice for the synthetic strategy of other heterocycles mainly depends on the availability of starting materials. The *β*-anomeric nucleoside intermediate was prepared using three different glycosylation methods. Only the Vorbrüggen glycosylation yielded the intermediate **57** as a pure *β*-anomer in 51% yield. The nucleobase-anion glycosylation furnished the corresponding nucleoside **58** in an inseparable anomeric mixture in total 61% yield. The Downey glycosylation under modified Mitsunobu conditions gave only traces of the desired product **59**. This is in agreement with the existing view that the Vorbrüggen procedure is the first choice. Derivatization and deprotection of the nucleoside intermediate **57** gave a series of eight different pyrazolo-fused deazapurine ribonucleosides **61a-h**.

These fused nucleoside derivatives exerted fluorescence with low to moderate quantum yields in methanol (1.4–7.7%). The methyl, amino, and methylsulfanyl derivatives (**61d,g,h**) exerted submicromolar cytotoxic effects *in vitro* against a panel of cancer and leukemia cell lines with low toxicity to non-proliferating fibroblasts. The same derivatives, as well as the methoxy derivative exerted submicromolar anti-HCV activities.

To access additional tricyclic heteroaryl-fused 7-deazapurine nucleobases, the C-H functionalization of 5-membered heterocycles through the formation of different aryl sulfonium salts was studied. These sulfonium salts would then serve as an alternative to heteroaryl iodides in the Negishi cross-coupling with the zincated 4,6-dichloropyrimidine **22**. The systematic study of 5-membered heterocycles with one or two heteroatoms contained twelve different examples. Each of them was subjected to the thianthrenation and dibenzothiophenation with thianthrene *S*-oxide (TT=O) and dibenzothiophene *S*-oxide (DBT=O), respectively, in the presence of TFAA. For both sulfoxides, only the corresponding sulfonium salts with pyrrole, 1-methylpyrrole, thiophene, and 1-methylpyrazole were formed. The other heterocycles were unreactive. The Negishi cross-coupling reaction between the methylpyrazol-4-yl-thianthrenium salt **63** and the zincated 4,6-dichloropyrimidine **22** did not yield the desired pyrazolyl pyrimidine cross-coupling product. The same reaction was performed also with the dibenzothiophenium salt **68** but only resulted in the decomposition of the sulfonium salt. The Negishi cross-coupling between the thiophen-2-yl dibenzothiophenium salt **72** and the zincated 4,6-dichloropyrimidine **22** resulted in the desired cross-coupling product **73** with 56% yield. The same reaction with 2-iodothiophene gave the same cross-coupling product **73** with 60–80% yield. To this day, the C-H functionalization of heterocycles through the formation of aryl sulfonium salts is a great concept, but stays limited to fused and biaryl heterocyclic ring systems.

The tetracyclic quinolino-fused 7-deazapurine was synthesized using the three-step cross-coupling and cyclization approach. Four different iodo(iso)quinolines (**79-82**) were subjected

to the Negishi cross-coupling requiring an increased amount of the zincated 4,6-dichloropyrimidine **22**. The reaction with 5-iodoquinoline gave the desired cross-coupling product **83** with 78% yield. The other iodo(iso)quinolines gave the corresponding cross-coupling products **84** and **85** in significant lower yields. Apparently the position of the nitrogen in the quinoline ring system correlates with the yield of the coupling reaction. The further apart the nitrogen position is from the iodo position, the better the outcome. The reason for this correlation was not investigated further but a side reaction during the transmetalation step is possible. The synthesis was continued only with the most successful cross-coupling product. After azidation, the cyclization required extensive optimization: The thermal cyclization gave the desired tetracyclic nucleobase **88** in only 11–18% yield. In a flow reactor, the thermal cyclization did not give the desired product but the amine side product **89** instead. The rhodium-catalyzed reaction did not give the desired product neither; and the photocyclization in TFA resulted in decomposition of the azide. Only the photocyclization in THF with pyrene, a singlet photosensitizer and triplet quencher, resulted in the formation of the quinolino-fused 7-deazapurine **88** with 26% yield. Although the Vorbrüggen glycosylation gave the benzoylated nucleoside intermediate **94** as a pure β-anomer with only 75% purity (but 52% yield), it was still better than the anion base glycosylation, which gave the nucleoside intermediate **95** in an anomeric mixture with only 29% total yield (β/α = 2:1). The benzoylated nucleoside was used for the final derivatization and deprotection of seven different quinolino-fused 7-deazapurine ribonucleoside derivatives which exerted fluorescence with moderate to excellent yields in methanol (3–51%).

The methyl, amino, and methoxy derivatives (**97d,f,g**) showed moderate cytotoxic effects *in vitro* against a panel of cancer and leukemia cell lines with low toxicity to non-proliferating fibroblasts. This makes them more potent than their naphtho-fused analogues[50] suggesting certain positive effect of a nitrogen atom in the fused ring. However, the quinolino moiety might be too bulky for the for the interaction with their biological target and the efficient intracellular phosphorylation required for the incorporation into DNA and/or RNA.

The amino derivative **97f** was triphosphorylated (59% yield) and used as an ATP analogue in *in vitro* transcription experiments with the T7 RNA polymerase. The corresponding full-length RNA strand was 35 nucleotides long, contained 7 modifications, and exerted only low fluorescence in water (1.1%).

5. Experimental

5.1. General Remarks

5.1.1. Materials and Experimental Methods

All reagents purchased from suppliers were of analytical grade and were used directly without further purification. Pre-dried compounds were dried under vacuum for at least 4 hours. Unless otherwise stated, all reactions were carried out under argon atmosphere. An oil bath or a metal heating block were used for reactions requiring heating. All solvents for reactions were anhydrous and of analytical grade from *Acros* and used without further purification. Solvents used for extraction and for chromatography were from *Lach-Ner* and used without prior distillation. Solvents used for washing after filtration of precipitated products were cooled to –18 °C resp. 4 °C for water prior to use. All products were dried under vacuum overnight prior to analytical characterization.

Thin-layer chromatography (TLC) was performed on *Merck silica gel 60 F-254 aluminum sheets*. Visualization was obtained by UV light (λ_{max} = 254 nm or 366 nm). High Pressure Liquid Chromatography – Mass Spectroscopy (HPLC-MS) for reaction control was performed on a *1260 Automated LC/UV Purification System* with an *InfinityLab Poroshell 120 EC-C18* column (3.0 × 50 mm, particle size 2.7 µm) coupled with a *6530 Accurate-Mass QTOF LC/MS* spectrometer from *Agilent Technologies Inc*. The solvent system 0.1% formic acid in water / MeCN at 0.400 mL min^{-1} flow rate was run (gradient from 95:5 to 0:100 within 8 min, 0:100 for 5 min, 95:5 for 3 min).

Flash chromatography (FC) was performed with SiO_2 (particle size 0.040–0.063 mm, 230–400 mesh) from either *Merck* or *Fluorochem* in a glass column (Ø = 8 cm) with the solvent gradient indicated in the corresponding procedures. The separation was directly monitored by connecting the column output to a UV monitor[126] (λ_{max} = 254 nm) before collecting. High-performance flash chromatography (HPFC) was conducted with a *Combi Flash R_f* instrument from *Teledyne Isco Inc.* using the same SiO_2 from either *Merck* or *Fluorochem* in refillable flash columns or using *HP C18 Redi Sep R_f gold* flash columns with the solvent gradient indicated in the corresponding procedures. Preparative high-pressure liquid chromatography (prep. HPLC) was performed on a *Waters 2535 Quaternary Gradient* System with a fraction collector.

5.1.2. Characterization

Melting points (m.p.) were measured by Bohunka Šperlichová at Charles University Prague on a *Büchi Melting Point B-545* apparatus using open glass capillaries and are uncorrected. Optical rotation of final nucleosides was measured by the analytical laboratory at IOCB Prague using an *AUTOPOL IV* automatic polarimeter from *Rudolph Research Analytical* at 20 °C and 589 nm. The sample concentration c is given in $g\,mL^{-1}$. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were measured on a *Bruker Avance III HD 400 MHz*, on a *Bruker Avance III 500 MHz* or on a *Bruker Avance III 600 MHz* spectrometer at 20 °C in $CDCl_3$ referenced to the residual solvent signal (δ_H = 7.26 ppm, δ_C = 77.16 ppm), in deuterated dimethyl sulfoxide (DMSO-d_6) referenced to the residual solvent signal (δ_H = 2.50 ppm, δ_C = 39.52 ppm], or in D_2O with *t*BuOD-d_{10} as the internal standard [δ_H = 1.25 ppm, δ_C = 31.6 ppm]. ^{19}F NMR spectra and ^{31}P NMR spectra were measured without any internal reference. Coupling constants (J) are given in Hz and the multiplets are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). $^{13}C\{^{1}H\}$ NMR experiments are broadband proton-decoupled and were performed using APT pulse sequence. DFQ-COSY, HSQC and HMBC experiments were used to assign the ^{1}H and ^{13}C NMR signals where required. ROESY experiments were used to confirm the relative stereochemistry of nucleosides **57**, **58**, **94**, and **95**. To simplify the assignment, the benzoyl group attached to the 2'-hydroxy group of the ribofuranose ring was given the letter A, the one attached to 3'-hydroxy group is considered B and the benzoyl group at the 5'-OH is called C (**Figure 24**). The Spectra can be found in the supporting information of the corresponding publications.[49,54] In these ROESY spectra for nucleosides **57**, **58**-*β*, **94**, **95**-*β* and **95**-*α*, the important relations for the determination of the stereochemistry are highlighted.

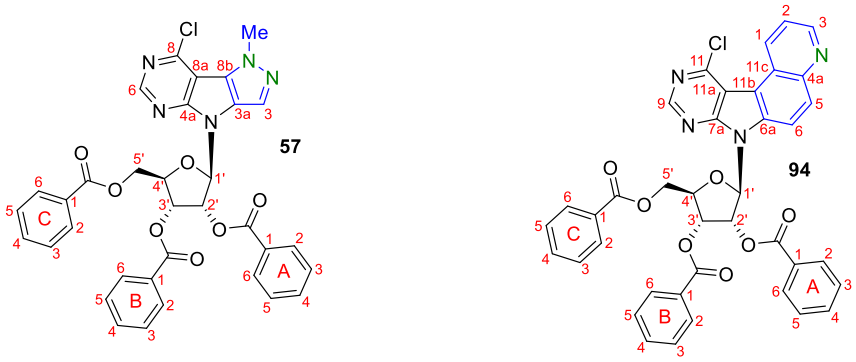


Figure 24: Numbering used in the assignment of NMR signals: examples of key intermediates **57** (left) and **94** (right).

Infrared spectra (IR) were recorded on a *Bruker ALPHA FT-IR* spectrometer with a *single-reflexion Platinum ATR* module. The compounds were measured in their initial state of appearance at 20 °C, and their absorption bands were reported in wavenumbers ($\tilde{\nu}$) in the range between 4000–400 cm^{-1}. Intensities are described as strong (s), medium (m) and weak (w). UV/Vis spectra were measured on a *Varian Cary 100 Bio* UV-Visible spectrophotometer in the range 250–800 nm using transparent 1.5 mL quartz cuvettes. Fluorescence spectra were recorded on a *Fluoromax 4* spectrofluorimeter from *HORIBA Scientific*. The sample concentration was adjusted to have an UV absorbance of 0.05–0.10. The excitation was performed at the absorption maximum with the highest wavelength λ_{abs} with the slit set at 2 nm. The emission spectra were recorded from λ_{abs} + *20 nm* to *2* × λ_{abs} – *20 nm* with a 2 nm slit opening. High-resolution mass spectrometry (HR-MS) was measured by the MS service at IOCB Prague on an *LTQ Orbitrap XL* instrument from Thermo Fisher Scientific using electrospray ionization (ESI) or on a *Waters GCT Premier* instrument using electron ionization (EI).

The purity of the final nucleosides **57a-h** (>95%) was confirmed by analytical HPLC on a *Waters 717 Autosampler* system with a *Waters 2996 Photodiode Array Detector* using a

Gemini 5 µm C18 110 Å column (250 × 4.60 mm) from *Phenomenex*. Samples were dissolved in DMSO (10 µL injection volume). Whereas the purity of the final nucleosides **94a-h** (>95%) was confirmed by UPLC-MS on an *Agilent 1260 Infinity II LC* system with an *Agilent 1260 Photodiode Array Detector* using a *KinetexEVO C18 100 Å* column (2.1 × 150 mm) from *Phenomenex*. Samples were dissolved in DMSO (1 µL injection volume).

5.1.3. Biochemistry

Single stranded DNA oligonucleotides for the preparation of the double stranded DNA template **35DNA_A7** were purchased from *Generi Biotech*. The *T7 Hight yield RNA Kit*, *DNase I*, *EDTA* (50 mM), and the *Monarch RNA purification kit* (50 µg) were purchased from *New England Biolabs*. RNase/DNase free solutions for biochemical reactions were prepared using MilliQ water, that was treated with DEPC and sterilized by autoclaving.

The precision RNA mass marker 10–100nt was purchased in *Future Synthesis*. The denaturating PAGE gel was analyzed by fluorescence (λ_{ex} = 532 nm) using *Typhoon FLA 9500* from *GE Healthcare Life Sciences*. LC-ESI-MS analysis of oligonucleotides was carried out on an *Agilent 1260 Infinity II LC* system with an *Agilent InfinityLab LS/MSD XT* Detector using a *BioZen C18 100 Å* column (2.1 × 150 mm) from *Phenomenex* with the mobile phases A (12.2 mM Et_3N, 300 mM HFIP in water) and B (12.2 mM Et_3N, 300 mM HFIP in 100% MeOH) and a gradient from 95:5 to 0:100 within 10 min. Deconvolutions of the HPLC-ESI-MS spectra were carried out using a *UniDec* program.

5.2. Experimental Procedures

5.2.1. Synthesis of pyrazolo-fused 7-Deazapurine Nucleosides

1-Methyl-4-nitro-1*H*-pyrazole (**42**)

A colorless solution of pre-dried 4-nitro-1*H*-pyrazole (**41**, 20.0 g, 177 mmol) in DMF (100 mL) was stirred at 20 °C for 15 min and treated with K_2CO_3 (29.4 g, 213 mmol) resulting in a white suspension. After adding iodomethane (13.2 mL, 212 mmol), the pale-yellow suspension was stirred at 20 °C for 18 hours. The resulting bright-yellow suspension was treated with water (200 mL) and extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine (600 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by FC (SiO_2; Hex/EtOAc, gradient 0 → 50% EtOAc) gave **42** (22.1 g, 98%) as a white solid.

R_f = 0.49 (SiO_2; Hex/EtOAc 1:1); m.p. = 91 °C (Lit.[127]: 91–92 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.95 (s, 3 H; N*Me*), 8.01 (s, 1 H; H-3), 8.12 ppm (s, 1 H; H-5); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 40.2 (N*Me*), 129.3 (C-5), 135.8 (C-3), 135.7 ppm (C-4); IR (ATR): $\tilde{\nu}$ = 3112 (m), 1504 (m), 1421 (m), 1398 (s), 1310 (s), 1129 (m), 999 (m), 968 (m), 885 (s), 818 (s), 753 (s), 692 (w), 648 cm^{-1} (w); HR MS (EI) for $C_4H_5N_3O_2^+$ [M]$^+$: calcd 127.0382, found 127.0383.

5-Chloro-1-methyl-4-nitro-1*H*-pyrazole (**43**)

LiHMDS (260 mL, 1.0 M in THF, 260 mmol) was added dropwise (over 50 min) to a stirring solution of **42** (22.04 g, 173.40 mmol) and hexachloroethane (42.6 g, 178.2 mmol) in DCM (340 mL). The resulting bordeaux-red solution was stirred at 20 °C for 4 hours and then treated with water (400 mL). After extraction, the organic layer was washed with satd $NaHCO_3$ (2 × 400 mL) and brine (400 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by FC (SiO_2; Hex/EtOAc, gradient 0 → 50% EtOAc) gave **43** (24.6 g, 88%) as an off-white solid.

R_f = 0.70 (SiO_2; Hex/EtOAc 1:1); m.p. = 67 °C (Lit.[128]: 59–60 °C); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.92 (s, 3 H; N*Me*), 8.14 ppm (s, 1 H; H-3); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 37.6 (N*Me*), 127.6 (C-5), 130.4 (C-4), 136.6 ppm (C-3); IR (ATR): $\tilde{\nu}$ = 3124 (w), 1493 (s), 1397 (s), 1312 (s), 1180 (m), 1129 (m), 1047 (w), 975 (m), 883 (m), 827 (s), 756 (s), 735 (m), 588 cm^{-1} (w); HR MS (EI) for $C_4H_4N_3O_2{}^{35}Cl^+$ [M(^{35}Cl)]$^+$: calcd 160.9992, found 160.9991; for $C_4H_4N_3O_2{}^{37}Cl^+$ [M(^{37}Cl)]$^+$: calcd 162.9963, found 162.9972.

Ethyl 2-cyano-2-(1-methyl-4-nitro-1*H*-pyrazol-5-yl)acetate (**44**)

A white suspension of pre-dried potassium *tert*-butoxide (14.6 g, 130.4 mmol) in 1,4-dioxane (120 mL) was treated with ethyl cyanoacetate (13.2 mL, 123.8 mmol) and stirred at 20 °C for 15 min. Pre-dried **43** (10.0 g, 61.9 mmol) was dissolved in 1,4-dioxane (80 mL) and added via syringe. The reaction mixture was stirred at 100 °C for 48 hours. This orange suspension was treated with water (~20 mL) resulting in a red solution; followed by addition of hydrochloric acid (~50 mL, 1 M in water) until pH = 2 was reached. After extraction with Et_2O (3 × 200 mL), the combined organic layers were washed with brine (400 mL), dried over $MgSO_4$ and concentrated *in vacuo* giving a brown oil. Excess of unreacted ethyl cyanoacetate was removed by vacuum distillation at 95 °C / 24 mbar prior to solidification at –18 °C during 1 week. The solid was then washed with an ice-cold DCM and dried *in vacuo* giving **44** (14.3 g, 97%) as colorless crystals.

R_f = 0.38 (SiO_2; DCM/MeOH 95:5); m.p. = 140 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.36 (t, $J_{2'',1''}$ = 7.2 Hz, 3 H; H-2''), 4.03 (s, 3 H; N*Me*), 4.38 (m, 2 H; H-1''), 6.18 (s, 1 H; H-1'), 8.16 ppm (s, 1 H; H-3); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 14.1 (C-2''), 33.1 (C-1'), 39.0 (N*Me*), 65.0 (C-1''), 111.7 (*C*N),129.7 (C-5), 133.4 (C-4), 136.4 (C-3), 161.4 ppm (*C*OO); IR (ATR): $\tilde{\nu}$ = 3118 (w), 2990 (w), 2945 (w), 2899 (w), 2262 (w), 1743 (s), 1560 (m), 1509 (s), 1497 (s), 1453 (m), 1445 (m), 1398 (m), 1310 (s), 1261 (s), 1221 (m), 1190 (m), 1027 (s), 1000 (w), 984 (m), 894 (w), 856 (m), 825 (s), 795 (w), 759 (m), 705 (w), 644 cm^{-1} (w); HR MS (ESI) for $C_9H_{10}N_4O_4Na^+$ [M + Na]$^+$: calcd 261.0594, found 261.0594.

Ethyl 5-amino-1-methyl-1,4-dihydropyrrolo[3,2-*c*]pyrazole-6-carboxylate (**45**)

A solution of **44** (3.93 g, 16.5 mmol) in glacial acetic acid (37 mL) was heated to 80 °C; and under an argon stream, treated with zinc dust (5.49 g, 83.9 mmol) in small portions in order to avoid extensive gas formation. After stirring at 95 °C for 2 hours, HPLC-MS showed completion of the reaction (t_R = 2.53 min, m/z = 209). The greenish suspension was cooled down and the zinc species was removed by filtration through a celite plug (5 cm) followed by washing with fresh acetic acid (~50 mL). The filtrate was concentrated *in vacuo* giving a brown oil. Neutralization with satd $NaHCO_3$ (~50 mL) resulted in precipitation of the cyclized product. Filtration and washing with cold water gave **45** (1.91 g, 55%) as a light-grey solid.

R_f = 0.33 (SiO_2; (DCM with 1% TEA)/MeOH 9:1); m.p. > 300 °C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.28 (t, $J_{2',1'}$ = 7.1 Hz, 3 H; H-2'), 3.96 (s, 3 H; N*Me*), 4.18 (q, $J_{1',2'}$ = 7.1 Hz, 2 H; H-1'), 6.40 (bs, 2 H; NH_2), 6.99 (s, 1 H; H-3), 10.01 ppm (bs, 1 H; NH); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 14.6 (C-2'), 38.1 (N*Me*), 58.4 (C-1'), 118.5 (C-3), 120.3 (C-3a), 134.3 (C-6a), 164.2 ppm (*C*OO); IR (ATR): $\tilde{\nu}$ = 3337 (broad w), 1652 (w), 1610 (w), 1427 (s), 1379 (m), 1322 m), 1278 (w), 1238 (w), 1121 (w), 1095 (w), 962 (w), 843 (m), 754 (m), 694 cm^{-1} (w); HR MS (ESI) for $C_9H_{13}N_4O_2^+$ [M]$^+$: calcd 209.1033, found 209.1033.

1-Methyl-4,7-dihydropyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidin-8(1*H*)-one (**46**)

Powdered compound **45** (1.0 g, 4.8 mmol) was treated with sodium methoxide (2.2 mL, 25 wt% in MeOH, 9.62 mmol) and sonicated for 10 min to improve solubility. After adding formamide (3.82 mL, 96.15 mmol), the solution was stirred at 90 °C for 48 hours, until HPLC-MS showed completion of the reaction (t_R = 1.07 min, m/z = 190). Ice was added; followed by addition of concd HCl (0.85 mL, 35 wt% in water, 9.6 mmol) resulting in precipitation. Filtration and washing with ice-cold water (100 mL) gave **46** (418 mg, 46%) as a brown solid.

R_f = 0.38 (SiO_2; DCM/MeOH 9:1); m.p. > 300 °C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.16 (s, 3 H; N*Me*), 7.46 (s, 1 H; H-3), 7.99 (s, 1 H; H-6), 11.75 (bs, 1 H; H-4), 12.19 ppm (bs, 1 H; H-7); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 38.4 (N*Me*), 93.9 (C-8a), 119.9 (C-3), 125.2 (C-3a), 132.0 (C-8b), 145.8 (C-6), 154.6 and 157.1 ppm (2 C; C-4a, C-8); IR (ATR): $\tilde{\nu}$ = 3130 (m), 3066 (m), 3017 (m), 2898 (m), 1669 (s), 1619 (m), 1584 (m), 1537 (m), 1495 (w), 1424 (w), 1362 (m), 1330 (s), 1291 (m), 1236 (w), 1105 (m), 1014 (m), 990 (m), 884 (m), 860 (m), 825 (m), 797 (m), 777 (m), 721 (m), 687 (m), 640 cm^{-1} (m); HR MS (ESI) for $C_8H_7N_5ONa^+$ [M + Na]$^+$: calcd 212.0543, found 212.0544.

8-Chloro-1-methyl-1,4-dihydropyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**47**)

Approach A: A suspension of **46** (451 mg, 2.38 mmol) in MeCN (4.5 mL) was treated with BTEA-Cl (1.09 g, 4.80 mmol) and *N*,*N*-dimethylaniline (0.33 mL, 2.60 mmol). After 15 min, freshly distilled $POCl_3$ (1.35 mL, 14.48 mmol) was added dropwise and the brown mixture was stirred at 90 °C for 75 min, until HPLC-MS showed completion of the reaction (t_R = 4.20 min, m/z = 208, 210). After concentration *in vacuo*, ice was added followed by ammonia (1 M in water) until pH 4–5 was reached, resulting in precipitation. Filtration and washing with ice-cold water (50 mL) gave crude **47** (425 mg, 86%, NMR pure) as a grey-brown solid.

Approach B: A yellow mixture of **51** (72.2 mg, 0.306 mmol) and 1,4-dibromobenzene (365 mg, 1.55 mmol) was heated to 170 °C. An argon balloon and airlock (filled with EtOAc) were connected to the reaction mixture. During the reaction under argon flow, an increased gas evolution can be observed due to the formation of N_2. After 30 min, the reaction was complete, and the flow rate returned back to normal, and the stirring mixture was cooled down. When the dark mixture reached approx. 80 °C, EtOAc was added before cooling down to r.t. completely. After loading the mixture onto silica, 1,4-dibromobenzene was washed directly from the solid depot in the pre-column with cHex/DCM (1:1) into a beaker prior to the purification by HPFC (SiO_2; cHex/DCM 1:1/EtOAc, gradient 0 → 50% EtOAc) which gave crude **47** (46.8 mg, 60% pure, 44%) as a white solid.

R_f = 0.29 (SiO_2; cHex/EtOAc 1:1); m.p. > 300 °C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.31 (s, 3 H; N*Me*), 7.72 (s, 1 H; H-3), 8.66 (s, 1 H; H-6), 12.46 ppm (bs, 1 H; N*H*); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 39.7 (N*Me*), 103.5 (C-8a), 119.9 (C-3), 126.3 (C-8b), 129.9 (C-3a), 152.0 (C-6), 150.0 and 157.2 ppm (2 C; C-4a, C-8); IR (ATR): $\tilde{\nu}$ = 3025 (w), 2851 (w), 1619 (w), 1575 (w), 1531 (m), 1436 (m), 1306 (w), 1234 (m), 1087 (s), 1015 (s), 982 (s), 967 (s), 939 (s), 819 (m), 797 (s), 777 (s), 632 (m), 614 cm^{-1} (m); HR MS (EI) for $C_8H_6N_5{}^{35}Cl^+$ [M(^{35}Cl)]$^+$: calcd 207.0312, found 207.0313; for $C_8H_6N_5{}^{37}Cl^+$ [M(^{37}Cl)]$^+$: calcd 209.0282, found 209.0271.

4,6-Dichloro-5-(1-methyl-1*H*-pyrazol-5-yl)pyrimidine (**49**)

$ZnCl_2$ (789 mg, 5.79 mmol) was pre-dried at 250 °C for 20 min and allowed to cool down under vacuum before being treated with a solution of (TMP)MgCl · LiCl (11.5 mL, 1.0 M solution in THF/toluene, 11.5 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of 4,6-dichloropyrimidine (**22**, 1.41 g, 9.45 mmol) in THF (8 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of 5-iodo-1-methyl-1*H*-pyrazole (**48**, 4.00 g, 19.24 mmol) and $Pd(PPh_3)_4$ (2.22 g, 1.92 mmol) in THF (8 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 18 hours. After adding water (2 mL), the mixture was loaded on silica and filtered through a silica plug (1 cm) with DCM (500 mL). Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 50% EtOAc) gave **49** (925.7 mg, 42%) as a yellow solid.

R_f = 0.41 (SiO_2; cHex/EtOAc 2:1); m.p. = 86 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.73 (s, 3 H; N*Me*), 6.39 (d, $J_{4',3'}$ = 2.0 Hz, 1 H; H-4'), 7.62 (d, $J_{3',4'}$ = 2.0 Hz, 1 H; H-3'), 8.86 ppm (s, 1 H; H-2); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 37.2 (N*Me*), 108.4 (C-4'), 124.9 (C-5), 133.4 (CH-5'), 139.3 (C-3'), 158.5 (C-2), 162.9 ppm (2 C; C-4, C-6); IR (ATR): $\tilde{\nu}$ = 3127 (w), 2919 (w), 2850 (w), 1572 (w), 1518 (m), 1505 (m), 1470 (w), 1436 (w), 1404 (m), 1378 (m), 1359 (m), 1291 (w), 1272 (w), 1235 (w), 1200 (w), 1166 (w), 1094 (w), 1063 (w), 1029 (w), 975 (w), 926 (w), 807 (s), 782 (m), 774 (m), 744 (w), 698 (m), 685 (w), 649 (w), 599 cm^{-1} (w); HR MS (ESI) for $C_8H_7N_4{}^{35}Cl_2^+$ [M(^{35}Cl) + H]$^+$: calcd 229.00478, found 229.00429; $C_8H_7N_4{}^{35}Cl^{37}Cl^+$ [M(^{35}Cl^{37}Cl) + H]$^+$: calcd 231.00183, found 231.00139; for $C_8H_7N_4{}^{37}Cl_2^+$ [M(^{37}Cl) + H]$^+$: calcd 232.9989, found 232.9988.

4-Azido-6-chloro-5-(1-methyl-1*H*-pyrazol-5-yl)pyrimidine (**51a**) / 7-chloro-8-(1-methyl-1*H*-pyrazol-5-yl)tetrazolo[1,5-*c*]pyrimidine (**51b**)

A solution of **49** (271 mg, 1.18 mmol) in THF (6 mL) was treated with sodium azide (77.6 mg, 1.18 mmol) and lithium chloride (52.8 mg, 1.24 mmol) and stirred under light exclusion at 20 °C for 26 hours. After adding water (1 mL), the mixture was concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **51** (196 mg, 70%) as a light-yellow oil.

R_f = 0.40 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR of **51a** (500 MHz, $CDCl_3$): δ = 3.73 (s, 3 H; N*Me*), 6.33 (d, $J_{4',3'}$ = 2.0 Hz, 1 H; H-4'), 7.60 (d, $J_{3',4'}$ = 2.0 Hz, 1 H; H-3'), 8.76 ppm (s, 1 H; H-2); ^{13}C{^{1}H} NMR of **51a** (125.7 MHz, $CDCl_3$): δ = 37.3 (N*Me*), 108.5 (C-4'), 113.7 (C-5), 132.3 (C-5'), 139.1 (C-3'), 158.4 (C-2), 162.3 and 163.2 ppm (2 C; C-4, C-6); *tautomer **51b** was not observed in $CDCl_3$*; ^{1}H NMR of **51a** (500 MHz, DMSO-d_6): δ = 3.67 (s, 3 H; N*Me*), 6.40 (d, $J_{4',3'}$ = 2.0 Hz, 1 H; H-4'), 7.54 (d, $J_{3',4'}$ = 2.0 Hz, 1 H; H-3'), 8.94 ppm (s, 1 H; H-2); ^{13}C{^{1}H} NMR of **51a** (125.7 MHz, DMSO-d_6): δ = 36.8 (N*Me*), 108.3 (C-4'), 113.2 C-5), 132.1 (C-5'), 138.3 (C-3'), 158.5 (C-2), 162.6 ppm (2 C; C-4, C-6); ^{1}H NMR of **51b** (500 MHz, DMSO-d_6): δ = 3.74 (s, 3 H; N*Me*), 6.63 (d, $J_{4',3'}$ = 2.0 Hz, 1 H; H-4'), 7.68 (d, $J_{3',4'}$ = 2.0 Hz, 1 H; H-3'), 10.35 ppm (s, 1 H; H-5); ^{13}C{^{1}H} NMR of **51b** (125.7 MHz, DMSO-d_6): δ = 37.6 (N*Me*), 109.0 (C-4'), 112.6 (c-8), 131.5 (C-5'), 138.7 (C-3'), 140.4 (C-5), 147.8 and 150.5 ppm (2 C; C-7, C-9); IR (ATR): $\tilde{\nu}$ = 3374 (broad w), 2931 (w), 2856 (w), 2322 (w), 2143 (s), 2082 (w), 1604 (w), 1581 (w), 1549 (m), 1530 (s), 1464 (w), 1403 (s), 1385 (m), 1369 (m), 1321 (m), 1294 (m), 1276 (m), 1242 (m), 1203 (m), 1180 (m), 1150 (m), 1079 (w), 1057 (w), 976 (m), 936 (w), 897 (m), 848 (w), 786 (m), 752 (m), 701 (m), 649 cm^{-1} (w); HR MS (EI) for $C_8H_6N_7{}^{35}Cl^+$ [M(^{35}Cl)]$^+$: calcd 235.0373, found 235.0374; for $C_8H_6N_7{}^{37}Cl^+$ [M(^{37}Cl)]$^+$: calcd 237.0344, found 237.0393.

4-Iodo-1-methyl-1*H*-pyrazole (**53**)

In an extra-large flask, NaH (4.91 g, 60 w% dispersion in mineral oil, 123 mmol) was washed with cHex (3 × 40 mL) followed by THF (2 × 50 mL) to remove the mineral oil. After each washing step, the volatiles were removed by vacuum. THF (340 mL) was added, and the resulting suspension was cooled to 0 °C (water/ice). A solution of 4-iodopyrazole (**52**, 19.8 g, 102 mmol) in THF (40 mL) was added dropwise to the suspension. After stirring at 20 °C for 3 hours, the reaction mixture was treated with iodomethane (12.7 mL, 204 mmol), stirred further at 20 °C for additional 12 hours, and then treated with water (600 mL). After extraction with Et2O (2 × 600 mL), the combined organic layers were washed with brine (600 mL), dried over Na_2SO_4 and concentrated *in vacuo* giving **53** (20.5 g, 96%) as ivory crystals. ^{1}H NMR of the product **53** showed that the removal of mineral oil prior to the reaction made a purification afterwards unnecessary.

R_f = 0.63 (SiO_2; cHex/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.92 (s, 3 H; N*Me*), 7.40 (s, 1 H; H-3), 7.49 ppm (s, 1 H; H-5), *this is in agreement with literature*.[96]

4,6-Dichloro-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidine (**54**)

$ZnCl_2$ (208 mg, 1.53 mmol) was pre-dried at 250 °C for 20 min and allowed to cool down under vacuum before being treated with a solution of (TMP)MgCl · LiCl (3.0 mL, 1.0 M solution in THF/toluene, 3.0 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of 4,6-dichloropyrimidine (**22**, 375 mg, 2.52 mmol) in THF (2 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of **53** (520 mg, 2.50 mmol) and $Pd(PPh_3)_4$ (291.6 mg, 0.25 mmol) in THF (2 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 18 hours. After adding water (0.5 mL), the mixture was loaded on silica. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 15% EtOAc) gave **54** (142 mg, 25%) as a yellow solid.

R_f = 0.68 (SiO_2; cHex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.01 (s, 3 H; N*Me*), 7.68 (s, 1 H; H-5'), 7.74 (s, 1 H; H-3'), 8.67 ppm (s, 1 H; H-2); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 39.31 (N*Me*), 111.91 (C-4'), 126.43 (C-5), 131.20 (CH-5'), 140.03 (C-3'), 155.49 (C-2), 161.14 ppm (2 C; C-4, C-6).

4-Azido-6-chloro-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidine (**55a**) / 7-chloro-8-(1-methyl-1*H*-pyrazol-4-yl)tetrazolo[1,5-*c*]pyrimidine (**55b**)

A solution of **16** (350 mg, 1.53 mmol) in DMF (3.82 mL) was treated with sodium azide (101 mg, 1.57 mmol) and lithium chloride (66.0 mg, 1.56 mmol) and stirred under light exclusion at 30 °C for 55 hours. Adding water (2 mL) resulted in precipitation. Filtration, and washing with cold water (~50 mL) gave **17** (320 mg, 89%) as a beige solid.

^{1}H NMR of **55b** (500 MHz, DMSO-d_6): δ = 4.02 (s, 3 H; N*Me*), 8.51 (s, 1 H; H-5'), 8.85 (s, 1 H; H-3'), 10.04 ppm (s, 1 H; H-2).

8-Chloro-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo-[2,3-*d*]pyrimidine (**57**)

A suspension of nucleobase **47** (406 mg, 1.96 mmol) in MeCN (18 mL) was treated with BSA (0.72 mL, 2.95 mmol), sonicated for 30 min to maximize solubility and stirred at 60 °C for 1 hour. A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (**30**, 1.99 g, 3.95 mmol; dried under vacuum at 60 °C for 6 hours) in MeCN (2 mL) was added, followed by TMSOTf (0.72 mL, 3.95 mmol). After stirring at 60 °C for 36 hours, the resulting mixture was treated with water (20 mL) and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with satd $NaHCO_3$ (100 mL) and water (100 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 50% EtOAc) gave **57** (407 mg, 51%) as a pearl-colored foam. A small amount was recrystallized from DCM/MeOH prior to characterization.

R_f = 0.64 (SiO_2; cHex/EtOAc 1:1); m.p. = 97 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.38 (s, 3 H; N*Me*), 4.68 (dd, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 3.6 Hz, 1 H; H-5'a), 4.82 (m, 1 H; H-4'), 4.85 (dd, $J_{5'b,5'a}$ = 12.0 Hz, $J_{5'b,4'}$ = 2.9 Hz, 1 H; H-5'b), 6.11 (dd, $J_{3',2'}$ = 5.9 Hz, $J_{3',4'}$ = 4.4 Hz, 1 H; H-3'), 6.33 (t, $J_{2',1'} = J_{2',3'}$ = 5.9 Hz, 1 H; H-2'), 6.88 (d, $J_{1',2'}$ = 5.9 Hz, 1 H; H-1'), 7.35 (m, 2 H; H-A3, H-A5), 7.42 (m, 2 H; H-B3, H-B5), 7.46 (m, 2 H; H-C3, H-C5), 7.53 (tt, $J_{A4,A3} = J_{A4,A5}$ = 7.5 Hz, $J_{A4,A2} = J_{A4,A6}$ = 1.3 Hz, 1 H; H-A4), 7.60 (m, 2 H; H-B4, H-C4), 7.67 (s, 1 H; H-3), 7.90 (dd, $J_{A2,A3} = J_{A6,A5}$ = 8.4 Hz, $J_{A2,A4} = J_{A6,A4}$ = 1.3 Hz, 2 H; H-A2, H-A6), 8.01 (dd, $J_{B2,B3} = J_{B6,B5}$ = 8.4 Hz, $J_{B2,B4} = J_{B6,B4}$ = 1.3 Hz, 2 H; H-B2, H-B6), 8.05 (dd, $J_{C2,C3} = J_{C6,C5}$ = 8.4 Hz, $J_{C2,C4} = J_{C6,C4}$ = 1.3 Hz, 2 H; H-C2, H-C6), 8.69 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 40.2 (N*Me*), 63.8 (C-5'), 71.3 (C-3'), 72.6 (C-2'), 80.1 (C-4'), 85.7 (C-1'), 106.0 (C-8a), 120.2 (C-3), 127.5 (C-8b), 128.5, 128.8 and 129.3

(3 C; C-A1, C-B1, C-C1), 128.6 (2 C; C-A3, C-A5), 128.7 and 128.8 (4 C; C-B3, C-B5, C-C3, C-C5), 129.0 (C-3a), 129.8, 129.9 and 130.0 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.7 and 133.9 (3 C; C-A4, C-B4, C-C4), 149.5 (C-8), 152.5 (C-6), 157.4 (C-4a), 165.2 (C=O A), 165.6 (C=O B), 166.3 ppm (C=O C); IR (ATR): $\tilde{\nu}$ = 2927 (very w), 1720 (m), 1601 (w), 1553 (w), 1495 (w), 1450 (w), 1372 (w), 1315 (w), 1262 (s), 1176 (w), 1093 (m), 1067 (m), 1024 (m), 978 (m), 936 (m), 805 (w), 706 (s), 686 (m), 616 cm^{-1} (m); HR MS (ESI) for $C_{34}H_{26}N_5O_7{}^{35}ClNa^+$ $[M(^{35}Cl) + Na]^+$: calcd 674.1413, found 674.1405; for $C_{34}H_{26}N_5O_7{}^{37}ClNa^+$ $[M(^{37}Cl) + Na]^+$: calcd 676.1389, found 676.1362.

8-Chloro-1-methyl-4-(2,3-*O*-isopropylidene-5-*O*-tert-butyldimethylsilyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-d]pyrimidine (**58-*β***) & *α*-anomer (**58-*α***)

A solution of the previously synthesized halosugar precursor, 2,3-*O*-isopropylidene-5-*O*-tert-butyldimethylsilyl-*β*-D-ribofuranose[74] (**31**, 117 mg, 0.37 mmol) in THF (1.8 mL) was cooled to –30 °C and treated with CCl_4 (47 µL, 0.48 mmol). Then, HMPT (86 µL, 0.48 mmol) was added dropwise. The resulting solution was stirred vigorously for 1 hour resulting in the desired halosugar. In a separate flask, a suspension of nucleobase **47** (50.0 mg, 0.24 mmol) in MeCN (1.5 mL) and powdered KOH (37.5 mg, 0.67 mmol) was treated with TDA-1 (77 µL, 0.24 mmol) and stirred for 30 min. After cooling it to 0 °C (water/ice), the halosugar solution was added via syringe. The resulting mixture was first stirred at 0 °C for 1 hour, then at 20 °C for 36 hours before being treated with water (0.25 mL). After concentration *in vacuo*, purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 20% EtOAc) gave an inseparable mixture of **58-*β***/**58-*α*** 1:0.7 (70.4 mg, 59%) as white sticky solid.

Collecting of several early fractions gave a small sample of pure *β*-anomer **58-*β*** (3.2 mg, 2%) as a white amorphous solid, which was used for characterization:

R_f = 0.50 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.00 (s, 3 H; Si*Me*$_2$), 0.03 (s, 3 H; Si*Me*$_2$), 0.86 (s, 9 H; SiC*Me*$_3$), 1.39 (s, 3 H; OC*Me*$_2$), 1.65 (s, 3 H; OC*Me*$_2$), 3.81 (dd, $J_{5'a,5'b}$ = 11.3 Hz, $J_{5'a,4'}$ = 3.9 Hz, 1 H; H-5'a), 3.86 (dd, $J_{5'b,5'a}$ = 11.3 Hz, $J_{5'b,4'}$ = 3.8 Hz, 1 H; H-5'b), 4.29 (q, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 3.7 Hz, 1 H; H-4'), 4.41 (s, 3 H; N*Me*), 4.98 (dd, $J_{3',2'}$ = 6.5 Hz, $J_{3',4'}$ = 3.6 Hz, 1 H; H-3'), 5.21 (dd, $J_{2',3'}$ = 6.6 Hz, $J_{2',1'}$ = 3.6 Hz, 1 H; H-2'), 6.49 (d, $J_{1',2'}$ = 3.6 Hz, 1 H; H-1'), 7.66 (s, 1 H; H-3), 8.71 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = -5.3 (Si*Me*$_2$), -5.2 (Si*Me*$_2$), 18.7 (Si*C*Me$_3$), 25.7 (OC*Me*$_2$), 26.1 (SiC*Me*$_3$), 27.5 (OC*Me*$_2$), 40.2 (N*Me*), 63.1 (C-5'), 80.6 (C-3'), 83.4 (C-2'), 85.4 (C-4'), 89.7 (C-1'), 105.6 (C-8a), 115.0 (O*C*Me$_2$), 121.1 (C-3), 127.3 (C-8b), 129.7 (C-3a), 149.4 (C-8), 152.5 (C-6), 156.7 ppm (C-4a); HR MS (ESI) for $C_{22}H_{33}N_5O_4Si^{35}Cl^+$ $[M(^{35}Cl) + H]^+$: calcd 494.1990, found 494.1992; for $C_{22}H_{32}N_5O_4Si^{35}ClNa^+$ $[M(^{35}Cl) + Na]^+$: calcd 516.1810, found 516.1812.

8-Chloro-1-methyl-4-(5-*O*-trityl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-d]pyrimidine (**59**)

A solution of 5-*O*-trityl-D-ribose[75] (**32**, 47 mg, 0.12 mmol) in MeCN (2 mL) was treated with P(*n*Bu)$_3$ (48 µL, 93.5%, 0.19 mmol) and ADDP (42 mg, 0.18 mmol). After stirring at 20 °C for 15 min, the homogenous orange solution turned into a heterogeneous white suspension forming the sugar epoxide *in situ*. Separately, a suspension of nucleobase **47** (50.0 mg, 0.24 mmol) in DMF (1.5 mL) was treated with NaH (9.6 mg, 60 w% dispersion in mineral oil, 0.24 mmol), stirred at 20 °C for 15 min, and then transferred via syringe to the epoxide mixture. After stirring at 20 °C for 12 h, the reaction mixture was neutralized with HCl (1 M in water). Concentration *in vacuo*, followed by co-evaporation with toluene multiple times gave a crude oil. The oil diluted with MeOH (~3 mL) and loaded on silica. Purification by HPFC (SiO_2; $CHCl_3$/MeOH, gradient 0 → 10% MeOH) gave a fraction containing the crude product **59** (4.2 mg).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.07–4.10 (m, 1 H; H-5'a), 4.20–4.25 (m, 1 H; H-5'b), 4.33 (s, 3 H; N*Me*), 4.59 (q, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 6.0 Hz, 1 H; H-4'), 5.32 (m, 1 H; H-3'), 5.52 (m, 1 H; H-2'), 6.35 (d, $J_{1',2'}$ = 6.2 Hz, 1 H; H-1'), 7.20–7.37 (m, 15 H; H-Ar), 7.59 (s, 1 H; H-3), 8.77 ppm (s, 1 H; H-6); HR MS (ESI) for $C_{32}H_{28}N_5O_4{}^{35}ClNa^+$ $[M + Na]^+$: calcd 604.1722, found 604.1726.

8-(Furan-2-yl)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]-pyrrolo[2,3-*d*]pyrimidine (**60a**)

A solution of **57** (300 mg, 0.46 mmol) in DMF (4.6 mL) was treated with $PdCl_2(PPh_3)_2$ (35.7 mg, 0.05 mmol) and 2-(tributylstannyl)furan (0.175 mL, 0.55 mmol). After stirring at 100 °C for 10 hours, DMF was removed *in vacuo*. EtOAc (2.5 mL) and a solution of KF (32 mg, 0.551 mmol) in MeOH (2.5 mL) were added. The mixture was concentrated *in vacuo* and filtered through a silica plug (5 cm) with cHex (100 mL) followed by EtOAc (500 mL). Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 100% EtOAc) gave **60a** (216 mg, 69%) as a pale-yellow foam.

R_f = 0.40 (SiO_2; cHex/EtOAc 1:1); m.p. = 71 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.27 (s, 3 H; N*Me*), 4.72 (m, 1 H; H-5'a), 4.81–4.87 (m, 2 H; H-4', H-5'b), 6.12 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.3 Hz, 1 H; H-3'), 6.33 (t, $J_{2',1'} = J_{2',3'}$ = 6.1 Hz, 1 H; H-2'), 6.76 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.7 Hz, 1 H; H-4-furyl), 6.99 (d, $J_{1',2'}$ = 6.3 Hz, 1 H; H-1'), 7.35, 7.42 and 7.46 (3 × m, 3 × 2 H; H-A3, H-A5, H-B3, H-B5, H-C3, H-C5), 7.48 (dd, $J_{3,4}$ = 3.5 Hz, $J_{3,5}$ = 0.9 Hz, 1 H; H-3-furyl), 7.53, 7.58 and 7.59 (3 × m, 3 H; H-A4, H-B4, HC4), 7.70 (s, 1 H; H-3), 7.75 (bd, $J_{5,4}$ = 1.7 Hz, 1 H; H-5-furyl), 7.91, 8.02 and 8.09 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.89 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 41.8 (N*Me*), 64.0 (C-5'), 71.3 (C-3'), 72.5 (C-2'), 80.1 (C-4'), 85.3 (C-1'), 101.5 (C-8a), 113.9 (C-4-furyl), 119.9 (C-3), 128.5, 128.9 and 129.4 (3 C; C-A1, C-B1, C-C1), 128.6 (C-8b), 128.6, 128.7 and 128.8 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 129.9 (C-3a), 129.9, 130.0 and 130.1 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.6, 133.8 and 133.9 (3 C; C-A4, C-B4, C-C4), 145.1 (C-5-furyl), 150.4 (C-2-furyl), 151.2 (C-6), 158.1 (C-4a), 165.2, 165.6 and 166.3 ppm (3 C; C=O), *C-8 and C-3-furyl hidden by noise*; IR (ATR): $\tilde{\nu}$ = 2956 (w), 1720 (s), 1599 (w), 1555 (w), 15332 (w), 1488 (w), 1442 (m), 1414 (w), 1315 (w), 1261 (s), 1176 (w), 1117 (m), 1092 (m), 1067 (m), 1024 (m), 1009 (m), 973 (m), 944 (w), 885 (w), 793 (m), 745 (w), 705 (s), 685 (w), 616 cm^{-1} (w); HR MS (ESI) for $C_{38}H_{30}N_5O_8^+$ $[M + H]^+$: calcd 684.2089, found 684.2086; for $C_{38}H_{29}N_5O_8Na^+$ $[M + Na]^+$: calcd 706.1908, found 706.1905.

8-(Furan-3-yl)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]-pyrrolo[2,3-*d*]pyrimidine (**60b**)

A solution of **57** (300 mg, 0.46 mmol) in toluene (3.1 mL) was treated with furan-3-ylboronic acid (77.1 mg, 0.69 mmol), K_2CO_3 (127 mg, 0.92 mmol) and $Pd(PPh_3)_4$ (54.88 mg, 0.05 mmol). After stirring at 100 °C for 48 hours, the suspension was filtered through a celite plug (2 cm). Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **60b** (101 mg, 32%) as a yellow film.

R_f = 0.56 (SiO_2; cHex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.81 (s, 3 H; N*Me*), 4.70 (m, 1 H; H-5'a), 4.83 (m, 1 H; H-4'), 4.84 (m, 1 H; H-5'b), 6.15 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.3 Hz, 1 H; H-3'), 6.38 (t, $J_{2',1'} = J_{2',3'}$ = 6.1 Hz, 1 H; H-2'), 6.85 (dd, $J_{4,5}$ = 1.8 Hz, $J_{4,2}$ = 0.8 Hz, 1 H; H-4-furyl), 6.98 (d, $J_{1',2'}$ = 6.2 Hz, 1 H; H-1'), 7.34, 7.40 and 7.45 (3 × t, J = 7.7 Hz, 3 × 2 H; H-A3, H-A5, H-B3, H-B5, H-C3, H-C5), 7.49–7.59 (3 × m, 3 H; H-A4, H-B4, H-C4), 7.61 (t, $J_{5,4} = J_{5,2}$ = 1.7 Hz, 1 H; H-5-furyl), 7.66 (s, 1 H; H-3), 7.91 (dd, $J_{2,5}$ = 1.5 Hz, $J_{2,4}$ = 0.8 Hz, 1 H; H-2-furyl), 7.92, 8.01 and 8.09 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.92 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 40.4 (N*Me*), 63.9 (C-5'), 71.3 (C-3'), 72.4 (C-2'), 79.8 (C-4'), 85.1 (C-1'), 105.9 (C-8a),

111.2 (C-4-furyl), 120.5 (C-3), 125.0 (C-3-furyl), 128.5, 128.6 and 128.7 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.8, 128.9, 129.1 and 129.4 (5 C; C-A1, C-B1, C-C1, C-3a, C-8b), 129.8 and 129.9 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.5, 133.8 and 133.8 (3 C; C-A4, C-B4, C-C4), 142.9 (C-2-furyl), 143.9 (C-5-furyl), 150.4 (C-8), 152.8 (C-6), 157.6 (C-4a), 165.2, 165.6 and 166.2 ppm (3 C; C=O); IR (ATR): $\tilde{\nu}$ = 2924 (w), 2852 (w), 1722 (s), 1602 (w), 1562 (w), 1525 (w), 1492 (w), 1451 (w), 1368 (w), 1315 (w), 1260 (s), 1177 (w), 1119 (m), 1089 (m), 1068 (m), 1024 (m), 1000 (m), 977 (m), 906 (w), 802 (w), 706 (s), 685 (m), 615 (w), 601 cm^{-1} (w); HR MS (ESI) for $C_{38}H_{30}N_5O_8^+$ $[M + H]^+$: calcd 684.2089, found 684.2086; for $C_{38}H_{29}N_5O_8Na^+$ $[M + Na]^+$: calcd 706.1908, found 706.1903.

8-(Benzofuran-2-yl)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**60c**)

A solution of **57** (301 mg, 0.46 mmol) in toluene (3.1 mL) was treated with benzofuran-2-ylboronic acid (112 mg, 0.69 mmol), K_2CO_3 (128.3 mg, 0.93 mmol) and $Pd(PPh_3)_4$ (54.7 mg, 0.05 mmol). After stirring at 100 °C for 48 hours, the suspension was filtered through a celite plug (2 cm). Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **60c** (127 mg, 38%) as a colorless film.

R_f = 0.74 (SiO_2; cHex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.31 (s, 3 H; N*Me*), 4.73 (m, 1 H; H-5'a), 4.84 (m, 1 H; H-4'), 4.85 (m, 1 H; H-5'b), 6.16 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.4 Hz, 1 H; H-3'), 6.40 (t, $J_{2',1'} = J_{2',3'}$ = 6.1 Hz, 1 H; H-2'), 7.01 (d, $J_{1',2'}$ = 6.2 Hz, 1 H; H-1'), 7.32–7.37 (m, 3 H; H-5-benzofuryl, H-C3, H-C5), 7.38–7.49 (m, 5 H; H-6-benzofuryl, H-A3, H-A5, H-B3, H-B5), 7.52 (m, 1 H; H-C4), 7.55–7.61 (m, 3 H; H-7-benzofuryl, H-A4, H-B4), 7.73 (s, 1 H; H-3), 7.76 (m, $J_{4,5}$ = 7.8 Hz, 1 H; H-4-benzofuryl), 7.79 (bs, 1 H; H-3-benzofuryl), 7.93, 8.02 and 8.09 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.93 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 41.7 (N*Me*), 64.0 (C-5'), 71.3 (C-3'), 72.4 (C-2'), 80.0 (C-4'), 85.3 (C-1'), 102.9 (C-8a), 110.0 (C-3-benzofuryl), 111.5 (C-7-benzofuryl), 119.9 (C-3), 122.8 (C-4-benzofuryl), 124.2 (C-5-benzofuryl), 126.6 (C-6-benzofuryl), 128.4, 128.8, 128.9 and 129.4 (6 C; C-3a, C-8b, C-3a-benzofuryl, C-A1, C-B1, C-C1), 128.6, 128.7 and 128.7 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 129.8 and 129.9 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.6, 133.7 and 133.8 (3 C; C-A4, C-B4, C-C4), 146.0 (C-8), 152.2 (C-6), 153.4 (C-2-benzofuryl), 155.5 (C-7a-benzofuryl), 158.4 (C-4a), 165.2, 165.6 and 166.3 ppm (3 C; C=O); IR (ATR): $\tilde{\nu}$ = 2969 (w), 2953 (w), 2882 (w), 2249 (w), 2180 (w), 2153 (w), 2042 (w), 2016 (w), 1987 (w), 1979 (w), 1970 (w), 1922 (w), 1870 (w), 1718 (m), 1583 (m), 1543 (s), 1498 (m), 1451 (w), 1431 (m), 1408 (m), 1364 (m), 1315 (m), 1245 (s), 1119 (m), 1081 (s), 1054 (s), 1046 (s), 1021 (s), 994 (s), 975 (s), 949 (m), 814 (m), 803 (m), 777 (m), 734 (m), 716 (s), 709 (s), 688 (m), 676 (m), 669 (m), 649 (m), 621 (m), 610 (s), 598 cm^{-1} (s); HR MS (ESI) for $C_{42}H_{32}N_5O_8^+$ $[M + H]^+$: calcd 734.2245, found 734.2239; for $C_{42}H_{31}N_5O_8Na^+$ $[M + H]^+$: calcd 756.2065, found 756.2060.

1,8-Dimethyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]-pyrimidine (**60d**)

A solution of **57** (249 mg, 0.38 mmol) in THF (10.1 mL) was treated with $Pd(PPh_3)_4$ (23.6 mg, 0.02 mmol) and Me_3Al (0.38 mL, 2.0 M in toluene, 0.78 mmol). After stirring at 65 °C for 3 hours, the resulting red-brown solution was treated with MeOH (1 mL) before being concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 25 → 75% EtOAc) gave **60d** (156 mg, 64%) as an off-white foam.

R_f = 0.19 (SiO_2; cHex/EtOAc 1:2); m.p. = 79 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.98 (s, 3 H; C*Me*), 4.30 (s, 3 H; N*Me*), 4.68 (m, 1 H; H-5'a), 4.80 (m, 1 H; H-4'), 4.82 (m, 1 H; H-5'b), 6.12 (dd, $J_{3',2'}$ = 5.9 Hz, $J_{3',4'}$ = 4.2 Hz, 1 H; H-3'), 6.43 (t, $J_{2',1'} = J_{2',3'}$ = 6.0 Hz, 1 H; H-2'), 6.93 (d, $J_{1',2'}$ = 6.2 Hz, 1 H; H-1'), 7.33 (m, 2 H; H-A3, H-A5), 7.40 (m, 2 H; H-B3, H-

B5), 7.45 (m, 2 H; H-C3, H-C5), 7.49–7.61 (m, 3 H; H-A4, H-B4, H-C4), 7.63 (s, 1 H; H-3), 7.90 (m, 2 H; H-A2, H-A6), 8.00 (m, 2 H; H-B2, H-B6), 8.07 (m, 2 H; H-C2, H-C6), 8.82 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 24.6 (C*Me*), 40.1 (N*Me*), 63.9 (C-5'), 71.3 (C-3'), 72.4 (C-2'), 79.8 (C-4'), 85.1 (C-1'), 106.4 (C-8a), 120.2 (C-3), 128.5 (C-3a), 128.5, 128.6 and 128.7 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.6, 128.9 and 129.2 (3 C; C-A1, C-B1, C-C1), 129.4 (C-8b), 129.8 and 129.9 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6); 133.5, 133.7 and 133.80 (3 C; C-A4, C-B4, C-C4), 152.8 (C-6), 156.5 (C-8), 157.0 (C-4a), 165.2 (C=O A), 165.6 (C=O B), 166.2 ppm (C=O C); IR (ATR): $\tilde{\nu}$ = 3060 (w), 2923 (w), 2853 (w), 1720 (m), 1601 (w), 1558 (w), 1499 (w), 1451 (m), 1315 (w), 1262 (s), 1177 (m), 1118 (m), 1091 (s), 1067 (m), 1024 (m), 978 (m), 803 (w), 784 (w), 707 (s), 615 (m), 540 cm^{-1} (s); HR MS (ESI) for $C_{35}H_{30}N_5O_7^+$ $[M + H]^+$: calcd 632.2140, found 632.2134; for $C_{35}H_{29}N_5O_7Na^+$ $[M + Na]^+$: calcd 654.1959, found 654.1953.

8-(*N*,*N*-Dimethylamino)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**60e**)

A suspension of **57** (136 mg, 0.21 mmol) in 2-propanol (6.71 mL) was treated with dimethylamine (0.155 mL, 2.0 M in THF, 0.31 mmol) and stirred at 60 °C for 24 hours. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 100% EtOAc) gave **60e** (63.9 mg, 47%) as a colorless film.

R_f = 0.54 (SiO_2; cHex/EtOAc 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 3.17 (s, 6 H; N*Me*$_2$), 4.22 (s, 3 H; N*Me*), 4.69 (m, 1 H; H-5'a), 4.78 (m, 1 H; H-4'), 4.79 (m, 1 H; H-5'b), 6.11 (dd, $J_{3',2'}$ = 6.0 Hz, $J_{3',4'}$ = 4.1 Hz, 1 H; H-3'), 6.32 (t, $J_{2',1'} = J_{2',3'}$ = 6.2 Hz, 1 H; H-2'), 6.93 (d, $J_{1',2'}$ = 6.4 Hz, 1 H; H-1'), 7.34 (m, 2 H; H-A3, H-A5), 7.39 (m, 2 H; H-B3, H-B5), 7.46 (m, 2 H; H-C3, H-C5), 7.52, 7.56 and 7.59 (3 × m, 3 H; H-A4, H-B4, H-C4), 7.63 (s, 1 H; H-3), 7.93 (m, 2 H; H-A2, H-A6), 7.99 (m, 2 H; H-B2, H-B6), 8.11 (m, 2 H; H-C2, H-C6), 8.51 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 39.7 (N*Me*), 41.3 (2 C; N*Me*$_2$), 64.1 (C-5'), 71.3 (C-3'), 72.4 (C-2'), 79.8 (C-4'), 84.9 (C-1'), 94.4 (C-8a), 121.2 (C-3), 126.3 (C-3a), 128.5 and 128.6 (4 C; C-A3, C-A5, C-B3, C-B5), 128.7, 128.9 and 129.5 (3 C; C-A1, C-B1, C-C1), 128.7 (2 C; C-C3, C-C5), 129.9 and 130.0 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 130.8 (C-8b), 133.6, 133.7 and 133.8 (3 C; C-A4, C-B4, C-C4), 152.1 (C-6), 158.2 (C-4a), 159.9 (C-8), 165.2 (C=O A), 165.6 (C=O B), 166.3 ppm (C=O C); IR (ATR): $\tilde{\nu}$ = 2953 (w), 1720 (m), 1584 (w), 1555 (w), 1493 (w), 1450 (w), 1435 (w), 1413 (w), 1367 (w), 1315 (w), 1259 (s), 1177 (w), 1085 (m), 1067 (m), 1024 (m), 993 (w), 973 (w), 948 (w), 835 (w), 803 (w), 733 (w), 705 (s), 686 (m), 611 cm^{-1} (w); HR MS (ESI) for $C_{36}H_{33}N_6O_7^+$ $[M + H]^+$: calcd 661.2405, found 661.2398; for $C_{36}H_{32}N_6O_7Na^+$ $[M + Na]^+$: calcd 683.2225, found 683.2217.

8-(Furan-2-yl)-1-methyl-4-(*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]-pyrimidine (**61a**)

A suspension of **60a** (137 mg, 0.20 mmol) in MeOH (10 mL) was treated with NaOMe (14 µL, 25 wt% in MeOH, 0.06 mmol) and stirred at 60 °C for 6 hours. After co-evaporation with MeOH (3 × 10 mL), purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **61a** (64.6 mg, 87%) as a light-beige solid.

R_f = 0.42 (SiO_2; DCM/MeOH 9:1); m.p. = 217 °C; $[\alpha]_D^{20}$ = 0 (*c* = 0.167 in DMSO); ^1H NMR (500 MHz, DMSO-d_6): δ = 3.59 and 3.62 (2 × dd, J_{gem} = 11.8 Hz, $J_{5',4'}$ = 4.0 Hz, 2 H; H-5'), 3.94 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.0 Hz, $J_{4',3'}$ = 2.3 Hz, 1 H; H-4'), 4.13 (dd, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 2.3 Hz, 1 H; H-3'), 4.22 (s, 3 H; N*Me*), 4.55 (bt, $J_{2',1'} = J_{2',3'}$ = 6.4 Hz, 1 H; H-2'), 5.08 (bs, 1 H; OH-5'), 5.24 (bs, 1 H; OH-3'), 5.33 (bs, 1 H; OH-2'), 6.41 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 6.87 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.7 Hz, 1 H; H-4-furyl), 7.45 (dd, $J_{3,4}$ = 3.4 Hz, $J_{3,5}$ = 0.8 Hz, 1 H; H-3-furyl), 7.92 (s, 1 H; H-3), 8.19 (dd, $J_{5,4}$ = 1.7 Hz, $J_{5,3}$ = 0.8 Hz, 1 H; H-5-furyl),

8.83 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 41.3 (N*Me*), 61.8 (C-5'), 70.6 (C-3'), 71.7 (C-2'), 85.2 (C-4'), 85.4 (C-1'), 100.2 (C-8a), 113.2 (C-4-furyl), 113.6 (C-3-furyl), 120.8 (C-3), 127.7 (C-8b), 129.6 (C-3a), 145.2 (C-8), 146.0 (C-5-furyl), 151.3 (C-2-furyl), 151.9 (C-6), 157.7 ppm (C-4a); IR (ATR): $\tilde{\nu}$ = 3248 (m), 3129 (m), 2923 (m), 2854 (m), 1728 (w), 1667 (w), 1596 (m), 1552 (m), 1531 (m), 1484 (m), 1444 (s), 1415 (s). 1319 (m), 1289 (s), 1258 (m), 1236 (m), 1206 (w), 1180 (w), 1124 (s), 1070 (m), 1021 (s), 968 (s), 936 (m), 883(s), 861 (m), 790 (s), 757 (s), 718 (s), 617 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 284 (10700), 323 (12000), 254 nm (29800 $M^{-1}cm^{-1}$); HR MS (ESI) for for $C_{17}H_{18}N_5O_5^+$ $[M + H]^+$: calcd 372.1303, found 372.1298; for $C_{17}H_{17}N_5O_5Na^+$ $[M + Na]^+$: calcd 394.1122, found 394.1120.

8-(Furan-3-yl)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]-pyrrolo[2,3-*d*]pyrimidine (**61b**)

A suspension of **60b** (80.9 mg, 0.12 mmol) in MeOH (6 mL) was treated with NaOMe (8 µL, 25 wt% in MeOH, 0.03 mmol) and stirred at 60 °C for 6 hours. Co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **61b** (31.6 mg, 72%) as an ocher-yellow film.

R_f = 0.38 (SiO_2; DCM/MeOH 9:1); $[\alpha]_D^{20}$ = –15.0 (c = 0.171 in DMSO); ^1H NMR (500 MHz, DMSO-d_6): δ = 3.58 (ddd, $J_{5'a,5'b}$ = 11.7 Hz, $J_{5'a,OH}$ = 5.1 Hz, $J_{5'a,4'}$ = 4.0 Hz, 1 H; H-5'a), 3.61 (ddd, $J_{5'b,5'a}$ = 11.7 Hz, $J_{5'b,OH}$ = 5.3 Hz, $J_{5'b,4'}$ = 4.1 Hz, 1 H; H-5'b), 3.81 (s, 3 H; N*Me*), 3.94 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.0 Hz, $J_{4',3'}$ = 2.3 Hz, 1 H; H-4'), 4.12 (btd, $J_{3',2'} = J_{3',OH}$ = 4.9 Hz, $J_{3',4'}$ = 2.3 Hz, 1 H; H-3'), 4.54 (btd, $J_{2',1'} = J_{2',OH}$ = 7.1 Hz, $J_{2',3'}$ = 5.2 Hz, 1 H; H-2'), 5.07 (bt, $J_{OH,5'a} = J_{OH,5'b}$ = 5.2 Hz, 1 H; OH-5'), 5.26 (bd, $J_{OH,3'}$ = 4.5 Hz, 1 H; OH-3'), 5.33 (bd, $J_{OH,2'}$ = 6.7 Hz, 1 H; OH-2'), 6.39 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.03 (dd, $J_{4,5}$ = 1.9 Hz, $J_{4,2}$ = 0.9 Hz, 1 H; H-4-furyl), 7.88 (s, 1 H; H-3), 7.94 (t, $J_{5,4} = J_{5,2}$ = 1.7 Hz, 1 H; H-5-furyl), 8.41 (dd, $J_{2,5}$ = 1.5 Hz, $J_{2,4}$ = 0.8 Hz, 1 H; H-2-furyl), 8.87 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 40.1 (N*Me*), 61.8 (C-5'), 70.7 (C-3'), 71.9 (C-2'), 85.2 (C-4'), 85.5 (C-1'), 104.4 (C-8a), 111.4 (C-4-furyl), 121.4 (C-3), 124.8 (C-3-furyl), 128.1 (C-8b), 128.8 (C-3a), 144.0 (C-2-furyl), 144.3 (C-5-furyl), 150.0 (C-8), 152.4 (C-6), 157.1 ppm (C-4a); IR (ATR): $\tilde{\nu}$ = 3346 (m), 3249 (m), 2925 (w), 1662 (w), 1602 (w), 1552 (m), 1499 (m), 1447 (m), 1416 (s), 1344 (s), 1317 (s), 1283 (m), 1253 (m), 1208 (m), 1161 (m), 1120 (s), 1083 (s), 1041 (s), 1008 (m), 980 (s), 873 (s), 834 (m), 822 (m), 795 (s), 745 (m), 717 (m), 682 (m), 649 (m), 615 (m), 600 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 301 (4600), 250 nm (24200 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{17}H_{18}N_5O_5^+$ $[M + H]^+$: calcd 372.1303, found 372.1230; for $C_{17}H_{17}N_5O_5Na^+$ $[M + Na]^+$: calcd 394.1122, found 394.1119.

8-(Benzofuran-2-yl)-1-methyl-4-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrazolo-[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**61c**)

A suspension of **60c** (121 mg, 0.17 mmol) in MeOH (8.25 mL) was treated with NaOMe (11 µL, 25 wt% in MeOH, 0.05 mmol) and stirred at 60 °C for 6 hours. Co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **61c** (69.6 mg, 98%) as a light yellow solid.

R_f = 0.47 (SiO_2; DCM/MeOH 9:1); m.p. = 257 °C; $[\alpha]_D^{20}$ = + 19.6 (c = 0.176 in DMSO); ^1H NMR (500 MHz, DMSO-d_6): δ = 3.58–3.67 (m, 2 H; H-5'), 3.96 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.0 Hz, $J_{4',3'}$ = 2.4 Hz, 1 H; H-4'), 4.14 (dd, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 2.4 Hz, 1 H; H-3'), 4.29 (s, 3 H; N*Me*), 4.57 (dd, $J_{2',1'}$ = 7.4 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 5.10 (m, 1 H; OH-5'), 5.20–5.52 (m, 2 H; OH-2', OH-3'), 6.44 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.41 (ddd, $J_{5,4}$ = 7.7 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,7}$ = 1.0 Hz, 1 H; H-5-benzofuryl), 7.50 (ddd, $J_{6,7}$ = 8.4 Hz, $J_{6,5}$ = 7.2 Hz, $J_{6,4}$ = 1.2 Hz, 1 H; H-6-benzofuryl), 7.87 (dq, $J_{4,5}$ = 7.7 Hz, $J_{4,6}$ = 1.1 Hz, 1 H; H-4-benzofuryl), 7.88 (dq, $J_{7,6}$ = 8.4 Hz, $J_{7,3} = J_{7,4} = J_{7,5}$ = 0.9 Hz, 1 H; H-7-benzofuryl), 7.90 (d, $J_{3,7}$ = 1.0 Hz,

1 H; H-3-benzofuryl), 7.97 (s, 1 H; H-3), 8.94 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 41.4 (N*Me*), 61.8 (C-5'), 70.6 (C-3'), 71.8 (C-2'), 85.3 (C-4'), 85.6 (C-1'), 101.5 (C-8a), 109.4 (C-3-benzofuryl), 111.9 (C-7-benzofuryl), 121.1 (C-3), 122.7 (C-4-benzofuryl), 124.1 (C-5-benzofuryl), 126.5 (C-6-benzofuryl), 127.7 (C-8b), 128.0 (C-3a-benzofuryl), 129.9 (C-3a), 145.2 (C-8), 152.0 (C-6), 153.4 (C-2-benzofuryl), 154.9 (C-7a-benzofuryl), 157.9 ppm (C-4a); IR (ATR): $\tilde{\nu}$ = 3240 (w), 3084 (w), 2920 (m), 2852 (w), 1739 (w), 1652 (w), 1597 (m), 1559 (m), 1530 (m), 1489 (w), 1447 (s), 1412 (m), 1351 (m), 1331 (m), 1291 (m), 1259 (m), 1215 (w), 1191 (w), 1173 (m), 1135 (m), 1118 (m), 1074 (s), 1038 (s), 1008 (s), 982 (s), 976 (s), 965 (m), 926 (m), 887 (m), 823 (s), 793 (s), 747 (s), 731 (m), 685 (m), 663 (m), 612 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 336 (19800), 258 nm (20900 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{21}H_{20}N_5O_5^+$ $[M + H]^+$: calcd 422.1459, found 422.1456; for $C_{21}H_{19}N_5O_5Na^+$ $[M + Na]^+$: calcd 444.1278, found 444.1276.

1,8-Dimethyl-4-(*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**61d**)

A suspension of **60d** (135 mg, 0.21 mmol) in MeOH (10 mL) was treated with NaOMe (15 µL, 25 wt% in MeOH, 0.07 mmol) and stirred at 60 °C for 4 hours. Co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **61d** (50.4 mg, 73%) as a white solid.

R_f = 0.33 (SiO_2; DCM/MeOH 9:1); m.p. = 286 °C; $[\alpha]_D^{20}$ = –50.3 (*c* = 0.173 in DMSO); ^1H NMR (500 MHz, DMSO-d_6): δ = 2.96 (s, 3 H; C*Me*), 3.53–3.62 (m, 2 H; H-5'), 3.91 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.1 Hz, $J_{4',3'}$ = 2.4 Hz, 1 H; H-4'), 4.10 (m, 1 H; H-3'), 4.29 (s, 3 H; N*Me*), 4.51 (td, $J_{2',1'} = J_{2',OH}$ = 6.9 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 5.09 (t, $J_{OH,5'a} = J_{OH,5'b}$ = 5.2 Hz, 1 H; OH-5'), 5.26 (d, $J_{OH,3'}$ = 4.3 Hz, 1 H; OH-3'), 5.32 (d, $J_{OH,2'}$ = 6.6 Hz, 1 H; OH-2'), 6.33 (d, $J_{1',2'}$ = 7.3 Hz, 1 H; H-1'), 7.79 (s, 1 H; H-3), 8.73 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 24.3 (C*Me*), 39.7 (N*Me*), 62.0 (C-5'), 70.8 (C-3'), 71.8 (C-2'), 85.2 (C-4'), 85.6 (C-1'), 105.1 (C-8a), 120.9 (C-3), 128.3 (C-3a), 128.5 (C-8b), 152.6 (C-6), 156.5 (C-4a), 157.0 ppm (C-8); IR (ATR): $\tilde{\nu}$ = 3213 (m), 2918 (w), 1606 (w), 1551 (m), 1503 (m), 1477 (m), 1455 (m), 1415 (m), 1374 (m), 1316 (m), 1244 (m), 1218 (m), 1124 (s), 1098 (s), 1077 (s), 1047 (s), 1020 (s), 983 (s), 931 (m), 882 (m), 854 (m), 828 (m), 785 (m), 720 (s), 647 (s), 617 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 281 (6600), 246 nm (39300 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{14}H_{18}N_5O_4^+$ $[M + H]^+$: calcd 320.1353, found 320.1351; for $C_{14}H_{17}N_5O_4Na^+$ $[M + Na]^+$: calcd 342.1173, found 342.1170.

1-Methyl-8-(*N*,*N*-dimethylamino)-4-(*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**61e**)

A suspension of **60e** (116 mg, 0.18 mmol) in MeOH (8.8 mL) was treated with NaOMe (12 µL, 25 wt% in MeOH, 0.05 mmol) and stirred at 60 °C for 6 hours. Concentration *in vacuo*, followed by co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **61e** (61.0 mg, 98%) as a light-beige film.

R_f = 0.38 (SiO_2; DCM/MeOH 9:1); $[\alpha]_D^{20}$ = + 16.1 (*c* = 0.261 in DMSO); ^1H NMR (500 MHz, DMSO-d_6): δ = 3.11 (s, 6 H; NMe_2), 3.55 (ddd, $J_{5'a,5'b}$ = 11.8 Hz, $J_{5'a,OH}$ = 5.1 Hz, $J_{5'a,4'}$ = 4.2 Hz, 1 H; H-5'a), 3.58 (ddd, $J_{5'b,5'a}$ = 11.8 Hz, $J_{5'b,OH}$ = 5.5 Hz, $J_{5'b,4'}$ = 4.2 Hz, 1 H; H-5'b), 3.88 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.2 Hz, $J_{4',3'}$ = 2.4 Hz, 1 H; H-4'), 4.09 (btd, $J_{3',2'} = J_{3',OH}$ = 4.9 Hz, $J_{3',4'}$ = 2.4 Hz, 1 H; H-3'), 4.18 (s, 3 H; N*Me*), 4.51 (td, $J_{2',1'} = J_{2',OH}$ = 7.1 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 5.04 (t, $J_{OH,5'a} = J_{OH,5'b}$ = 5.3 Hz, 1 H; OH-5'), 5.27 (d, $J_{OH,3'}$ = 4.5 Hz, 1 H; OH-3'), 5.27 (d, $J_{OH,2'}$ = 6.9 Hz, 1 H; OH-2'), 6.27 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.79 (s, 1 H; H-3), 8.36 ppm (s, 1 H; H-6); $^{13}C\{^1H\}$ NMR (125.7 MHz, DMSO-d_6): δ = 39.8 (N*Me*), 40.5 (2 C; NMe_2), 61.9 (C-5'), 70.6 (C-3'), 71.8 (C-2'), 85.0 (C-4'), 85.5 (C-1'), 92.6 (C-8a), 121.8 (C-3), 126.2 (C-3a), 129.6 (C-8b), 151.8 (C-6), 157.7 (C-4a), 159.6 ppm (C-8); IR (ATR): $\tilde{\nu}$ = 3261 (broad w), 2925 (w), 2855 (w), 1732 (w), 1636 (w), 1594 (m), 1559 (m),

1496 (w), 1441 (m), 1412 (m), 1391 (m), 1317 (w), 1263 (m), 1093 (s), 1044 (s), 995 (s), 974 (m), 942 (m), 787 (s), 710 (s), 594 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 305 nm (7400 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{15}H_{21}N_6O_4^+$ $[M + H]^+$: calcd 349.1619, found 349.1617; for $C_{15}H_{20}N_6O_4Na^+$ $[M + Na]^+$: calcd 371.1438, found 371.1436.

8-Amino-1-methyl-4-(*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**61f**)

In a screw-cap pressure glass tube, a solution of nucleoside **57** (201 mg, 0.31 mmol) in 1,4-dioxane (1 mL) was treated with aq. ammonia (2.5 mL, 25 wt% in water, 16.23 mmol). After stirring at 120 °C for 24 hours (HPLC-MS: t_R = 0.82 min, m/z = 321), the solution was cooled down and concentrated *in vacuo*. Recrystallisation from DCM/MeOH (9:1, 2.5 mL), followed by filtration and rigorous washing with ice-cold DCM/MeOH solution (9:1) gave **61f** (79.3 mg, 81%) as an ocher-yellow solid.

R_f = 0.15 (SiO_2; DCM/MeOH 9:1); m.p. = 264 °C; $[\alpha]_D^{20}$ = –25.7 (c = 0.198 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.53 and 3.57 (2 dd, J_{gem} = 11.7 Hz, $J_{5',4'}$ = 4.2 Hz, 2 H; H-5'), 3.87 (td, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.2 Hz, $J_{4',3'}$ = 2.5 Hz, 1 H; H-4'), 4.08 (dd, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 2.5 Hz, 1 H; H-3'), 4.22 (s, 3 H; N*Me*); 4.48 (dd, $J_{2',1'}$ = 7.2 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 4.78–5.53 (m, 3 H; OH-2', OH-3', OH-5'), 6.20 (d, $J_{1',2'}$ = 7.3 Hz, 1 H; H-1'), 6.76 (bs, 2 H; NH_2), 7.64 (s, 1 H; H-3), 8.21 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 39.1 (N*Me*), 61.9 (C-5'), 70.7 (C-3'), 71.8 (C-2'), 84.8 (C-4'), 85.5 (C-1'), 89.7 (C-8a), 120.6 (C-3), 126.2 (C-3a), 129.1 (C-8a), 153.3 (C-6), 156.2 (C-8), 157.3 ppm (C-4a); IR (ATR, neat): $\tilde{\nu}$ = 3229 (m), 3042 (m), 2935 (m), 2898 (m), 1643 (s), 1612 (m), 1570 (m), 1524 (m), 1505 (m), 1487 (m), 1435 (m), 1359 (m), 1328 (m), 1278 (m), 1239 (m), 1193 (m), 1127 (m), 1101 (s), 1064 (s), 1013 (s), 990 (s), 976 (s), 945 (m), 905 (m), 870 (m), 789 (s), 737 (s), 725 (m), 626 (s), 610 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 281 nm (7000 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{13}H_{17}N_6O_4^+$ $[M + H]^+$: calcd 321.1306, found 321.1303; for $C_{13}H_{16}N_6O_4Na^+$ $[M + Na]^+$: calcd 343.1125, found 343.1122.

8-Methoxy-1-methyl-4-(*β*-D-ribofuranosyl)pyrazolo[3',4':4,5]pyrrolo[2,3-*d*]pyrimidine (**61g**)

A suspension of nucleoside **57** (201 mg, 0.31 mmol) in MeOH (20 mL) was treated with sodium methoxide (0.15 mL, 25 wt% in MeOH, 0.66 mmol) and stirred at 25 °C for 76 hours, followed by concentration *in vacuo*. Purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **61g** (82.1 mg, 80%) as a white solid.

R_f = 0.54 (SiO_2; DCM/MeOH 9:1); m.p. = 207 °C; $[\alpha]_D^{20}$ = –37.6 (c = 0.173 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.53–3.62 (m, 2 H; H-5'), 3.91 (td, $J_{4',5'a}$ = $J_{4',5'b}$ = 4.1 Hz, $J_{4',3'}$ = 2.4 Hz, 1 H; H-4'), 4.11 (dd, $J_{3',2'}$ = 5.3 Hz, $J_{3',4'}$ = 2.4 Hz, 1 H; H-3'), 4.14 (s, 3 H, O*Me*), 4.16 (s, 3 H; N*Me*), 4.51 (dd, $J_{2',1'}$ = 7.3 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 5.00–5.43 (m, 3 H; OH-2', OH-3', OH-5'), 6.28 (d, $J_{1',2'}$ = 7.3 Hz, 1 H; H-1'), 7.77 (s, 1 H; H-3), 8.54 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 38.4 (N*Me*), 54.5 (O*Me*), 62.0 (C-5'), 70.8 (C-3'), 72.2 (C-2'), 85.2 (C-4'), 85.9 (C-1'), 91.5 (C-8a), 121.6 (C-3), 126.8 (C-3a), 128.4 (C-8a), 152.9 (C-6), 158.1 (C-4a), 161.3 ppm (C-8); IR (ATR): $\tilde{\nu}$ = 3197 (w), 2919 (w), 1723 (m), 1679 (w), 1602 (w), 1569 (w), 1537 (w), 1450 (m), 1315 (w), 1266 (s), 1177 (w), 1121 (s), 1096 (m), 1067 (m), 1046 (m), 1025 (s), 987 (m), 973 (m), 940 (w), 803 (w), 786 (w), 707 (s), 686 (m), 620 (m), 600 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 271 nm (9300 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{14}H_{17}N_5O_5Na^+$ $[M + Na]^+$: calcd 358.1122, found 358.1126.

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

A solution of **57** (30.5 mg, 0.05 mmol) in 1,4-dioxane (2 mL) was treated with NaSMe (17.3 mg, 0.32 mmol) and stirred at 60 °C for 14 hours. Purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **61h** (8.2 mg, 51%) as a beige oil.

R_f = 0.56 (SiO_2; DCM/MeOH 9:1); $[\alpha]_D^{20}$ = –23.3 (*c* = 0.031 in DMSO); ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ = 2.71 (s, 3 H; S*Me*), 3.53–3.63 (m, 2 H; H-5'), 3.91 (td, $J_{4',5'a} = J_{4',5'b}$ = 4.7 Hz, $J_{4',3'}$ = 2.3 Hz, 1 H; H-4'), 4.10 (td, $J_{3',2'} = J_{3',OH}$ = 4.8 Hz, $J_{3',4'}$ = 2.3 Hz, 1 H; H-3'), 4.36 (s, 3 H; N*Me*), 4.51 (btd, $J_{2',1'} = J_{2',OH}$ = 7.0 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H; H-2'), 5.07 (t, $J_{OH,5'a} = J_{OH,5'b}$ = 5.3 Hz, OH-5'), 5.24 (d, $J_{OH,3'}$ = 4.5 Hz, 1 H; OH-3'), 5.32 (d, $J_{OH,2'}$ = 6.6 Hz, 1 H; OH-2'), 6.31 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.83 (s, 1 H; H-3), 8.73 ppm (s, 1 H; H-6); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-*d*$_6$): δ = 12.3 (S*Me*), 40.1 (N*Me*), 61.8 (C-5'), 70.7 (C-3'), 77.9 (C-2'), 85.3 (C-4'), 85.6 (C-1'), 102.6 (C-8a), 121.3 (C-3), 127.8 and 127.9 (2 C; C-3a, C-8b), 152.1 (C-6), 154.8 (C-4a), 158.5 ppm (C-8); IR (ATR): $\tilde{\nu}$ = 2964 (w), 1718 (m), 1583 (w), 1555 (w), 1491 (w), 1451 (w), 1413 (w), 1246 (m), 1177 (w), 1084 (m), 1066 (m), 1024 (w), 992 (w), 972 (w), 946 (w), 842 (w), 793 (w), 734 (w), 703 (s), 685 (m), 611 cm^{-1} (w); UV/Vis (MeOH): λ_{max} (ε) = 303 nm (5000 M^{-1}cm^{-1}); HR MS (ESI) for $C_{14}H_{18}N_5O_4S^+$ $[M + H]^+$: calcd 352.1074, found 352.1071; for $C_{14}H_{17}N_5O_4SNa^+$ $[M + Na]^+$: calcd 374.0894, found 374.0891.

5.2.2. Preparation of Aryl Sulfonium Salts

The sulfoxides TT=O, TFT=O and DBT=O were synthesized according to published procedures and ^{1}H NMR measurements of all products were in agreement with the literature.[65,67c]

5-(1-Methyl-1*H*-pyrazol-4-yl)-5*H*-thianthren-5-ium tetrafluoroborate (**63**)

A solution of 1-methyl-1*H*-pyrazole (**62**, 0.50 mL, 5.96 mmol) in MeCN (6.0 mL) under ambient atmosphere was cooled to 0 °C (water/ice) and treated with $HBF_4{\cdot}OEt_2$ (0.97 mL, 7.13 mmol) resulting in a colorless solution. After the addition of TT=O (1.46 g, 5.97 mmol), the white suspension was treated with TFAA (2.55 mL, 17.9 mmol) resulting in a violet-blue solution which was allowed to slowly warm up to 25 °C over 1 hour and then stirred for 48 hours. After treating the solution with satd $NaHCO_3$ (0.5 mL), the solution was stirred for 5 min prior to concentration *in vacuo*. The resulting oil was diluted with DCM (25 mL) and washed with satd $NaHCO_3$ (25 mL) and aq. $NaBF_4$ (10 wt% in water, 4 × 25 mL). The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by HPFC (SiO_2; DCM/MeOH gradient, 0 → 10% MeOH) gave **63** (1.96 g, 86%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.91 (s, 3 H; N*Me*), 7.64 (btd, $J_{3,2} = J_{3,4} = 7.7$ Hz, $J_{3,1} = 1.4$ Hz, 2 H; H-3, H-7), 7.73 (td, $J_{2,1} = J_{2,3} = 7.7$ Hz, $J_{2,4} = 1.4$ Hz, 2 H; H-2, H-8), 7.74 (s, 1 H; H-3'), 7.84 (dd, $J_{1,2} = 7.9$ Hz, $J_{1,3} = 1.4$ Hz, 2 H; H-1, H-9), 8.29 (dd, $J_{4,3} = 8.0$ Hz, $J_{4,2} = 1.4$ Hz, 2 H; H-4, H-6), 8.63 ppm (s,1 H; H-5'); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 40.15 (N*Me*), 100.17 (C-4'), 121.29 (2 C; C-4a, C-5a), 130.07 (2 C; C-1, C-9), 130.22 (2 C; C-3, C-7), 133.09 (2 C; C-4, C-6), 134.25 (2 C; C-2, C-8), 135.42 (2 C; C-9a, C-10a), 136.80 (C-5'), 139.61 ppm (C-3'); ^{19}F NMR (470.4 MHz, $CDCl_3$): δ = –176.16 ppm (s, 4 F; BF_4^-).

5-(1-methyl-1*H*-pyrazol-4-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (**68**)

Under ambient atmosphere, a white suspension of DBT=O (301 mg, 1.50 mmol) in MeCN (4.5 mL) was treated with 1-methyl-1*H*-pyrazole (**62**, 0.13 mL, 1.53 mmol) and cooled to –78 °C (acetone/dry ice). TFAA (0.32 mL, 6.10 mmol) was added dropwise, and the mixture was stirred at –78 °C for 2 hours. Then, the reaction mixture was allowed to slowly warm-up over the course of 2 hours and continued stirring at 20 °C for 48 hours. The now transparent solution was treated with water (0.5 mL) and stirred for 5 min prior to concentration *in vacuo*. Purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **68** (464 mg, 82%) as a light-yellow solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.84 (s, 3 H; N*Me*), 7.74 (btd, $J_{3,2} = J_{3,4} = J_{7,6} = J_{7,8} = 7.7$ Hz, $J_{3,1} = J_{7,9} = 1.2$ Hz, 2 H; H-3, H-7), 7.76 (d, $J_{3',5'} = 0.8$ Hz, 1 H; H-3'), 7.93 (td, $J_{2,1} = J_{2,3} = J_{8,7} = J_{8,9} = 7.6$ Hz, $J_{2,4} = J_{8,6} = 1.1$ Hz, 2 H; H-2, H-8), 8.31 (bd, $J_{4,3} = J_{6,7} = 8.1$ Hz, 2 H; H-4, H-6), 8.33 (s, 1 H; H-5'), 8.48 ppm (dd, $J_{1,2} = 7.9$ Hz, $J_{1,3} = 1.2$ Hz, 2 H; H-1, H-9); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 39.33 (N*Me*), 102.30 (C-2'), 117.43 (q, $J_{C,F}$ = 301.6 Hz; F_3CCOO), 124.28 (2 C; C-1, C-9), 127.65 (2 C; C-4, C-6), 131.12 (2 C; C-3, C-7), 133.52 (2 C; C-2, C-8), 134.76 (2 C; C-4a, C-5a), 133.73 (C-5'), 138.39 (2 C; C-9a, C-9b), 140.45 (C-3'), 157.55 ppm (q, $J_{C,F}$ = 30.1 Hz; F_3CCOO); ^{19}F NMR (470.4 MHz, DMSO-d_6): δ = –73.40 ppm (s, 3 F; F_3CCOO).

5-(1*H*-Pyrrol-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (**71**)

Under ambient atmosphere, a white suspension of DBT=O (301 mg, 1.50 mmol) in MeCN (4.5 mL) was treated with 1*H*-pyrrole (0.11 mL, 1.55 mmol) and cooled to –78 °C (acetone/dry ice). TFAA (0.32 mL, 6.10 mmol) was added dropwise, and the mixture was stirred at –78 °C for 2 hours. Then, the reaction mixture was allowed to slowly warm-up over

the course of 2 hours and continued stirring at 20 °C for 48 hours. The resulting solution was treated with water (0.5 mL) and stirred for 5 min prior to concentration *in vacuo*. Purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **71** (40 mg, 7%) as a light film.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 6.35 (btd, $J_{4',3'} = J_{4',5'}$ = 3.3 Hz, $J_{4',NH}$ = 0.9 Hz, 1 H; H-4'), 7.18 (dt, $J_{3',4'}$ = 3.9 Hz, $J_{3',5'} = J_{3',NH}$ = 1.5 Hz, 1 H; H-3'), 7.31 (btd, $J_{5',4'} = J_{5,NH}$ = 2.5 Hz, $J_{5',3'}$ = 1.7 Hz, 1 H; H-5'), 7.72 (btd, $J_{3,2} = J_{3,4} = J_{7,6} = J_{7,8}$ = 7.7 Hz, $J_{3,1} = J_{7,9}$ = 1.2 Hz, 2 H; H-3, H-7), 7.93 (td, $J_{2,1} = J_{2,3} = J_{8,7} = J_{8,9}$ = 7.6 Hz, $J_{2,4} = J_{8,6}$ = 1.1 Hz, 2 H; H-2, H-8), 8.19 (bd, $J_{4,3} = J_{6,7}$ = 8.1 Hz, 2 H; H-4, H-6), 8.49 (dd, $J_{1,2} = J_{9,8}$ = 7.8 Hz, $J_{1,3} = J_{9,7}$ = 1.2 Hz, 2 H; H-1, H-9), 12.12 ppm (bs, 1 H; N*H*); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 104.07 (C-2'), 111.28 (C-4'), 117.42 (q, $J_{C,F}$ = 301.2 Hz, F$_3$*C*COO), 123.17 (C-3'), 124.35 (2 C; C-1, C-9), 127.44 (2 C; C-4, C-6), 130.07 (C-5'), 131.01 (2 C; C-3, C-7), 133.09 (2 C; C-4a, C-5a), 133.63 (2 C; C-2, C-8), 138.79 (2 C; C-9a, C-9b), 157.69 ppm (q, $J_{C,F}$ = 30.5 Hz, F$_3$C*C*OO); ^{19}F NMR (470.4 MHz, DMSO-d_6): δ = –73.44 ppm (s, 3 F; *F*$_3$CCOO).

5-(Thiophen-2-yl)-5*H*-dibenzo[*b*,*d*]thiophen-5-ium 2,2,2-trifluoroacetate (**72**)

Under ambient atmosphere, a white suspension of DBT=O (301 mg, 1.50 mmol) in MeCN (4.5 mL) was treated with thiophene (0.13 mL, 1.61 mmol) and cooled to –78 °C (acetone/dry ice). TFAA (0.32 mL, 6.10 mmol) was added dropwise, and the mixture was stirred at –78 °C for 2 hours. Then, the reaction mixture was allowed to slowly warm-up over the course of 2 hours and continued stirring at 20 °C for 48 hours. The resulting solution was treated with water (0.5 mL) and stirred for 5 min prior to concentration *in vacuo*. Purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **72** (517 mg, 91%) as a grey solid.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.31 (dd, $J_{4',5'}$ = 5.2 Hz, $J_{4',3'}$ = 3.9 Hz, H-4'), 7.76 (bddd, $J_{3,4} = J_{7,6}$ = 8.0 Hz, $J_{3,2} = J_{7,8}$ = 7.5 Hz, $J_{3,1} = J_{7,9}$ = 1.2 Hz, 2 H; H-3, H-7), 7.96 (td, $J_{2,1} = J_{2,3} = J_{8,7} = J_{8,9}$ = 7.6 Hz, $J_{2,4} = J_{8,6}$ = 1.1 Hz, 2 H; H-2, H-8), 8.06 (dd, $J_{5',4'}$ = 5.2 Hz, $J_{5',3'}$ = 1.4 Hz, 1 H; H-5'), 8.31 (dd, $J_{3',4'}$ = 3.9 Hz, $J_{3',5'}$ = 1.4 Hz, H-3'), 8.41 (bd, $J_{4,3} = J_{6,7}$ = 8.0 Hz, 2 H; H-4, H-6), 8.50 ppm (dd, $J_{1,2} = J_{9,8}$ = 7.8 Hz, $J_{1,3} = J_{9,7}$ = 1.1 Hz, 2 H; H-1, H-9); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 117.38 (q, $J_{C,F}$ = 300.2 Hz; F$_3$*C*COO), 124.41 (2 C; C-1, C-9), 124.59 (C-2'), 128.03 (2 C; C-4, C-6), 129.30 (C-4'), 131.31 (2 C; C-3, C-7), 134.03 (2 C; C-2, C-8), 135.44 (2 C; C-4a, C-5a), 137.49 (C-5'), 138.24 (2 C; C-9a, C-9b), 140.29 (C-3'), 157.68 (q, $J_{C,F}$ = 30.4 Hz; F$_3$C*C*OO); ^{19}F NMR (470.4 MHz, DMSO-d_6): δ = –73.45 ppm (s, 3 F; *F*$_3$CCOO).

4,6-Dichloro-5(thiophen-2-yl)pyrimidine (**73**)

$ZnCl_2$ (215 mg, 1.58 mmol) was treated with a solution of (TMP)MgCl · LiCl (3.20 mL, 1.0 M solution in THF/toluene, 3.20 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of 4,6-dichloropyrimidine (**22**, 391 mg, 2.63 mmol) in THF (2 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of **72** (500 mg, 1.31 mmol) and $Pd(PPh_3)_4$ (122 mg, 0.11 mmol) in THF (4.1 mL) and MeCN (3 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 18 hours. After adding water (0.5 mL), the mixture concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 1% EtOAc) gave **73** (171 mg, 56%) as a white solid.

^{1}H NMR is in agreement with literature.[48]

5.2.3. Synthesis of (iso)quinolino-fused 7-Deazapurine Nucleosides

5-Iodoquinoline (**79**)

A suspension of 5-aminoquinoline (**90**, 7.27 g, 49.4 mmol) in water (165 mL) under ambient atmosphere was cooled to 0 °C (water/ice) and treated with concd HCl (23.3 mL, 35 wt% in water, 264 mmol) resulting in a red solution. After stirring for 30 min, a solution of $NaNO_2$ (11.6 g, 164 mmol) in water (85.6 mL) was added slowly, followed by stirring for further 30 min resulting in a brown solution. A suspension of CuI (11.5 g, 59.5 mmol) in water (144 mL) was slowly added in small portions under vigorous stirring to avoid excessive gas and foam formation. The mixture was then stirred further until the foaming subsided completely. Only then, HI (71.7 mL, 57 wt% in water, 545 mmol) was added followed by further stirring at 0 °C for 15 min. Then, the mixture was stirred at 60 °C for 3 hours. The mixture was poured over ice, its pH was increased to ~10 with aq. NaOH (4 M in water) and stirred for 20 min before being extracted with EtOAc (3 × 500 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by HPFC (SiO_2; Hex/EtOAc, gradient 0 → 20% EtOAc) gave **79** (9.38 g, 75%) as an off-white solid.

R_f = 0.51 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR is in agreement with literature.[112]

5-Iodoisoquinoline (**80**)

A suspension of 5-aminoisoquinoline (**91**, 14.4 g, 100 mmol) in water (41.2 mL) was treated with concd HCl (41.2 mL, 35 wt% in water, 467 mmol) and cooled to –10 °C (ice/acetone). After 30 min, $NaNO_2$ (8.23 g, 119 mmol) in water (31.0 mL) was added slowly resulting in a clear brown solution. After further 15 min, KI (33.4 g, 201 mmol) in water (41.0 mL) was added slowly. Then, the mixture was stirred at 100 °C for 2 hours. After cooling to r.t., the mixture was neutralized with aq. NH_3 resulting in precipitation. Immediate filtration, washing with cold water and purification by HPFC (SiO_2; DCM/MeOH, gradient 0 → 10% MeOH) gave **80** (11.9 g, 47%) as brown solid.

R_f = 0.37 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR is in agreement with literature.[113]

5-(4,6-Dichloropyrimidin-5-yl)quinoline (**83**)

$ZnCl_2$ (2.44 g, 17.9 mmol) was pre-dried at 250 °C for 20 min and allowed to cool down under vacuum before being treated with a solution of (TMP)MgCl · LiCl (35.7 mL, 1.0 M solution in THF/toluene, 35.7 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of 4,6-dichloropyrimidine (**22**, 4.43 g, 29.7 mmol) in THF (5.0 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of **79** (3.79 g, 14.9 mmol) and $Pd(PPh_3)_4$ (1.72 g, 1.49 mmol) in THF (20.0 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 24 hours. After adding water (2 mL), the mixture was loaded on silica. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **83** (3.19 g, 78%) as a pale-yellow solid.

R_f = 0.45 (SiO_2; cHex/EtOAc 2:1); m.p. = 179 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83 (dd, $J_{6,7}$ = 7.2 Hz, $J_{6,8}$ = 1.0 Hz, 1 H; H-6), 7.91 (dd, $J_{3,4}$ = 8.6 Hz, $J_{3,2}$ = 5.1 Hz, 1 H; H-3), 8.20 (dd, $J_{7,8}$ = 8.7 Hz, $J_{7,6}$ = 7.2 Hz, 1 H; H-7), 8.30 (d, $J_{4,3}$ = 8.5 Hz, 1 H; H-4), 9.01 (s, 1 H; H-2'), 9.09 (d, $J_{8,7}$ = 8.7 Hz, 1 H; H-8), 9.15 ppm (dd, $J_{2,3}$ = 5.1 Hz, $J_{2,4}$ = 1.5 Hz, 1 H; H-2); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 122.30 (C-3), 124.89 (C-8), 126.73 (C-4a), 129.31 (C-5'), 131.68 (C-5), 132.00 (C-6), 134.39 (C-7), 139.65 (C-8a), 141.50 (C-4), 144.21 (C-2), 158.86 (C-2'), 162.41 ppm (2 C; C-4', C-6'); IR (ATR, neat): $\tilde{\nu}$ = 3053 (w), 2991 (w), 2750–1700 (br w), 1601 (w), 1571 (w), 1548 (w), 1511 (m), 1500 (s), 1403 (s), 1377 (m), 1351 (m), 1311 (w), 1225 (m), 1162 (w), 1095 (w), 1036 (w), 1022 (w), 977 (w), 954 (m), 846 (w), 829 (w), 802 (s), 784 (s), 743 (m), 663 (m), 642 (w), 589 (w), 572 (w), 540 (w), 510 (w), 473 (w),

459 (w), 441 (w), 429 cm^{-1} (w); HR MS (ESI) for $C_{13}H_8N_3{}^{35}Cl_2{}^+$ $[M(^{35}Cl) + H]^+$: calcd 276.0095, found 276.0089; for $C_{13}H_8N_3{}^{35}Cl{}^{37}Cl^+$ $[M(^{35}Cl{}^{37}Cl) + H]^+$: calcd 278.0066, found 278.0058; for $C_{13}H_8N_3{}^{37}Cl_2{}^+$ $[M(^{37}Cl) + H]^+$: calcd 280.0036, found 280.0028.

5-(4,6-dichloropyrimidin-5-yl)isoquinoline (**84**)

$ZnCl_2$ (215 mg, 1.56 mmol) was pre-dried at 250 °C for 20 min and allowed to cool down under vacuum before being treated with a solution of (TMP)MgCl · LiCl (3.2 mL, 1.0 M solution in THF/toluene, 3.2 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of **22** (392 mg, 2.63 mmol) in THF (0.5 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of **80** (336 mg, 1.32 mmol) and $Pd(PPh_3)_4$ (154 mg, 0.13 mmol) in THF (1.7 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 24 hours. After adding water (0.5 mL), the mixture was loaded on silica. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **84** (182 mg, 50%) as a orange solid.

R_f = 0.46 (SiO_2; cHex/EtOAc 2:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.12 (bd, $J_{3,4}$ = 5.9 Hz, 1 H; H-3), 7.65 (dd, $J_{6,7}$ = 7.1 Hz, $J_{6,8}$ = 1.1 Hz, 1 H; H-6), 7.76 (dd, $J_{7,8}$ = 8.2 Hz, $J_{7,6}$ = 7.2 Hz, 1 H; H-7), 8.16 (bd, $J_{8,7}$ = 8.3 Hz, 1 H; H-8), 8.55 (d, $J_{4,3}$ = 5.9 Hz, 1 H; H-4), 8.93 (s, 1 H; H-2'), 9.39 ppm (bs, 1 H; H-1); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ = 116.94 (C-3), 127.11 (C-7), 128.79 (C-8a), 129.65 (C-5'), 129.85 (C-8), 131.96 (C-6), 133.30 (C-4a), 144.46 (C-4), 153.45 (C-1), 157.94 (C-2'), 162.56 ppm (2 C; C-4', C-6').

8-(4,6-dichloropyrimidin-5-yl)isoquinoline (**85**)

$ZnCl_2$ (215 mg, 1.56 mmol) was pre-dried at 250 °C for 20 min and allowed to cool down under vacuum before being treated with a solution of (TMP)MgCl · LiCl (3.2 mL, 1.0 M solution in THF/toluene, 3.2 mmol) and stirred at 20 °C for 24 hours. The solution was cooled to 0 °C (water/ice) and treated with a solution of **22** (392 mg, 2.63 mmol) in THF (0.5 mL). After 1 hour, the solution was stirred at 20 °C for an additional hour resulting in the zincated pyrimidine. Separately, a solution of **81** (335 mg, 1.31 mmol) and $Pd(PPh_3)_4$ (153 mg, 0.13 mmol) in THF (1.7 mL) was pre-stirred at 20 °C for 20 min before addition to the zincated pyrimidine. The resulting solution was stirred at 65 °C for 24 hours. After adding water (0.5 mL), the mixture was loaded on silica. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **85** (160 mg, 44%) as a mustard-yellow solid.

R_f = 0.32 (SiO_2; cHex/EtOAc 2:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 7.51 (dd, $J_{7,6}$ = 7.1 Hz, $J_{7,5}$ = 1.1 Hz, 1 H; H-7), 7.79 (bdd, $J_{4,3}$ = 5.7 Hz, $J_{4,5}$ = 1.0 Hz, 1 H; H-4), 7.83 (dd, $J_{6,5}$ = 8.4 Hz, $J_{6,7}$ = 7.1 Hz, 1 H; H-6), 8.01 (bdt, $J_{5,6}$ = 8.4 Hz, $J_{5,4}$ = $J_{5,7}$ = 1.1 Hz, 1 H; H-5), 8.63 (d, $J_{3,4}$ = 5.7 Hz, 1 H; H-3), 8.79 (bs, 1 H; H-1), 8.95 ppm (s, 1 H; H-2'); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ =121.25 (C-4), 125.59 (C-8a), 128.84 (C-5), 129.09 (C-7), 130.24 (C-6), 131.05 (C-8), 131.17 (C-5'), 136.33 (C-4a), 143.84 (C-3), 149.00 (C-1), 158.00 (CH-2'), 162.44 ppm (2 C; C-4', C-6'); HR MS (EI) for $C_{13}H_7N_3{}^{35}Cl_2{}^+$ $[M(^{35}Cl)]^+$: calcd 275.0017, found 275.0010; for $C_{13}H_7N_3{}^{35}Cl{}^{37}Cl^+$ $[M(^{35}Cl{}^{37}Cl)]^+$: calcd 276.9988, found 276.9982; for $C_{13}H_7N_3{}^{37}Cl_2{}^+$ $[M(^{37}Cl)]^+$: calcd 278.9958, found 278.9952.

5-(4-Azido-6-chloropyrimidin-5-yl)quinoline (**87a**) / 5-(7-Chlorotetrazolo[1,5-*c*]pyrimidin-8-yl)quinoline (**87b**)

A solution of **83** (4.07 g, 14.7 mmol) in THF (60.0 mL) was treated with sodium azide (957 mg, 14.7 mmol) and lithium chloride (628 mg, 14.8 mmol) and stirred under light exclusion at 20 °C for 24 hours. After adding water (1 mL), the mixture was concentrated *in*

vacuo. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 50% EtOAc) gave **87** (3.65 g, 88%) as an off-white solid.

R_f = 0.64 (SiO_2; cHex/EtOAc 1:1); m.p. = 154–158 °C (decomp.); ^{1}H NMR of **87a** (500 MHz, $CDCl_3$): δ = 7.43 (dd, $J_{3,4}$ = 8.5 Hz, $J_{3,2}$ = 4.2 Hz, 1 H; H-3), 7.45 (dd, $J_{6,7}$ = 7.1 Hz, $J_{6,8}$ = 1.0 Hz, 1 H; H-6), 7.70 (bd, $J_{4,3}$ = 8.5 Hz, 1 H; H-4), 7.83 (dd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.1 Hz, 1 H; H-7), 8.28 (bd, $J_{8,7}$ = 8.5 Hz, 1 H; H-8), 8.82 (s, 1 H; H-2'), 8.99 ppm (dd, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.6 Hz, 1 H; H-2); ^{13}C{^{1}H} NMR of **87a** (125.7 MHz, $CDCl_3$): δ = 120.86 (C-5'), 121.97 (C-3), 126.24 (C-4a), 128.65 (C-6), 129.30 (C-7), 129.39 (C-5), 131.15 (C-8), 133.15 (C-4), 148.02 (C-8a), 150. 70 (C-2'), 147.59 (C-2), 157.83 (C-2'), 161.82 and 163.04 ppm (2 C; C-4', C-6'); ***87b** was not observed in $CDCl_3$*; ^{1}H NMR of **87a** (500 MHz, DMSO-d_6): δ = 7.53 (dd, $J_{3,4}$ = 8.5 Hz, $J_{3,2}$ = 4.2 Hz, 1 H; H-3), 7.62 (dd, $J_{6,7}$ = 7.1 Hz, $J_{6,8}$ = 1.2 Hz, 1 H; H-6), 7.88 (dd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.1 Hz, 1 H; H-7), 7.98 (ddd, $J_{4,3}$ = 8.5 Hz, $J_{4,2}$ = 1.6 Hz, $J_{4,8}$ = 0.9 Hz, 1 H; H-4), 8.15 (dt, $J_{8,7}$ = 8.5 Hz, $J_{8,6}$ = $J_{8,4}$ = 1.0 Hz, 1 H; H-8), 8.97 (dd, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.7 Hz, 1 H; H-2), 8.99 ppm (s, 1 H; H-2'); ^{13}C{^{1}H} NMR of **87a** (125.7 MHz, DMSO-d_6): δ = 120.46 (C-5'), 122.22 (C-3), 125.84 (C-4a), 128.59 (C-6), 129.24 (C-7), 129.59 (C-5), 130.34 (C-8), 133.35 (C-4), 147.53 (C-8a), 150.89 (C-2), 157.86 (C-2'), 160.43 and 162.35 ppm (2 C; C-4', C-6'); ^{1}H NMR of **87b** (500 MHz, DMSO-d_6): δ = 7.52 (dd, $J_{3,4}$ = 8.5 Hz, $J_{3,2}$ = 4.1 Hz, 1 H; H-3), 7.77 (dd, $J_{6,7}$ = 7.1 Hz, $J_{6,8}$ = 1.2 Hz, 1 H; H-6), 7.99 (dd, $J_{7,8}$ = 8.5 Hz, $J_{7,6}$ = 7.1 Hz, 1 H; H-7), 8.08 (ddd, $J_{4,3}$ = 8.5 Hz, $J_{4,2}$ = 1.6 Hz, $J_{4,8}$ = 1.0 Hz, 1 H; H-4), 8.27 (dt, $J_{8,7}$ = 8.5 Hz, $J_{8,6}$ = $J_{8,4}$ = 1.1 Hz, 1 H; H-8), 9.00 (dd, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.7 Hz, 1 H; H-2), 10.41 ppm (s, 1 H; H-5'); ^{13}C{^{1}H} NMR of **87b** (125.7 MHz, DMSO-d_6): δ = 120.00 (C-8'), 122.11 (C-3), 125.95 (C-4a), 128.57 (C-5), 128.82 (C-6), 129.33 (C-7), 131.07 (C-8), 133.72 (C-4), 139.91 (C-5'), 147.29 (C-9'), 147.59 (C-8a), 151.13 (C-2), 151.58 ppm (C-7'); IR (ATR, neat): $\tilde{\nu}$ = 2294 (w), 2203 (w), 2137 (s), 2041 (w), 1599 (w), 1571 (w), 1550 (w), 1524 (s), 1499 (m), 1425 (w), 1402 (s), 1358 (s), 1316 (m), 1305 (s), 1225 (w), 1200 (w), 1178 (w), 1145 (s), 1078 (w), 1058 (w), 1023 (w), 978 (w), 954 (m), 919 (w), 896 (s), 847 (w), 830 (w), 802 (s), 793 (s), 771 (s), 751 (m), 736 (m), 694 (w), 663 (w), 637 (m), 586 (m), 539 (m), 510 (m), 492 (w), 459 (w), 437 cm^{-1} (m); HR MS (ESI) for $C_{13}H_8N_6{}^{35}Cl^+$ [M(^{35}Cl) + H]$^+$: calcd 283.0499, found 283.0495; for $C_{13}H_8N_6{}^{37}Cl^+$ [M(^{37}Cl) + H]$^+$: calcd 285.0470, found 285.0464.

11-Chloro-7*H*-pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**88**)

Two batches were prepared at the same time. For each batch, a solution of **87** (300 mg, 1.06 mmol) and pyrene (215 mg, 1.06 mmol) in THF (42.4 mL) was irradiated under UV light (254 nm, 4 W) at r.t. under ambient atmosphere. Every 12 hours, the reaction mixtures were sonicated for 30 seconds to remove any precipitation from the light source. After 72 hours, the batches were combined, concentrated *in vacuo* and purified by HPFC (SiO_2; DCM/EtOAc, gradient 0 → 100% EtOAc) giving **88** (138 mg, 26%) as a light-brown solid.

R_f = 0.11 (SiO_2; cHex/EtOAc 1:1); m.p. = 170–185 °C (decomp.); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.69 (dd, 1 H, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 4.2 Hz; H-2), 8.00 (bd, 1 H, $J_{6,5}$ = 9.0 Hz; H-6), 8.19 (bd, 1 H, $J_{5,6}$ = 9.0 Hz; H-5), 8.80 (s, 1 H; H-9), 8.90 (dd, 1 H, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.5 Hz; H-3), 9.83 (bd, 1 H, $J_{1,2}$ = 8.7 Hz; H-1), 13.43 ppm (s, 1 H; N*H*); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 110.43 (C-11b), 112.79 (C-11a), 116.10 (C-6), 121.56 (C-2), 123.70 (C-11c), 131.58 (C-5), 133.51 (C-1), 136.95 (C-6a), 145.22 (C-4a), 147.59 (C-3), 150.54 (C-11), 152.39 (C-9), 155.33 ppm (C-4a); IR (ATR, neat): $\tilde{\nu}$ = 3500–2250 (br w), 1727 (m), 1684 (m), 1590 (m), 1562 (m), 1542 (m), 1503 (m), 1465 (m), 1439 (m), 1412 (m), 1385 (m), 1367 (m), 1306 (m), 1234 (s), 1188 (m), 1161 (m), 1094 (m), 1055 (m), 1016 (m), 978 (m), 947 (s), 845 (m), 806 (s), 785 (s), 767 (s), 693 (m), 663 (s), 631 (s), 586 (s), 542 (s), 509 (s), 468 (m), 435 (s), 425 cm^{-1} (m); HR MS (ESI) for $C_{13}H_8N_4{}^{35}Cl^+$ [M(^{35}Cl) + H]$^+$: calcd 255.0438, found 255.0432; for $C_{13}H_8N_4{}^{37}Cl^+$ [M(^{37}Cl) + H]$^+$: calcd 257.0408, found 257.0402.

5-(4-Azido-6-chloropyrimidin-5-yl)isoquinoline (**92a**) / 5-(7-Chlorotetrazolo[1,5-*c*]pyrimidin-8-yl)isoquinoline (**92b**)

A solution of **84** (340 mg, 1.23 mmol) in THF (6.2 mL) was treated with sodium azide (80.0 mg, 1.23 mmol) and lithium chloride (56.2 mg, 1.23 mmol) and stirred under light exclusion at 20 °C for 24 hours. After adding water (0.5 mL), the mixture was concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 50% EtOAc) gave **92** (222 mg, 60%) as an off-white solid.

^{1}H NMR of **92a** (400 MHz, $CDCl_3$): δ = 7.16 (dt, $J_{3,4}$ = 6.0 Hz, $J_{3,1}$ = 1.0 Hz, 1 H; H-3), 7.61 (dd, $J_{6,7}$ = 7.2 Hz, $J_{6,8}$ = 1.2 Hz, 1 H; H-6), 7.75 (dd, $J_{7,8}$ = 8.3 Hz, $J_{7,6}$ = 7.2 Hz, 1 H; H-7), 8.13 (dt, $J_{8,7}$ = 6.4 Hz, $J_{8,6}$ = 1.2 Hz, 1 H; H-8), 8.53 (d, $J_{4,3}$ = 6.0 Hz, 1 H; H-4), 8.84 (s, 1 H; H-2'), 9.36 ppm (d, $J_{1,3}$ = 1.0 Hz, 1 H; H-1).

11-Chloro-7-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]-quinoline (**94**)

A suspension of nucleobase **88** (221 mg, 0.87 mmol) in MeCN (26.5 mL) was treated with BSA (0.32 mL, 1.31 mmol) and stirred at 60 °C for 15 min. The sugar **30** (876 mg, 1.74 mmol; dried under vacuum at 60 °C for 6 h) was added under argon flow, followed by TMSOTf (0.32 mL, 1.74 mmol). After being stirred at 60 °C for 48 h, the resulting mixture was treated with water (25 mL), concentrated *in vacuo* to remove MeCN and extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with saturated $NaHCO_3$ (50 mL) and water (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 60% EtOAc) gave crude **94** (419 mg, 75% pure, 52%) as a light grey foam. A small amount was repurified by preparative TLC (C18; pure MeCN) resulting in a white foam prior to characterization.

R_f = 0.40 (SiO_2; cHex/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.76 (dd, $J_{5'a,5'b}$ = 12.3 Hz, $J_{5'a,4'}$ = 3.9 Hz, 1 H; H-5'a), 4.87 (dt, $J_{4',3'}$ = 6.1 Hz, $J_{4',5'a}$ = $J_{4',5'b}$ = 3.4 Hz, 1 H; H-4'), 4.99 (dd, $J_{5'b,5'a}$ = 12.3 Hz, $J_{5'b,4'}$ = 2.9 Hz, 1 H; H-5'b), 6.44 (t, $J_{3',2'}$ = $J_{3',4'}$ = 6.3 Hz, 1 H; H-3'), 6.67 (dd, $J_{2',3'}$ = 6.5 Hz, $J_{2',1'}$ = 5.1 Hz, 1 H; H-2'), 7.02 (d, $J_{1',2'}$ = 5.1 Hz, 1 H; H-1'), 7.34, 7.42 and 7.45 (3 × m, 3 × 2 H; H-A3, H-A5, H-B3, H-B5, H-C3, H-C5), 7.53, 7.59 and 7.61 (3 × m, 3 H; H-A4, H-B4, H-C4), 7.61 (dd, $J_{2,1}$ = 8.9 Hz, $J_{2,3}$ = 4.1 Hz, 1 H; H-2), 7.88, 8.03 and 8.07 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.12 (d, $J_{5,6}$ = 9.3 Hz, 1 H; H-5), 8.17 (d, $J_{6,5}$ = 9.3 Hz, 1 H; H-6), 8.77 (s, 1 H; H-9), 8.97 (dd, $J_{3,2}$ = 4.1 Hz, $J_{3,1}$ = 1.3 Hz, 1 H; H-3), 10.00 ppm (d, $J_{1,2}$ = 8.9 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 63.35 (C-5'), 70.89 (C-3'), 73.03 (C-2'), 80.23 (C-4'), 87.01 (C-1'), 113.13 (C-11b), 114.65 (C-6), 114.83 (C-11a), 121.63 (C-2), 124.53 (C-11c), 128.64 (C-A1), 128.66, 128.71 and 128.77 (3 × 2 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.92 and 129.49 (2 C; C-B1, C-C1), 129.85, 129.93 and 130.00 (3 × 2 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 132.72 (C-5), 133.71, 133.87 and 133.90 (3 C; C-A4, C-B4, C-C4), 134.95 (C-1), 136.77 (C-6a), 146.15 (C-4a), 148.75 (C-3), 152.28 (C-11), 152.40 (C-9), 155.40 (C-7a), 165.37, 165.60 and 166.27 ppm (3 C; C=O); IR (ATR, neat): $\tilde{\nu}$ = 2921 (w), 2851 (w), 1721 (m), 1601 (w), 1585 (w), 1560 (w), 1533 (w), 1518 (w), 1467 (w), 1450 (w), 1406 (w), 1375 (w), 1314 (w), 1262 (s), 1243 (m), 1176 (w), 1157 (w), 1090 (m), 1068 (m), 1024 (m), 945 (w), 847 (w), 810 (m), 787 (m), 768 (w), 707 (s), 686 (m), 617 (w), 546 (w), 509 (w), 461 (w), 436 (w), 421 cm^{-1} (w); HR MS (ESI) for $C_{39}H_{28}N_4O_7{}^{35}Cl^+$ [M(^{35}Cl) + H]$^+$: calcd 699.1647, found 699.1634; for $C_{39}H_{28}N_4O_7{}^{37}Cl^+$ [M(^{37}Cl) + H]$^+$: calcd 701.1617, found 701.1610.

11-Chloro-7-(2,3-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**95-*β***) / *α*-anomer (**95-*α***)

A solution of the previously synthesized halosugar precursor, 2,3-*O*-isopropylidene-5-*O*-*tert*-butyldimethylsilyl-D-ribofuranose[74] (**31**, 180 mg, 0.59 mmol) in THF (2.9 mL) was cooled to −30 °C and treated with CCl_4 (77 µL, 0.79 mmol). Then, HMPT (141 µL, 0.79 mmol) was added dropwise. The resulting solution was stirred vigorously for 1 h, resulting in the desired halosugar. In a separate flask, a suspension of **88** (100 mg, 0.40 mmol) in MeCN (2.5 mL) and powdered KOH (44.5 mg, 0.79 mmol) was treated with TDA-1 (0.13 mL, 0.40 mmol) and stirred for 30 min. After being cooled to 0 °C, the halosugar solution was added via syringe. The resulting mixture was first stirred at 0 °C for 30 min and then at r.t. for 36 h before being treated with water (0.25 mL). After concentration *in vacuo*, purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave the pure *β*-anomer **95-*β*** (14.4 mg, 7%), a light beige solid, which was used for characterization, together with a mixed fraction containing an anomeric mixture **95-*β*/-*α*** 0.8:0.2 (32.4 mg, 15%) as a light-yellow solid and **95-*α*** (16.4 mg, 8%) as a sticky yellow solid. Additionally, some starting material **88** (16.0 mg, 16%) was recovered.

***β*-anomer (95-*β*):** R_f = 0.55 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.11 and 0.12 (2 × s, 2 × 3 H; Si*Me*$_2$), 0.95 (s, 9 H; C*Me*$_3$), 1.39 and 1.70 (2 × s, 2 × 3 H; OC*Me*$_2$), 3.91 (dd, $J_{5'a,5'b}$ = 11.4 Hz, $J_{5'a,4'}$ = 3.7 Hz, 1 H; H-5'a), 4.01 (dd, $J_{5'b,5'a}$ = 11.4 Hz, $J_{5'b,4'}$ = 3.3 Hz, 1 H; H-5'b), 4.31 (q, $J_{4',3'}$ = $J_{4',5'a}$ = $J_{4',5'b}$ = 3.7 Hz, 1 H; H-4'), 5.21 (dd, $J_{3',2'}$ = 6.9 Hz, $J_{3',4'}$ = 4.3 Hz, 1 H; H-3'), 5.41 (dd, $J_{2',3'}$ = 6.9 Hz, $J_{2',1'}$ = 4.0 Hz, 1 H; H-2'), 6.92 (d, $J_{1',2'}$ = 4.0 Hz, 1 H; H-1'), 7.62 (dd, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 8.28 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.32 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.84 (s, 1 H; H-9), 8.98 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 10.02 ppm (dd, $J_{1,2}$ = 8.7 Hz, $J_{1,3}$ = 1.6 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = –5.25 and –5.10 (2 C; Si*Me*$_2$), 18.71 (Si*C*Me$_3$), 25.68 and 27.56 (2 C; OC*Me*$_2$), 26.17 (Si*C*Me$_3$), 62.69 (C-5'), 80.00 (C-3'), 82.87 (C-2'), 85.32 (C-4'), 88.82 (C-1'), 113.07 (C-11b), 114.38 (C-11a), 115.54 (O*C*Me$_2$), 115.99 (C-6), 121.56 (C-2), 124.47 (C-11c), 132.52 (C-5), 134.91 (C-1), 136.58 (C-6a), 146.14 (C-4a), 148.65 (C-3), 152.08 (C-11), 152.43 (C-9), 155.32 ppm (C-7a); IR (ATR, neat): $\tilde{\nu}$ = 2929 (w), 2856 (w), 1737 (very w), 1610 (w), 1588 (w), 1559 (w), 1531 (w), 1518 (m), 1468 (m), 1440 (w), 1427 (w), 1382 (w), 1372 (w), 1316 (w), 1242 (m), 1211 (m), 1136 (m), 1080 (s), 1007 (m), 974 (w), 946 (w), 929 (m), 888 (w), 832 (s), 810 (s), 777 (s), 670 (w), 627 (w), 567 (w), 546 (w), 512 (w), 469 (w), 432 cm^{-1} (w); HR MS (ESI) for $C_{27}H_{34}N_4O_4{}^{35}ClSi^+$ [M(^{35}Cl) + H]$^+$: calcd 541.20379, found 541.20359; for $C_{27}H_{34}N_4O_4{}^{37}ClSi^+$ [M(^{37}Cl) + H]$^+$: calcd 543.20084, found 543.20082; $C_{27}H_{33}N_4O_4{}^{35}ClSiNa^+$ [M(^{35}Cl) + Na]$^+$: calcd 563.18573, found 563.18567; for $C_{27}H_{33}N_4O_4{}^{37}ClSiNa^+$ [M(^{37}Cl) + Na]$^+$: calcd 565.18278, found 565.18286.

***α*-anomer (95-*α*):** R_f = 0.44 (SiO_2; cHex/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.16 and 0.19 (2 × s, 2 × 3 H; Si*Me*$_2$), 1.03 (s, 9 H; C*Me*$_3$), 1.27 and 1.34 (2 × s, 2 × 3 H; OC*Me*$_2$), 3.91 (dd, $J_{5'a,5'b}$ = 10.9 Hz, $J_{5'a,4'}$ = 1.8 Hz, 1 H; H-5'a), 4.01 (dd, $J_{5'b,5'a}$ = 10.9 Hz, $J_{5'b,4'}$ = 2.4 Hz, 1 H; H-5'b), 4.66 (t, $J_{4',5'a}$ = $J_{4',5'b}$ = 2.2 Hz, 1 H; H-4'), 5.04–5.08 (m, 2 H; H-2', H-3'), 7.45 (m, 1 H; H-1'), 7.57 (dd, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 8.24 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.57 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.76 (s, 1 H; H-9), 8.93 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 10.00 ppm (dm, $J_{1,2}$ = 8.7 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = –5.33 and –5.34 (2 C; Si*Me*$_2$), 18.35 (Si*C*Me$_3$), 23.51 and 25.38 (2 C; OC*Me*$_2$), 26.08 (Si*C*Me$_3$), 66.24 (C-5'), 80.79 (C-3'), 82.49 (C-2'), 83.07 (C-4'), 88.78 (C-1'), 112.55 (C-11b), 113.47 (O*C*Me$_2$), 113.95 (C-11a), 120.07 (C-6), 121.08 (C-2), 124.25 (C-11c), 130.88 (C-5), 134.88 (C-1), 38.37 (C-6a), 146.02 (C-4a), 148.15 (C-3), 151.60 (C-11), 152.12 (C-9), 154.86 ppm (C-7a); IR (ATR, neat): $\tilde{\nu}$ = 2929 (w), 2856 (w), 1610 (w), 1588 (w), 1558 (w), 1532 (m), 1515 (m), 1469 (m), 1438 (w), 1383 (w), 1375 (w), 1337 (w), 1316 (w), 1272 (w), 1241 (s), 1206 (m), 1161 (m), 1124 (m), 1076 (s), 1053 (m), 1026 (w), 989 (m), 973 (m), 939 (m), 912 (m), 881 (w), 833 (s), 810 (s), 777 (s), 728 (s), 674 (w), 640 (w), 608 (w), 578

(w), 544 (w), 515 (w), 472 (w), 440 cm^{-1} (w); HR MS (ESI) for $C_{27}H_{34}N_4O_4{}^{35}ClSi^+$ $[M(^{35}Cl) + H]^+$: calcd 541.20379, found 541.20366; for $C_{27}H_{34}N_4O_4{}^{37}ClSi^+$ $[M(^{37}Cl) + H]^+$: calcd 543.20084, found 543.20080; $C_{27}H_{33}N_4O_4{}^{35}ClSiNa^+$ $[M(^{35}Cl) + Na]^+$: calcd 563.1857, found 563.1856; for $C_{27}H_{33}N_4O_4{}^{37}ClSiNa^+$ $[M(^{37}Cl) + Na]^+$: calcd 565.1828, found 565.1829.

11-(Furan-2-yl)-7-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**96a**)

A solution of the crude nucleoside **94** (427 mg, 75%, 0.46 mmol) in DMF (4.6 mL) was treated with $PdCl_2(PPh_3)_2$ (32.4 mg, 0.05 mmol) and 2-(tributylstannyl)furan (0.18 mL, 0.55 mmol) and stirred at 100 °C for 24 hours. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **96a** (85.3 mg, 26%) as a yellow film.

R_f = 0.39 (SiO_2; cHex/EtOAc 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 4.78 (dd, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 4.2 Hz, 1 H; H-5'a), 4.89 (ddd, $J_{4',3'}$ = 6.3 Hz, $J_{4',5'a}$ = 4.1 Hz, $J_{4',5'b}$ = 3.1 Hz, 1 H; H-4'), 4.99 (dd, $J_{5'b,5'a}$ = 12.2 Hz, $J_{5'b,4'}$ = 3.0 Hz, 1 H; H-5'b), 6.48 (t, $J_{3',2'} = J_{3',4'}$ = 6.3 Hz, 1 H; H-3'), 6.71 (bdd, $J_{2',3'}$ = 6.5 Hz, $J_{2',1'}$ = 4.9 Hz, 1 H; H-2'), 6.75 (dd, $J_{4,3}$ = 3.5 Hz, $J_{4,5}$ = 1.8 Hz, 1 H; H-4-furyl), 6.99 (d, $J_{1',2'}$ = 4.9 Hz, 1 H; H-1'), 7.25 (d, $J_{3,4}$ = 3.5 Hz, H-3-furyl), 7.34 (m, 2 H; H-A3, H-A5), 7.39 (m, 1 H; H-6), 7.42 and 7.45 (2 × m, 2 × 2 H; H-B3, H-B5, H-C3, H-C5), 7.53 (m, 1 H; H-A4), 7.56–7.63 (m, 3 H, H-5-furyl, H-B4, H-C4), 7.85 (m, 1 H; H-1), 7.90, 8.04 and 8.08 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.23 (d, $J_{6,5}$ = 9.2 Hz, H-6), 8.35 (bm, 1 H; H-5), 8.87 (dd, $J_{3,2}$ = 4.5 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.01 ppm (s, 1 H; H-9); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 63.51 (C-5'), 71.03 (C-3'), 73.24 (C-2'), 80.24 (C-4'), 87.03 (C-1'), 111.57 (C-11a), 113.30 (C-4-furyl), 114.01 (C-3-furyl), 114.11 (C-11b), 116.10 (C-6), 120.84 (C-2), 124.78 (C-11c), 128.7 (C-A1), 128.67, 128.71 and 128.77 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.94 and 129.52 (2 C; C-B1, C-C1), 129.86, 129.94 and 130.00 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.69, 133.86 and 133.91 (3 C; C-A4, C-B4, C-C4), 137.44 (C-6a), 145.18 and 145.20 (2 C; C-3, C-5-furyl), 150.00 (C-11), 151.97 (C-2-furyl), 153.19 (C-9), 155.76 (C-7a), 165.47, 165.61 and 166.32 ppm (3 C; C=O), *3 C (C-1, C-4a, C-5) not detected*; IR (ATR, neat): $\tilde{\nu}$ = 3065 (w), 2919 (w), 2852 (w), 1724 (s), 1601 (w), 1584 (w), 1562 (m), 1538 (w), 1518 (m), 1491 (w), 1464 (w), 1451 (m), 1375 (w), 1315 (w), 1264 (s), 1177 (w), 1161 (w), 1116 (s), 1093 (s), 1069 (m), 1025 (m), 932 (w), 885 (w), 850 (w), 813 (w), 798 (w), 751 (s), 708 (m), 687 (w), 633 (w), 617 (w), 595 (w), 548 (w), 463 (w), 444 (w), 416 cm^{-1} (w); HR MS (ESI) for $C_{43}H_{31}N_4O_8^+$ $[M + H]^+$: calcd 731.2142, found 731.2133; for $C_{43}H_{30}N_4O_8Na^+$ $[M + Na]^+$: calcd 753.1961, found 753.1953.

11-(Benzofuran-2-yl)-7-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]-pyrrolo[3,2-*f*]quinoline (**96c**)

A solution of the crude nucleoside **94** (300 mg, 75%, 0.32 mmol) in toluene (4.3 mL) was treated with benzofuran-2-ylboronic acid (105 mg, 0.65 mmol), K_2CO_3 (114 mg, 0.82 mmol) and $Pd(PPh_3)_4$ (38.2 mg, 0.33 mmol). After stirring at 100 °C for 24 hours, the suspension was filtered through a celite plug (2 cm). Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 25% EtOAc) gave **96c** (207 mg, 82%) as a bright-yellow solid.

R_f = 0.62 (SiO_2; cHex/EtOAc 1:1); m.p. = 93°C; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.80 (dd, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 4.0 Hz, 1 H; H-5'a), 4.88 (m, 1 H; H-4'), 5.00 (dd, $J_{5'b,5'a}$ = 12.2 Hz, $J_{5'b,4'}$ = 2.9 Hz, 1 H; H-5'b), 6.49 (t, $J_{3',2'} = J_{3',4'}$ = 6.1 Hz, 1 H; H-3'), 6.74 (dd, $J_{2',3'}$ = 6.5 Hz, $J_{2',1'}$ = 5.3 Hz, 1 H; H-2'), 6.94 (dd, $J_{2,1}$ = 8.6 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 7.08 (d, $J_{1',2'}$ = 5.3 Hz, 1 H; H-1'), 7.29 (m, 1 H; H-7-benzofuryl), 7.32–7.39 (m, 4 H; H-A3, H-A5, H-5-benzofuryl, H-6-benzofuryl), 7.41 (m, 2 H; H-B3, H-B5), 7.46 (m, 2 H; H-C3, H-C5), 7.53 (m, 1 H; H-A4), 7.56 (d, $J_{3,LR}$ = 0.9 Hz, 1 H; H-3-benzofuryl), 7.59 and 7.60 (2 × m, 2 × 1 H; H-B4, H-C4), 7.66 (bd, $J_{1,2}$ = 8.6 Hz, 1 H; H-1), 7.76 (m, 1 H; H-4-benzofuryl), 7.92 (m, 2 H;

H-A2, H-A6), 8.04 (m, 2 H; H-B2, H-B6), 8.07 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.12 (m, 2 H; H-C2, H-C6), 8.17 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-6), 8.80 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.07 ppm (s, 1 H; H-9); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 63.62 (C-5'), 71.04 (C-3'), 73.03 (C-2'), 80.25 (C-4'), 86.69 (C-1'), 110.18 (C-3-benzofuryl), 112.26 (C-7-benzofuryl), 112.41 (C-11a), 114.04 (C-11b), 114.71 (C-6), 120.60 (C-2), 122.53 (C-4-benzofuryl), 123.93 (C-5-benzofuryl), 124.33 (C-11c), 126.43 (C-6-benzofuryl), 128.35 (C-3a-benzofuryl), 128.64 and 128.69 (4 C; C-A3, C-A5, C-B3, C-B5), 128.74 (C-A1), 128.77 (2 C; C-C3, C-C5), 129.00 (C-B1), 129.57 (C-C1), 129.91, 129.95 and 131.01 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 132.11 (C-5), 133.64 and 133.83 (3 C, C-A4, C-B4, C-C4), 134.43 (C-1), 137.31 (C-6a), 145.86 (C-4a), 148.44 (C-3), 149.48 (C-11), 152.70 (C-9), 153.66 (C-2-benzofuryl), 155.48 (C-7a-benzofuryl), 155.89 (C-7a), 165.40 (C=O A), 165.63 (C=O B), 166.35 ppm (C=O C); IR (ATR, neat): $\tilde{\nu}$ = 3062 (w), 2932 (w), 1723 (s), 1601 (m), 1585 (w), 1561 (m), 1533 (w), 1516 (m), 1464 (w), 1450 (m), 1424 (w), 1377 (w), 1315 (m), 1257 (s), 1165 (m), 1109 (s), 1092 (s), 1068 (s), 1025 (m), 933 (w), 885 (m), 851 (w), 813 (w), 797 (m), 751 (w), 708 (s), 687 (m), 634 (w), 616 (w), 578 (w), 547 (w), 516 (w), 492 (w), 445 (w), 430 (w), 407 cm^{-1} (w); HR MS (ESI) for $C_{47}H_{33}N_4O_8^+$ $[M + H]^+$: calcd 781.2298, found 781.2299; for $C_{47}H_{32}N_4O_8Na^+$ $[M + Na]^+$: calcd 803.2118, found 803.2119.

11-Methyl-7-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**96d**)

A solution of the crude nucleoside **94** (268 mg, 75%, 0.29 mmol) in THF (7.2 mL) was treated with $Pd(PPh_3)_4$ (18.2 mg, 0.02 mmol) and Me_3Al (0.43 mL, 2.0 M in toluene, 0.86 mmol). After stirring at 65 °C for 3 hours, the resulting mixture was treated with MeOH (1 mL) before being concentrated *in vacuo*. Purification by HPFC (SiO_2; cHex/EtOAc, gradient 0 → 50% EtOAc) gave **96d** (81.6 mg, 42%) as a brown sticky solid.

R_f = 0.39 (SiO_2; cHex/EtOAc 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ = 3.32 (s, 3 H; C*Me*), 4.77 (dd, $J_{5'a,5'b}$ = 12.2 Hz, $J_{5'a,4'}$ = 4.0 Hz, 1 Hz; H-5'a), 4.85 (bddd, $J_{4',3'}$ = 6.0 Hz, $J_{4',5'a}$ = 4.0 Hz, $J_{4',5'b}$ = 2.9 Hz, 1 Hz; H-4'), 4.98 (dd, $J_{5'b,5'a}$ = 12.2 Hz, $J_{5'b,4'}$ = 2.9 Hz, 1 Hz; H-5'b), 6.47 (t, $J_{3',2'}$ = $J_{3',4'}$ = 6.3 Hz, 1 Hz; H-3'), 6.69 (dd, $J_{2',3'}$ = 6.6 Hz, $J_{2',1'}$ = 5.1 Hz, 1 Hz; H-2'), 7.05 (d, $J_{1',2'}$ = 5.1 Hz, 1 Hz; H-1'), 7.33, 7.40 and 7.46 (3 × m, 3 × 2 H: H-A3, H-A5, H-B3, H-B5, H-C3, H-C5), 7.53, 7.58 and 7.61 (3 × m, 3 × 1 H, H-A4, H-B4, H-C4), 7.58 (dd, $J_{2,1}$ = 8.7 Hz, $J_{2,3}$ = 4.2 Hz, H-2), 7.90, 8.02 and 8.10 (3 × m, 3 × 2 H; H-A2, H-A6, H-B2, H-B6, H-C2, H-C6), 8.06 (d, $J_{5,6}$ = 9.2 Hz, H-5), 8.17 (d, $J_{6,5}$ = 9.2 Hz, 1 Hz; H-6), 8.92 (s, 1 H; H-9), 8.95 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 Hz; H-3), 9.23 ppm (bd, $J_{1,2}$ = 8.7 Hz, 1 Hz; H-1); $^{13}C\{^1H\}$ NMR (125.7 MHz, $CDCl_3$): δ = 28.89 (C*Me*), 63.58 (C-5'), 70.97 (C-3'), 73.03 (C-2'), 80.07 (C-4'), 86.69 (C-1'), 114.66 (C-11b), 114.93 (C-6), 115.10 (C-11a), 121.37 (C-2), 124.35 (C-11c), 128.62, 128.67 and 128.74 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.75, 129.00 and 129.58 (3 C; C-A1, C-B1, C-C1), 129.90, 129.93 and 129.99 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 131.29 (C-5), 133.27 (C-1), 133.62 and 133.80 (3 C; C-A4, C-B4, C-C4), 136.12 (C-4a), 146.05 (C-4a), 148.30 (C-3), 153.01 (C-9), 154.49 (C-7a), 159.80 (C-11), 165.38, 165.61 and 166.34 ppm (3 C; C=O); IR (ATR, neat): $\tilde{\nu}$ = 3062 (w), 2923 (w), 2853 (w), 1719 (m), 1601 (w), 1584 (w), 1554 (w), 1519 (w), 1492 (w), 1468 (w), 1451 (w), 1376 (w), 1314 (w), 1261 (s), 1176 (m), 1116 (m), 1092 (s), 1067 (s), 1024 (m), 1000 (m), 939 (m), 803 (w), 757 (w), 706 (s), 617 (m), 600 (m), 540 (m), 481 (m), 423 (m), 402 cm^{-1} (m); HR MS (ESI) for $C_{40}H_{31}N_4O_7^+$ $[M + H]^+$: calcd 679.2193, found 679.2183; for $C_{40}H_{30}N_4O_7Na^+$ $[M + Na]^+$: calcd 701.2012, found 701.2003.

11-(*N*,*N*-dimethylamino)-7-(2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranosyl)pyrimido[5',4':4,5]-pyrrolo[3,2-*f*]quinoline (**96e**)

A suspension of the crude nucleoside **94** (250.9 mg, 75%, 0.27 mmol) in 2-propanol (10.7 mL) was treated with dimethylamine (0.27 mL, 2.0 M in THF, 0.54 mmol) and stirred at 60 °C for 24 hours. Purification by HPFC (SiO_2; Hex/EtOAc, gradient 0 → 30% EtOAc) gave **96e** (126.4 mg, 64%) as a sunflower-yellow solid.

R_f = 0.40 (SiO_2; cHex/EtOAc 1:1); m.p. = 240 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.08 (s, 6 H; N*Me*$_2$), 4.78 (dd, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 4.1 Hz, 1 Hz; H-5'a), 4.83 (m, 1 Hz; H-4'), 4.96 (dd, $J_{5'b,5'a}$ = 12.0 Hz, $J_{5'b,4'}$ = 2.9 Hz, 1 Hz; H-5'b), 6.47 (t, $J_{3',2'}$ = $J_{3',4'}$ = 6.2 Hz, 1 Hz; H-3'), 6.67 (bdd, $J_{2',3'}$ = 6.5 Hz, $J_{2',1'}$ = 5.2 Hz, 1 Hz; H-2'), 7.01 (d, $J_{1',2'}$ = 5.2 Hz, 1 Hz; H-1'), 7.34 (m, 2 Hz; H-A3, H-A5), 7.38 (m, 2 H; H-B3, H-B5), 7.46 (m, 2 H; H-C3, H-C5), 7.52 (dd, $J_{2,1}$ = 8.5 Hz, $J_{2,3}$ = 4.2 Hz, 1 Hz; H-2), 7.52 (m, 1 H; H-A4), 7.56 (m, 1 H; H-B4), 7.60 (m, 1 H; H-C4), 7.92 (m, 2 H; H-A2, H-A6), 7.94 (d, $J_{5,6}$ = 9.2 Hz, 1 Hz; H-5), 8.00 (m, 2 H; H-B2, H-B6), 8.10 (d, $J_{6,5}$ = 9.2 Hz, 1 Hz; H-6), 8.12 (m, 2 H, H-C2, H-C6), 8.61 (s, 1 H; H-9), 8.90 (dd, $J_{7,6}$ = 4.2 Hz, $J_{7,5}$ = 1.6 Hz, 1 Hz; H-7), 9.12 ppm (bdd, $J_{1,2}$ = 8.6 Hz, $J_{1,3}$ = 1.5 Hz, H-1); ^{13}C{^{1}H} NMR (125.7 MHz, $CDCl_3$): δ = 41.76 and 41.86 (N*Me*$_2$), 63.82 (C-5'), 71.03 (C-3'), 73.13 (C-2'), 80.07 (C-4'), 86.66 (C-1'), 102.70 (C-11a), 114.70 (C-6), 115.15 (C-11b), 120.57 (C-2), 124.50 (C-11c), 128.60, 128.62 and 128.74 (6 C; C-A3, C-A5, C-B3, C-B5, C-C3, C-C5), 128.88 (C-A1), 129.04 (C-B1), 129.17 (C-5), 129.65 (C-C1), 129.94, 129.95 and 129.98 (6 C; C-A2, C-A6, C-B2, C-B6, C-C2, C-C6), 133.56 and 133.73 (3 C; C-A4, C-B4, C-C4), 134.03 (C-6a), 134.89 (C-1), 145.79 (C-4a), 148.16 (C-3), 152.58 (C-9), 155.96 (C-7a), 163.13 (C-11), 165.38 (C=O A), 165.59 (C=O B), 166.40 ppm (C=O C); IR (ATR, neat): $\tilde{\nu}$ = 2920 (w), 2852 (w), 1724 (m), 1561 (m), 1517 (w), 1450 (w), 1420 (m), 1372 (w), 1315 (w), 1261 (s), 1176 (m), 1091 (s), 1068 (s), 1024 (s), 950 (m), 879 (w), 854 (w), 798 (m), 708 (s), 686 (m), 650 (m), 617 (m), 555 (m), 451 (s), 408 cm^{-1} (m); HR MS (ESI) for $C_{41}H_{34}N_5O_7^+$ $[M + H]^+$: calcd 708.2458, found 708.2458; for $C_{41}H_{33}N_5O_7Na^+$ $[M + Na]^+$: calcd 730.2278, found 730.2278.

11-(Furan-2-yl)-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97a**)

A suspension of **96a** (94.6 mg, 0.13 mmol) in MeOH (6.5 mL) was treated with NaOMe (14 µL, 25 wt% in MeOH, 0.08 mmol) and stirred at 60 °C for 24 hours. Concentration *in vacuo*, followed by co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97a** (14.6 mg, 27%) as a brown film.

R_f = 0.44 (SiO_2; DCM/MeOH 9:1); $[\alpha]_D^{20}$ = –65.7 (c = 0.023 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.68–3.84 (m, 2 H; H-5'), 4.07 (q, $J_{4',3'}$ = $J_{4',5'}$ = 3.4 Hz, 1 H; H-4'), 4.31 (m, 1 H; H-3'), 4.86 (m, 1 H; H-2'), 5.19–5.43 (m, 3 H; OH-2', OH-3', OH-5'), 6.70 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 6.89 (dd, $J_{4,3}$ = 3.4 Hz, $J_{4,5}$ = 1.8 Hz, 1 H; H-4-furyl), 7.336 (bd, $J_{3,4}$ = 3.4 Hz, 1 H; H-3-furyl), 7.344 (bdd, $J_{2,1}$ = 8.6 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 7.50 (bdd, $J_{1,2}$ = 8.7 Hz, $J_{1,3}$ = 1.6 Hz, 1 H; H-1), 7.92 (dd, $J_{5,4}$ = 1.9 Hz, $J_{5,3}$ = 0.7 Hz, 1 H; H-5-furyl), 8.16 (d, $J_{5,6}$ = 9.3 Hz, 1 H; H-5), 8.61 (d, $J_{6,5}$ = 9.3 Hz, 1 H; H-6), 8.83 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.03 ppm (s, 1 H; H-9); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 61.57 (C-5'), 70.05 (C-3'), 71.24 (C-2'), 85.79 (C-4'), 87.09 (C-1'), 110.72 (C-11a), 112.98 (C-11b), 113.20 and 113.34 (2 C; C-3-furyl, C-4-furyl), 117.06 (C-6), 120.94 (C-2), 123.48 (C-11c), 130.69 (C-5), 133.05 (C-1), 136.68 (C-6a), 145.03 (C-4a), 145.82 (C-5-furyl), 148.06 (C-3), 148.95 (C-11), 151.65 (C-2-furyl), 152.34 (C-9), 155.43 ppm (C-7a); IR (ATR, neat): $\tilde{\nu}$ = 3500–2500 (br w), 1669 (w), 1592 (m), 1558 (m), 1518 (m), 1466 (m), 1426 (m), 1372 (w), 1324 (m), 1242 (m), 1160 (m), 1119 (m), 1090 (m), 1045 (s), 1022 (s), 999 (s), 929 (m), 884 (m), 822 (m), 799 (m), 761 (m), 626 (m), 593 (m), 547 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 255 (27500), 330 nm (5400 M^{-1}cm^{-1}); HR MS (ESI) for $C_{22}H_{19}N_4O_5^+$ $[M + H]^+$: calcd 419.1356, found 419.1353; for $C_{22}H_{18}N_4O_5Na^+$ $[M + Na]^+$: calcd 441.1175, found 441.1172.

11-(Benzofuran-2-yl)-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97c**)

A suspension of **96c** (180 mg, 0.23 mmol) in MeOH (12.8 mL) was treated with NaOMe (14 µL, 25 wt% in MeOH, 0.08 mmol) and stirred at 60 °C for 18 hours. Concentration *in vacuo*, followed by co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97c** (51.4 mg, 48%) as a bright yellow solid.

R_f = 0.46 (SiO_2; DCM/MeOH 9:1); m.p. = 124 °C; $[\alpha]_D^{20}$ = 46.4 (*c* = 0.115 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.76 (bdt, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,OH} = J_{5',4'}$ = 4.3 Hz, 1 H; H-5'a), 3.81 (ddd, $J_{5'b,5'a}$ = 12.0 Hz, $J_{5'b,OH}$ = 4.7 Hz, $J_{5'b,4'}$ = 3.4 Hz, 1 H; H-5'b), 4.09 (q, $J_{4',3'} = J_{4',5'}$ = 3.3 Hz, 1 H; H-4'), 4.33 (dt, $J_{3',2'}$ = 6.1 Hz, $J_{3',OH} = J_{3',4'}$ = 3.1 Hz, 1 H; H-3'), 4.88 (q, $J_{2',1'} = J_{2',OH} = J_{2',3'}$ = 6.0 Hz, 1 H; H-2'), 5.28–5.33 (m, 2 H; OH-3', OH-5'), 5.40 (d, $J_{OH,2'}$ = 6.1 Hz, 1 H; OH-2'), 6.74 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 6.96 (dd, $J_{6,5}$ = 8.5 Hz, $J_{6,7}$ = 4.2 Hz, 1 H; H-6), 7.34 (m, 1 H; H-7-benzofuryl), 7.39–7.44 (m, 2 H; H-5-benzofuryl, H-6-benzofuryl), 7.50 (bddd, $J_{1,2}$ = 8.6 Hz, $J_{1,3}$ = 1.7 Hz, $J_{1,5}$ = 0.7 Hz, 1 H; H-1), 7.82 (d, $J_{3,LR}$ = 0.9 Hz, 1 H; H-3-benzofuryl), 7.90 (m, 1 H; H-4-benzofuryl), 8.20 (dd, $J_{5,6}$ = 9.2 Hz, $J_{5,1}$ = 0.7 Hz, 1 H; H-5), 8.66 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.78 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.13 ppm (s, 1 H; H-9); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 61.59 (C-5'), 70.07 (C-3'), 71.30 (C-2'), 85.84 (C-4'), 87.13 (C-1'), 109.59 (C-3-benzofuryl), 111.20 (C-11a), 111.84 (C-7-benzofuryl), 112.85 (C-11b), 117.09 (C-6), 120.45 (C-2), 122.70 (C-4-benzofuryl), 123.39 (C-11c), 123.97 (C-5-benzofuryl), 126.42 (C-6-benzofuryl), 127.99 (C-3a-benzofuryl), 131.03 (C-5), 133.19 (C-1), 137.04 (C-6a), 144.99 (C-4a), 148.14 (C-3), 148.56 (C-11), 152.39 (C-9), 153.48 (C-2-benzofuryl), 154.66 (C-7a-benzofuryl), 155.57 ppm (C-7a); IR (ATR, neat): $\tilde{\nu}$ = 3278 (br w), 2922 (w), 1655 (w), 1590 (w), 1559 (m), 1536 (m), 1517 (s), 1467 (m), 1447 (m), 1427 (m), 1370 (w), 1346 (w), 1299 (m), 1256 (m), 1167 (m), 1115 (s), 1088 (s), 1045 (s), 1017 (m), 960 (m), 932 (m), 915 (m), 883 (m), 853 (m), 815 (s), 796 (s), 751 (s), 697 (m), 634 (m), 612 (s), 580 (s), 568 (m), 548 (s), 518 (s), 498 (m), 485 (m), 451 (m), 432 (s), 419 (m), 405 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 259 (38200), 355 (13600 M^{-1}cm^{-1}); HR MS (ESI) for $C_{26}H_{21}N_4O_5^+$ [M + H]$^+$: calcd 469.1512, found 469.1510; for $C_{26}H_{20}N_4O_5Na^+$ [M + Na]$^+$: calcd 491.1331, found 491.1330.

11-Methyl-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97d**)

A suspension of **96d** (60.0 mg, 0.08 mmol) in MeOH (4.4 mL) was treated with NaOMe (5 µL, 25 wt% in MeOH, 0.04 mmol) and stirred at 60 °C for 18 hours. Concentration *in vacuo*, followed by co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97d** (17.0 mg, 53%) as an off-white solid.

R_f = 0.34 (SiO_2; DCM/MeOH 9:1); m.p. = 256 °C; $[\alpha]_D^{20}$ = –47.9 (*c* = 0.073 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.30 (s, 3 H; C*Me*), 3.73 (bdd, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,OH}$ = 5.5 Hz, $J_{5'a,4'}$ = 3.8 Hz, 1 H; H-5'a), 3.77 (ddd, $J_{5'b,5'a}$ = 11.9 Hz, $J_{5'b,OH}$ = 5.1 Hz, $J_{5'b,4'}$ = 3.2 Hz, 1 H; H-5'b), 4.04 (q, $J_{4',3'} = J_{4',5'}$ = 3.4 Hz, 1 H; H-4'), 4.29 (td, $J_{3',2'} = J_{3',OH}$ = 5.3 Hz, $J_{3',4'}$ = 3.1 Hz, 1 H; H-3'), 4.83 (q, $J_{2',1'} = J_{2',OH} = J_{2',3'}$ = 6.5 Hz, 1 H; H-2'), 5.24 (d, $J_{OH,3'}$ = 4.8 Hz, 1 H; OH-3'), 5.27–5.31 (m, 2 H; OH-2', OH-5'), 6.70 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.74 (dd, $J_{2,1}$ = 8.6 Hz, $J_{2,3}$ = 4.1 Hz, 1 H; H-2), 8.18 (bd, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.61 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.91 (s, 1 H; H-9), 8.94 (dd, $J_{3,2}$ = 4.1 Hz, $J_{3,1}$ = 1.5 Hz, 1 H; H-3), 9.32 ppm (bd, $J_{1,2}$ = 8.6 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 28.28 (C*Me*), 61.57 (C-5'), 70.00 (C-3'), 71.12 (C-2'), 85.65 (C-4'), 86.98 (C-1'), 113.56 and 113.68 (2 C; C-11a, C-11b), 117.03 (C-6), 121.52 (C-2), 123.44 (C-11c), 130.10 (C-5), 133.29 (C-1), 135.70 (C-6a), 145.15 (C-4a), 148.03 (C-3), 152.48 (C-9), 154.08 (C-7a); 159.82 ppm (C-11); IR (ATR, neat): $\tilde{\nu}$ = 3481 (w), 3225m–2275 (bw), 1587 (w), 1559 (m), 1521 (s), 1470 (m), 1441 (m), 1407 (w), 1370 (m), 1321 (m), 1307 (w), 1270 (w), 1245 (m),

1174 (m), 1124 (m), 1110 (m), 1080 (m), 1058 (s), 1033 (s), 1024 (s), 966 (m), 946 (w), 922 (m), 882 (m),858 (w), 819 (s), 790 (m), 771 (w), 729 (w), 688 (w), 660 (m), 632 (w), 603 (m), 581 (w), 564 (w), 538 (m), 514 (m), 438 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 259 (50400), 319 (12900), 353 nm (4700 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{19}H_{19}N_4O_4^+$ $[M + H]^+$: calcd 367.1406, found 367.1403; for $C_{19}H_{18}N_4O_4Na^+$ $[M + Na]^+$: calcd 389.1226, found 389.1223.

11-(*N*,*N*-dimethylamino)-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97e**)

A suspension of **96e** (100 mg, 0.14 mmol) in MeOH (7.1 mL) was treated with NaOMe (16 µL, 25 wt% in MeOH, 0.08 mmol) and stirred at 60 °C for 24 hours. Concentration *in vacuo*, followed by co-evaporation with MeOH (3 × 10 mL) and purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97e** (35.5 mg, 64%) as a light-yellow solid.

R_f = 0.42 (SiO_2; DCM/MeOH 9:1); m.p. = 240 °C; $[\alpha]_D^{20}$ = –5.0 (c = 0.119 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.03 and 3.05 (2 × s, 2 × 3 H; NMe_2), 3.70 (ddd, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,OH}$ = 6.0 Hz, $J_{5'a,4'}$ = 3.8 Hz, 1 H; H-5'a), 3.76 (ddd, $J_{5'b,5'a}$ = 11.9 Hz, $J_{5'b,OH}$ = 4.8 Hz, $J_{5'b,4'}$ = 3.2 Hz, 1 H; H-5'b), 4.03 (q, $J_{4',3'} = J_{4',5'}$ = 3.3 Hz, 1 H; H-4'), 4.27 (dt, $J_{3',2'}$ = 6.3 Hz, $J_{3',4'} = J_{3',OH}$ = 3.3 Hz, 1 H; H-3'), 4.87 (q, $J_{2',1'} = J_{2',OH} = J_{2',3'}$ = 6.3 Hz, 1 H; H-2'), 5.25 (d, $J_{OH,3'}$ = 4.4 Hz, 1 H; OH-3'), 5.31 (d, $J_{OH,2'}$ = 6.1 Hz, 1 H; OH-2'), 5.38 (t, $J_{OH,5'}$ = 5.4 Hz, 1 H; OH-5'), 6.58 (d, $J_{1',2'}$ = 7.3 Hz, 1 H; H-1'), 7.68 (dd, $J_{6,5}$ = 8.6 Hz, $J_{6,7}$ = 4.2 Hz, 1 H; H-2), 8.07 (d, $J_{9,10}$ = 9.2 Hz, 1 H; H-5), 8.47 (d, $J_{10,9}$ = 9.2 Hz, 1 H; H-6), 8.55 (s, 1 H; H-9), 8.90 (dd, $J_{7,6}$ = 4.2 Hz, $J_{7,5}$ = 1.7 Hz, 1 H; H-3), 9.07 ppm (dm, $J_{5,6}$ = 8.6 Hz,1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 40.99 and 41.40 (2 C; NMe_2), 61.76 (C-5'), 70.20 (C-3'), 71.28 (C-2'), 85.67 (C-4'), 87.28 (C-1'), 101.06 (C-11a), 113.82 (C-11b), 116.54 (C-6), 120.80 (C-2), 123.50 (C-11c), 128.18 (C-5), 133.80 (C-6a), 134.15 (C-1), 144.99 (C-4a), 147.86 (C-3), 151.94 (C-9), 155.52 (C-7a), 162.41 ppm (C-11); IR (ATR, neat): $\tilde{\nu}$ = 3500–2000 (br w), 1561 (s), 1519 (s), 1462 (m), 1418 (m), 1396 (m), 1373 (m), 1352 (m), 1304 (m), 1284 (m), 1234 (m), 1182 (m), 1156 (m), 1121 (s), 1054 (s), 1031 (s), 993 (m), 958 (m), 919 (m), 901 (m), 877 (m), 858 (s), 817 (s), 797 (m), 736 (m), 714 (m), 694 (m), 661 (m), 634 (s), 599 (s), 584 (s), 553 (s), 540 (s), 522 (m), 484 (m), 444 (m), 431 (m), 415 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 256 (29800), 298 (7700), 339 (7900), 359 nm (7100 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{20}H_{22}N_5O_4^+$ $[M + H]^+$: calcd 396.1672, found 396.1669; for $C_{20}H_{21}N_5O_4Na^+$ $[M + Na]^+$: calcd 418.1491, found 418.1488.

11-Amino-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97f**)

In a screw-cap pressure glass tube, a solution of the crude nucleoside **94** (245 mg, 75%, 0.26 mmol) in 1,4-dioxane (0.9 mL) was treated with aq. ammonia (2.0 mL, 25 wt% in water, 13.14 mmol). After being stirred at 120 °C for 24 h, the solution was cooled and concentrated *in vacuo*. Purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97f** (50.0 mg, 52%) as a beige solid.

R_f = 0.31 (SiO_2; DCM/MeOH 9:1); m.p. = 210–235 °C (decomp.); $[\alpha]_D^{20}$ = 26.3 (c = 0.024 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.69 (ddd, $J_{5'a,5'b}$ = 11.9 Hz, $J_{5'a,OH}$ = 6.3 Hz, $J_{5'a,4'}$ = 3.6 Hz, 1 H; H-5'a), 3.76 (ddd, $J_{5'b,5'a}$ = 11.9 Hz, $J_{5'b,OH}$ = 4.6 Hz, $J_{5'b,4'}$ = 3.1 Hz, 1 H; H-5'b), 4.02 (q, $J_{4',3'} = J_{4',5'a} = J_{4',5'b}$ = 3.2 Hz, 1 H; H-4'), 4.25 (td, $J_{3',2'} = J_{3',OH}$ = 5.0 Hz, $J_{3',4'}$ = 2.8 Hz, 1 H; H-3'), 4.85 (q, $J_{2',1'} = J_{2',OH} = J_{2',3'}$ = 6.6 Hz, 1 H; H-2'), 5.20 (d, $J_{OH,3'}$ = 4.8 Hz, 1 H; OH-3'), 5.26 (d, $J_{OH,2'}$ = 6.7 Hz, 1 H; OH-2'), 5.47 (bt, $J_{OH,5'a} = J_{OH,5'b}$ = 5.5 Hz, 1 H; OH-5'), 6.54 (d, $J_{1',2'}$ = 7.4 Hz, 1 H; H-1'), 7.24 (bs, 2 H; NH_2), 7.66 (dd, $J_{2,1}$ = 8.5 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 8.03 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.37 (s, 1 H; H-9), 8.41 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.88 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.19 ppm (bd, $J_{1,2}$ = 8.5 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 61.82 (C-5'), 70.26 (C-3'), 71.23 (C-2'), 85.68 (C-4'), 87.21 (C-1'), 98.72 (C-11a), 114.42 (C-11b), 116.32 (C-6), 120.66

(C-2), 122.90 (C-11c), 127.61 (C-5), 133.75 (C-1), 133.76 (C-6a), 144.99 (C-4a), 147.60 (C-3), 153.38 (C-9), 154.87 (C-7a), 159.13 ppm (C-11); IR (ATR, neat): $\tilde{\nu}$ = 3600–22500 (br w), 1633 (m), 1589 (m), 1574 (m), 1558 (m), 1522 (m), 1465 (m), 1441 (m), 1368 (m), 1317 (m), 1288 (m), 1188 (m), 1117 (s), 1035 (s), 961 (m), 930 (m), 903 (m), 888 (m), 859 (w), 811 (m), 793 (s), 767 (m), 697 (m), 672 (s), 602 (s), 578 (s), 541 (s), 511 (s), 466 (s), 424 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 291 (13600), 330 nm (6800 $M^{-1}cm^{-1}$); UV/Vis (water): λ_{max} (ε) = 290 (11200), 333 nm (5700 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{18}H_{18}N_5O_4^+$ $[M + H]^+$: calcd 368.1359, found 368.1351; for $C_{18}H_{17}N_5O_4Na^+$ $[M + Na]^+$: calcd 390.1178, found 390.1171.

11-Methoxy-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97g**)

A suspension of the crude nucleoside **94** (208 mg, 75%, 0.22 mmol) in MeOH (17.8 mL) was treated with sodium methoxide (0.25 mL, 30 wt% in MeOH, 1.31 mmol) and stirred at 60 °C for 4 h. Purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97g** (24.7 mg, 22%) as a beige solid.

R_f = 0.58 (SiO_2; DCM/MeOH 9:1); m.p. = 190–205 °C (decomp.); $[\alpha]_D^{20}$ = –36.9 (c = 0.096 in DMSO); ^{1}H NMR (600 MHz, DMSO-d_6): δ = 3.72 (dd, 1 H, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 3.8 Hz; H-5'a), 3.97 (dd, 1 H, $J_{5'b,5'a}$ = 12.0 Hz, $J_{5'b,4'}$ = 3.3 Hz; H-5'b), 4.04 (q, 1 H, $J_{4',3'}$ = $J_{4',5'}$ = 3.4 Hz; H-4'), 4.29 (dd, 1 H, $J_{3',2'}$ = 5.8 Hz, $J_{3',4'}$ = 3.1 Hz; H-3'), 4.34 (s, 3 H; O*Me*), 4.84 (dd, 1 H, $J_{2',1'}$ = 7.3 Hz, $J_{2',3'}$ = 5.8 Hz; H-2'), 6.65 (d, 1 H, $J_{1',2'}$ = 7.3 Hz; H-1'), 7.71 (dd, 1 H, $J_{2,1}$ = 8.6 Hz, $J_{2,3}$ = 4.2 Hz; H-2), 8.11 (dd, 1 H, $J_{5,6}$ = 9.2 Hz, $J_{5,1}$ = 0.7 Hz; H-5), 8.55 (d, 1 H, $J_{6,5}$ = 9.2 Hz; H-6), 8.73 (s, 1 H; H-9), 8.92 (dd, 1 H, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.7 Hz; H-3), 9.77 ppm (ddd, 1 H, $J_{1,2}$ = 8.6 Hz, $J_{1,3}$ = 1.6 Hz, $J_{1,5}$ = 0.7 Hz; H-1); *OH-2', OH-3', OH-5' not detectable due to water content in the NMR sample*; ^{13}C{^{1}H} NMR (150.9 MHz, DMSO-d_6): δ = 54.59 (O*Me*), 61.62 (C-5'), 70.05 (C-3'), 71.32 (C-2'), 85.69 (C-4'), 87.35 (C-1'), 100.45 (C-11b), 113.40 (C-11a), 116.88 (C-6), 121.70 (C-2), 123.45 (C-11c), 128.91 (C-5), 133.95 (C-1), 134.35 (C-6a), 145.07 (C-4a), 148.24 (C-3), 153.18 (C-9), 155.62 (C-7a), 162.63 ppm (C-11); IR (ATR, neat): $\tilde{\nu}$ = 3203 (br m), 2929 (br m), 1673 (br w), 1592 (m), 1573 (m), 1556 (s), 1521 (s), 1468 (s), 1442 (m), 1429 (m), 1375 (m), 1311 (s), 1272 (m), 1207 (m), 1181 (m), 1115 (s), 1068 (s), 1039 (s), 984 (s), 960 (s), 918 (m), 887 (m), 857 (m), 812 (s), 793 (s), 715 (m), 685 (m), 636 (s), 586 (m), 542 (s), 506 (s), 466 (m), 435 (m), 410 cm^{-1} (m); UV/Vis (MeOH): λ_{max} (ε) = 255 (38000), 282 (16300), 315 nm (9900 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{19}H_{19}N_4O_5^+$ $[M + H]^+$: calcd 383.1356, found 383.1351; for $C_{19}H_{18}N_4O_5Na^+$ $[M + Na]^+$: calcd 405.1175, found 405.1171.

11-Methylthio-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline (**97h**)

A solution of the crude nucleoside **94** (200.3 mg, 75%, 0.22 mmol) in THF (8.6 mL) was treated with NaSMe (92.3 mg, 1.32 mmol) and stirred at 60 °C for 18 h. Purification by HPFC (C18; water/MeOH, gradient 0 → 100% MeOH) gave **97h** (24.5 mg, 29%) as a pale-yellow solid.

R_f = 0.50 (SiO_2; DCM/MeOH 9:1); m.p. = 228–242 °C (decomp.); $[\alpha]_D^{20}$ = –18.0 (*c* = 0.067 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.84 (s, 3 H, S*Me*), 3.73 (bdd, $J_{5'a,5'b}$ = 12.0 Hz, $J_{5'a,4'}$ = 3.7 Hz, 1 H; H-5'a), 3.79 (d, $J_{5'b,5'a}$ = 12.0 Hz, 1 H; H-5'b), 4.04 (q, $J_{4',3'}$ = $J_{4',5'}$ = 3.4 Hz, 1 H; H-4'), 4.29 (dd, $J_{3',2'}$ = 5.9 Hz, $J_{3',4'}$ = 3.1 Hz, 1 H; H-3'), 4.81 (dd, $J_{2',1'}$ = 7.3 Hz, $J_{2',3'}$ = 5.9 Hz, 1 H; H-2'), 5.23–5.47 (m, 3 H; OH-2', OH-3', OH-5'), 6.71 (d, $J_{1',2'}$ = 7.3 Hz, 1 H; H-1'), 7.74 (dd, $J_{2,1}$ = 8.6 Hz, $J_{2,3}$ = 4.2 Hz, 1 H; H-2), 8.17 (bd, $J_{5,6}$ = 9.3 Hz, 1 H; H-5), 8.62 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.89 (s, 1 H; H-9), 8.94 (dd, $J_{3,2}$ = 4.2 Hz, $J_{3,1}$ = 1.6 Hz, 1 H; H-3), 9.95 ppm (dm, $J_{1,2}$ = 8.7 Hz, 1 H; H-1); ^{13}C{^{1}H} NMR (125.7 MHz, DMSO-d_6): δ = 13.86 (S*Me*); 61.54 (C-5'), 69.96 (C-3'), 71.24 (C-2'), 85.73 (C-4'), 87.15 (C-1'), 111.69 (C-11a), 113.26 (C-11b), 117.10 (C-6), 120.83 (C-2), 122.88 (C-11c), 130.15 (C-5), 133.82 (C-1), 135.15 (C-6a), 145.24 (C-4a), 148.12 (C-3), 151.84 (C-9), 152.80 (C-6a),

161.70 ppm (C-11); IR (ATR, neat): $\tilde{\nu}$ = 3600–2000 (m), 1665 (w),1591 (w), 1547 (m), 1516 (s), 1465 (m), 1437 (m), 1413 (m), 1369 (m), 1337 (m), 1303 (m), 1281 (w), 1260 (s), 1214 (m), 1178 (m), 1160 (m), 1123 (s), 1080 (s), 1067 (s), 1001 (s), 958 (m), 932 (s), 913 (m), 886 (m), 852 (m), 838 (s), 812 (s), 785 (s), 725 (m), 687 (s), 672 (s), 650 (s), 627 (s), 579 (s), 542 (s), 517 (s), 459 (s), 434 (s), 419 cm^{-1} (s); UV/Vis (MeOH): λ_{max} (ε) = 252 (36600), 329 (10100), 359 nm (6600 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{19}H_{19}N_4O_4S^+$ $[M + H]^+$: calcd 399.1127, found 399.1125; for $C_{19}H_{18}N_4O_4SNa^+$ $[M + Na]^+$: calcd 421.0946, found 421.0945.

11-Amino-7-(*β*-D-ribofuranosyl)pyrimido[5',4':4,5]pyrrolo[3,2-*f*]quinoline 5'-*O*-Triphosphate Bistriethylammonium Salt (**98**, **A^{Q}TP**)

A solution of **97f** (25.1 mg, 0.07 mmol; dried under vacuum at 45 °C overnight) in $PO(OMe)_3$ (1.0 mL) was cooled to 0 °C and treated with $POCl_3$ (13 µL, 0.14 mmol). After 2 hours, the solution turned sunflower yellow and TLC showed the completion of the transformation towards the monophosphate (R_f = 0.00; SiO_2, DCM/MeOH 9:1). In a separate flask, a solution of bis(tributylammonium) pyrophosphate (187 mg, 0.34 mmol) in MeCN (1.0 mL) was treated with NBu_3 (0.08 mL, 0.34 mmol) and stirred for 5 min before being transferred to the monophosphate solution via syringe. The resulting pale yellow solution was stirred at 0 °C (water/ice) for 1 hour until TLC showed the completion of the reaction (R_f = 0.32; SiO_2, *i*PrOH/water/NH_4OH 11:2:7). The mixture was concentrated *in vacuo* at 38 °C, co-evaporated with water (2 × 10 mL) and dissolved in water (25 mL) followed by washing with $CHCl_3$ (3 × 25 mL) to remove any traces of $PO(OMe)_3$. Purification by prep. HPLC (Sepharose; 21.2 × 165 mm, flow rate = 12 mL min^{-1}, water / 800 mM TEAB, gradient 100:0 for 5 min, from 100:0 to 0:100 within 60 min) gave triphosphate **98** (32.4 mg, 59%) as a pale yellow solid.

prep. HPLC: t_R = 30 min (Sepharose, 21.2 × 165 mm, flow rate = 12 mL min^{-1}, water / 800 mM TEAB, gradient 100:0 for 5 min, from 100:0 to 0:100 within 60 min); ^{1}H NMR (500 MHz, D_2O): δ = 4.40 (m, 1 H; H-4'), 4.42–4.48 (m, 2 H; H-5'), 4.70 (dd, $J_{3',2'}$ = 6.4 Hz, $J_{3',4'}$ = 4.1 Hz, 1 H; H-3'), 4.88 (t, $J_{2',1'}$ = $J_{2',3'}$ = 6.8 Hz, 1 H; H-2'), 6.33 (d, $J_{1',2'}$ = 7.1 Hz, 1 H; H-1'), 7.36 (m, 1 H; H-2), 7.69 (d, $J_{5,6}$ = 9.2 Hz, 1 H; H-5), 8.02 (s, 1 H; H-11), 8.04 (d, $J_{6,5}$ = 9.2 Hz, 1 H; H-6), 8.23 (bd, $J_{1,2}$ = 7.9 Hz, 1 H; H-1), 8.55 ppm (m, 1 H; H-3); $^{13}C\{^1H\}$ NMR (125.7 MHz, D_2O): δ = 67.45 (d, $J_{C,P\alpha}$ = 5.6 Hz, 1 C; C-5'), 71.16 (C-3'), 73.20 (C-2'), 85.18 (d, $J_{C,P\alpha}$ = 9.1 Hz, 1 C; C-4'), 88.85 (C-1'), 99.91 (C-11a), 115.86 (C-11b), 119.14 (C-6), 123.02 (C-2), 123.79 (C-11c), 128.14 (C-5), 134.89 (C-6a), 135.87 (C-1), 144.58 (C-4a), 148.72 (C-3), 154.30 (C-9), 155.61 (C-7a), 158.71 ppm (C-11); ^{31}P NMR (202.4 MHz, D_2O): δ = –21.74 (t, $J_{\beta,\alpha}$ = $J_{\beta,\gamma}$ = 19.6 Hz, 1 P; P$_\beta$), –10.59 (d, $J_{\alpha,\beta}$ = 19.5 Hz, 1 P; P$_\alpha$), –7.56 ppm (d, $J_{\gamma,\beta}$ = 19.7 Hz, 1 P; P$_\gamma$); UV/Vis (water): λ_{max} (ε) = 258 (21000), 289 (12200), 333 nm (6600 $M^{-1}cm^{-1}$); HR MS (ESI) for $C_{18}H_{19}N_5O_{13}P_3^+$ $[M - H]^+$: calcd 606.01922, found 606.01923.

5.2.4. Biochemistry

Preparation of dsDNA template for transcription (**35DNA_A7**)

A solution of complementary single stranded DNA oligonucleotide (100 µM each) in water was heated up to 95 °C for 5 min in a thermal cycler, and then slowly cooled down to 25 °C. The resulting dsDNA (50 µM) was used as the template **35DNA_A7** for the transcription reaction.

Transcription experiment with T7 polymerase

Four *in vitro* transcription reactions were performed in parallel using the *HiScribe T7 High yield RNA synthesis Kit*: positive control, negative control, modification and negative control for spectroscopy.

Each reaction mixture (100 µL) contained Tris buffer (40 mM, pH 7.9), the three natural NTPs (7.5 mM each), the dsDNA template **35DNA_A7** (1 µg) and the T7 RNA polymerase (7.5 µL). Additionally, the positive control contained natural ATP (7.5 mM), the negative control contained water instead of ATP or **A^{Q}TP**, the modification contained **A^{Q}TP** (7.5 mM) and the negative control for spectroscopy contained the modified **A^{Q}TP** (7.5 mM) but no T7 polymerase. All 4 reaction mixtures were incubated at 37 °C for 16 hours. Then, the reactions were stopped by treatment with DNase I (0.1 U/µL) at 37 °C for 15 min followed by treatment with EDTA (0.05 M) at 70 °C for 10 min. Afterwards, the mixtures were purified by the *Monarch RNA Cleanup Kit* (50 µg) resulting in solutions of 40 µL each.

Aliquots of the first 3 reactions (50 ng) were separated by denaturating PAGE (20%) with urea at 23 mV for 1 h and visualized by fluorescence imaging.

Aliquots of the positive control (**35RNA_A7**) and the modified RNA (**35RNA_A^{Q}7**) were analyzed by UPLC-ESI-MS confirming the full transcription without any misincorporation (see **Figure 25** and **Figure 26** in the Appendix).

6. References

1 Meurer, F.; Do, H. T.; Sadowski, G.; Held, C. Standard Gibbs energy of metabolic reactions: II. Glucose-6-phosphatase reaction and ATP hydrolysis. *Biophys. Chem.* **2017**, *223*, 30–38. DOI: 10.1016/j.bpc.2017.02.005

2 (a) Serezani, C. H.; Ballinger, M. N.; Aronoff, D. M.; Peters-Golden, M. Cyclic AMP. *Am. J. Respir. Cell Mol. Biol.* **2008**, *39*, 127–132. DOI: 10.1165/rcmb.2008-0091tr

(b) Yarwood, S. J. Special Issue on "New Advances in Cyclic AMP Signalling" – An Editorial Overview. *Cells* **2020**, *9*, 2274. DOI: 10.3390/cells9102274

3 Pietrocola, F.; Galluzzi, L.; Bravo-San Pedro, J. M.; Madeo, F.; Kroemer, G. Acetyl Coenzyme A: A Central Metabolite and Second Messenger. *Cell Metab.* **2015**, *21*, 805–821. DOI: 10.1016/j.cmet.2015.05.014

4 (a) Pollak, N.; Dölle, C.; Ziegler, M. The power to reduce: pyridine nucleotides – small molecules with a multitude of functions. *Biochem J.* **2007**, *402*. 205–218. DOI: 10.1042/BJ20061638
(b) Ying, W. NAD+/NADH and NADP+/NADPH in Cellular Functions and Cell Death: Regulation and Biological Consequences. *Antioxid. Redox Signaling* **2008**, *10*, 179–206. DOI: 10.1089/ars.2007.1672
(c) Sellés Vidal, L.; Kelly, C. L.; Mordaka, P. M.; Heap, J. T. Review of NAD(P)H-dependent oxidoreductases: Properties, engineering and application. *Biochim. Biophys. Acta, Proteins Proteomics* **2018**, *1866*, 327–347. DOI: 10.1016/j.bbapap.2017.11.005

5 McCrown, P. J.; Ruszkowska, A.; Kunkler, C. N.; Breger, K.; Hulewicz, J. P.; Wang, M. C.; Springer, N. A.; Brown, J. A. Naturally occurring modified ribonucleosides *Wiley Interdiscip. Rev.: RNA* **2020**, *11*, e1595. DOI: 10.1002/wrna.1595

6 (a) De Coen, L. M.; Heugebaert, T. S. A.; García, D.; Stevens, C. V. Synthetic Entries to and Biological Activity of Pyrrolopyrimidines. *Chem. Rev.* **2016**, *116*, 80–139. DOI: 10.1021/acs.chemrev.5b00483
(b) Shelton, J.; Lu, X.; Hollenbaugh, J. A.; Cho, J. H.; Amblard, F.; Schinazi, R. F. Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev.* **2016**, *116*, 14379–14455. DOI: 10.1021/acs.chemrev.6b00209

7 (a) Périgaud, C.; Gosselin, G.; Imbach, J. L. Nucleoside Analogues as Chemotherapeutic Agents: A Review. *Nucleosides Nucleotides* **1992**, *11*, 903 – 945. DOI: 10.1080/07328319208021748
(b) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discovery* **2013**, *12*, 447–464. DOI: 10.1038/nrd4010
(c) Seley-Radtke, K. L.; Yates, M. K. The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold. *Antiviral Res.* **2018**, *154*, 66–86. DOI: 10.1016/j.antiviral.2018.04.004.
(d) Yates, M. K.; Seley-Radtke, K. L. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. *Antiviral Res.* **2019**, *162*, 5–21. DOI: 10.1016/j.antiviral.2018.11.016.

8 Segovia, M. C.; Chacra, W.; Gordon, S. C. Adefovir dipivoxil in chronic hepatitis B: history and current uses. *Expert Opin. Pharmacother.* **2012**, 13, 245–254. DOI: 10.1517/14656566.2012.649727

9 Cladribine. In *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]*; National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda; 2012-. https://www.ncbi.nlm.nih.gov/books/NBK548555/ (last reviewed October 2017)

10 Liliemark, J. The Clinical Pharmacokinetics of Cladribine. *Clin. Pharmacokinet.* **1997**, *32*, 120–131. DOI: 10.2165/00003088-199732020-00003

11 (a) Leist, T.; Weissert, R. Cladribine: Mode of Action and Implications for Treatment of Multiple Sclerosis. *Clin. Neuropharmacol.* **2011**, *34*, 28–35. DOI: 10.1097/WNF.0b013e318204cd90
(b) Otto, G. The story of cladribine reaches its climax. Nature Milestones, December 12, 2018. https://www.nature.com/articles/d42859-018-00029-1 (accessed 2024-03-30).

12 Bonate, P. L.; Arthaud, L.; Cantrell, W. R.; Stephenson, K.; Secrist, J. A.; Weitman, S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. *Nat. Rev. Drug Discovery* **2006**, *5*, 855–863. DOI: 10.1038/nrd2055

13 Hood, M. A.; Finley, R.S. Fludarabine: A Review. *DICP, Ann. Pharmacother.* **1991**, *25*, 518–524. DOI:10.1177/106002809102500512

14 World Health Organization. *World Health Organization model list of essential medicines: 21st list 2019*. World Health Organization, 2019. https://iris.who.int/handle/10665/325771 (accessed 2024-03-30).

15 Julander, J. G.; Demarest, J. F.; Taylor, R.; Gowen, B. B.; Walling, D. M.; Mathis, A.; Babu, Y. S. An update on the progress of galidesivir (BCX4430), a broad-spectrum antiviral. *Antiviral Res.* **2021**, *195*, 105180. DOI: 10.1016/j.antiviral.2021.105180

16 Blair, H. A. Remdesivir: A Review in COVID-19. *Drugs* **2023**, *83*, 1215–1237. DOI: 10.1007/s40265-023-01926-0

17 (a) Wassner, C.; Bradley, N.; Lee, Y. A Review and Clinical Understanding of Tenofovir: Tenofovir Disoproxil Fumarate versus Tenofovir Alafenamide. *J. Int. Assoc. Provid. AIDS Care* **2020**, *19*, 2325958220919231. DOI: 10.1177/2325958220919231
(b) Martin, P.; Lau, D. T.-Y.; Nguyen, M. H.; Janssen, H. L.A.; Dieterich, D. T.; Peters, M. G.; Jacobson, I. M. A Treatment Algorithm for the Management of Chronic Hepatitis B Virus Infection in the United States: 2015 Update. *Clin. Gastroenterol. Hepatol.* **2015**, *13*, 2071–2087. DOI: 10.1016/j.cgh.2015.07.007

18 De Coen, L. M.; Heugebaert, T. S. A.; García, D.; Stevens, C. V. Synthetic Entries to and Biological Activity of Pyrrolopyrimidines. *Chem. Rev.* **2016**, *116*, 80–139. DOI: 10.1021/acs.chemrev.5b00483

19 Perlíková, P.; Hocek, M. Pyrrolo[2,3-d]pyrimidine (7-deazapurine) as a privileged scaffold in design of antitumor and antiviral nucleosides. *Med. Res. Rev.* **2017**, *37*, 1429–1460. DOI: 10.1002/med.21465

20 (a) Replacing N1: Kimoto, M.; Moriyama, K.; Yokoyama, S.; Hirao, I. Cytostatic evaluations of nucleoside analogs related to unnatural base pairs for a genetic expansion system. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5582–5585. DOI: 10.1016/j.bmcl.2007.07.088
(b) Replacing N3: Nauš, P.; Kuchař, M.; Hocek, M. Cytostatic and Antiviral 6-Arylpurine Ribonucleosides IX. Synthesis and Evaluation of 6-Substituted 3-

Deazapurine Ribonucleosides. *Collect. Czech. Chem. Commun.* **2008**, *73*, 665–678. DOI: 10.1135/cccc20080665
(c) Substitution in C2: Hocek, M.; Holý, A.; Dvořáková, H. Cytostatic 6-Arylpurine Nucleosides IV. Synthesis of 2-Substituted 6-Phenylpurine Ribonucleosides. *Collect. Czech. Chem. Commun.* **2002**, *67*, 325–335. DOI: 10.1135/cccc20020325

21 Smith, C. G.; Lummis, W. L.; Grady, J. E. An Improved Tissue Culture Assay II. Cytotoxicity Studies with Antibiotics, Chemicals, and Solvents. *Cancer Res* **1959**, *19*, 847–852.

22 Acs, G.; Reich, E.; Mori, M. Biological and biochemical properties of the analogue antibiotic tubercidin. *Proc. Natl. Acad. Sci. USA*. **1964**, *52*, 493–501. DOI: 10.1073/pnas.52.2.493

23 Tolman, R. L.; Robins, R. K.; Townsend, L. B. Pyrrolo[2,3-*d*]pyrimidine Nucleoside Antibiotics. Total Synthesis and Structure of Toyocamycin, Unamycin B, Vengicide, Antibiotic E-212, and Sangivamycin (BA-90912). *J. Am. Chem. Soc.* **1968**, *90*, 524–526. DOI: 10.1021/ja01004a076

24 Greenhill, J. V. Pyrroles with Fused Six-membered Heterocyclic Rings: (ii) *b*- and *c*-Fused. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Vol. 4; Pergamon, 1984; pp. 497 – 529. DOI: 10.1016/B978-008096519-2.00059-X

25 Loomis, C. R.; Bell, R. M. Sangivamycin, a nucleoside analogue, is a potent inhibitor of protein kinase C. *J. Biol. Chem.* **1988**, *263*, 1682–1692. DOI: 10.1016/S0021-9258(19)77930-7

26 McCarty, R. M.; Bandarian, V. Deciphering Deazapurine Biosynthesis: Pathway for Pyrrolopyrimidine Nucleosides Toyocamycin and Sangivamycin. *Chem. Biol. (Oxford, UK)* **2008**, *15*, 790–798. DOI: 10.1016/j.chembiol.2008.07.012.

27 Wu, R.; Smidansky, E. D.; Oh, H. S.; Takhampunya, R.; Padmanabhan, R.;Cameron, C. E.; Peterson, B. R. Synthesis of a 6-Methyl-7-deaza Analogue of Adenosine That Potently Inhibits Replication of Polio and Dengue Viruses. *J. Med. Chem.* **2010**, *53*, 7958–7966. DOI: 10.1021/jm100593s

28 Drexler, J.; Groth, U. Trifluoromethylated Nucleosides: A Building Block Approach to Cytotoxic Adenosine Analogues. *Eur. J. Org. Chem.* **2014**, 6314–6320. DOI: 10.1002/ejoc.201402755

29 Nauš, P.; Caletková, O.; Konečný, P.; Džubák, P.; Bogdanová, K.; Kolář, M.; Vrbková, J.; Slavětínská, L.; Tloušťová, E.; Perlíková, P.; Hajdúch, M.; Hocek, M. Synthesis, Cytostatic, Antimicrobial, and Anti-HCV Activity of 6-Substituted 7-(Het)aryl-7-deazapurine Ribonucleosides. *J. Med. Chem.* **2014**, *57*, 1097–1110. DOI: 10.1021/jm4018948

30 Nauš, P.; Pohl, R.; Votruba, I.; Džubák, P.; Hajdúch, M.; Ameral, R.; Birkuš, G.; Wang, T.; Ray, A. S.; Mackman, R.; Cihlar, T.; Hocek, M. 6-(Het)aryl-7-Deazapurine Ribonucleosides as Novel Potent Cytostatic Agents. *J. Med. Chem.* **2010**, *53*, 460–470. DOI: 10.1021/jm901428k

31 Spáčilová, P.; Nauš, P.; Pohl, R.; Votruba, I.; Snášel, J.; Zábranská, H.; Pichová, I.; Ameral, R.; Birkuš, G.; Cihlář, T.; Hocek, M. *Cyclo*Sal-phosphate Pronucleotides of Cytostatic 6-(Het)aryl-7-deazapurine Ribonucleosides: Synthesis, Cytostatic Activity, and Inhibition of Adenosine Kinases. *ChemMedChem*, **2010**, *5*, 1386–1396. DOI: 10.1002/cmdc.201000192

32 Perlíková, P.; Krajczyk, A.; Doleželová, E.; Slapničková, M.; Milisavljevic, N.; Poštová Slavětínská, L.; Tloušťová, E.; Gurská, S.; Džubák, P.; Hajdúch, M.; Zíková, A.;

Hocek, M. Synthesis and Antitrypanosomal Activity of 6-Substituted 7-Methyl-7-deazapurine Nucleosides. *ACS Infect. Dis.* **2021**, *7*, 917–926. DOI: 10.1021/acsinfecdis.1c00062

33 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.; Birkuš, G.; Cihlar, T.; Hocek, M. Synthesis and Significant Cytostatic Activity of 7-Heteroaryl-7-deazaadenosines. *J. Med. Chem.* **2011**, *54*, 5498–5507. DOI: 10.1021/jm2005173

34 Perlíková, P.; Rylová, G.; Nauš, P.; Elbert, T.; Tloušťová, E.; Bourderioux, A.; Poštová Slavětínská, L.; Motyka, K.; Doležal, D.; Znojek, P.; Nová, A.; Harvanová, M.; Džubák, P.; Šiller, M.; Hlaváč, J.; Hajdúch, M.; Hocek, M. 7-(2-Thienyl)-7-Deazaadenosine (AB61), a New Potent Nucleoside Cytostatic with a Complex Mode of Action. *Mol. Cancer Ther.* **2016**, *15*, 922−937. DOI: 10.1158/1535-7163.MCT-14-0933

35 Snášel, J.; Nauš, P.; Dostál, J.; Hnízda, A.; Fanfrlík, J.; Brynda, J.; Bourderioux, A.; Dušek, M.; Dvořáková, H.; Stolaříková, J.; Zábranská, H.; Pohl, R.; Konečný, P.; Džubák, P.; Votruba, I.; Hajdúch, M.; Řezáčová, P.; Veverka, V.; Hocek, M.; Pichová, I. Structural Basis for Inhibition of Mycobacterial and Human Adenosine Kinase by 7-Substituted 7-(Het)aryl-7-deazaadenine Ribonucleosides. *J. Med. Chem.* **2014**, 57, 8268–8279. DOI: 10.1021/jm500497v

36 Dolloff, N. G.; Allen, J. E.; Dicker, D. T.; Aqui, N.; Vogl, D.; Malysz, J.;Talamo, G.; El-Deiry, W. S. Sangivamycin-like Molecule 6 Exhibits Potent Anti-Multiple Myeloma Activity through Inhibition of Cyclin-Dependent Kinase-9. *Mol. Cancer Ther.* **2012**, *11*, 2321–2330. DOI: 10.1158/1535-7163.MCT-12-0578

37 Stellrecht, C. M.; Chen, L. S. Transcription Inhibition as a Therapeutic Target for Cancer. *Cancers* **2011**, *3*, 2072–6694. DOI: 10.3390/cancers3044170

38 Cho, S.-J.; Lee, S.-S.; Kim, Y.-J.; Park, B.-D.; Choi, J.-S.; Liu, L. H.; Ham, Y.-M.; Moon Kim, B.; Lee, S.-K. Xylocydine, a novel Cdk inhibitor, is an effective inducer of apoptosis in hepatocellular carcinoma cells *in vitro* and *in vivo*. *Canc. Lett.* **2010**, *287*, 196–206. DOI: 10.1016/j.canlet.2009.06.011

39 Xiao, C.; Sun, C.; Han, W.; Pan, F.; Dan, Z.; Li, Y.; Song, Z.-G.; Jin, Y.-H. Synthesis of 6-(het) ary Xylocydine analogues and evaluating their inhibitory activities of CDK1 and CDK2 *in vitro*. *Bioorg. Med. Chem.* **2011**, *19*, 7100–7110. DOI: 10.1016/j.bmc.2011.10.003

40 Shen, W.; Kim, J.-S.; Hilfinger, J. Expedient Total Synthesis of Triciribine and Its Prodrugs. *Synth. Commun.* **2012**, *42*, 358–374. DOI: 10.1080/00397911.2010.524342

41 Leonard, N. J.; Sprecker, M. A.; Morrice, A. G. Defined dimensional changes in enzyme substrates and cofactors. Synthesis of lin-benzoadenosine and enzymic evaluation of derivatives of the benzopurines. *J. Am. Chem. Soc.* **1976**, *98*, 3987–3994. DOI: 10.1021/ja00429a040

42 Seley-Radtke, K. L.; Zhang, Z.; Wauchope, O. R.; Zimmermann, S. C.; Ivanov, A.; Korba, B. Hetero-expanded Purine Nucleosides. Design, Synthesis and Preliminary Biological Activity. *Nucleic Acids Symp. Ser.* **2008**, *52*, 635–636. DOI: 10.1093/nass/nrn321

43 Zhou, L.; Amblard, F.; Zhang, H.; McBrayer, T. R.; Detorio, M. A.; Whitaker, T.; Coats, S. J.; Schinazi, R. F. Synthesis and evaluation of Janus type nucleosides as potential HCV NS5B polymerase inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3385–3388. DOI: 10.1016/j.bmcl.2013.03.077

44 Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Yu-Jen Lee, Y.-J.; Hocek, M. Synthesis and biological activity of benzo-fused 7-deazaadenosine analogues. 5- and 6-substituted 4-amino- or 4-alkylpyrimido[4,5-*b*]indole ribonucleosides. *Bioorg. Med. Chem.* **2013**, *21*, 5362–5372. DOI: 10.1016/j.bmc.2013.06.011

45 Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y.-L.; Yokokawa, F.; Shi, P.-Y.; Hocek, M. Synthesis and antiviral activity of 4,6-disubstituted pyrimido[4,5-*b*]indole ribonucleosides. *Bioorg. Med. Chem.* **2012**, *20*, 6123–6133. DOI: 10.1016/j.bmc.2012.08.021

46 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. Synthesis and Cytotoxic and Antiviral Activity Profiling of All-Four Isomeric Series of Pyrido-Fused 7-Deazapurine Ribonucleosides. *Chem. – Eur. J.* **2020**, *26*, 13002–13015. DOI: 10.1002/chem.202001124

47 Tokarenko, A.; Lišková, B.; Smoleń, S.; Táborská, N.; Tichý, M.; Gurská, S.; Perlíková, P.; Frydrych, I.; Tloušťová, E.; Znojek, P.; Mertlíková-Kaiserová, H.; Poštová Slavětínská, L.; Pohl, R.; Klepetářová, B.;Khalid, N.-U.-A.; Wenren, Y.; R. Laposa, R. R.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Cytotoxic and Antiviral Profiling of Pyrrolo- and Furo-Fused 7‑Deazapurine Ribonucleosides. *J. Med. Chem.* **2018**, *61*, 9347−9359. DOI: 10.1021/acs.jmedchem.8b01258

48 Tichý, M.; Smoleń, S.; Tloušťová, E.; Pohl, R.; Oždian, T.; Hejtmánková, K.; Lišková, B.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Cytostatic and Antiviral Profiling of Thieno-Fused 7-Deazapurine Ribonucleosides. *J. Med. Chem.* **2017**, *60*, 2411–2424. DOI: 10.1021/acs.jmedchem.6b01766

49 Fleuti, M.; Bártová, K.; Poštová Slavětínská, L.; Tloušťová, E.; Tichý, M.; Gurská, S.; Pavliš, P.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Biological Profiling of Pyrazolo-Fused 7-Deazapurine Nucleosides. *J. Org. Chem.* **2020**, *85*, 10539−10551. DOI: 10.1021/acs.joc.0c00928

50 Ghosh, K.; Perlíková, P.; Havlíček, V.; Yang, C.; Pohl, R.; Tloušťová, E.; Hodek, J.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. Isomeric Naphtho-Fused 7-Deazapurine Nucleosides and Nucleotides: Synthesis, Biological Activity, Photophysical Properties and Enzymatic Incorporation to Nucleic Acids. *Eur. J. Org. Chem.* **2018**, *37*, 5092–5108. DOI: 10.1002/ejoc.201800165

51 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. DOI: 10.1021/acs.joc.0c00927

52 Yang, C.; Tichý, M.; Poštová Slavětínská, L.; Vaiedelich, E.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Biological Profiling of Benzofuro-Fused 7-Deazapurine Nucleosides. *Eur. J. Org. Chem.* **2023**, *26*, e202300723. DOI: 10.1002/ejoc.202300723

53 Yang, C.; Poštová Slavětínská, L.; 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. DOI: 10.1021/jacs.2c07517

54 Fleuti, M.; Sanchez-Quirante, T.; Poštová Slavětínská, L.; Tloušťová, E.; Tichý, M.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. Synthesis and Biological Profiling of Quinolino-Fused 7-Deazapurine Nucleosides. *ACS Omega*, **2024**, *manuscript under revision*.

55 (a) Showalter, H. D. H.; Bridges, A. J.; Zhou, H.; Sercel, A. D.; McMichael, A.; Fry, D. W. Tyrosine Kinase Inhibitors. 16. 6,5,6-Tricyclic Benzothieno[3,2-*d*]pyrimidines and Pyrimido[5,4-*b*]- and -[4,5-*b*]indoles as Potent Inhibitors of the Epidermal Growth Factor Receptor Tyrosine Kinase. *J. Med. Chem.* **1999**, *42*, 5464–5474. DOI: 10.1021/jm9903949
(b) Gangjee, A.; Zaware, N.; Raghavan, S.; Ihnat, M.; Shenoy, S.; Kisliuk, R. L. Single Agents with Designed Combination Chemotherapy Potential: Synthesis and Evaluation of Substituted Pyrimido[4,5-*b*]indoles as Receptor Tyrosine Kinase and Thymidylate Synthase Inhibitors and as Antitumor Agents. *J. Med. Chem.* **2010**, *53*, 1563–1578. DOI: 10.1021/jm9011142
(c) Reader, J. C.; Matthews, T. P.; Klair, S.; Cheung, K.-M. J.; Scanlon, J.; Proisy, N.; Addison, G.; Ellard, J.; Piton, N.; Taylor, S.; Cherry, M.; Fisher, M.; Boxall, K.; Burns, S.; Walton, M. I.; Westwood, I. M.; Hayes, A.; Eve, P.;Valenti, M.; de Haven Brandon, A.;Box, G.; van Montfort, R. L. M.; Williams, D. H.; Aherne, G. W.; Raynaud, F. I.; Eccles, S. A.; Garrett, M. D.; Collins, I. Structure-Guided Evolution of Potent and Selective CHK1 Inhibitors through Scaffold Morphing. *J. Med. Chem.* **2011**, *54*, 8328−8342. DOI: 10.1021/jm2007326

56 Grob, C. A.; Weissbach, O. Zur Herstellung von Oxindol-Derivaten aus o-Chlor-nitrobenzolen und Cyanessigsäure- bzw. Malonsäure-Estern. *Helv. Chim. Acta* **1961**, *44*, 1748–1753. DOI: 10.1002/hlca.19610440630

57 Wang, Z. Niementowski Reaction. In *Comprehensive Organic Name Reactions and Reagents*, 1st ed.; John Wiley & Sons Inc., 2010; pp 2054–2057. DOI: 10.1002/9780470638859.conrr462

58 Mechanism with Et_4NCl instead of BTEA-Cl: Robins, M. J.; Uznański, B. Nucleic Acid Related Compounds. 33. Conversions of adenosine and guanosine to 2,6-dichloro, 2-amino-6-chloro, and derived purine nucleosides. *Can. J. Chem.* **1981**, *59*, 2601–2607. DOI: 10.1139/v81-374

59 Smirnova, N. B.; Postovskii, I. Y.; Vereshchagina, N. N.; Lundina, I. B.; Mudretsova, I. I. New Cases of Tetrazole-Azide Tautomeric Transformations. *Chem. Heterocycl. Comp*. **1970**, *4*, 130–131. DOI: 10.1007/bf00478105

60 (a) Wentrup, C. Carbenes and Nitrenes in Heterocyclic Chemistry: Intramolecular Reactions. *Adv. Heterocycl. Chem.* **1981**, 28, 231–361. DOI: 10.1016/S0065-2725(08)60044-6
(b) Allen, L. A. T.; Natho, P. Trends in Carbazole Synthesis – An Update (2013–2023). *Org. Biomol. Chem.* **2023**, *21*, 8956–8974. DOI: 10.1039/d3ob01605f

61 (a) Sakamoto, T.; Kondo, Y.; Yamanaka, H. Studies on Pyrimidine Derivatives. 14. Synthesis of Pyrimidines fused with Five-membered Heterocycles by Cross-coupling of 5-Iodopyrimidines with Phenylacetylene and Styrene. *Chem. Pharm. Bull.* **1982**, 30, 2417–2420. DOI: 10.1248/cpb.30.2417
(b) Kondo, Y.; Watanabe, R.; Sakamoto, T.; Yamanaka, H. Condensed Heteroaromatic Ring-systems, 16. Synthesis of Pyrrolo-[2,3-*d*]pyrimidine Derivatives. *Chem. Pharm. Bull.* **1989**, *37*, 2933–2936. DOI: 10.1248/cpb.37.2933

62 Stokes, B. J.; Jovanović, B.; Dong, H.; Richert, K. J.; Riell, R. D.; Driver, T. G. Rh_2(II)-Catalyzed Synthesis of Carbazoles from Biaryl Azides. *J. Org. Chem.* **2009**, *74*, 3225–3228. DOI: 10.1021/jo9002536

63 Zhou, F.; Liu, S.; Santarsiero, B.; Wink, D. J.; Boudinet, D.; Facchetti, A.; Driver, T. Synthesis and Properties of New *N*-Heteroheptacenes for Solution-Based Organic Field Effect Transistors. *Chem. Eur. J.* **2017**, *23*, 12542–12549. DOI: 10.1002/chem.201701966

64 (a) Hyatt, H. A.; Swenton, J. S. A Facile Synthesis of 9*H*-Pyrimido [4,5-*b*]indole *via* Photolysis of 4-Azido-5-phenylpyrimidine. *J. Heterocycl. Chem.* **1972**, *9*, 409–410. DOI: 10.1002/jhet.5570090240
(b) Kondo, Y.; Watanabe, R.; Sakamoto, T.; Yamanaka, H. Condensed Heteroaromatic Ring-systems, 16. Synthesis of Pyrrolo-[2,3-*d*]pyrimidine Derivatives. *Chem. Pharm. Bull.* **1989**, *37*, 2933–2936. DOI: 10.1248/cpb.37.2933

65 Berger, F.; Plutschack, M. B.; Riegger, J.; Yu, W.; Speicher, S.; Ho, M.; Frank, N.; Ritter, T. Site-selective and versatile aromatic C-H functionalization by thianthrenation. *Nature* **2019**, *567*, 223–228. DOI: 10.1038/s41586-019-0982-0

66 (a) Engl, P. S.; Häring, A. P.; Berger, F.; Berger, G.; Pérez-Bitrián, A.; Ritter, T. C–N Cross-Couplings for Site-Selective Late-Stage Diversification via Aryl Sulfonium Salts. *J. Am. Chem. Soc.* **2019**, *141*, 13346–13351. DOI: 10.1021/jacs.9b07323
(b) Sang, R.; Korkis, S. E.; Su, W.; Ye, F.; Engl, P. S.; Berger, F.; Ritter, T. Site-Selective C−H Oxygenation via Aryl Sulfonium Salts. *Angew. Chem. Int. Ed.* **2019**, *58*, 1433–7851. DOI: 10.1002/anie.201908718
(c) Li, J.; Chen, J.: Sang, R.; Ham, W.-S.; Plutschack, M. B.; Berger, F.; Chabbra, S.; Schnegg, A.; Genicot, C.; Ritter, T. Photoredox catalysis with aryl sulfonium salts enables site-selective late-stage fluorination. *Nat. Chem.* **2020**, *12*, 56–62. DOI: 10.1038/s41557-019-0353-3
(d) Alvarez, E. M.; Karl, T.; Berger, F.; Torkowski, L.; Ritter, T. Late-Stage Heteroarylation of Hetero(aryl)sulfonium Salts Activated by α-Amino Alkyl Radicals. *Angew. Chem. Int. Ed.* **2021**, *60*, 13609–13613. DOI: 10.1002/anie.202103085
(e) Juliá, F.; Shao, Q.; Duan, M.; Plutschack, M. B.; Berger, F.; Mateos, J.; Lu, C.; Xue, X.-S.; Houk, K. N.; Ritter, T. High Site Selectivity in Electrophilic Aromatic Substitutions: Mechanism of C-H Thianthrenation. *J. Am. Chem. Soc.* **2021**, *143*, 16041–16054. DOI: 10.1021/jacs.1c06281
(f) Berger, F.; Ritter, T. Site-Selective Late-Stage C–H Functionalization via Thianthrenium Salts. *Synlett* **2022**, *33*, 339–345. DOI: 10.1055/s-0040-1706034
(g) Chen, X.-Y.; Wu, Y.; Wang, P. Recent Advances in Thianthrenation/ Phenoxathiination Enabled Site-Selective Functionalization of Arenes. *Synthesis* **2022**, *54*, 3928–3940. DOI: 10.1055/s-0041-1737493

67 (a) Kafuta, K.; Korzun, A.; Böhm, M.; Golz, C.; Alcarazo, M. Synthesis, Structure, and Reactivity of 5-(Aryl)dibenzothiophenium Triflates. *Angew. Chem. Int. Ed.* **2020**, *59*, 1950–1955. DOI: 10.1002/anie.201912383
(b) Xu, P.; Zhao, D.; Berger, F.; Hamad, A.; Rickmeier, J.; Petzold, R.; Kondratiuk, M.; Bohdan, K.; Ritter, T. Site-Selective Late-Stage Aromatic [^{18}F]Fluorination via Aryl Sulfonium Salts. *Angew. Chem. Int. Ed.* **2020**, *59*, 1956–1960. DOI: 10.1002/anie.201912567
(c) Berger, F.; Alvarez, E. M.; Frank, N.; Bohdan, K.; Kondratiuk, M.; Torkowski, L.; Engl, P. S.; Barletta, J.; Ritter, T. *Cine*-Substitutions at Five-Membered Hetarenes Enabled by Sulfonium Salts. *Org. Lett.* **2020**, *22*, 5671–5674. DOI: 10.1021/acs.orglett.0c02067

68 Kaspar, F.; Stone, M. R. L.; Neubauer, P.; Kurreck, A. Route efficiency assessment and review of the synthesis of *β*-nucleosides via *N*-glycosylation of nucleobases. *Green Chem.* **2021**, *23*, 37–50. DOI: 10.1039/d0gc02665d

69 Seela, F.; Peng, X. Progress in 7-Deazapurine – Pyrrolo[2,3-*d*]pyrimidine – Ribonucleoside Snythesis. *Curr. Top. Med. Chem.* **2006**, *6*, 867–892. DOI: 10.2174/156802606777303649

70 (a) Kim, Y. A.; Sharon, A.; Chu, C. K.; Rais, R. H.; Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. Structure-activity relationships of 7-deaza-6-benzylthioinosine analogues as ligands of Toxoplasma gondii adenosine kinase. *J. Med. Chem.* **2008**, *51*, 3934–3945. DOI: 10.1021/jm800201s
(b) Konč, J.; Tichý, M.; Pohl, R.; Hodek, J.; Džubák, P.; Hajdúch, M.; Hocek, M., Sugar modified pyrimido[4,5-*b*]indole nucleosides: synthesis and antiviral activity. *MedChemComm* **2017**, *8*, 1856–1862. DOI: 10.1039/C7MD00319F

71 (a) Downey, A. M.; Richter, C.; Pohl, R.; Mahrwald, R.; Hocek, M. Direct One-Pot Synthesis of Nucleosides from Unprotected or 5-*O*-Monoprotected d-Ribose. *Org. Lett.* **2015**, *17*, 4604–4607. DOI: 10.1021/acs.orglett.5b02332
(b) Downey, A. M.; Pohl, R.; Roithová, J.; Hocek, M. Synthesis of Nucleosides through Direct Glycosylation of Nucleobases with 5-*O*-Monoprotected or 5-Modified Ribose: Improved Protocol, Scope, and Mechanism. *Chem. – Eur. J.* **2017**, *23*, 3910–3917. DOI: 10.1002/chem.201604955

72 Vorbrüggen, H.; Lagoja, I. M.; Herdewijn, P. Synthesis of Ribonucleosides by Condensation Using Trimethylsilyl Triflate. *Curr. Prot. Nucleic Acid Chem.* **2006**, *27*, 1.13.1-1.13.16. DOI: 10.1002/0471142700.nc0113s27

73 Vorbrüggen, H. Some recent trends and progress in nucleoside synthesis. *Acta Biochim. Pol.* **1996**, *43*, 25–36. DOI: 10.18388/abp.1996_4514

74 (a) Step 1: Michel, B. Y.; Strazewski, P. Synthesis of (−)-neplanocin A with the highest overall yield via an efficient Mitsunobu coupling. *Tetrahedron* **2007**, *63*, 9836–9841. DOI: 10.1016/j.tet.2007.06.100
(b) Step 2: Kim, Y. A.; Sharon, A.; Chu, C. K.; Rais, R. H.; Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. Structure−Activity Relationships of 7-Deaza-6-benzylthioinosine Analogues as Ligands of *Toxoplasma gondii* Adenosine Kinase. *J. Med. Chem.* **2008**, *51*, 3934–3945. DOI: 10.1021/jm800201s

75 Kam, B. L., Oppenheimer, N. J. Selective tritylation: a general, one-step, method for synthesis of 5-*O*-trityl-d-pentofuranoses. *Carbohydr. Res.* **1979**, *69*, 308–310. DOI: 10.1016/S0008-6215(00)85783-3

76 Wang, Z. Zemplén Deacetylation. In *Comprehensive Organic Name Reactions and Reagents*, 1st ed.; John Wiley & Sons, 2010; pp 3123–3128. DOI: 10.1002/9780470638859.conrr691

77 (a) Burgess, K.; Cook, D. Syntheses of Nucleoside Triphosphates. *Chem. Rev.* **2000**, *100*, 2047–2060. DOI: 10.1021/cr990045m
(b) Hollenstein, M. Nucleoside Triphosphates — Building Blocks for the Modification of Nucleic Acids. *Molecules* **2012**, *17*, 13569–13591. DOI: 10.3390/molecules171113569

78 (a) Galmarini, C.; Popowycz, F.; Joseph, B. Cytotoxic Nucleoside Analogues: Different Strategies to Improve their Clinical Efficacy. *Curr. Med. Chem.* **2008**, *15*, 1072–1082. DOI: 10.2174/092986708784221449

(b) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat. Rev. Drug Discovery* **2013**, *12*, 447–464. DOI: 10.1038/nrd4010
(c) Shelton, J.; Lu, X.; Hollenbaugh, J. A.; Cho, J. H.; Amblard, F.; Schinazi, R. F. Metabolism, Biochemical Actions, and Chemical Synthesis of Anticancer Nucleosides, Nucleotides, and Base Analogs. *Chem. Rev.* **2016**, *116*, 14379–14455. DOI: 10.1021/acs.chemrev.6b00209
(d) Kataev, V. E.; Garifullin, B. F. Antiviral nucleoside analogs. *Chem. Heterocycl. Compd.* **2021**, *57*, 326–341. DOI: 10.1007/s10593-021-02912-8

79 Perlíková, P.; Rylová, G.; Nauš, P.; Elbert, T.; Tloušťová, E.; Bourderioux, A.; Poštová Slavětínská, L.; Motyka, K.; Doležal, D.; Znojek, P.; Nová, A.; Harvanová, M.; Džubák, P.; Šiller, M.; Hlaváč, J.; Hajdúch, M.; Hocek, M. 7-(2-Thienyl)-7-Deazaadenosine (AB61), a New Potent Nucleoside Cytostatic with a Complex Mode of Action. *Mol. Cancer Ther.* **2016**, *15*, 922−937. DOI: 10.1158/1535-7163.MCT-14-0933

80 Han, S. J.; Kim, H. T.; Joo J. M. Direct C−H Alkenylation of Functionalized Pyrazoles. *J. Org. Chem.* **2016**, *81*, 689−698. DOI: 10.1021/acs.joc.5b02398

81 Li, P.; Zheng, H.; Snyder, G.; Wennogle, L. P.; Hendrick, J. Organic Compounds. WO 2016/090380 A1, 2016-06-09.

82 (a) Goikhman, R.; Jacques, T. L.; Sames, D. C−H Bonds as Ubiquitous Functionality: A General Approach to Complex Arylated Pyrazoles via Sequential Regioselective C-Arylation and N-Alkylation Enabled by SEM-Group Transposition. *J. Am. Chem. Soc.* **2009**, *131*, 3042–3048. DOI: 10.1021/ja8096114
(b) Wong, S.-M.; Kwong, F.-Y. Chapter 3 – Nondirected C–H Bond Functionalizations of (Hetero)arenes. In: *Strategies for Palladium-Catalyzed Non-Directed and Directed C-H Bond Functionalization*, 1st ed.; Kapdi, A. R., Maiti, D., Eds.; Elsevier, 2017; pp. 80. DOI: 10.1016/B978-0-12-805254-9.00003-7

83 *Ethyl cyanoacetate, 98%*; CAS RN: 105-56-6; E18425; rev. 6.9; Sigma-Aldrich Chemie GmbH, Eschenstrasse 5, D-82024 Taufkirchen, Germany, 2023-10-27. https://www.sigmaaldrich.com/DE/en/sds/aldrich/e18425 (accessed 2024-03-02)

84 The ED-XRF analysis was performed by the *Analytical Laboratory* under S. Matějková at IOCB Prague.

85 Activation of zinc dust by stirring in diluted HCl (1 M in water), followed by washing with water, EtOH, Et_2O, and drying under reduced pressure for two days.
Smith, C. R. Activated Zinc Dust. *Synlett* **2009**, *9*, 1522–1523. DOI: 10.1055/s-0029-1217181

86 (a) Schneller, S. W.; Clough, F. W. Synthesis of Pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine Derivatives. *J. Heterocycl. Chem.* **1974**, 11, 975–977. DOI: 10.1002/jhet.5570110622
(b) Bridges, A. J.; Zhou, H. Synthesis of [1]Benzothieno[3,2-d]pyrimidines Substituted with Electron Donating Substituents on the Benzene Ring. *J. Heterocycl. Chem.* **1997**, 34, 1163–1172. DOI: 10.1002/jhet.5570340412
(c) Wang, S.; Zhao, Y.; Zhou, B.; Aguilar, A.; Liu, L.; Bai, L.; McEachern, D.; Dun, D.; Wen, B.; Luo, R.; Zhao, T.; Chinnaiyan, A.; Asangani, I. A.; Stuckey, J.; Meagher, J. L.; Ran, X. 9*H*-Pyrimido[4,5-*b*]indoles and Related Analogs as Bet Bromodomain Inhibitors. WO 2015/131005 A1, 2015-09-03.
(d) Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y.-L.; Yokokawa, F.; Shi, P.-Y.; Hocek, M. Synthesis and antiviral activity of 4,6-disubstituted pyrimido[4,5-*b*]indole

ribonucleosides. *Bioorg. Med. Chem.* **2012**, *20*, 6123–6133. DOI: 10.1016/j.bmc.2012.08.021
(e) Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Lee, Y.-J.; Hocek, M. Synthesis and biological activity of benzo-fused 7-deazaadenosine analogues. 5- and 6-substituted 4-amino- or 4-alkylpyrimido[4,5-*b*]indole ribonucleosides. *Bioorg. Med. Chem.* **2013**, *21*, 5362–5372. DOI: 10.1016/j.bmc.2013.06.011

87 (a) Lowinger, T.; Shimazaki, M.; Sato, H.; Tanaka, K.; Tsuno, N.; Marx, K.; Yamamoto, M.; Urbahns, K.; Ganter, F.; Okigami, H.; Nakashima, K.; Takshita, K.; Bacon, K.; Komura, H.; Yoshida, N. Pyrimido[4,5-*b*]indole derivatives. WO 2003/037898 A1, 2003-05-08.
(b) Sato, H.; Inoue, T.; Ly, T.-W.; Muramatsu, A.; Shimazaki, M.; Urbahns, K.; Ganter, F.; Okigami, H.; Bacon, K. B.; Komura, H.; Yoshida, N.; Tsuno, N. 4-Phenyl-pyrimido[4,5-b]indole Derivatives. WO 2004/058764 A1, 2014-07-15.
(c) Reader, J. C.; Matthews, T. P.; Klair, S.; Cheung, K.-M. J.; Scanlon, J.; Proisy, N.; Addison, G.; Ellard, J.; Piton, N.; Taylor, S.; Cherry, M.; Fisher, M.; Boxall, K.; Burns, S.; Walton, M. I.; Westwood, I. M.; Hayes, A.; Eve, P.; Valenti, M.; de Haven Brandon, A.; Box, G.; van Montfort, R. L. M.; Williams, D. H.; Aherne, G. W.; Raynaud, F. I.; Eccles, S. A.; Garrett, M. D.; Collins, I. Structure-Guided Evolution of Potent and Selective CHK1 Inhibitors through Scaffold Morphing. *J. Med. Chem.* **2011**, *54*, 8328–8342. DOI: 10.1021/jm2007326

88 Hill, P. J.; Abibi, A.; Albert, R.; Andrews, B.; Gagnon, M. M.; Gao, N.; Grebe, T.; Hajec, L. I.; Huang, J.; Livchak, S.; Lahiri, S. D.; McKinney, D. C.; Thresher, J.; Wang, H.; Olivier, N.; Buurman, E. T. Selective Inhibitors of Bacterial t-RNA-(N^1G37) Methyltransferase (TrmD) That Demonstrate Novel Ordering of the Lid Domain. *J. Med. Chem.* **2013**, *56*, 7278–7288. DOI: 10.1021/jm400718n

89 (a) Cai, X.; Qian, C.; Gould, S. Fused Bicyclic Pyrimidines as PTK Inhibitors containing a Zinc Binding Moiety. WO 2008/033745 A2, 2008-03-20.
(b) Kaspersen, S. J.; Sørum, C.; Willassen, V.; Fuglseth, E.; Kjøbli, E.; Bjørkøy, G.; Sundby, E.; Hoff, B. H. Synthesis and *in vitro* EGFR (ErbB1) tyrosine kinase inhibitory activity of 4-*N*-substituted 6-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-amines. *Eur. J. Med. Chem.* **2011**, *46*, 6002–6014. DOI: 10.1016/j.ejmech.2011.10.012

90 Gangjee, A.; Namjoshi, O. A.; Ihnat, M. A.; Buchanan, A. The contribution of a 2-amino group on receptor tyrosine kinase inhibition and antiangiogenic activity in 4-anilinosubstituted pyrrolo[2,3-*d*]pyrimidines. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3177–3181. DOI: 10.1016/j.bmcl.2010.03.064

91 (a) Anderson, R.; Hsiao, Y. Y. Isothiazoles. III. Synthesis of Isothiazolo[5,4-*d*]-pyrimidines. *J. Heterocycl. Chem.* **1975**, *12*, 883–887. DOI: 10.1002/jhet.5570120514
(b) Hubschwerlen, C.; Pflieger, P.; Specklin, J. L.; Gubernator, K.; Gmuender, H.; Angehrn, P.; Kompis, I. Pyrimido[1,6-*a*]benzimidazoles: a new class of DNA gyrase inhibitors. *J. Med. Chem.* **1992**, *35*, 1385–1392. DOI: 10.1021/jm00086a006

92 Nauš, P.; Caletková, O.; Konečný, P.; Džubák, P.; Bogdanová, K.; Kolář, M.; Vrbková, J.; Slavětínská, L.; Tloušťová, E.; Perlíková, P.; Hajdúch, M.; Hocek, M. Synthesis, Cytostatic, Antimicrobial, and Anti-HCV Activity of 6-Substituted 7-(Het)aryl-7-deazapurine Ribonucleosides. *J. Med. Chem.* **2014**, *57*, 1097–1110. DOI: 10.1021/jm4018948

93 Mosrin, M.; Knochel, P. Regio- and Chemoselective Metalation of Chloropyrimidine Derivatives with TMPMgCl·LiCl and $TMP_2Zn{\cdot}2MgCl_2{\cdot}2LiCl$. *Chem. – Eur. J.* **2009**, *15*, 1468–1477. DOI: 10.1002/chem.200801831

94 Treitler, D. S.; Leung, S. How Dangerous Is Too Dangerous? A Perspective on Azide Chemistry. *J. Org. Chem.* **2022**, *87*, 11293–11295. DOI: 10.1021/acs.joc.2c01402

95 (a) Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. Organic Azides: An Exploding Diversity of a Unique Class of Compounds. *Angew. Chem. Int. Ed.* **2005**, *44*, 5188 – 5240. DOI: 10.1002/anie.200400657
(b) *Azides – Research Interest of the Bräse group at the Karlsruhe Institute of Technology*. https://www.ioc.kit.edu/braese/english/1165.php (accessed 2024-03-30)
(c) *Information of Azide Compounds – Department of Environmental Health & Safety at the Stanford University*. https://ehs.stanford.edu/reference/information-azide-compounds (accessed 2024-03-30)

96 Ahlburg, N. L.; Doppleb, O.; Hillrichs, K.; Namyslo, J. C.; Hübner, E. G.; Schmidt, A. Deprotonation of 4-ethynylpyrazolium salts. *Heterocycles* **2008**, *96*, 1203–1215. DOI: 10.3987/com-18-13916

97 Ku, T.; Lopresti, N.; Shirley, M.; Mori, M.; Marchant, J.; Heng, X.; Botta, M.; Summers, M. F.; Seley-Radtke, K. L. Synthesis of distal and proximal fleximer base analogues and evaluation in the nucleocapsid protein of HIV-1. *Bioorg. Med. Chem.* **2019**, *27*, 2883 – 2892. DOI: 10.1016/j.bmc.2019.05.019

98 Meijer, E. W.; Nijhuis, S.; van Vroonhoven, F. C. B. M. Poly-1,2-azepines by the photopolymerization of phenyl azides. Precursors for conducting polymer films. *J. Am. Chem. Soc.* **1988**, *110*, 7209–7210. DOI: 10.1021/ja00229a043

99 Kaspar, F.; Stone, M. R. L.; Neubauer, P.; Kurreck, A. Route efficiency assessment and review of the synthesis of *β*-nucleosides via *N*-glycosylation of nucleobases. *Green Chem.* **2021**, *23*, 37–50. DOI: 10.1039/d0gc02665d

100 Konč, J.; Tichý, M.; Pohl, R.; Hodek, J.; Džubák, P-; Hajdúch, M.; Hocek, M. Sugar modified pyrimido[4,5-*b*]indole nucleosides: synthesis and antiviral activity. *Med. Chem. Commun.* **2017**, *8*, 1856 – 1862. DOI: 10.1039/c7md00319f

101 This reaction was performed in cooperation with Michal Tichý. Neither him nor me were able to reproduce these results in several attempts.

102 Salomon, C. J.; Danelon, G. O.; Mascaretti, O. A. A Practical Method for the Disposal of Organotin Residues from Reaction Mixtures. *J. Org. Chem.* **2000**, *65*, 9220–9222. DOI: 10.1021/jo000525+

103 Leibner, J. E.; Jacobus, J. Facile Product Isolation from Organostannane Reductions of Organic Halides. *J. Org. Chem.* **1979**, *44*, 449–450. DOI: 10.1021/jo01317a032

104 These reactions were performed by Chao Yang.

105 Tichý, M. Synthesis of novel types of annulated deazapurine nucleosides with potential biological activity. Ph.D. Dissertation, Charles University Prague, 2015; pp 78. https://dspace.cuni.cz/handle/20.500.11956/64609 (accessed 2024-03-30)

106 These reactions were performed in cooperation with Dr. Jiří Rybáček from the research group under Dr. I. Starý at IOCB Prague using their flow reactor set-up.

107 Nejedlý, J.; Šámal, M.; Rybáček, J.; Tobrmanová, M.; Szydlo, F.; Coudret, C.; Neumeier, M.; Vacek, J.; Vacek Chocholoušová, J.; Buděšínský, M.; Šaman, D.; Bednárová, L.; Sieger, L.; Stará, I. G.; Starý, I. Synthesis of Long Oxahelicenes by Polycyclization in a Flow Reactor. *Angew. Chem. Int. Ed.* **2017**, *56*, 5839–5843. DOI: 10.1002/anie.201700341

108 Strieth-Kalthoff, F.; James, M. J.; Teders, M.; Pitzera, L.; Glorius, F. Energy transfer catalysis mediated by visible light: principles, applications, directions. *Chem. Soc. Rev.* **2018**, *47*, 7190–7202. DOI: 10.1039/c8cs00054a

109 Frackowiak, D. The Jablonski diagram. *J. Photochem. Photobiol., B* **1988**, *2*, 399–401. DOI: 10.1016/1011-1344(88)85060-7

110 Baris, N.; Dračínský, M.; Tarábek, J.; Filgas, J.; Slavíček, P.; Ludvíková, L.; Boháčová, S.; Slanina, T.; Klepetářová, B.; Beier, P. Photocatalytic Generation of Trifluoromethyl Nitrene for Alkene Aziridination. *Angew. Chem. Int. Ed.* **2024**, 63, e202315162. DOI: 10.1002/anie.202315162

111 (a) Reiser, A.; L. J. Leyshon, L. J. Spin state of photogenerated phenylnitrene. *J. Am. Chem. Soc.* **1971**, *93*, 4051–4052. DOI: 10.1021/ja00745a042
(b) Lindley, J. M.; McRobbie, I. M.; Meth-Cohn, O.; Suschitzky, H. Competitive Cyclisations of Singlet and Triplet Nitrenes. Part 5. Mechanism of Cyclisation of 2-Nitrenobiphenyls and Related Systems. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2194–2204. DOI: 10.1039/P19770002194

112 Choo, H.; Min, S.-J.; Kim, Y. K.; Ryu, H.; Seo, H.; Kim, J.; Jang, J.; Lim. S.; Kim, Y.; Hwang, Y. J.; Cho, Y. S. Quinoline Derivatives for Inhibiting Histone Methyltransferases and Use Thereof. US 2017/0354650 A1, 2017-12-14.

113 Gamage, S. A.; Spicer, J. A.; Rewcastle, G. W.; Milton, J.; Sohal, S.; Dangerfield, W.; Mistry, P.; Vicker, N.; Charlton, P. A.; Denny, W. A. Structure−Activity Relationships for Pyrido-, Imidazo-, Pyrazolo-, Pyrazino-, and Pyrrolophenazinecarboxamides as Topoisomerase-Targeted Anticancer Agents. *J. Med. Chem.* **2002**, *45*, 740–743. DOI: 10.1021/jm010330+

114 Shou, W. G.; Li, J.; Guo, T.; Lin, Z.; Jia, G. Ruthenium-Catalyzed Intramolecular Amination Reactions of Aryl- and Vinylazides. *Organometallics* **2009**, *28*, 6847–6854. DOI: 10.1021/om900275j

115 (a) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. Design of Base-discriminating Fluorescent Nucleoside and Its Application to T/C SNP Typing. *J. Am. Chem. Soc.* **2003**, *125*, 9296−9297. DOI: 10.1021/ja035408l
(b) Okamoto, A.; Tanaka, K.; Fukuta, T.; Saito, I. Cytosine Detection by a Fluorescein-Labeled Probe Containing Base-Discriminating Fluorescent Nucleobase. *ChemBioChem.* **2004**, *5*, 958−963. DOI: 10.1002/cbic.200400010
(c) Bosáková, A.; Perlíková, P.; Tichý, M.; Pohl, R.; Hocek, M. 6-Aryl-4-amino-pyrimido[4,5-*b*]indole 2'-deoxyribonucleoside triphosphates (benzo-fused 7-deaza-dATP analogues): Synthesis, fluorescent properties, enzymatic incorporation into DNA and DNA-protein binding study. *Bioorg. Med. Chem.* **2016**, *24*, 4528–4535. DOI: 10.1016/j.bmc.2016.07.054

116 Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U., Relative and absolute determination of fluorescence quantum yields of transparent samples. *Nat. Protoc.* **2013,** *8*, 1535–1550.

117 Eaton, D. F. Reference materials for fluorescence measurement. *Pure Appl. Chem.* **1988**, *60*, 1107–1114. DOI: 10.1351/pac198860071107

118 Nosková, V.; Džubák, P.; Kuzmina, G.; Ludkova, A.; Stehlik, D.; Trojanec, R.; Janostakova, A.; Korinkova, G.; Mihal, V.; Hajduch, M. *In vitro* chemoresistance profile and expression/function of MDR associated proteins in resistant cell lines derived from CCRF-CEM, K562, A549 and MDA MB 231 parental cells. *Neoplasma* **2002**, *49*, 418−425.

119 Aslantürk, Ö. S. *In Vitro* Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. In *Genotoxicity – A Predictable Risk to our Actual World*; Larramendy, M.; Soloneski, S., Eds.; IntechOpen: London, 2018, pp 1–17.

120 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* [Online]; Sittampalan, G. S.; Grossman, A.; Brimacombe, K., et al., Eds.; Eli Lilly & Company and the National Center for Advancing Translational Sciences: Bethesda (MD), 2019; Chapter 3.2, pp 289–313. https://www.ncbi.nlm.nih.gov/books/NBK144065/ (accessed 2024-03-30).

121 Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd M. R. Evaluation of a Soluble Tetrazolium/Formazan Assay for Cell Growth and Drug Sensitivity in Culture Using Human and Other Tumor Cell Lines. *Cancer Res.* **1988**, *48*, 4827–4833.

122 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. Preclinical Characterization of the Novel Hepatitis C virus NS3 Protease Inhibitor GS-9451. *Antimicrob. Agents Chemother.* **2014**, *58*, 647–653.

123 (a) Burgess, K.; Cook, D. Syntheses of Nucleoside Triphosphates. *Chem. Rev.* **2000**, *100*, 2047–2060. DOI: 10.1021/cr990045m; (b) Hollenstein, M. Nucleoside Triphosphates — Building Blocks for the Modification of Nucleic Acids. *Molecules* **2012**, *17*, 13569–13591. DOI: 10.3390/molecules171113569

124 This experimental work was performed by Tania Sánchez Quirante at IOCB Prague.

125 Milisavljevič, N.; Perlíková, P.; Pohl, R.; Hocek, M. Enzymatic Synthesis of Base-Modified RNA by T7 RNA Polymerase. A Systematic Study and Comparison of 5-Substituted Pyrimidine and 7-Substituted 7-Deazapurine Nucleoside Triphosphates as Substrates. *Org. Biomol. Chem.* **2018**, *16*, 5800–5807. Doi: 10.1039/C8OB01498A

126 "Simple UV detector for liquid chromatography", produced by the *Development Center* at IOCB Prague (https://developmentcenter.group.uochb.cz/en/products, accessed 2024-03-30)

127 Ravi, P.; Gore, G. M.; Sikder, A. K.; Tewari, S. P. Silica–Sulfuric Acid Catalyzed Nitrodeiodination of Iodopyrazoles. *Synth. Commun.* **2012**, *42*, 3463–3471. DOI: 10.1080/00397911.2011.584261

128 Ferguson, I. J.; Schofield, K.; Barnett, J. W.; Grimmett, M. R. Studies in azole chemistry. Part 2. Nitration of 1,4,5-trimethylimidazole 3-oxide and 1-methylpyrazole 2-oxide, and some reactions of the products. *J. Chem. Soc., Perkin Trans. 1* **1977**, 672−675. DOI: 10.1039/p19770000672

129 Kozma, I. Z.; Krok, P.; Riedle, E. Direct measurement of group-velocity mismatch and derivation of the refractive index dispersion for a variety of solvents in the ultraviolet. *J. Opt. Soc. Am. B* **2005**, *22*, 1479–1485. DOI: 10.1364/AO.12.000555
Values retrieved from: Polyanskiy, M. RefractiveIndex.INFO – refractive index database. https://refractiveindex.info/?shelf=organic&book=methanol&page=Kozma (accessed 2024-03-30)

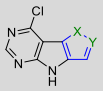
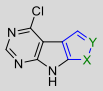
130 Hale, G. M.; Querry, M. R. Optical constants of water in the 200 nm to 200 μm wavelength region. *Appl. Opt.* **1973**, *12*, 555–563. DOI: 10.1364/JOSAB.22.001479
Values retrieved from: Polyanskiy, M. RefractiveIndex.INFO – refractive index database. https://refractiveindex.info/?shelf=3d&book=liquids&page=water (accessed 2024-03-30).

7. Appendix

7.1. Accessibility of 5-membered Heterocycles through their iodo-substituted Derivatives

In chapter 3.1.2., I have discussed the use of iodo-substituted heterocycles as a coupling partner for the zincated 4,6-dichloropyrimidine during the Negishi cross-coupling reaction. Unfortunately, not all the iodo derivatives are commercially available (**Table A1**) or accessible in one or two steps with nearly quantitative yield. Hence, using aryl sulfonium salts potential alternatives to the iodo derivative in the Negishi cross-coupling reaction was explored in **Chapter 3.2**.

Table A1. Commercial availability of 5-membered heterocycles with iodo-substitution: The price of the largest available package size was taken into account and recalculated for 1 g. All prices are given in Euro (1 EUR ≈ 25.30 CZK) and the corresponding supplier is given in brackets.

 Iodo derivative for:		
	4.12 EUR/g (BLDpharm) *CAS 34091-51-5*	0.48 EUR/g (BLDpharm) *CAS 39806-90-1*
	not available in Europe[a] *CAS 1934714-58-5*	23.57 EUR/g (Fluorochem) *CAS 847490-69-1*
	226.90 EUR/g (Enamine) *CAS 49602-30-4*	215.00 EUR/g (Enamine) *CAS 49602-28-0*
	35.20 EUR/g (BLDpharm) *CAS 71759-88-1*	5.98 EUR/g (BLDpharm) *CAS 71759-87-0*
	not available in Europe[a] *CAS 2174001-43-3*	not available in Europe[a] *CAS 122474-18-0*
	423.60 EUR/g (Enamine) *CAS 108306-61-2*	639 EUR/g (Enamine) *CAS 108306-60-1*

Last Update: March 2024. [a] not available from Apollo Scientific, BLDpharm, Enamine, Fluorochem, Sigma-Aldrich (Merck), TCI Europe or Thermofisher (Fisher Scientific).

7.2. HPLC Purity of the final Ribonucleosides

7.2.1. Pyrazolo-fused 7-Deazapurine Ribonucleosides

The purity of final nucleosides **61a-h** was confirmed by analytical HPLC on a *Waters 717 Autosampler* system with a *Waters 2996 Photodiode Array Detector* using a *Gemini 5 µm C_{18} 110 Å* column (250 × 4.60 mm) from *Phenomenex*. Samples were dissolved in DMSO (10 µL injection volume). Two methods were used (flow 1 mL/min):

A) water/MeOH gradient from 95:5 to 50:50 within 10 min, from 50:50 to 0:100 within 10 min, 0:100 for 10 min;
B) water/MeCN gradient from 95:5 to 50:50 within 10 min, from 50:50 to 0:100 within 10 min, 0:100 for 10 min.

The chromatogram was analyzed at 254 nm. After automatic integration, peaks with lower than 0.10% area were dismissed as noise.

Table A2. HPLC purity of the final nucleosides **61a-h**.

	Method A (water → MeOH)		Method B (water → MeCN)	
Compound	Retention time t_R [min]	Purity [%]	Retention time t_R [min]	Purity [%]
61a	29.86	99.38	24.78	98.77
61b	28.53	98.87	24.24	99.14
61c	33.39	95.79	27.42	96.73
61d	26.42	99.63	22.75	99.60
61e	27.95	94.69	23.65	95.10
61f	25.15	94.99	7.79	96.30
61g	28.80	98.87	23.89	98.89
61h	30.53	96.33	24.91	95.42

7.2.2. Quinolino-fused 7-Deazapurine Ribonucleosides

The purity of final nucleosides **97a-h** was confirmed by analytical UPLC on an *Agilent 1260 Infinity II LC* system with a *Agilent 1260 Photodiode Array Detector* using a *KinetexEVO C18 100 Å* column (2.1 × 150 mm) from *Phenomenex*. Samples were dissolved in DMSO (1 µL injection volume). Two methods were used (flow 0.2 mL/min):

A) water (0.1% formic acid)/MeOH gradient 100:0 for 0.5 min, from 100:0 to 0:100 within 7 min, 0:100 for 2 min, from 0:100 to 100:0 within 0.1 min, 100:0 for 4 min

B) water (0.1% formic acid)/MeCN gradient 100:0 for 0.5 min, from 100:0 to 0:100 within 7 min, 0:100 for 2 min, from 0:100 to 100:0 within 0.1 min, 100:0 for 4 min

Table A3. HPLC purity of final nucleosides **97a-h**.

	Method A (water → MeOH)		Method B (water → MeCN)	
Compound	Retention time t_R [min]	Purity [%]	Retention time t_R [min]	Purity [%]
97a	8.30	95.14	6.47	95.42
97c	9.59	95.45	7.38	96.10
97d	7.19	99.26	5.87	98.70
97e	7.58	96.24	6.16	97.48
97f	6.43	96.47	5.58	95.15
97g	7.87	99.22	7.87	99.24
97h	8.96	95.13	7.57	96.79

7.3. UV/Vis and Fluorescence Spectra

7.3.1. Heteroaryl-fused 7-deazapurine ribonucleosides

UV/Vis spectra of the final nucleosides were measured in methanol (HPLC grade) on a *Varian Cary 100 Bio* UV-Visible spectrophotometer in the range 250–800 nm using transparent 1.5 mL quartz cuvettes. The absorption coefficient ε was calculated using the Lambert-Beer equation $A = c \cdot l \cdot \varepsilon$, where A is the absorbance of the sample, c the exact concentration of the sample, l the length of the cuvette (1 cm). The samples were measured at three different concentrations and its average absorption coefficient was calculated.

Fluorescence spectra were recorded on a *Fluoromax 4* spectrofluorimeter from *HORIBA Scientific*. The sample concentration was adjusted to have a UV absorbance of 0.05–0.10. The excitation was performed at the absorption maximum with the highest wavelength λ_{abs} with the slit set at 2 nm. The emission spectra were recorded from $\lambda_{abs} + 20\ nm$ to $2 \times \lambda_{abs} - 20\ nm$ with a 2 nm slit opening. For each nucleoside **25a–h** and **58a–h**, the sample for measuring optical rotation in DMSO was used as a stock solution. Then, three samples with the same dilution in MeOH were prepared and measured.

For nucleosides with an absorption maximum above 300 nm, their fluorescence quantum yield Φ_f was determined with quinine sulfate as a standard (equation S1),[116]

$$\Phi_{f,x} = \Phi_{f,QS}\left[\frac{F_x\left(1-10^{-A_x}\right)}{F_{QS}\left(1-10^{-A_{QS}}\right)}\right]\frac{\eta_x^2}{\eta_{QS}^2} \qquad \text{(Eq. S1)}$$

where $\Phi_{f,x}$ is the quantum yield of the sample x, $\Phi_{f,QS}$ is the quantum yield of the standard quinine sulfate in 0.1 M H_2SO_4 (Lit.[117]: 0.546), F_x and F_{QS} are the integrals of the emission spectra between $\lambda_{abs} + 20\ nm$ to $2 \times \lambda_{abs} - 20\ nm$, A_x and A_{QS} are the absorbance coefficients maxima during the UV/Vis measurement ($\lambda_{abs} = \lambda_{ex}$), η_x is the refractive index of methanol at the excitation wavelength,[129] η_{QS} is the refractive index of water at the excitation wavelength.[130]

Standard deviations for the extinction coefficient ε and for the fluorescence quantum yield Φ_f are calculated using the *STEV.P* function in excel.

7.3.2. Products from *in vitro* Transcription

The nucleoside **58f**, the triphosphate **59** and all biochemistry products were measured in MilliQ water as described above. The fluorescence quantum yield Φ_f was determined with an excitation wavelength of $\lambda_{ex} = 333$ nm and a 2 nm slit opening. The emission spectra were recorded from 353 nm to 646 nm with the same 2 nm slit opening.

7.4. Biochemistry

7.4.1. MS spectra of oligonucleotides

LC-ESI-MS analysis of oligonucleotides was carried out on an *Agilent 1260 Infinity II LC* system with an *Agilent InfinityLab LS/MSD XT* Detector using a *BioZen C_{18} 100 Å* column (2.1 × 150 mm) from *Phenomenex* with the mobile phases A (12.2 mM Et_3N, 300 mM HFIP in water) and B (12.2 mM Et_3N, 300 mM HFIP in 100% MeOH) and a gradient from 95:5 to 0:100 within 10 min. Deconvolutions of the LC-ESI-MS spectra were carried out using a *UniDec* program.

Positive Control: 35RNA_A7

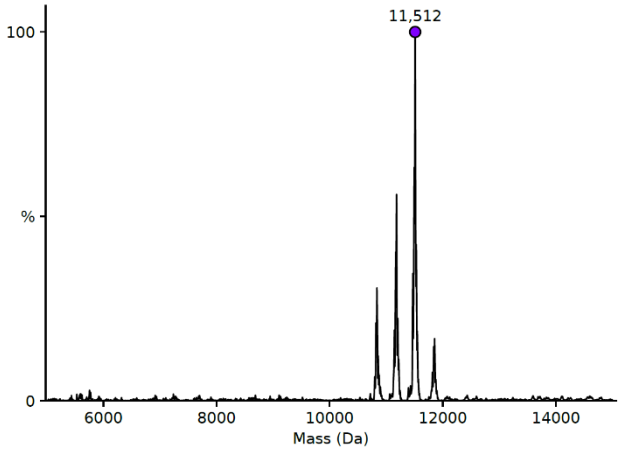


Figure 25: MS spectrum after LC-ESI-MS analysis of the positive control (**35RNA_A7**) from the IVT experiment. Deconvoluted mass spectrum.

	Sequence	Calcd. Mass [Da]	Found mass [Da]
35RNA_A7	5'-pppGGGCUUGC**A**CGUG**AA**UCGCUCUU**AA**UGGA**U**CGCG**A**	11471	11512 [+K^+]

Modified RNA: 35RNA_A^Q7

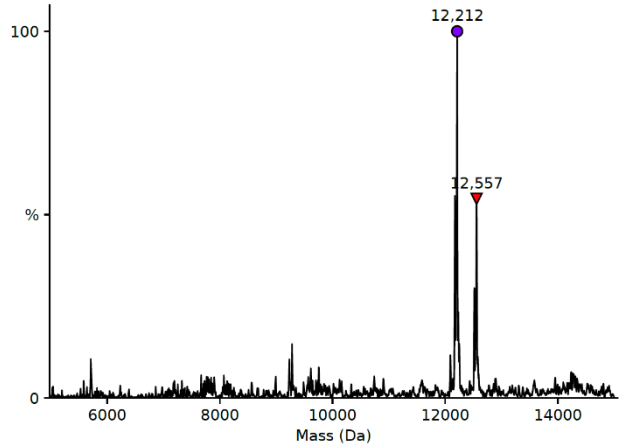


Figure 26: MS spectrum after LC-ESI-MS analysis of the modified **35RNA_A^Q7** from the IVT experiment. Deconvoluted mass spectrum.

	Sequence	Calcd. Mass [Da]	Found mass [Da]
35RNA_A^Q7	5'-pppGGGCUUGCA^QCGUG$A^Q$$A^Q$UCGCUCUU$A^Q$$A^QUGGA^Q$$A^Q$UCGCG$A^Q$	12174	12212 [+K^+] 12557 [+G,+K^+]