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C6-*O*-Alkylated 7-deaza inosine nucleoside analogues: Discovery of potent and selective anti-sleeping sickness agents

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Abstract

African trypanosomiasis, a deadly infectious disease caused by the protozoan *Trypanosoma brucei* spp., is spread to new hosts by bites of infected tsetse flies. Currently approved therapies all have their specific drawbacks, prompting a search for novel therapeutic agents. *T. brucei* lacks the enzymes necessary to forge the purine ring from amino acid precursors, rendering them dependent on the uptake and interconversion of host purines. This dependency renders analogues of purines and corresponding nucleosides an interesting source of potential anti-*T. brucei* agents. In this study, we synthesized and evaluated a series of 7-substituted 7-deazainosine derivatives and found that 6-*O*-alkylated analogues in particular showed highly promising *in vitro* activity with EC_{50} values in the mid-nanomolar range. SAR investigation of the *O*-alkyl chain showed that antitrypanosomal activity increased, and also cytotoxicity, with alkyl chain length, at least in the linear alkyl chain series. However, this could be attenuated by introducing a terminal branch point, resulting in the highly potent and selective analogues, **36**, **37** and **38**. No resistance related to transporter mediated-uptake could be identified, earmarking several of these analogues for further *in vivo* follow-up studies.

Keywords

Inosine nucleoside analogues

Trypanosoma brucei

Parasite nucleoside transporter

Introduction

Sleeping sickness or human African trypanosomiasis (HAT) is an infectious disease that is classified by the WHO as a Neglected Tropical Disease (NTD) and is confined to the African subcontinent. The disease-causing pathogen is the protozoan parasite *Trypanosoma brucei* spp., of which *T. b. gambiense* and *T. b. rhodesiense* are infectious to humans. Being a vector-transmitted disease, the incidence maps geographically with the natural habitats of the tsetse fly, *i.e.* in sub-Saharan Africa.[1]

Currently available therapies are not without limitations, ranging from being ineffective against CNS-stage disease (pentamidine and suramin), requiring parenteral administration (pentamidine, suramin, nifurtimox-eflornithine combination therapy (NECT) and melarsoprol) to inherent toxicity (melarsoprol is an organo-arsenical).[2-3] These shortcomings add additional layers of difficulty to reach the goal of HAT elimination set by the WHO.[4-5] Recently, the completion of a phase-2/3 non-inferiority clinical trial with fexinidazole[6] and subsequent approval of this oral drug for HAT represents new hope for patients, as well as people living in regions where they are at risk of contracting the disease. Unfortunately, fexinidazole shows cross-resistance to the other nitro group containing drug nifurtimox.[7-8] Additionally, fexinidazole treatment suffers from a high pill burden,[6] highlighting the need for further drug discovery efforts to identify new potential chemotherapeutic intervention options.[1, 9]

African trypanosomes markedly differ from their mammalian hosts with respect to the means by which they meet their demand for purine and purine nucleoside building blocks, which they need in great quantities to sustain their high replication rate. These parasites lack the enzymes for *de novo* purine synthesis from amino acid precursors and therefore depend on uptake and subsequent interconversion of available purines from the host.[10-11] *T. brucei* is capable of growing on virtually every purine source, showing a large degree of possible interconversion in the purine salvage pathway enzymatic machinery.[12-15] This biochemical difference between parasite and host could be leveraged to discover purine and purine nucleoside analogues with antitrypanosomal activities,[11] a strategy that already afforded several promising derivatives, such as tubercidin (**2**)[11, 14] and cordycepin (**4,** Figure 1),[14, 16] two so-called naturally occurring 'nucleoside antibiotics'. However, tubercidin suffers from high toxicity

against mammalian cells[17] and cordycepin is readily deaminated[18] to the corresponding 3'-deoxyinosine, which displays much weaker anti-*T. brucei* activity.[16, 18-19]

Recently, our group has re-examined tubercidin (7-deazaadenosine) and several 7-substituted analogues.[17] Although the cytotoxicity of tubercidin is impairing its further development as a trypanocide, we demonstrated that the introduction of aromatic substituents at position 7 conferred potent antitrypanosomal activity while reducing cytotoxicity (e.g. analogue **3**). Furthermore, the combination of structural elements of tubercidin (*in casu* its 7-deazapurine nucleobase) with the sugar ring of cordycepin (3'-deoxyadenosine), led to the discovery of the highly potent antitrypanosomal agent **5**, which was able to clear an established CNS-stage infection in mice.[20] These examples showcase the potential of purine nucleoside analogues to identify potent (and selective) antitrypanosomal agents.

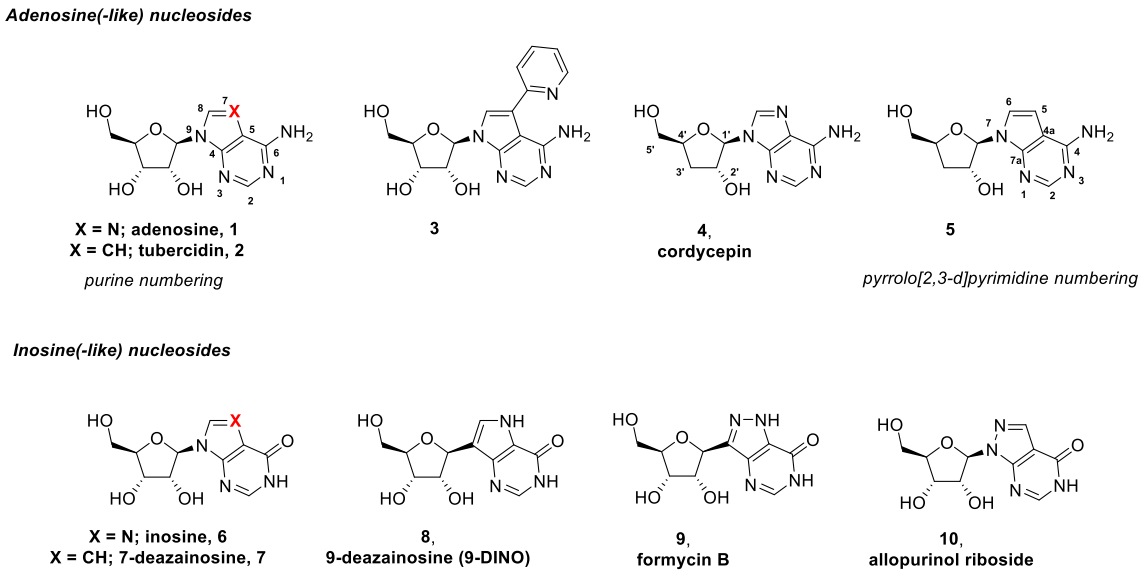


Figure 1: Overview of reported purine nucleoside analogues with activity against African trypanosomes.

The analogues mentioned above are all structurally related to adenosine, being 6-aminopurine or 7-deazapurine nucleosides (Figure 1, upper line), while on the other hand, derivatives related to their 6-oxo congener inosine (Figure 1, lower line), have been less extensively explored. Nonetheless, previous studies identified several potent inosine-like nucleoside analogues with interesting anti-*T. brucei*

activity,[21-23] of which 9-deazainosine (**8**)[22, 24] and Formycin B (**9**)[22, 25] are noteworthy. Recently, 7-cyano-7-deazainosine has been discovered as a potent activator of *T. brucei* protein kinase A (PKA), albeit that it did not display antitrypanosomal effects *in vitro*.[26]

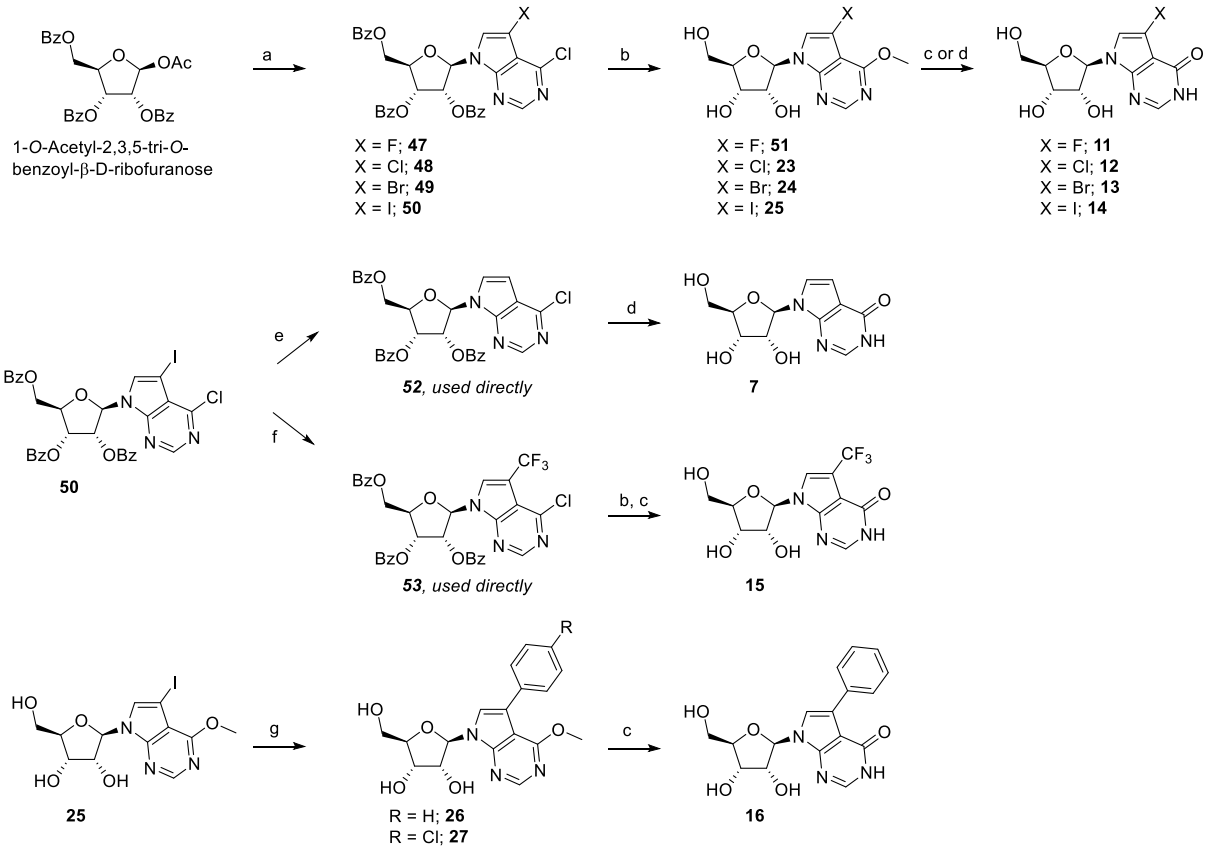
Inosine(-like) nucleosides, are preferentially taken up by the trypanosomal P1-nucleoside transporter, which can be inferred from previously described recognition elements for the P1 and P2 transporter systems.[10, 27-29] This would constitute a significant benefit regarding the development of (cross-)resistance, as several adenosine(-like) nucleoside analogues have been shown to be predominantly concentrated inside the trypanosome by the P2 transporter,[19, 28] which is encoded by a single non-essential gene,[30] and therefore represents a known resistance factor (also *vide infra*).[31-33]

Building on our recent discovery of 7-substituted 7-deazaadenosine analogues as potent anti-*T. brucei* agents,[17] we became interested in the evaluation of 7-substituted inosine nucleoside analogues. In the present manuscript, we elaborate on their synthesis and assess their anti-*T. brucei* activity against *T. b. brucei* and *T. b. rhodesiense*. Additionally, we provide *in vitro* data on P2 knock-out *T. brucei* parasites, demonstrating that this transporter does not significantly contribute to drug sensitivity, thereby rendering the development of cross-resistance with diamidine and melaminophenyl arsenical drugs such as pentamidine, diminazene or melarsoprol unlikely.

Results and discussion

Chemistry

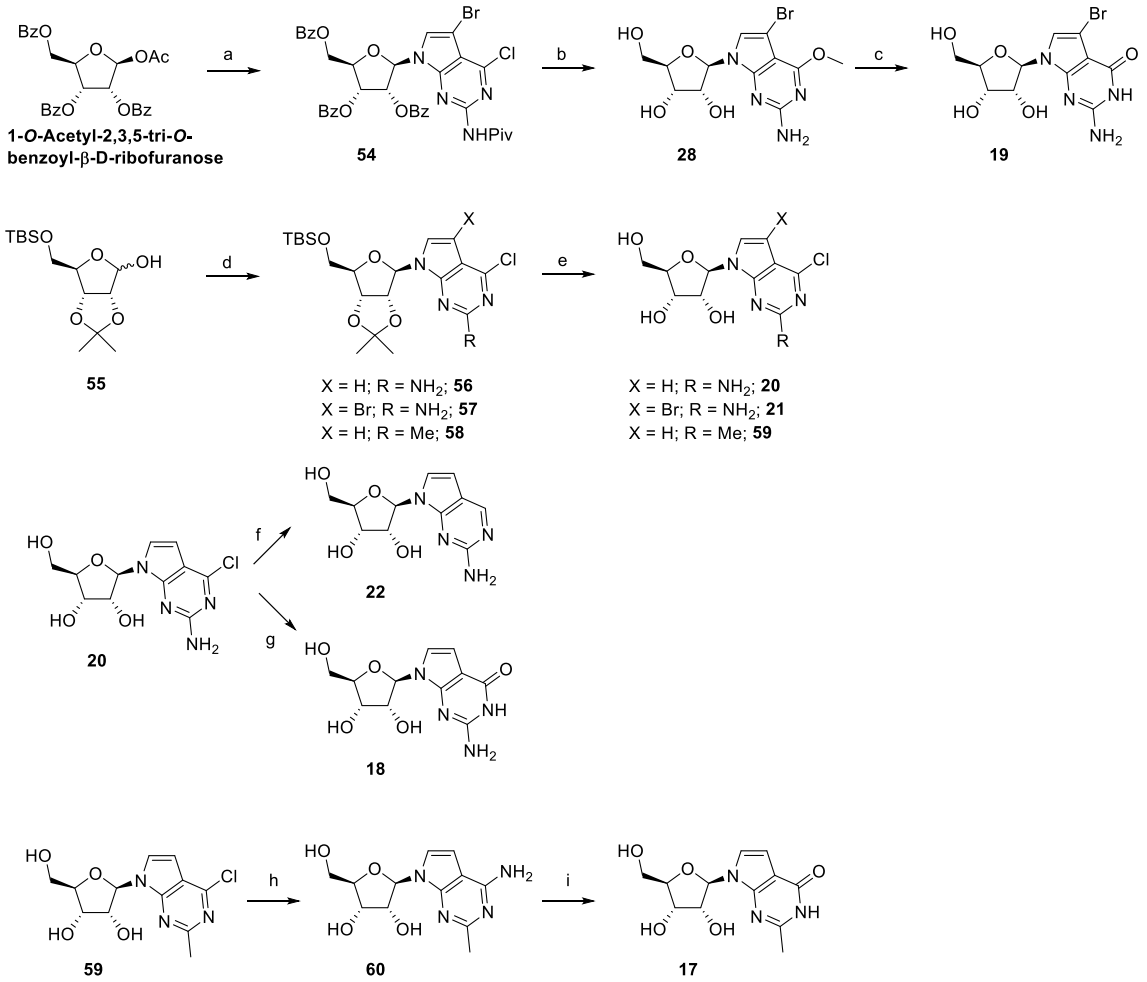
The synthesis of nucleoside analogues **7**-**46** is depicted in Scheme 1-4. 7-Deazainosine derivatives **11**-**14** were prepared employing Vorbrüggen glycosylation of appropriately substituted pyrrolo[2,3-*b*]pyrimidines with commercially available 1-*O*-acetyl-tri-*O*-benzoylribofuranose (Scheme 1).[34] Then, the 6-chloride was displaced with sodium methoxide, which also cleaved the benzoyl protecting groups. The 6-oxo group was revealed by either TMSI-mediated demethylation or nucleophilic displacement with NaOH. To obtain 7-deazainosine **7**, the 7-iodide was removed from **50** by halogen/magnesium exchange with Knochel's Turbo Grignard reagent,[35-36] followed by protic quench. One-pot nucleophilic aromatic substitution and deprotection with aqueous NaOH furnished **7**. The 7-trifluoromethyl substituted analogue **15** was prepared by reacting **50** with *in situ* prepared $CuCF_3$ in DMF/NMP, employing the method we recently reported for related pyrrolo[2,3-*b*]pyridine nucleoside analogues.[37] 7-Aryl substituents were introduced on **25** by Suzuki reaction.[17, 38-39] Analogue **26** was then demethylated to give inosine analogue **16**.



Scheme 1: Reagents and conditions: 5-substituted pyrrolo[2,3-*d*]pyrimidine (X=F,[40-41] Cl,[42] Br[42] or I[42]), BSA, TMSOTf, MeCN, 80 °C; b) NaOMe, MeOH, 65% (F), 57% (Cl), 67% (Br), 54% (I); c) TMSCl, NaI, MeCN, 67% (**11**), 80% (2 steps, **15**), 58% (**16**); d) 2M aq. NaOH, 1,4-dioxane, reflux, 55% (**7**), 33% (**12**), 58% (**13**), 54% (**14**); e) i. 1.3M iPrMgCl·LiCl/THF, THF; ii. iPrOH; f) $TMSCF_3$, CuI, KF, NMP/DMF (1:1), 36%; g) arylboronic acid, Na_2CO_3, $Pd(OAc)_2$, TPPTS, MeCN/water (1/2), 100 °C, 91% (**26**), 33% (**27**).

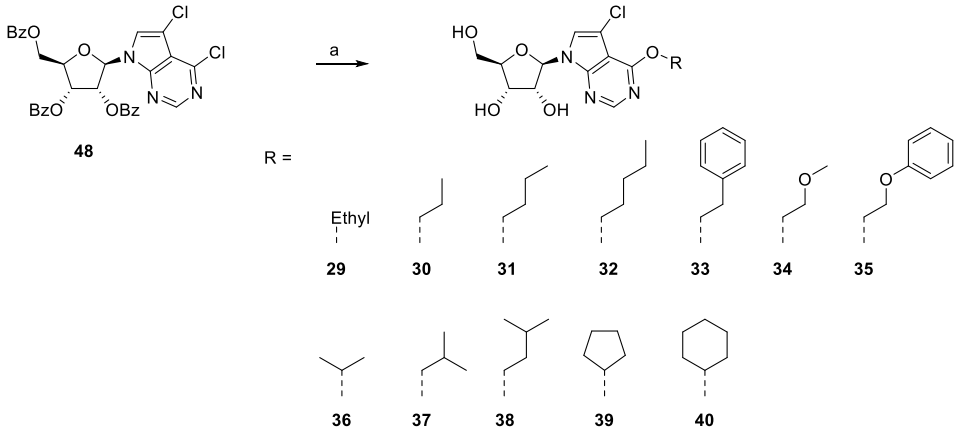
Nucleosides with a 2-substituted 6-oxo-7-deazapurine purine ring **17**-**19** (Scheme 2) were either synthesized via Vorbrüggen coupling [43] (**19**) or anion glycosylation[44] (**20**, **21** and **59**), followed by removal of all sugar protecting groups, and additional nucleophilic aromatic substitution for **19**. The 6-chloride of **20** was removed by hydrogenation with Pd/C, to give **22**, or substituted with NaOH to give **18**. In order to prepare derivative **17**, nucleophilic aromatic substitution with NaOH was first attempted on **59**, which unexpectedly failed to deliver any of the desired product. Therefore, **59** was first converted into its corresponding 6-amino analogue **60** in a sequence that employed substitution with NaN_3 and

Staudinger reduction under conditions that were reported previously,[45-47] albeit that an elevated temperature and prolonged reaction time were required to effect 6-substitution. Then, a diazotization reaction on **60** furnished the desired 2-methyl substituted 7-deazainosine derivative **17**.



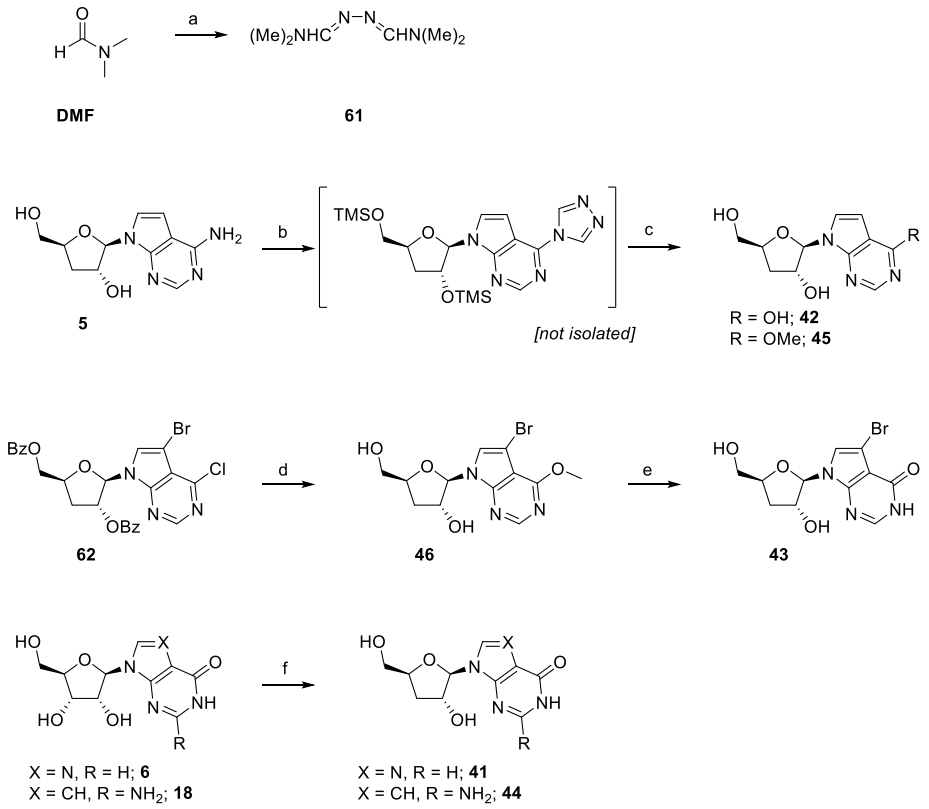
Scheme 2: Reagents and conditions: a) 2-pivaloylamino-4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine,[43, 48] BSA, TMSOTf, MeCN, 50 °C, 65 %; b) NaOMe, MeOH, reflux, 56 %; c) 2M aq. NaOH, 1,4-dioxane, reflux, 54 %; d) i. $P(NMe_2)_3$, CCl_4, THF, -78 °C; ii) 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine or 2-amino-4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine[43] or 2-methyl-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine,[35, 49] NaH, MeCN, 47% (**56**), 37% (**57**), 48% (**58**); e) 95% aq. TFA, 48% (**20**), 59% (**21**), 43% (**59**); f) Pd/C, H_2, NaOAc, MeOH; g) aq. NaOH, 1,4-dioxane; h) i. NaN_3, DMF, 80 °C; ii. 1M PMe_3 in THF, water, 62 % (2 steps); i) $NaNO_2$, AcOH, water, 64%.

The synthesis of various 6-*O*-alkyl substituted nucleoside analogues (**29** – **40**, Scheme 3) was achieved by suspending intermediate **48** in the appropriate alcohol and treating it with the corresponding alkoxide, prepared by dissolving metallic sodium in said alcohol or by treating it with NaOtBu.



Scheme 3: Reagents and conditions: a) RONa, ROH, 20 – 80 %.

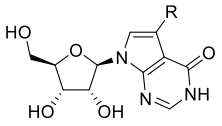
The synthesis of 3'-deoxyribofuranose nucleoside analogues is depicted in Scheme 4. Analogues **42** and **45** were prepared from **5**[20, 50], via a two-step one pot sequence. The 6-amino group was transformed into a 1,2,4-triazol-4-yl leaving group,[51-52] with **61** (prepared from DMF and $SOCl_2$),[52-53] and directly converted to the desired nucleoside analogues **42** and **45**. Alternatively, the 7-bromo functionalized derivative **7**[39] was reacted with sodium methoxide yielding **46**, which after TMSI-mediated demethylation gave rise to 3'-deoxyinosine analogue **43**. 3'-Deoxyinosine (**41**) and 3'-deoxy-7-deazaguanosine (**44**) were prepared from the corresponding ribonucleosides, in a three-step sequence. First, the vicinal diol was transformed into the 3'-*xylo*-iodide upon reaction with α-acetoxyisobutyrylchloride in the presence of NaI.[50, 54-55] Then, de-iodination was effected by catalytic hydrogenation with $Pd(OH)_2/C$, after which ammonolysis furnished the desired analogues.



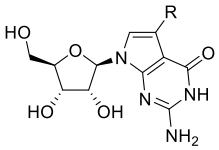
Scheme 4: Reagents and conditions: a) 1. $SOCl_2$, aq. NH_2NH_2; 2. aq. NaOH; b) **61**, TMSCl, pyridine, reflux; c) appropriate nucleophile: 1M aq. NaOH, 20 % (**42**); 0.5M NaOMe/MeOH, 66 % (**45**); d) NaOMe, MeOH, 50 °C, 69 %; e) NaI, TMSCl, MeCN, rt, 21 %; f) i) α-acetoxyisobutyryl chloride, NaI, MeCN, ii) $Pd(OH)_2/C$, H_2, NaOAc, MeOH, iii) 7N NH_3, MeOH, 33% (**41**), 21% (**44**).

Biological evaluation

All final nucleoside analogues were evaluated *in vitro* against *T. b. brucei* and *T. b. rhodesiense*, and assayed for cytotoxicity in MRC-5 human fibroblasts. The results are depicted in Table 1-3.



Inosine analogues



Guanosine analogues

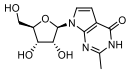
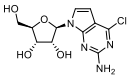
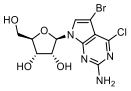
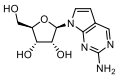
Cpd.	R / structure	*T. b. brucei* EC_{50} (µM)	*T. b. rhodesiense* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI *T. brucei*	SI *T. brucei rhod.*
			Inosine analogues			
7	H	4.2 ± 0.85	19.1 ± 0.4	31.3 ± 1.6	7.4	1.6
11	F	0.10 ± 0.01	1.06 ± 0.07	2.03 ± 0.64	21	1.9
12	Cl	10.7 ± 3.1	25.1 ± 3.0	>64.0	6.0	2.5
13	Br	52.4 ± 11.7	29.2 ± 0.8	>64.0	1.2	2.2
14	I	57.3 ± 6.7	23.9 ± 1.1	>64.0	1.1	2.7
15	CF_3	>64.0	10.9	3.28	0.1	0.3
16	phenyl	*32.2*	*9.4*	*>64.0*	>2	>6.8
17		*>64.0*	*4.7*	*>64.0*	--	>13
			Guanosine analogues			
18	H	*>64.0*	*12.9*	*>64.0*	--	>5.0
19	Br	2.01 ± 0.04	0.49 ± 0.01	>64.0	>31	>130
20		0.49 ± 0.004	0.18 ± 0.08	>64.0	>131	>347
21		0.13 ± 0.003	0.047 ± 0.023	40.2 ± 9.6	316	847
22		*>64.0*	*>64.0*	*>64.0*	--	--
Suramin	--	0.028 ± 0.009	0.033 ± 0.005	ND	--	--

Table 1: Evaluation of drug sensitivity of nucleoside analogues against bloodstream forms of *T. b. brucei* and *T. b. rhodesiense*. Cytotoxicity was assayed against the human MRC-5 fibroblast cell line.

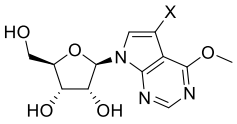
Values represent mean ± SEM, which originated from 2–3 independent experiments and are expressed in µM. Values in *italics* represent the result of a single determination. SI, *in vitro* selectivity index, being the ratio of the EC_{50} for the MRC-5 cells and the EC_{50} of the trypanosome species.

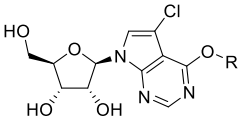
Our investigation started with 7-deazainosine (**7**, Table 1), which was previously reported to exhibit antitrypanosomal effects.[21] This analogue exhibited low micromolar *in vitro* potency (EC_{50} = 4.2 ± 0.85 µM) against *T. b. brucei* and was only moderately active against *T. b. rhodesiense*, which prompted the evaluation of several 7-substituted analogues (**11**-**16**). Introduction of a halogen atom in the 7-position led to a significant loss of activity, with the exception of 7-fluoro substituted analogue (**11**), although this derivative also displayed significant cytotoxicity against MRC-5 fibroblasts. The addition of a phenyl ring (**16**), nor a trifluoromethyl group (**15**) was able to produce any improvement of the *in vitro* antitrypanosomal activity, which contrasts with the observed SAR for the corresponding 6-aminopurine nucleoside analogues.[17]

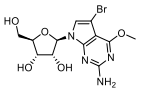
The introduction of a substituent in the 2-position such as a methyl (**17**) or an amino group (giving rise to guanosine analogue **18**), revealed that neither was tolerated. On the other hand, adding a 7-bromide to 7-deazaguanosine **18** did restore antitrypanosomal activity (*i.e.* analogue **19**), and this analogue also lacked *in vitro* cytotoxicity against MRC-5 cells. Interestingly, the evaluation of synthetic intermediates **20** and **21** having a 6-chloride, showed remarkable antitrypanosomal activity with excellent selectivity indexes. Removal of the 6-substituent of **20** (*i.e.* analogue **22**) resulted in the complete loss of any *in vitro* activity against either trypanosome species. These results led us to evaluate 6-OMe substituted nucleoside analogues **23**-**25** and **26** (Table 2), which were prepared *en route* to the inosine analogues.

All three halogenated analogues (**23**, **24** and **25**) displayed sub-micromolar potency against *T. brucei* parasites (Table 2), while having virtually no cytotoxicity. On the 7-deaza-6-*O*-methylinosine scaffold, a 7-phenyl group or a 7-*para*-chlorophenyl substituent was not tolerated. Analogue **28** also showed sub-micromolar antitrypanosomal activity, albeit with only modest selectivity. Encouraged by the results with the 6-*O*-methylated analogues, we decided to further explore the SAR in this series by varying the

6-*O*-alkyl group. In this series, we chose a chloride as the preferred substituent in the 7-position, as we reasoned that such derivatives might potentially be chemically more stable than the 7-bromide and 7-iodide equivalents.





Cpd.	R or X / Structure	*T. b. brucei* EC_{50} (µM)	*T. b. rhodesiense* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI *T. brucei*	SI *T. brucei rhod.*
23	X=Cl	0.16 ± 0.02	0.036 ± 0.003	[32.0; >64]	>200	>888
24	X=Br	0.19 ± 0.02	0.036 ± 0.003	>64.0	>336	>1777
25	X=I	0.22 ± 0.004	0.053 ± 0.003	38.4 ± 1.0	168	708
26	X=Phe	>64.0	>64.0	>64.0	--	--
27	X=4Cl-Phe	>64.0	22.3	61.1	--	2.7
28		0.13 ± 0.00	0.077 ± 0.007	3.24 ± 0.01	24	42
29	R=ethyl	0.12 ± 0.01	0.057 ± 0.04	[33.0; >64]	>275	>578
30	R=propyl	0.081 ± 0.05	0.076 ± 0.050	2.81 ± 1.6	34	37
31	R=butyl	0.069 ± 0.040	0.036 ± 0.010	1.32 ± 0.09	19	37
32	R=pentyl	0.088 ± 0.026	0.032 ± 0.001	0.10 ± 0.03	1.1	3.2
33	R=ethyl-phenyl	0.095 ± 0.03	0.030 ± 0.002	0.31 ± 0.42	3.3	10.3
34	R=2-methoxy-ethyl	0.17 ± 0.03	0.22 ± 0.07	4.13 ± 1.5	25	18.8
35	R=2-phenoxy-ethyl	0.13 ± 0.009	0.16 ± 0.05	43.2 ± 20.8	329	263
36	R=i-propyl	0.090 ± 0.04	0.10 ± 0.08	>64.0	>711	>640
37	R=i-butyl	0.071 ± 0.041	0.030 ± 0.0004	49.5 ± 14.6	694	1671
38	R=i-pentyl	0.058 ± 0.028	0.029 ± 0.0008	>64.0	>1099	>2195

39	R=c-pentyl	0.20 ± 0.0008	0.12 ± 0.003	38.1 ± 20.0	191	326
40	R=c-hexyl	0.40 ± 0.03	0.38 ± 0.13	>64.0	>161	>168
Suramin	--	0.028 ± 0.009	0.033 ± 0.005	ND	--	--

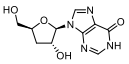
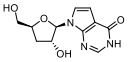
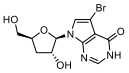
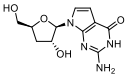
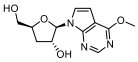
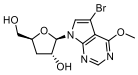
Table 2: Evaluation of drug sensitivity of nucleoside analogues against bloodstream forms of *T. b. brucei* and *T. b. rhodesiense*. Cytotoxicity was assayed against the human MRC-5 fibroblast cell line. Values represent mean ± SEM, which originated from 2–3 independent experiments and are expressed in µM. SI, *in vitro* selectivity index, being the ratio of the EC_{50} for the MRC-5 cells and the EC_{50} of the trypanosome species.

Varying the length of the 6-*O*-alkyl group from methyl (**23**) to pentyl (**32**) showed that antitrypanosomal activity increased with the chain length. Unfortunately, the selectivity dropped progressively to almost none when the *n*-alkyl group was larger than two carbon units. The introduction of an extra phenyl group (2-phenylethyl analogue **33**) increased anti-*T. brucei* potency relative to the *O*-ethyl analogue **29**, but this compound also displayed cytotoxicity at sub-micromolar concentrations. The 2-methoxyethyl analogue **34** actually displayed identical activity as the ethyl analogue, but again with lower selectivity, whereas the 2-phenoxyethyl analogue **35** actually had a favourable SI, with comparable antitrypanosomal activity to the *O*-ethyl analogue **29**.

For branched-chain alkyl groups, the antitrypanosomal activity also increased with growing chain length (EC_{50} = 0.090 µM for i-propyl **36** to EC_{50} = 0.058 µM for i-pentyl **38**), but in contrast to the linear alkyl groups the selectivity index remained very high, as compounds displayed virtually no cytotoxicity against MRC-5 fibroblast cells. Finally, cyclic alkyl groups **39** (c-pentyl) and **40** (c-hexyl), which can be considered as modified isopropyl analogues, did not display increased antitrypanosomal activity with respect to **36**, but the toxicity remained very low. From this SAR exploration, analogue **38** appeared as most potent, with a roughly three-fold increase in *in vitro* anti-*T. brucei* activity, compared to the initially discovered **23**.

Finally, we investigated the effects of removing the hydroxyl group at the 3'-position of the sugar moiety, a strategy that in the 7-deazaadenosine series led to a significant increase in antitrypanosomal

activity.[20, 39] 3'-Deoxyinosine[23] **41** was confirmed to elicit low micromolar activity against *T. brucei* (Table 3). In the case of 7-deazainosine (**7**), however, 3'-deoxygenation (*i.e.* analogue **42**), failed to improve antitrypanosomal activity. Interestingly, the addition of a 7-bromide led to an increase in *in vitro* activity regardless of 3'-deoxygenation (compare matched pairs **7** *vs.* **13** (Table 1) and **42** *vs.* **43**), while for the 6-*O*-alkylated derivatives removal of the 3'-hydroxyl group was clearly not tolerated (compare **24** *vs.* **46**).

Cpd.	Structure	*T. b. brucei* EC_{50} (µM)	*T. b. rhodesiense* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI *T. brucei*	SI *T. brucei rhod.*
41		1.1 ± 0.2	6.7 ± 0.6	>64.0	56.1	9.5
42		29.6 ± 0.1	2.9 ± 0.8	>64.0	>2	>22
43		8.1 ± 0.03	2.3 ± 0.1	>64.0	>7	>28
44		*>64.0*	*>64.0*	*>64.0*	--	--
45		>64.0	>64.0	>64.0	--	--
46		2.6 ± 0.6	3.7 ± 1.6	2.7 ± 1.1	1	0.7
Suramin	--	0.028 ± 0.009	0.033 ± 0.005	ND	--	--

Evaluation of drug sensitivity of nucleoside analogues against bloodstream forms of *T. b. brucei* and *T. b. rhodesiense*. Cytotoxicity was assayed against the human MRC-5 fibroblast cell line. Values represent mean ± SEM, which originated from 2–3 independent experiments and are expressed in µM. Values in *italics* represent the result of a single determination. SI, *in vitro* selectivity index, being the ratio of the EC_{50} for the MRC-5 cells and the EC_{50} of the trypanosome species.

To further investigate the potential of these analogues as trypanocides, we assayed selected analogues against a panel of drug resistant *T. brucei* strains (TbAT1-KO, B48 and ISMR1; Table 4). Given the polar nature of nucleoside analogues, their uptake by *T. brucei* parasites is expected to be mediated by

purine transporters with no significant contribution from simple diffusion.[10] When the relevant transporter is non-essential for survival of the parasite, as is the case for the P2 aminopurine transporter,[30-31] it constitutes a potential resistance factor. Comparison of the potency of **23**, **24**, **29** and **36** for wild-type (Lister-427) versus TbAT1-KO and B48 cell lines (both of which lack the P2 transporter), revealed that the activity of none of these analogues is greatly impacted by the loss of the P2 transporter, particularly when compared to tubercidin (7-deazaadenosine), which is greatly impacted by the loss of the transporter.[17, 28] Based on known recognition elements for both P1 and P2 transporter systems,[10, 27-29] it would indeed seem unlikely that these *O*-alkylated inosine analogues are good substrates for the P2 transporter, which mainly recognizes an amidine or amidine-like moiety, as present in pentamidine, diminazene, the melamine moiety of melarsoprol and 6-aminopurines.

Compound	Lister-427 EC_{50} (µM)	TbAT1-KO EC_{50} (µM)	RF	B48 EC_{50} (µM)	RF	ISMR1 EC_{50} (µM)	RF
Tubercidin, 2[17]	0.15 ± 0.03	2.61 ± 0.70	17.2	4.3 ± 1.3	28.7	1.7 ± 0.5	11.1
23	0.10 ± 0.01	0.057 ± 0.005	0.57	0.057 ± 0.002	0.57	0.084 ± 0.015	0.84
24	0.117 ± 0.006	0.083 ± 0.013	0.71	0.090 ± 0.010	0.77	0.099 ± 0.016	0.85
29	0.079 ± 0.006	0.113 ± 0.011	1.43	0.134 ± 0.007	1.70	0.194 ± 0.037	2.45
36	0.184 ± 0.008	0.198 ± 0.014	1.08	0.188 ± 0.014	1.02	0.27 ± 0.01	1.47
Pentamidine	0.011 ± 0.001	0.018 ± 0.002	1.8	0.99 ± 0.16	94.6	0.14 ± 0.04	13.8
Diminazene	0.42 ± 0.06	4.5 ± 0.9	10.6	7.2 ± 1.6	16.9	2.9 ± 0.4	6.9
Isometamidium	0.65 ± 0.09	0.75 ± 0.14	1.2	0.56 ± 0.13	0.85	3.1 ± 0.5	4.8

Table 4: *In vitro* anti-trypanosomal evaluation against three drug-resistant *T. b. brucei* cell lines. EC_{50} values are given as the mean and SEM of three independent determinations (in µM). RF = Resistance factor: ratio of EC_{50} between resistant and reference (Lister-427) cell line. TbAT1-KO: *T. brucei* cell line lacking the TbAT1/P2 transporter gene. B48: pentamidine, diminazene and melaminophenyl

arsenical resistant cell line. ISMR1: isometamidium resistant cell line. The reported values for tubercidin were taken from reference [27].

Next, to gain a more in-depth understanding of the potential interaction of these analogues (**23**, **29** and **36**) with the P1 and/or P2 transporter, we performed competition experiments with [^{3}H]-adenosine (Figure 2 and Table 5). In order to measure P1-mediated uptake, B48 cells were employed, which lack the P2 transporter. Uptake via the P2 transporter was measured in a B48 cell line that stably overexpresses *TbAT1*, and uptake experiments were performed in the presence of 100 µM inosine to block P1-mediated transport.[56]

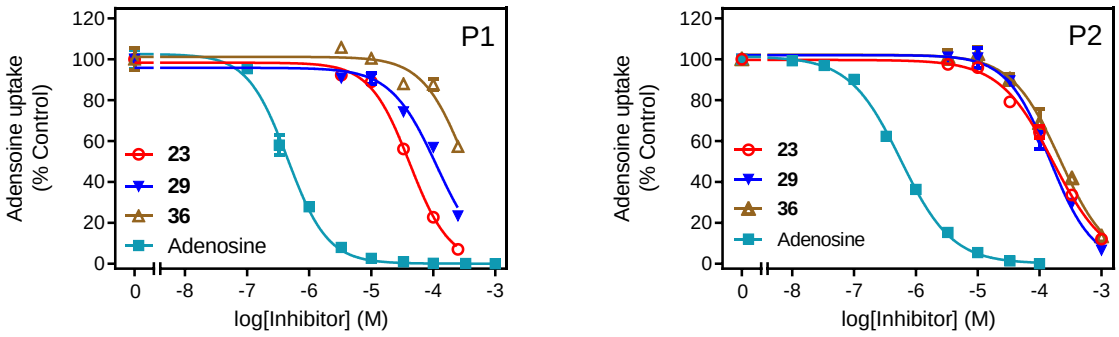


Figure 2: Transporter binding experiments of selected nucleoside analogues with P1 and P2 of bloodstream form *T. brucei*. The transport of 50 nM [^{3}H]-adenosine by bloodstream forms of *T. b. brucei* strain B48 (panel P1) or B48 expressing TbAT1/P2 (panel P2) was measured in the absence or presence of increasing concentration of nucleoside analogues. The panels each show a single experiment, performed in triplicate, that is representative of three identical but independent experiments. Error bars are SEM and when not shown fall within the symbol.

Compound	P1 transporter			P2 transporter		
	K_m or K_i	ΔG^0	$\delta(\Delta G^0)$	K_m or K_i	ΔG^0	$\delta(\Delta G^0)$
Adenosine	**0.12 ± 0.02**	-39.4	--	**0.53 ± 0.02**	-35.8	--
Tubercidin, 2[27]	78 ± 6.4	-23.4	15.4	3.8 ± 0.7	-30.9	4.9
23	35.5 ± 4.1	-25.4	14.0	149.0 ± 26.0	-21.8	14.0
29	99.0 ± 9.1	-22.9	16.5	138.8 ± 20.8	-22.0	13.8
36	284.5 ± 29.7	-20.6	19.2	245.7 ± 49.5	-20.6	15.2

Table 5: Kinetic parameters for P1 and P2 mediated uptake of selected nucleoside analogues (also see Figure 2). K_m (bold) or K_i values are given in µM, and ΔG^0 in kJ/mol. $\delta(\Delta G^0)$ was calculated with respect to the value for adenosine. The reported values for tubercidin were taken from reference [27].

From the uptake experiments it was shown that none of the *O*-alkylated analogues was a good P1 substrate, since all three analogues lost ≥14 kJ/mol in binding affinity compared to adenosine (Table 5). This indicates the loss of at least one H-bond interaction with the transporter, and is somewhat unexpected given the presence of three recognition elements: OH-3', OH-5' and N-3.[27] Indeed, the nitrogen atom in the 7-position, considered to be the fourth recognition element, has been replaced by a substituted carbon (compare with tubercidin, **2**), but we have previously found the addition of a halogen or heteroaryl group in this position to be able to restore, at least partially, P1-binding in 6-aminopurine nucleoside analogues.[17, 20] This holds true for **23**, but not for analogues **29** and **36**, which have a larger 6-*O*-alkyl substituent. It is noteworthy that the gradual increase in steric bulk at the 6-position impacted P1-transporter engagement to such an extent (K_i *O*-Me < *O*-Eth < *O*-iPr). We therefore hypothesize that a steric clash with the transporter protein is responsible for the reduced P1 binding affinity, even for **23**, having a 6-OMe substituent. A similar observation was made in a study on C2/N6 disubstituted adenosine analogues,[57] albeit that the presence of a 2-substituent in these two analogues confounds a direct matched pair analysis.

Concerning P2-mediated uptake, all three assayed analogues displayed affinities in the high micromolar range, which is expected, as the main recognition element for P2, being the N1-C6-NH_2 amidine

functionality, has been replaced. The binding affinity for the i-propyl-substituted analogue **36** was found to be somewhat lower than that of the other two analogues (~1.4 kJ/mol), but this difference was less pronounced than the equivalent $\delta(\Delta G^0)$ observed for the P1 transporter.

Conclusion

In the present study, we have investigated the antitrypanosomal effects of a series of 7-deazainosine nucleoside analogues. It was found that 7-substituted 7-deazainosine analogues had weak *in vitro* activity against *T. brucei* parasites. However, 6-*O*-Alkylated 7-deazainosine analogues which were prepared as synthetic intermediates, proved highly active trypanocides. Structure-activity relationship investigation pointed out that the size of the alkyl group positively correlated with anti-*T. brucei* activity, but so did the cytotoxicity against mammalian cells in the *n*-alkyl series. This could be circumvented by introducing an alkyl chain branch, identifying a 6-*O*-isopentyl substituent (derivative **38**) as the most potent analogue, while being completely devoid of toxicity against MRC-5 fibroblast cells at the highest concentration tested. Additionally, we have shown that these *O*-alkylated inosine derivatives are preferentially taken up by the P1 transporter, and hence do not suffer from P2-transporter based (cross)-resistance with other nucleoside analogues such as tuberidin or clinically used diamidines and melaminophenyl arsenicals. Several analogues identified in this work are now ready to proceed to evaluation in animal models of HAT.

Experimental section

Chemistry

Reagents and solvents were obtained from commercial sources of analytical grade. Unless otherwise specified, they were used as received. Nucleoside derivatives **5**[20], **47**[34], **48**[34], **49**[34], **50**[34] and **62**[39] were prepared according to published procedures. Moisture sensitive reactions were performed under either argon or nitrogen atmosphere. Unless otherwise described, reactions were carried out at ambient temperature. Analytical TLC analysis to monitor reactions was performed on Machery-Nagel® precoated F254 aluminum plates. Plates were visualized by UV followed by development by basic aq. $KMnO_4$ or sulfuric acid-anisaldehyde spray. Column chromatography was performed using Macherey-Nagel® 60M silica (40-63 µm) or on a Reveleris X2 (Grace/Büchi) automated Flash unit, with pre-packed silica columns. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts (δ) are given in ppm and spectra are referenced to the residual solvent peak. Coupling constants are given in Hz.). For ^{19}F-NMR spectra, signals were referenced to the lock resonance frequency of $CDCl_3$ or DMSO-d_6, according to IUPAC referencing ($CFCl_3$ set to $\delta = 0$ ppm). Exact mass measurements were done on a Waters LCT Premier XE™ Time of Flight (ToF) mass spectrometer with a standard electrospray (ESI) and modular Lockspray™ interface. Samples were infused in a MeCN/water (1:1) + 0.1 % formic acid mixture at 100 µL/min. Melting points were determined on a Büchi-545 apparatus and are uncorrected. Purity of all final compounds was assessed by means of analytical LC-MS on a Waters AutoPurification system with an ACQUITY QDa (mass; 100 – 1000 amu) and 2998 Photodiode Array (220 – 400 nm) using a Waters Cortecs® C18 (2.7 µm 100x4.6mm) column. A standard gradient consisting of HCOOH in H_2O (0.2 %, v/v)/MeCN at a flow rate of 1.44 mL/min, 95:05 to 00:100 in 6.5 minutes was used for all samples. All obtained final compounds had purity > 95 %, as assayed by analytical HPLC (UV); unless otherwise specifically mentioned.

4-Oxo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (7)[58] **52** (0.30 g, 0.50 mmol) was dissolved in 1,4-dioxane (2 mL). 2 N aq. NaOH (2 mL) was added, and the reaction was heated to reflux. After 2 hours, the reaction was cooled down to room temperature and neutralized to pH 7 via the addition of 4

M aq. HCl. Celite® (2 g) was added, the mixture concentrated *in vacuo*, and the residue purified by flash column chromatography (2 → 20% MeOH/DCM) to afford **7** as a white solid (0.093 g, 0.35 mmol) in 55% yield over 2 steps (starting from **50**). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47 - 3.75 (2H, m, H-5', H-5''), 3.88 (dd, *J* = 7.3, 3.8 Hz, 1H, H-4'), 3.98 - 4.11 (m, 1H, H-3'), 4.24 - 4.42 (m, 1H, H-2'), 5.01 (t, *J* = 5.0 Hz, 1H, OH-5'), 5.13 (br. s., 1H, OH-2'), 5.31 (br. s., 1H, OH-3'), 6.02 (d, *J* = 6.2 Hz, 1H, H-1'), 6.53 (d, *J* = 3.5 Hz, 1H, H-7), 7.37 (d, *J* = 3.5 Hz, 1H, H-6), 7.91 (s, 1H, H-2), 11.89 (br. s, 1H, NH). Spectral data are in accordance with literature values.[58]

4-Oxo-5-fluoro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11)[34] **51** (0.078 g, 0.26 mmol, 1.0 eq.) was dissolved in MeCN (5 mL). NaI (0.20 g, 1.3 mmol, 5.0 eq.) was added, followed by TMSCl (0.16 mL, 1.3 mmol, 5.0 eq.). The reaction was stirred for 3 h at room temperature, neutralized via the addition of aq. sat. $NaHCO_3$ (1 mL), and diluted with MeOH. Celite ® (2 g) was added, and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (5 → 20% MeOH/DCM) to afford **11** as a white solid (0.050 g, 0.18 mmol, 67%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.44 - 3.68 (m, 2H, H-5', H-5''), 3.86 (dd, *J* = 7.3, 3.8 Hz, 1H, H-4'), 3.98 - 4.10 (m, 1H, H-3'), 4.23 (s, 1H, H-2'), 5.01 (t, *J* = 5.0 Hz, 1H, OH-5'), 5.14 (br. s., 1H, OH-2'), 5.34 (br. s, 1H, OH-3'), 6.06 (dd, *J* = 6.0, 1.9 Hz, 1H, H-1'), 7.33 (d, *J* = 1.8 Hz, 1H, H-6), 7.92 (s, 1H, H-2), 12.05 (br. s, 1H, NH) ppm. Spectral data are in accordance with literature values.[34]

4-Oxo-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (12)[34] **23** (0.080 g, 0.25 mmol, 1 eq.) was suspended in 1,4-dioxane (4.0 mL, 13.3 mL/mmol SM) and 2 M aq. NaOH solution (15 mL, 89 mL/mmol SM) was added. The resulting mixture was refluxed for 4 h and cooled to ambient temperature. After neutralization with 3 M aq. HCl solution the mixture was evaporated till dryness, re-suspended in MeOH and adsorbed onto Celite®. Purification by column chromatography (8 → 20 % MeOH/DCM) gave **12** (0.025 g, 0.083 mmol) as a white powder in 33 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 12.0, 4.8, 4.2 Hz, 1H, H-5''), 3.61 (ddd, *J* = 12.0, 5.1, 3.9 Hz, 1H, H-5'), 3.88 (dd, *J* = 6.9, 3.9 Hz, 1H, H-4'), 4.04 – 4.08 (m, 1H, H-3'), 4.27 (q, *J* = 5.7 Hz, 1H, H-2'), 5.04 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.14 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.36 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.02 (d, *J* =

5.7 Hz, 1H, H-1'), 7.53 (s, 1H, H-6), 7.94 (s, 1H, H-2), 12.11 (s, 1H, NH). Spectral data are in accordance with literature values.[34]

4-Oxo-5-bromo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (13)[34] **24** (0.033 g, 0.090 mmol, 1.0 eq.) was dissolved in 1,4-dioxane (2 mL). 2 N aq. NaOH (2 mL) was added, and the reaction was heated to reflux. After 2 h, the reaction was cooled down to room temperature and neutralized to pH 7 via the addition of 4 M aq. HCl. Celite® (2 g) was added, the mixture concentrated *in vacuo*. The residue was purified by flash column chromatography (10% MeOH → DCM and 20% MeOH/DCM) to afford **13** as a white solid (0.018 g, 0.050 mmol) in 58% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.45 - 3.73 (m, 2H, H-5', H-5''), 3.88 (d, *J* = 3.5 Hz, 1H, H-4'), 4.06 (dd, *J* = 8.2, 4.7 Hz, 1H, H3'), 4.28 (dd, *J* = 6.2, 5.3 Hz, 1H, H2'), 5.05 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.15 (d, *J* = 5.0 Hz, 1H, OH-2'), 5.37 (d, *J* = 6.2 Hz, 1H, OH-3'), 6.01 (d, *J* = 6.2 Hz, 1H, H-1'), 7.56 (s, 1H, H-6), 7.94 (s, 1H, H-2), 12.04 (br. s, 1H, NH) ppm. Spectral data are in accordance with literature values.[34]

4-Oxo-5-iodo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (14)[34] 50 (0.27 g, 0.37 mmol) was dissolved in 1,4-dioxane (2 mL). 2 N aq. NaOH (2 mL) was added, and the reaction was heated to reflux. After 2 hours, the reaction was cooled down to room temperature and neutralized to pH 7 via the addition of 4M aq. HCl. Celite® (2 g) was added, the mixture concentrated *in vacuo*, and the residue purified by flash column chromatography twice (first 8% MeOH/DCM and 25% MeOH/DCM; then 2 → 20% MeOH/DCM) to afford **14** as a white solid (0.080 g, 0.20 mmol) in 54% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 - 3.67 (m, 2H, H-5', H-5''), 3.87 (dd, *J* = 7.0, 3.8 Hz, 1H, H-4'), 3.99 - 4.14 (m, 1H, H3'), 4.28 (dd, *J* = 11.1, 6.2 Hz, 1H, H2'), 5.03 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.12 (d, *J* = 4.7 Hz, 1H, OH-2'), 5.34 (d, *J* = 6.4 Hz, 1H, OH-3'), 5.99 (d, *J* = 6.2 Hz, 1H, H-1'), 7.57 (s, 1H, H-6), 7.93 (s, 1H, H-2), 12.06 (br. s., 1H, NH). Spectral data are in accordance with literature values.[34]

4-Oxo-5-trifluoromethyl-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (15) 53 (0.10 g, 0.15 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (1 mL). MeOH (4 mL) was added, followed by NaOMe (5.4 M in MeOH, 0.4 mL). After 1 h, TLC analysis indicated completion of the reaction. The reaction was neutralized to pH 7 via the addition of 4M aq. HCl. Celite® (2 g) was added and the mixture was concentrated *in vacuo*. The residue was purified via flash column chromatography (0 → 1%

MeOH/DCM) and the resulting product was dissolved in MeCN (3 mL). NaI (0.11 g, 0.75 mmol, 5.0 eq.) was added, followed by TMS-Cl (0.095 mL, 0.75 mmol, 5.0 eq.). The reaction was stirred for 3 hours at room temperature, neutralized via the addition of aq. sat. $NaHCO_3$ (1 mL), and diluted with MeOH. Celite® (2 g) was added, and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (0 → 20% MeOH/DCM) to afford **15** as a white solid (0.040 g, 0.12 mmol) in 80% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 - 3.74 (m, 2H, H-5', H-5''), 3.92 (dd, *J* = 7.0, 3.5 Hz, 1H, H-4'), 4.09 (br. s., 1H, H-3'), 4.34 (dd, *J* = 10.3, 4.7 Hz, 1H, H-2'), 5.10 (t, *J* = 5.1 Hz, 1H, OH-5'), 5.16 (br. s., 1H, OH-2'), 5.43 (d, *J* = 5.3 Hz, 1H, OH-3'), 6.05 (d, *J* = 5.9 Hz, 1H, H-1'), 8.04 (d, *J* = 1.2 Hz, 1H, H-6), 8.05 (s, 1H, H-2), 12.19 (br. s, 1H, NH). ^{19}F NMR (282 MHz, DMSO-d_6) δ -55.72. ^{13}C NMR (75 MHz, DMSO-d_6) δ 61.1 (C-5'), 70.2 (C-3'), 74.5 (C-2'), 85.4 (C-4'), 87.2 (C-1'), 104.4 (C-4a), 107.9 (q, *J* = 38 Hz, C-5), 122.5 (q, *J* = 5.8 Hz, C-6), 123.4 (q, *J* = 266 Hz, CF_3), 146.0 (C-2), 148.9 (C-7a), 156.4 (C-4) ppm. HRMS (ESI): calculated for $C_{12}H_{13}F_3N_3O_5$ ($[M+H]^+$): 336.0802, found: 336.0813.

4-Oxo-5-phenyl-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (16)[35] **26** (0.031 g, 0.090 mmol, 1.0 eq.) was dissolved in MeCN (3 mL). NaI (0.065 g, 0.43 mmol, 5.0 eq.) was added, followed by TMSCl (0.055 mL, 0.43 mmol, 5.0 eq.). The reaction was stirred for 4 h at room temperature, neutralized via the addition of aq. sat. $NaHCO_3$ (1 mL), and diluted with MeOH. Celite ® (2 g) was added, and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography (0 → 20% MeOH/DCM) to afford **16** as a white solid (0.017 g, 0.050 mmol) in 58% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.49 - 3.71 (m, 2H, H-5', H-5''), 3.90 (dd, *J* = 7.0, 3.5 Hz, 1H, H-4'), 4.10 (dd, *J* = 8.5, 4.7 Hz, 1H, H-3'), 4.37 (dd, *J* = 11.4, 5.9 Hz, 1H, H-2'), 5.04 (t, *J* = 5.6 Hz, 1H, OH-5'), 5.13 (d, *J* = 5.0 Hz, 1H, OH-2'), 5.36 (d, *J* = 6.2 Hz, 1H, OH-3'), 6.10 (d, *J* = 5.9 Hz, 1H, H-1'), 7.18 - 7.27 (m, 1H, H_{Phe}), 7.29 - 7.42 (m, 2H, H_{Phe}), 7.71 (s, 1H, H-6), 7.86 - 7.93 (m,2H, H_{Phe}), 7.95 (s, 1H, H-2), 11.97 (br. s, 1H, NH) ppm. Spectral data are in accordance with literature values.[35]

2-Methyl-4-oxo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (17) $NaNO_2$ (0.069 g, 1.0 mmol, 10.0 eq.) was added to a solution of **60** (0.028 g, 0.10 mmol, 1.0 eq.) in H_2O (4 mL) and AcOH (1 mL). After stirring for 10 days, the mixture was concentrated *in vacuo* and co-evaporated with toluene three

times. The residue was dissolved in MeOH, adsorbed onto Celite® and purified via flash column chromatography (0 → 20% MeOH/DCM) to afford **17** as a white solid (0.018 g, 0.060 mmol, 64% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.33 (s, 3H, CH_3), 3.43 - 3.70 (m, 2H, H-5', H-5''), 3.87 (dd, *J* = 7.0, 3.8 Hz, 1H, H-4'), 4.07 (m, 1H, H-3'), 4.34 (dd, *J* = 11.1, 5.6 Hz, 1H, H-2'), 5.06 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.10 - 5.15 (m, 1H, OH-2'), 5.27 (d, *J* = 6.2 Hz, 1H, OH-3'), 5.97 (d, *J* = 6.4 Hz, 1H, H-1'), 6.46 (d, *J* = 3.5 Hz, 1H, H-5), 7.26 (d, *J* = 3.5 Hz, 1H, H-6), 11.88 (br. s., 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9 (CH_3), 61.7 (C-5'), 70.7 (C-3'), 74.0 (C-2'), 85.1 (C-4'), 86.8 (C-1'), 102.1 (C-5), 106.0 (C-4a), 120.6 (C-6), 148.3 (C-7a), 153.0 (C-2), 158.9 (C-4). HRMS (ESI): calculated for $C_{12}H_{16}N_3O_5$ ($[M+H]^+$): 282.1085, found: 282.1078.

2-Amino-4-oxo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (18)[44] A solution of **20** (0.11 g, 0.35 mmol, 1.0 eq.) in 0.5 N NaOH (8 mL) was refluxed for 2 hours. After cooling to room temperature, the reaction was neutralized to pH 7 via the addition of 4M aq. HCl. The mixture was concentrated *in vacuo* and the residue taken up in MeOH, adsorbed onto Celite® and purified by flash column chromatography (20% MeOH/DCM and 40% MeOH/DCM) to afford **18** as a white solid (0.043 g, 0.15 mmol) in 44% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.41 - 3.62 (m,2H, H-5', H-5''), 3.80 (dd, *J* = 7.6, 3.8 Hz, 1H, H-4'), 4.02 (dd, *J* = 8.5, 4.4 Hz, 1H, H-3'), 4.23 (dd, *J* = 11.7, 5.9 Hz, 1H, H-2'), 4.96 (t, *J* = 5.6 Hz, 1H, OH-5'), 5.03 (d, *J* = 4.4 Hz, 1H, OH-2'), 5.22 (d, *J* = 6.2 Hz, 1H, OH-3'), 5.86 (d, *J* = 6.2 Hz, 1H, H-1'), 6.26 (d, *J* = 4.1 Hz, 1H, H-5), 6.30 (br. s, 2H, NH_2), 6.92 (d, *J* = 3.5 Hz, 1H, H-6), 10.42 (s, 1H, NH). Spectral data are in accordance with literature values.[44]

2-Amino-4-oxo-5-bromo-*N7*-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (19)[43] **28** (0.054 g, 0.14 mmol) was suspended in 1,4-dioxane (2.0 mL, 13.3 mL/mmol SM). Next, 2M aq. NaOH sol. (13 mL, 89 mL/mmol SM) was added and the mixture heated till reflux for 4 h. after cooling to ambient temperature, the mixture was neutralized by concentrated HCl and evaporated. The residue was purified by column chromatography 15 → 22 % MeOH/$CHCl_3$ to give, after precipitation from water, **19** (0.028 g, 0.078 mmol) as a yellowish solid in 54 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.45 – 3.59 (m, 2H, H-5', H-5''), 3.80 (dd, *J* = 7.2, 3.9 Hz, 1H, H-4'), 4.00 – 4.04 (m, 1H, H-3'), 4.20 (dd, *J* = 11.4, 6.3 Hz, 1H, H-2'), 5.01 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.10 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.30 (d, *J* = 6.3 Hz, 1H,

OH-2'), 5.85 (d, *J* = 6.3 Hz, 1H, H-1'), 6.65 (br. s, 2H, NH_2), 7.08 (s, 1H, H-6), 10.73 (br. s, 1H, NH). HRMS (ESI): calculated for $C_{11}H_{12}BrN_4O_5$ ($[M-H]^-$): 358.9997, found: 358.9935. Spectral data are in accordance with literature values.[43]

2-Amino-4-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (20)[44] **56** (0.40 g, 0.87 mmol, 1.0 eq.) is dissolved in TFA (4.5 mL). H_2O (0.5 mL) was added, and the mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo* and co-evaporated three times with toluene to remove all TFA. The residue was taken up in MeOH, adsorbed onto Celite® and purified by flash column chromatography (0 → 10% MeOH/DCM). The residue was then lyophilized to afford **20** as a white solid (0.13 g, 0.42 mmol) in 48 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.52 (dd, *J* = 11.7, 4.1 Hz, 1H, H-5'), 3.56 (dd, *J* = 12.0, 4.1 Hz, 1H, H-5''), 3.84 (dd, *J* = 7.3, 4.1 Hz, 1H, H-4'), 4.04 (dd, *J* = 5.0, 3.2 Hz, 1H, H-3'), 4.30 (t, *J* = 5.7 Hz, 1H, H-2'), 4.79 - 5.51 (m, 3H, OH-2', OH-3', OH-5'), 5.98 (d, *J* = 6.2 Hz, 1H, H-1'), 6.36 (d, *J* = 3.5 Hz, 1H, H-5), 6.68 (br. s., 2H, NH_2), 7.37 (d, *J* = 4.1 Hz, 1H, H-6). Spectral data are in accordance with literature values.[44]

2-Amino-5-bromo-4-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (21)[43] **57** (0.26 g, 0.48 mmol, 1.0 eq.) is dissolved in TFA (4.5 mL). H_2O (0.5 mL) was added, and the mixture was stirred for 1 h at room temperature. The mixture was concentrated *in vacuo* and co-evaporated three times with toluene to remove all TFA. The residue was taken up in MeOH, adsorbed onto Celite® and purified by flash column chromatography (2 → 12% MeOH/DCM). The residue was then lyophilized to afford **21** as a white solid (0.11 g, 0.28 mmol) in 59% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 - 3.73 (m, 2H, H-5', H-5''), 3.85 (dd, *J* = 7.3, 4.1 Hz, 1H, H-4'), 4.05 (dd, *J* = 7.6, 3.8 Hz, 1H, H-3'), 4.29 (dd, *J* = 11.7, 5.9 Hz, 1H, H-2'), 5.01 (t, *J* = 5.3 Hz, 1H, OH-5'), 5.10 (d, *J* = 4.4 Hz, 1H, OH-2'), 5.33 (d, *J* = 5.9 Hz, 1H, OH-3'), 6.00 (d, *J* = 6.4 Hz, 1H, H-1'), 6.92 (br. s, 2H, NH_2), 7.61 (s, 1H, H-6). Spectral data are in accordance with literature values.[43]

2-Amino-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (22)[59] **20** (0.064 g, 0.21 mmol, 1.0 eq.) was dissolved in MeOH (10 mL) and NH_4OH (0.5 mL) was added. Next, the flask was purged with N_2, after which a cat. amount of Pd/C was added. Next, the N_2-atmosphere was exchanged for H_2 (balloon; no bubbling) and the mixture stirred for 4 hours. The mixture was filtered over Celite®, and the filtrate

concentrated *in vacuo*. The residue was dissolved in MeOH, adsorbed onto Celite® and purified by flash column chromatography (0 → 30% MeOH/DCM) to afford **22** as a white solid (0.016 g, 0.060 mmol, 28% yield). ^{1}H NMR (300 MHz, DMSO-d_6): 3.43 - 3.73 (m, 2H, H-5', H-5''), 3.84 (dd, *J* = 7.3, 4.1 Hz, 1H, H-4'), 3.95 - 4.14 (m, 1H, H-3'), 4.33 (br. s., 1H, H-2'), 4.99 (br. s., 1H, OH-5'), 5.08 (br. s., 1H, OH-2'), 5.26 (br. s., 1H, OH-3'), 6.02 (d, *J* = 6.4 Hz, 1H, H-1'), 6.23 (s,2H, NH_2), 6.37 (d, *J* = 3.8 Hz, 1H, H-5), 7.28 (d, *J* = 3.8 Hz, 1H, H-6), 8.46 (s, 1H, H-4) ppm. Spectral data are in accordance with literature values.[59]

4-Methoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (23)[34] **48**[34] (0.32 g, 0.5. mmol, 1 eq.) was suspended in MeOH (12 mL) and 5.4 M NaOMe/MeOH (1 mL) was added. The resulting mixture was heated at 50 °C for 2 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with c.HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 10 % MeOH/EA to give **23** (0.10 g, 0.28 mmol) as a white solid in 57 % yield. Melting point: 171 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 (ddd, *J* = 12.0, 5.7, 3.9, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.1, 3.9 Hz, 1H, H-5'), 3.91 (q, *J* = 3.9 Hz, 1H, H-4'), 4.07 (s, 3H, OCH_3), 4.05 – 4.11 (m, 1H, H-3'), 4.36 (dd, *J* = 11.4, 6.0 Hz, 1H, H-2'), 5.07 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.38 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.16 (d, *J* = 6.0 Hz, 1H, H-1'), 7.85 (s, 1H, H-6), 8.47 (s, 1H, H-2). Spectral data are in accordance with literature values.[34]

4-Methoxy-5-bromo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (24)[34] **49**[34] (0.25 g, 0.37 mmol, 1 eq.) was suspended in 0.5 M NaOMe/MeOH (7.5 mL). The resulting mixture was heated at 50 °C for 3 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with c.HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 5 % MeOH/DCM to give **24** (0.090 g, 0.25 mmol) as a white solid in 67 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 (ddd, *J* = 12.0, 5.7, 3.9 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.9 Hz, 1H, H-4'), 4.05 (s, 3H, OCH_3), 4.06 – 4.11 (m, 1H, H-3'), 4.34 – 4.39 (m, 1H, H-2'), 5.08 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.38 (d,

J = 6.3 Hz, 1H, OH-2'), 6.16 (d, *J* = 6.3 Hz, 1H, H-1'), 7.88 (s, 1H, H-6), 8.46 (s, 1H, H-2). Spectral data are in accordance with literature values.[34]

4-Methoxy-5-iodo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (25)[34] NaOMe (5.4 M in MeOH, 1 mL) was added to **50**[34] (0.56 g, 0.77 mmol, 1.0 eq.) in MeOH (10 mL). After 3 hours, the reaction mixture was neutralized with 4 M HCl to pH 7. Celite® (2g) was added, the mixture was concentrated *in vacuo*, and the residue purified by flash column chromatography (0 → 6% MeOH/DCM) to afford **25** as a white solid (0.17 g, 0.42 mmol) in 54% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.59 (m, 2H, 5-H', 5-H''), 3.90 (dd, *J* = 6.4, 3.2 Hz, 1H, H-4'), 4.00 - 4.17 (m, 4H, OCH_3 + H-3'), 4.37 (dd, *J* = 11.7, 5.9 Hz, 1H, H-2'), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.15 (d, *J* = 4.7 Hz, 1H, OH-2'), 5.35 (d, *J* = 6.2 Hz, 1H, OH-3'), 6.13 (d, *J* = 6.2 Hz, 1H, H-1'), 7.88 (s,1H, H-5), 8.44 (s, 1H, H-6). Spectral data are in accordance with literature values.[34]

4-Methoxy-5-phenyl-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (26)[35] **25** (0.060 g, 0.15 mmol, 1.0 eq.), phenylboronic acid (0.027 g, 0.22 mmol, 1.5 eq.), Na_2CO_3 (0.047 g, 0.44 mmol, 3.0 eq.), $Pd(OAc)_2$ (0.002 g, 0.007 mmol, 0.05 eq.) and TPPTS (0.010 g, 0.018 mmol, 0.12 eq.) were added to a 10 mL round-bottom flask equipped with a stir bar. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, degassed MeCN (1 mL) and H_2O (2 mL) were added to the solids under argon. After 5 min of stirring, the mixture was heated to 100 °C on a pre-heated heating block. After 1 hour, the mixture was cooled to ambient temperature, and neutralized (pH ~ 7) with 1 M aq. HCl. Celite® (2 g) was added, the mixture concentrated *in vacuo*, and the residue purified by flash column chromatography (0 → 12% MeOH/DCM) to afford **26** as a white solid (0.048 g, 0.13 mmol) in 91% yield. ^{1}H NMR (300 MHz, CD_3OD-d_4) δ 3.76 (dd, *J* = 12.3, 3.2 Hz, 1H, H-5'), 3.88 (dd, *J* = 12.2, 2.8 Hz, 1H, H-5''), 4.07 (3H, s, OCH_3), 4.13 (dd, *J* = 6.2, 2.9 Hz, 1H, H-4'), 4.33 (dd, *J* = 5.4, 3.4 Hz, 1H, H-3'), 4.65 (t, *J* = 5.7 Hz, 1H, H-2'), 6.22 (d, *J* = 6.2 Hz, 1H, H-1'), 7.20 - 7.34 (m, 1H, H_{Phe}), 7.34 - 7.46 (m, 2H, H_{Phe}), 7.55 - 7.75 (m, 3H, $2H_{Phe}$ & H-6), 8.40 (s, 1H, H-2). Spectral data are in accordance with literature values.[35]

4-Methoxy-5-(4-chlorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (27) 25 (0.060 g, 0.15 mmol, 1.0 eq.), 4-chlorophenylboronic acid (0.035 g, 0.22 mmol, 1.5 eq.), Na_2CO_3 (0.047 g, 0.44

mmol, 3.0 eq.), $Pd(OAc)_2$ (0.002 g, 0.007 mmol, 0.05 eq.) and TPPTS (0.010 g, 0.018 mmol, 0.12 eq.) were added to a 10 mL round-bottom flask, equipped with a stir bar. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, degassed MeCN (1 mL) and H_2O (2 mL) were added to the solids under argon. After 5 min of stirring, the mixture was heated to 100 °C on a pre-heated heating block. After 1 hour, the mixture was cooled to ambient temperature, and neutralized (pH ~ 7) with 1 M aq. HCl. Celite® (2 g) was added, the mixture concentrated in vacuo, and the residue purified by flash column chromatography (0→12% MeOH/DCM) to afford **27** as a white solid (0.019 g, 0.050 mmol) in 33% yield. ^{1}H NMR (300 MHz, CD_3OD-d_4) δ 3.76 (dd, *J* = 12.3, 3.2 Hz, 1H, H-5'), 3.88 (dd, *J* = 12.3, 2.9 Hz, 1H, H-5''), 4.07 (s, 3H, OCH_3), 4.13 (dd, *J* = 6.2, 2.9 Hz, 1H, H-4'), 4.32 (dd, *J* = 5.3, 3.2 Hz, 1H, H-3'), 4.64 (t, *J* = 5.7 Hz, 1H, H-2'), 6.21 (d, *J* = 6.2 Hz, 1H, H-1'), 7.31 - 7.44 (m, 2H, H_{Phe}), 7.59 - 7.67 (m, 2H, H_{Phe}), 7.69 (s, 1H, H-6), 8.40 (s, 1H, H-2). ^{13}C NMR (75 MHz, CD_3OD-d_4) δ 54.4 ($O\underline{C}H_3$), 63.4 (C-5'), 72.5 (C-3'), 75.9 (C-2'), 87.2 (C-4'), 90.8 (C-1'), 105.5 (C-6), 124.6 (C_{Phe}), 129.3 (C_{Phe}), 131.5 (C_{Phe}), 133.7 (C_{Phe}), 152.0 (C-2), 165.0 (C-4) ppm. Three quaternary carbons could not be detected (C-5, C-4a, C-7a). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_5$ ($[M+H]^+$): 392.1008, found: 392.1000.

2-Amino-4-methoxy-5-bromo-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (28)[43] **54** (1.0 g, 1.3 mmol, 1 eq.) was dissolved in 0.5 M NaOMe/MeOH sol. and heated to reflux for 3 h. After cooling to ambient temperature, the mixture was neutralized with 3 M aq. HCl sol. and evaporated till dryness. The residue was purified by column chromatography 2.5 → 5 % MeOH/DCM to give **28** (0.27 g, 0.72 mmol) as a yellow solid in 56 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5''), 3.58 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5'), 3.82 (dd, *J* = 7.2, 4.2 Hz, 1H, H-4'), 3.93 (s, 3H, OCH_3), 4.01 – 4.05 (m, 1H, H-3'), 4.26 (dd, *J* = 11.7, 6.3 Hz, 1H, H-2'), 4.99 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.05 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.26 (d, *J* = 6.0 Hz, 1H, OH-2'), 5.97 (d, *J* = 6.6 Hz, 1H, H-1'), 6.42 (br. s, 2H, NH_2), 7.29 (s, 1H, H-6). HRMS (ESI): calculated for $C_{12}H_{16}BrN_4O_5$ ($[M+H]^+$): 375.0299, found: 375.0304. Spectral data are in accordance with literature values.[43]

4-Ethoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (29) 48 (0.19 g, 0.30 mmol, 1 eq.) was suspended in EtOH (6.9 mL) and 20 % (W/V) NaOEt/EtOH (0.10 mL) was added. The resulting

mixture was heated at 50 °C for 2 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with concentrated HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 10 % MeOH/DCM to give **29** (0.020 g, 0.061 mmol) as a white solid in 20 % yield. Melting point: 140 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.39 (t, *J* = 6.9 Hz, 3H, CH_3), 3.54 (ddd, *J* = 12.0, 5.7, 3.9 Hz, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.11 (m, 1H, H-3'), 4.32 – 4.38 (m, 1H, H-2'), 4.55 (q, *J* = 6.9 Hz, 2H, OCH_2), 5.07 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.37 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.15 (d, *J* = 6.3 Hz, 1H, H-1'), 7.83 (s, 1H, H-6), 8.44 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.3 (CH_3), 61.4 (C-5'), 62.2 (OCH_2), 70.4 (C-3'), 74.1 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 121.7 (C-6), 150.6 (C-7a), 151.6 (C-2), 161.9 (C-4). HRMS (ESI): calculated for $C_{13}H_{17}ClN_3O_5$ ($[M+H]^+$): 330.0851, found: 330.0774.

4-Propoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (30) 48 (0.19 g, 0.30 mmol, 1 eq.) was suspended in 0.5 M NaOPr/nPrOH solution (7.0 mL) [Na^0 wire (0.12 g) was added to nPrOH (10 mL) under argon and stirred vigorously until the wire was fully dissolved]. The resulting mixture was heated at 50 °C for 3 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with concentrated HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 8 % MeOH/DCM to give **30** (0.040 g, 0.12 mmol) as a white solid in 39 % yield. Melting point: 134 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02 (t, *J* = 7.5 Hz, 3H, CH_3), 1.74 – 1.85 (m, 2H, CH_2), 3.51 – 3.58 (m, 1H, H-5''), 3.60 – 3.67 (m, 1H, H-5'), 3.91 (q, *J* = 3.9 Hz, 1H, H-4'), 4.09 (br. s, 1H, H-3'), 4.35 (br. s, 1H, H-2'), 4.46 (t, *J* = 6.3 Hz, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.17 (br. s, 1H, OH-3'), 5.38 (br. s, 1H, OH-2'), 6.15 (d, *J* = 6.3 Hz, 1H, H-1'), 7.83 (s, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 10.3 (CH_3), 21.7 (CH_2), 61.4 (C-5'), 67.7 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 121.7 (C-6), 150.6 (C-7a), 151.7 (C-2), 162.1 (C-4). HRMS (ESI): calculated for $C_{14}H_{19}ClN_3O_5$ ($[M+H]^+$): 344.1008, found: 344.0921.

4-Butoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (31) 48 (0.19 g, 0.30 mmol, 1 eq.) was suspended in 0.5 M NaOBu/nBuOH solution (7.0 mL) [Na^0 wire (0.12 g) was added to nBuOH

(10 mL) under argon and stirred vigorously until the wire was fully dissolved]. The resulting mixture was heated at 50 °C for 3 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with concentrated HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 8 % MeOH/DCM to give **31** (0.065 g, 0.18 mmol) as a white solid in 61 % yield. Melting point: 156 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.95 (t, *J* = 7.5 Hz, 3H, CH_3), 1.42 – 1.54 (m, 2H, $OCH_2CH_2\underline{CH_2}$), 1.72 – 1.81 (m, 2H, $OCH_2\underline{CH_2}CH_2$), 3.54 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.9 Hz, 1H, H-4'), 4.09 (dd, *J* = 8.1, 4.5 Hz, 1H, H-3'), 4.35 (dd, *J* = 11.0, 6.0 Hz, 1H, H-2'), 4.50 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.37 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.15 (d, *J* = 6.3 Hz, 1H, H-1'), 7.83 (s, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 13.6 (CH_3), 18.7 ($OCH_2CH_2\underline{CH_2}$), 30.3 ($OCH_2\underline{CH_2}CH_2$), 61.4 (C-5'), 65.9 ($OCH_2$), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 121.6 (C-6), 150.6 (C-7a), 151.6 (C-2), 162.0 (C-4). HRMS (ESI): calculated for $C_{14}H_{19}ClN_3O_5$ ($[M+H]^+$): 358.1164, found: 358.1164.

4-Pentoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (32) n-Pentanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1 M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **32** (0.065 g, 0.18 mmol) as a white solid in 59 % yield. Melting point: 140 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.89 (t, *J* = 7.2 Hz, 3H, CH_3), 1.30 – 1.49 (m, 4H, CH_2), 1.74 – 1.83 (m, 2H, CH_2), 3.54 (ddd, *J* = 12.0, 5.4, 3.9 Hz, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.11 (m, 1H, H-3'), 4.35 (q, *J* = 5.7 Hz, 1H, H-2'), 4.50 (t, *J* = 6.9 Hz, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.17 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.38 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.15 (d, *J* = 6.0 Hz, 1H, H-1'), 7.83 (s, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 13.9 (CH_3), 21.8 (CH_2), 27.6 (CH_2), 27.9 (CH_2), 61.4 (C-5'), 68.2 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 121.7 (C-6), 150.6 (C-7a), 151.6 (C-2), 162.0 (C-4). HRMS (ESI): calculated for $C_{16}H_{23}ClN_3O_5$ ($[M+H]^+$): 372.1321, found: 372.1313.

4-Phenethoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (33) 2-Phenylethanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3h. After cooling to ambient temperature, the mixture was neutralized with 1 M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **33** (0.10 g, 0.25 mmol) as a white solid in 84 % yield. Melting point: 154 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.10 (t, *J* = 6.6 Hz, 2H, $PhCH_2$), 3.51 – 3.58 (m, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.4, 4.5 Hz, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.06 – 4.11 (m, 1H, H-3'), 4.34 (q, *J* = 5.4 Hz, 1H, H-2'), 4.69 (t, *J* = 6.6 Hz, 1H, OCH_2), 5.07 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.37 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.14 (d, *J* = 6.0 Hz, 1H, H-1'), 7.18 – 7.38 (m, 5H, H_{Phe}), 7.83 (s, 1H, H-6), 8.44 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (CH_2), 61.4 (C-5'), 67.0 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.66, 102.75, 121.8 (C-6), 126.3 (C_{Phe}), 128.3 (2C, C_{Phe}), 129.1 (2C, C_{Phe}), 138.3 (C_{Phe}), 150.6 (C-7a), 151.6 (C-2), 161.8 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_5$ ($[M+H]^+$): 406.1164, found: 406.1164.

4-(2-Methoxyethoxy)-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (34) 2-Methoxyethanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1 M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **34** (0.055 g, 0.15 mmol) as a white solid in 51 % yield. Melting point: 155 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.33 (s, 3H, OCH_3), 3.55 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.1, 3.9 Hz, 1H, H-5'), 3.72 – 3.76 (m, 2H, OCH_2), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.11 (m, 1H, H-3'), 4.35 (q, *J* = 5.7 Hz, 1H, H-2'), 4.62 – 4.65 (m, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.17 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.38 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.16 (d, *J* = 6.3 Hz, 1H, H-1'), 7.85 (s, 1H, H-6), 8.44 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 58.4, 61.4 (C-5'), 65.7, 70.0, 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 121.8

(C-6), 150.7 (C-7a), 151.7 (C-2), 161.8 (C-4). HRMS (ESI): calculated for $C_{14}H_{19}ClN_3O_6$ ([M+H]$^+$): 360.0957, found: 360.0976.

4-(2-Phenoxyethoxy)-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (35) 2-Phenoxyethanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1 M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **35** (0.060 g, 0.14 mmol) as a white solid in 84 % yield. Melting point: 115 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 (ddd, J = 12.0, 5.7, 3.9 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.11 (m, 1H, H-3'), 4.35 (q, *J* = 6.0 Hz, 1H, H-2'), 4.38 – 4.41 (m, 2H, PhOCH_2), 4.83 – 4.86 (m, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.17 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.38 (d, *J* = 6.3 Hz, 1H, OH-5'), 6.16 (d, *J* = 6.3 Hz, 1H, H-1'), 6.91 – 7.01 (m, 3H, H_{Phe}), 7.25 – 7.32 (m, 2H, H_{Phe}), 7.85 (s, 1H, H-6), 8.47 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.3 (C-5'), 64.9 (OCH_2), 65.9 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.7, 102.8, 114.6 (2C, C_{Phe}), 120.8 (C_{Phe}), 121.9 (C-6), 129.5 (2C, C_{Phe}), 150.7 (C-7a), 151.5 (C-2), 158.3 (C_{Phe}), 161.7 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_6$ ([M+H]$^+$): 422.1113, found: 422.1136.

4-Isopropoxy-5-chloro-(N7-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (36) **48** (0.19 g, 0.30 mmol, 1 eq.) was suspended in 0.5 M Na-iOPr/iPrOH solution (7.0 mL). [Na^0 wire (0.12 g) was added to iPrOH (10 mL) under argon and stirred vigorously until the wire was fully dissolved] The resulting mixture was heated at 50 °C for 3 h, after which LC/MS analysis showed full conversion of the starting material. Next, the mixture was neutralized with concentrated HCl solution and evaporated till dryness. The residue was purified by column chromatography 0 → 8 % MeOH/DCM to give **36** (0.037 g, 0.11 mmol) as a white solid in 36 % yield. Melting point: 127 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.38 (dd, *J* = 6.3, 2.1 Hz, 6H, 2 x CH_3), 3.54 (ddd, *J* = 12.0, 5.7, 3.9 Hz, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5'), 3.90 (q, *J* = 3.9 Hz, 1H, H-4'), 4.09 (dd, *J* = 8.1, 4.8 Hz, 1H, H-3'), 4.35 (dd, *J* = 11.4, 6.3 Hz, 1H, H-4'), 5.08 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.36 (d, *J* =

6.3 Hz, 1H, OH-2'), 5.52 (sept., *J* = 6.3 Hz, 1H, CH), 6.14 (d, *J* = 6.0 Hz, 1H, H-1'), 7.81 (s, 1H, H-6), 8.42 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.8 (2C, CH_3), 61.4 (C-5'), 69.3 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.87, 102.90, 121.6 (C-6), 150.7 (C-7a), 151.6 (C-2), 161.7 (C-4). HRMS (ESI): calculated for $C_{14}H_{19}ClN_3O_5$ ($[M+H]^+$): 344.1008, found: 344.0901.

4-Isobutoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (37) 2-Methyl-propan-1-ol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **37** (0.088 g, 0.25 mmol) as a white solid in 82 % yield. Melting point: 184 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.02 (d, *J* = 6.9 Hz, 6H, CH_3), 2.10 (sept., *J* = 6.6 Hz, 1H, CH), 3.53 – 3.57 (br. s, 1H, H-5''), 3.62 – 3.66 (br. s, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.08 – 4.11 (m, 1H, H-3'), 4.29 (d, *J* = 6.3 Hz, 2H, OCH_2), 4.35 (t, *J* = 5.7 Hz, 1H, H-2'), 5.08 (br. s, 1H, OH-5'), 5.19 (br. s, 1H, OH-3'), 5.40 (br. s, 1H, OH-2'), 6.15 (d, *J* = 6.0 Hz, 1H, H-1'), 7.84 (s, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 18.9 (2C, CH_3), 27.5 (CH), 61.4 (C-5'), 70.4 (OCH_2), 72.0 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.9 (C-1'), 102.75, 102.81, 121.7 (C-6), 150.8 (C-7a), 151.7 (C-2), 162.1 (C-4). HRMS (ESI): calculated for $C_{15}H_{21}ClN_3O_5$ ($[M+H]^+$): 358.1164, found: 358.1172.

4-Isopentyloxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (38) Isoamylalcohol (4 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **38** (0.085 g, 0.23 mmol) as a white solid in 76 % yield. Melting point: 150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.95 (d, *J* = 6.6 Hz, 6H, CH_3), 1.68 (q, *J* = 6.6 Hz, 2H, CH_2), 1.83 (sept., *J* = 6.9 Hz, 1H, CH), 3.54 (ddd, *J* = 12.0, 5.7, 3.9 Hz, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.11 (m, 1H, H-3'), 4.35

(q, *J* = 6.0 Hz, 1H, H-2'), 4.54 (t, *J* = 6.6 Hz, 2H, OCH_2), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.16 (d, *J* = 5.1 Hz, 1H, OH-3'), 5.37 (d, *J* = 6.6 Hz, 1H, OH-2'), 6.15 (d, *J* = 5.7 Hz, 1H, H-1'), 7.83 (s, 1H, H-6), 8.44 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.4 (2C, CH_3), 24.7 (CH), 37.0 (CH_2), 61.4 (C-5'), 64.7 (OCH_2), 70.4 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.72, 102.79, 121.7 (C-6), 150.6 (C-7a), 151.6 (C-2), 162.0 (C-4). HRMS (ESI): calculated for $C_{16}H_{23}ClN_3O_5$ ($[M+H]^+$): 372.1321, found: 372.1314.

4-Cyclopentoxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (39) Cyclopentanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **39** (0.030 g, 0.081 mmol) as a white solid in 27 % yield. Melting point: 176 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.58 – 1.85 (m, 6H, CH_2), 1.91 – 2.03 (m, 2H, CH_2), 3.51 – 3.57 (m, 1H, H-5''), 3.60 – 3.66 (m, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.10 (m, 1H, H-3'), 4.35 (t, J = 5.1 Hz, 1H, H-2'), 5.08 (t, *J* = 5.1 Hz, 1H, OH-5'), 5.19 (br. s, 1H, OH-3'), 5.40 (br. s, 1H, OH-2'), 5.62 – 5.68 (m, 1H, OCH), 6.14 (d, *J* = 6.0 Hz, 1H, H-1'), 7.81 (s, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.29 (CH_2), 23.32 (CH_2), 32.36 (CH_2), 32.39 (CH_2), 61.4 (C-5'), 70.4 (OCH), 74.2 (C-3'), 76.7 (C-2'), 85.3 (C-4'), 86.8 (C-1'), 102.9, 103.1, 121.6 (C-6), 150.6 (C-7a), 151.6 (C-2), 161.7 (C-6). HRMS (ESI): calculated for $C_{16}H_{21}ClN_3O_5$ ($[M+H]^+$): 370.1164, found: 370.1174.

4-Cyclohexyloxy-5-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (40) Cyclohexanol (4.0 mL) was added to solid NaOtBu (0.12 g, 1.2 mmol, 4 eq.) and stirred under inert atmosphere. Then, **48** (0.19 g, 0.30 mmol, 1eq.) was added and the resulting mixture heated to 70 °C for 2 – 3 h. After cooling to ambient temperature, the mixture was neutralized with 1M aq. HCl solution and evaporated till dryness. The residue was adsorbed onto Celite® from MeOH solution and purified by column chromatography 0.5 → 6 % MeOH/DCM to give **40** (0.025 g, 0.065 mmol) as a yellowish solid in 22 % yield. Melting point: 183 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.37 – 1.54 (m, 4H, CH_2), 1.60 – 1.79

(m, 4H, CH_2), 1.89 – 1.96 (m, 2H, CH_2), 3.52 – 3.56 (m, 1H, H-5''), 3.61 – 3.66 (m, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.10 (m, 1H, H-3'), 4.35 (t, *J* = 5.7 Hz, 1H, H-2'), 5.08 (br. s, 1H, OH-5'), 5.17 (br. s, 1H, OH-3'), 5.38 (br. s, 1H, OH-2'), 5.32 – 5.40 (m, 1H, CH), 6.14 (d, *J* = 6.0 Hz, 1H, H-1'), 7.82 (s, 1H, H-6), 8.41 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.7 (2C), 25.1, 30.8 (2C), 61.4 (C-5'), 70.4 (OCH), 73.2 (C-3'), 74.2 (C-2'), 85.3 (C-4'), 86.9 (C-1'), 102.9, 103.0, 121.6 (C-6), 150.7 (C-7a), 151.7 (C-2), 161.5 (C-2). HRMS (ESI): calculated for $C_{17}H_{22}ClN_3O_5$ ($[M+H]^+$): 384.1321, found: 384.1330.

3'-Deoxyinosine (41)[60] NaI (1.1 g, 7.5 mmol, 10 eq.) was dissolved in anhydrous MeCN (7.5 mL) and stirred for 5 min under argon. Next, α-acetoxyisobutyrylchloride (0.38 mL, 2.6 mmol, 3.5 eq.) was added, giving a white precipitate. The mixture was stirred vigorously for another 5 – 10 min, after which inosine (0.20 g, 0.75 mmol, 1 eq.) was added in one portion. The resulting mixture was stirred for 1.5 h after which TLC showed full conversion of SM. The mixture was poured in aq. sat. $NaHCO_3$/aq. sat. $Na_2S_2O_3$ (30 mL) solution. Next, $CHCl_3$ (30 mL) was added, and the layers separated. The water layer was extracted with $CHCl_3$ (30 mL) twice more. Organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was dissolved in MeOH (3 mL) and 1M aq. NaOAc solution (1 mL) was added. Next, the flask was purged with N_2, after which a cat. amount of $Pd(OH)_2$/C was added. Next, the N_2-atmosphere was exchanged for H_2 (balloon; no bubbling) and the mixture stirred overnight. Next, the mixture was purged with N_2 to remove residual H_2-gas and filtered over a pad of Celite®. The mixture was evaporated till dryness and partitioned between EtOAc (25 mL) and aq. sat. $NaHCO_3$/aq. sat. $Na_2S_2O_3$ solution (1:1, 20 mL). Layers were separated, and the water layer extracted twice more with EOAc (25 mL). Organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was dissolved in 7 N NH_3 in MeOH and stirred overnight. The solvent was removed, and the residue purified by column chromatography (2 → 20 % MeOH/DCM) to afford **41** as a white solid (0.062 g, 0.25 mmol) in 33% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.90 (1H, ddd, *J* = 13.2, 6.2, 2.1 Hz, H-3'), 2.21 (1H, ddd, *J* = 13.2, 9.4, 5.6 Hz, H-3''), 3.53 (1H, m, H-5'), 3.69 (1H, m, H-5''), 4.15 - 4.43 (1H, m, H-4'), 4.50 (1H, br. s., H-2'), 5.03 (1H, br. s., OH-5'), 5.69 (1H, br. s., OH-3'), 5.86 (1H,

d, *J* = 2.1 Hz, H-1'), 8.06 (1H, s, H-2), 8.33 (1H, s, H-8), 12.04 (1H, br. s, NH). Spectral data are in accordance with literature values.[60]

4-Oxo-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (42) **5**[20] (0.060 g, 0.24 mmol, 1 eq.) was added to a 5-mL flame-dried round bottom flask equipped with a stir bar. Then, Next, **61**[52-53] (2 eq.) was added, followed by anhydrous pyridine (6 mL/mmol SM). After complete dissolution of the solids, TMSCl (1.91 eq.) was added and the mixture heated at 100°C till full conversion of the starting material was observed (which was generally 24 h) The mixture was cooled to ambient temperature and another portion of TMSCl (2 eq.) was added. After stirring for 15 min, the mixture was evaporated till dryness. Next, the residue was taken up in ice-cold DCM and successively washed with brine/aq. sat. $NaHCO_3$ and twice brine/aq. 1 M HCl. The organic layer was dried over Na_2SO_4, filtered and evaporated. The intermediate N6-(1,2,4-triazol-4-yl)-nucleoside was directly used in nucleophilic displacement reactions without further purification: 3.0 mL of aq. 1 M NaOH was added and stirred until full conversion was observed, yielding **42** (0.012 g, 0.048 mmol) as a white solid in 20 % yield. (Purification: 2 → 12 % MeOH/DCM). Melting point: 248 – 250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.90 (ddd, *J* = 13.2, 6.0, 3.0 Hz, 1H, H-3''), 2.17 (ddd, *J* = 13.2, 9.0, 6.0 Hz, 1H, H-3'), 3.50 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.63 (ddd, *J* = 11.7, 5.7, 3.6 Hz, 1H, H-5'), 4.08 – 4.31 (m, 1H, H-4'), 4.32 – 4.38 (m, 1H, H-2'), 4.94 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.56 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.01 (d, *J* = 2.4 Hz, 1H, H-1'), 6.50 (d, *J* = 3.6 Hz, 1H, H-5), 7.36 (d, *J* = 3.6 Hz, 1H, H-6), 7.91 (s, 1H, H-2), 11.94 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.8 (C-3'), 62.8 (C-5'), 75.3 (C-2'), 80.1 (C-4'), 90.4 (C-1'), 102.2 (C-5), 108.2 (C-4a), 120.8 (C-6), 143.7 (C-7a), 147.1 (C-2), 158.2 (C-4). HRMS (ESI): calculated for $C_{11}H_{14}N_3O_4$ ($[M+H]^+$): 252.0979, found: 252.0979.

4-Oxo-5-bromo-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (43) **46** (0.050 g, 0.15 mmol) was suspended in anhydrous MeCN (5.0 mL, 30 mL/mmol SM) and NaI (0.087 g, 0.58 mmol, 4 eq.) was added. Next, TMSCl (0.075 mL, 0.58 mmol, 4 eq.) was added and the mixture stirred at ambient temperature till full conversion was observed by LC/MS (~approx. 1.5 h). The mixture was evaporated till dryness and co-evaporated with MeOH. Purification by column chromatography (1 → 15 % MeOH/DCM) gave **43** (0.010 g, 0.030 mmol) as a white solid in 21 % yield. Melting point: 268 °C. ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 1.86 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.18 (ddd, *J* = 13.2, 8.7, 5.7 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.4, 3.3 Hz, 1H, H-5'), 4.25 – 4.75 (m, 2H, H-4', H-2'), 5.00 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.59 (d, *J* = 4.5 Hz, 1H, OH-2'), 5.99 (d, *J* = 2.4 Hz, 1H, H-1'), 7.56 (s, 1H, H-6), 7.94 (s, 1H, H-2), 12.09 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.2 (C-3'), 62.4 (C-5'), 75.3 (C-2'), 80.4 (C-4'), 90.0 (C-1'), 90.3 (C-5), 106.0 (C-4a), 120.4 (C-6), 144.8 (C-2), 146.5 (C-7a), 157.2 (C-4). HRMS (ESI): calculated for $C_{11}H_{13}BrN_3O_4$ ($[M+H]^+$): 330.0084, found: 330.0080.

2-Amino-4-oxo-7-(3-deoxy-β-D-ribofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine (44)[61] NaI (0.21 g, 1.4 mmol, 10 eq.) was dissolved in anhydrous MeCN (5 mL) and stirred for 5 min under argon. Next, α-acetoxyisobutyrylchloride (0.072 mL, 0.47 mmol, 3.5 eq.) was added, giving a white precipitate. The mixture was stirred vigorously for another 5 – 10 min, after which **18** (0.040 g, 0.14 mmol, 1.0 eq.) was added in one portion. The resulting mixture was stirred for 1.5 h after which TLC showed full conversion of SM. The mixture was poured in aq. sat. $NaHCO_3$/aq. sat. $Na_2S_2O_3$ (30 mL) solution. Next, $CHCl_3$ (30 mL) was added, and the layers separated. The water layer was extracted with $CHCl_3$ (30 mL) twice more. Organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was dissolved in MeOH (3 mL) and 1M aq. NaOAc solution (1 mL) was added. Next, the flask was purged with N_2, after which a cat. amount of $Pd(OH)_2$/C was added. Next, the N_2-atmosphere was exchanged for H_2 (balloon; no bubbling) and the mixture stirred overnight. Next, the mixture was purged with N_2 to remove residual H_2-gas and filtered over a pad of Celite®. The mixture was evaporated till dryness and partitioned between EtOAc (25 mL) and aq. sat. $NaHCO_3$/aq. sat. $Na_2S_2O_3$ solution (1:1, 20 mL). Layers were separated, and the water layer extracted twice more with EOAc (25 mL). Organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was dissolved in 7N NH_3 in MeOH and stirred overnight. The solvent was removed, and the residue purified by column chromatography (4 → 20 % MeOH/DCM) to afford **44** as a white solid (0.0080 g, 0.030 mmol) in 21% yield. ^{1}H NMR (300 MHz, D_2O) δ 2.13 (ddd, *J* = 13.5, 6.4, 3.5 Hz, 1H, H-3'), 2.27 (ddd, *J* = 14.6, 8.5, 6.4 Hz, 1H, H-3''), 3.66 (dd, *J* = 12.6, 5.0 Hz, 1H, H-5'), 3.78 (dd, *J* = 12.0, 3.2 Hz, 1H, H-5''), 4.39 -

4.54 (m, 1H, H-4'), 4.58 - 4.69 (m, 1H, H-2'), 5.94 (d, *J* = 3.2 Hz, 1H, H-1'), 6.48 (d, *J* = 3.8 Hz, 1H, H-5), 6.94 (d, *J* = 3.8 Hz, 1H, H-6). Spectral data are in accordance with literature values.[61]

4-Methoxy-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (45) 45 was prepared as described for **42**: **5**[20] (0.063 g, 0.25 mmol) was derivatized and the intermediate reacted with 6 mL of 0.5 M NaOMe/MeOH, which yielded **45** (0.044 g, 0.16 mmol) as a white solid in 66 % yield. (Purification: 0.5 → 6 % MeOH/DCM). Melting point: 153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.92 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.22 (ddd, *J* = 12.9, 8.7, 6.0 Hz, 1H, H-3'), 3.52 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.7, 3.6 Hz, 1H, H-5'), 4.04 (s, 3H, OMe), 4.27 – 4.35 (m, 1H, H-4'), 4.39 – 4.45 (m, 1H, H-2'), 4.98 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.59 (d, *J* = 4.5 Hz, 1H, H-1'), 6.14 (d, *J* = 2.7 Hz, 1H, H-1'), 6.56 (d, *J* = 3.6 Hz, 1H, H-5), 7.66 (d, *J* = 3.6 Hz, 1H, H-6), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 53.4 (OCH_3), 62.7 (C-5'), 75.1 (C-2'), 80.1 (C-4'), 90.4 (C-1'), 98.4 (C-5), 105.0 (C-4a), 124.5 (C-6), 150.5 (C-2), 151.3 (C-7a), 162.2 (C-4). HRMS (ESI): calculated for $C_{12}H_{16}N_3O_4$ ($[M+H]^+$): 266.1135, found: 266.1132.

4-Methoxy-5-bromo-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (46) 62[39] (0.16 g, 0.32 mmol) was suspended in 0.5 M NaOMe/MeOH solution (7 mL) and heated at 50 °C for 1.5 h, after which the solution was cooled to ambient temperature and neutralized with aq. 0.5 M HCl. Then, the mixture was evaporated till dryness and purified by column chromatography (1 → 5 % MeOH/DCM) to give **46** (0.077 g, 0.22 mmol) as a white solid in 69 % yield. Melting point: 164 – 166°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.89 (ddd, *J* = 12.9, 6.0, 3.0 Hz, 1H, H-3''), 2.22 (ddd, *J* = 12.9, 8.7, 6.0 Hz, 1H, H-3'), 3.44 – 3.54 (m, 1H, H-5''), 3.66 – 3.72 (m, 1H, H-5'), 4.06 (s, 3H, OCH_3), 4.28 – 4.35 (m, 1H, H-4'), 4.40 (br. s, 1H, H-2'), 5.04 (br. s, 1H, OH-5'), 5.62 (br. s, 1H, OH-2'), 6.13 (d, *J* = 2.1 Hz, 1H, H-1'), 7.89 (s, 1H, H-6), 8.46 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.1 (C-3'), 53.8 (OCH_3), 62.4 (C-5'), 75.1 (C-2'), 80.4 (C-4'), 86.1 (C-5), 90.4 (C-1'), 104.0 (C-4a), 124.0 (C-6), 150.4 (C-7a), 151.3 (C-2), 162.2 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}BrN_3O_4$ ($[M+H]^+$): 344.0240, found: 344.0231.

4-Methoxy-5-fluoro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (51)[34] **47**[34] (0.25 g, 0.40 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (1 mL). MeOH (4 mL) was added, followed by NaOMe (5.4 M

in MeOH, 0.4 mL). After 2 h, TLC analysis indicated completion of the reaction. The reaction mixture was neutralized to pH 7 via the addition of 1M aq. HCl. Celite® (2 g) was added and the mixture was concentrated *in vacuo*. The residue was purified via flash column chromatography (0 → 1% MeOH/DCM) to afford **51** as a colourless oil (0.078 g, 0.26 mmol) in 65% yield. Spectral data were in accordance with literature values.[34] HRMS (ESI): calculated for $C_{12}H_{15}FN_3O_5$ ($[M+H]^+$): 300.0990, found: 300.0993.

4-Chloro-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (52) A solution of **50** (0.46 g, 0.64 mmol, 1.0 eq.) in THF (10 mL) was cooled to -10°C. iPrMgCl·LiCl (1.3 M in THF, 0.61 mL, 0.79 mmol, 1.25 eq.) was added dropwise. After 30 minutes, the reaction was poured into a separation funnel containing sat. aq. NH_4Cl (30 mL). The mixture was extracted with CH_2Cl_2 (3 x 50 mL), the combined organic phases dried over Na_2SO_4 and concentrated *in vacuo*. The residue was used as such in the next reaction.

4-Chloro-5-trifluoromethyl-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (53) $TMSCF_3$ (0.19 mL, 1.3 mmol, 3.0 eq.) was added dropwise over the course of 1 hour to a suspension of CuI (0.521 g, 1.30 mmol, 1.0 eq.) and KF (0.076 g, 1.3 mmol, 3.0 eq.) in a mixture of dry degassed DMF/NMP 1:1 (3 mL). when all solids had dissolved, **50** (0.31 g, 0.43 mmol, 1.0 eq.) in dry degassed DMF/NMP 1:1 (3 mL) was added, and the mixture was heated to reflux. After 2 hours, LC/MS analysis showed full conversion of the starting material, and the reaction was cooled to room temperature. The mixture was diluted with EtOAc (15 mL) and H_2O (5 mL) and the solids were filtered off over Celite®. The filter cake was washed extensively with additional EtOAc (3 x 25 mL), and the combined filtrates were transferred to a separation funnel. Additional water (40 mL) was added, the phases separated, and the organic phase washed twice more with water (25 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified via flash column chromatography (0 → 30 % EtOAc in petroleum ether) to afford **53** as a colourless oil (0.10 g, 0.15 mmol) in 36% yield. HRMS (ESI): calculated for $C_{33}H_{24}ClF_3N_3O_7$ ($[M+H]^+$): 666.1249, found: 666.1244.

2-Pivaloylamino-4-chloro-5-bromo-N7-(2',3',5'-tri-*O*-benzoyl)-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (54)[43] 2-Pivaloylamino-4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine[48] (0.67 g, 2.0 mmol, 1 eq.) was suspended in anhydrous MeCN (14 mL, 7 mL/mmol SM) under argon. Then, BSA (0.59 mL, 2.4 mmol, 1.21 eq.) was added. The mixture was stirred for ~10 min at ambient temperature, after which a solution was obtained. Next, a first portion of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.68 g, 1.3 mmol, 0.67 eq.) was added, followed by TMSOTf (0.47 mL, 2.6 mmol, 1.3 eq.). The resulting mixture was heated at 50 °C, and every 8 h another portion of glycosyl donor (0.67 eq. to surmount to 2 eq.) was added. After 24 h at 50°C, the mixture was cooled to ambient temperature, DCM and sat. aq. $NaHCO_3$ added. The layers were separated, and the water layer extracted twice more with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Next, the residue was purified by column chromatography 100 % DCM to give **54** (1.0 g, 1.3 mmol) as a white foam in 65 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.66 (dd, *J* = 11.7, 5.7 Hz, 1H, H-5''), 4.77 – 4.88 (m, 2H, H-4', H-5'), 6.37 (dd, *J* = 6.0, 3.9 Hz, 1H, H-3'), 6.45 (t, *J* = 6.0 Hz, 1H, H-2'), 6.53 (d, *J* = 3.9 Hz, 1H, H-1'), 7.40 – 7.49 (m, 6H, OBz), 7.60 – 7.68 (m, 3H, OBz), 7.89 – 7.94 (m, 6H, OBz), 8.07 (s, 1H, H-6), 10.38 (br. s, 1H, NH). Spectral data are in accordance with literature values.[43]

2-Amino-4-chloro-N7-(2',3'-O-isopropylidene-5'-O-tertbutyldimethylsilyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (56) 5-*O*-tert-Butyldimethylsilyl-2,3-*O*-isopropylidene-α,β-D-ribofuranose[47] (0.64 g, 2.0 mmol, 1.0 eq.) was dissolved in THF (8.0 mL). The solution was cooled to -78 °C, after which CCl_4 (0.29 mL, 3.0 mmol, 1.5 eq.) was added, followed by dropwise addition of hexamethylphosphorus triamide (0.40 mL, 2.2 mmol, 1.1 eq.). After 15 minutes, the temperature was raised to -45°C to prevent solidification of the reaction mixture. After 30 more minutes, TLC analysis (Petroleum ether/EtOAc 8:2) indicated full conversion of the starting material. The temperature was further raised to 0°C and added to a premixed (30 min.) suspension of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (0.67 g, 4.0 mmol, 2.0 eq.) and NaH (60 wt. % in mineral oil, 0.16 g, 4.0 mmol, 2.0 eq.) in MeCN (30 mL). The reaction mixture was stirred overnight and concentrated *in vacuo* until most of the MeCN was removed. Water (30 mL) and EtOAc (50 mL) were added, and the mixture was transferred to a separatory funnel. The phases were separated and the aqueous layer extracted twice more

with EtOAc (50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, adsorbed onto Celite® and purified by flash column chromatography (Petroleum ether/EtOAc 85:15 and 8:2) to afford **56** as a yellow sticky solid (0.40 g, 0.87 mmol) in 44% yield. HRMS (ESI): calculated for $C_{20}H_{32}ClN_4O_4Si$ ($[M+H]^+$): 455.1876, found: 455.1874.

2-Amino-4-chloro-5-bromo-N7-(2',3'-O-isopropylidene-5'-O-tertbutyldimethylsilyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (57) 5-*O*-tert-Butyldimethylsilyl-2,3-*O*-isopropylidene-α,β-D-ribofuranose[47] (0.41 g, 1.3 mmol, 1.0 eq.) was dissolved in THF (6 mL). The solution was cooled to -78 °C, after which CCl_4 (0.19 mL, 2.0 mmol, 1.5 eq.) was added, followed by dropwise addition of hexamethylphosphorus triamide (0.26 mL, 1.4 mmol, 1.1 eq.). After 15 minutes, the temperature was raised to -45°C to prevent solidification of the reaction mixture. After 30 more minutes, TLC analysis (Petroleum ether/EtOAc 8:2) indicated full conversion of the starting material. The temperature was further raised to 0°C and added to a premixed (30 min.) suspension of 2-amino-4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine[43] (0.322 g, 1.3 mmol, 1.0 eq.), powdered KOH (0.12 g, 2.2 mmol, 1.7 eq.) and TDA-1 (0.042 mL, 0.13 mmol, 0.1 eq.) in MeCN (8 mL). The reaction mixture was stirred overnight and filtered over Celite®. The filtrate was concentrated *in vacuo*, and the residue dissolved in CH_2Cl_2, adsorbed onto Celite® and purified by flash column chromatography (0 → 20% EtOAc in petroleum ether) to afford **57** as a colourless oil (0.26 g, 0.48 mmol) in 37% yield. HRMS (ESI): calculated for $C_{20}H_{31}BrClN_4O_4Si$ ($[M+H]^+$): 533.0981, found: 533.0997.

2-Methyl-4-chloro-N7-(2',3'-O-isopropylidene-5'-O-tertbutyldimethylsilyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (58) 5-*O*-tert-Butyldimethylsilyl-2,3-*O*-isopropylidene-α,β-D-ribofuranose[47] (1.47 g, 4.63 mmol, 1.5 eq.) was dissolved in THF (20 mL). The solution was cooled to -78 °C, after which CCl_4 (0.672 mL, 6.94 mmol, 2.25 eq.) was added, followed by dropwise addition of hexamethylphosphorus triamide (0.926 mL, 5.09 mmol, 1.65 eq.). After 15 minutes, the temperature was raised to -45 °C to prevent solidification of the reaction mixture. After 30 more minutes, TLC analysis (Petroleum ether/EtOAc 8:2) indicated full conversion of the starting material. The temperature was further raised to 0 °C and added to a pre-mixed (30 min) suspension of 2-methyl-4-chloro-7*H*-

pyrrolo[2,3-*d*]pyrimidine (0.472 g, 3.09 mmol, 1.0 eq.), powdered KOH (0.42 g, 7.5 mmol, 2.5 eq.) and TDA-1 (0.49 mL, 0.55 mmol, 0.5 eq.) in MeCN (20 mL). The reaction mixture was stirred overnight and poured into a separation funnel containing sat. aq. NH_4Cl (60 mL), H_2O (30 mL). The mixture was extracted twice with toluene (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2, adsorbed onto Celite® and purified by flash column chromatography (5 % EtOAc in petroleum ether) to afford **58** as a colourless oil (0.68 g, 1.5 mmol) in 48 % yield. HRMS (ESI): calculated for $C_{21}H_{33}ClN_3O_4Si$ ($[M+H]^+$): 454.1923, found: 454.1934.

2-Methyl-4-chloro-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (59)[62] **58** (0.68 g, 1.5 mmol, 1.0 eq.) was dissolved in TFA (4.5 mL). H_2O (0.5 mL) was added, and the mixture was stirred for 1 h at room temperature. The mixture was concentrated *in vacuo* and co-evaporated three times with toluene to remove all TFA. The residue was taken up in MeOH, adsorbed onto Celite® and purified by flash column chromatography (0 → 10% MeOH/DCM). The residue was then lyophilized to afford **59** as a white solid (0.19 g, 0.64 mmol) in 43% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.64 (s, 3H, CH_3), 3.55 (dd, *J* = 12.0, 4.0 Hz, 1H, H-5'), 3.63 (dd, *J* = 12.0, 4.1 Hz, 1H, H-5''), 3.93 (q, *J* = 3.8 Hz, 1H, H-4'), 4.11 (dd, *J* = 5.1, 3.1 Hz, 1H, H-3'), 4.42 (dd, *J* = 6.3, 5.1 Hz, 1H, H-2'), 6.17 (d, *J* = 6.4 Hz, 1H, H-1'), 6.67 (d, *J* = 3.8 Hz, 1H, H-5), 7.87 (d, *J* = 3.8 Hz, 1H, H-6). Spectral data are in accordance with literature values.[62]

2-Methyl-4-amino-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (60)[62] A mixture of **59** (0.11 g, 0.37 mmol, 1.0 eq.) and NaN_3 (0.049 g, 0.75 mmol, 2.0 eq.) in DMF (3 mL) was heated to 80 °C. After 5 hours, the reaction was cooled to room temperature. PMe_3 (1.0M in THF, 0.93 mL, 0.93 mmol, 2.5 eq.) and H_2O (0.1 mL) were added and the reaction was stirred for another 3 hours. The mixture was concentrated *in vacuo*, and co-evaporated with toluene three times. The residue was dissolved in MeOH, pre-absorbed onto Celite® and purified by flash column chromatography (2 → 20% MeOH/DCM) to afford **60** as a white solid (0.065 g, 0.23 mmol) in 62% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 3.52 (dd, *J* = 12.0, 3.8 Hz, 1H, H-5'), 3.62 (dd, *J* = 12.0, 4.1 Hz, 1H, H-5''), 3.91 (dd, *J* = 5.9, 3.8 Hz, 1H, H-4'), 4.08 (dd, *J* = 5.0, 2.6 Hz, 1H, H-3'), 4.45 (dd, *J* = 6.2, 5.0 Hz, 1H, H-2'), 5.95 (d, *J*

= 6.4 Hz, 1H, H-1'), 6.63 (d, *J* = 3.5 Hz, 1H, H-5), 7.32 (d, *J* = 3.8 Hz, 1H, H-6), 7.44 (br. s., 2H, NH_2). Spectral data are in accordance with literature values.[35]

1,2-Bis[(dimethylamino)methylene]hydrazine (61)[52-53] Anhydrous DMF (75 mL, 70.8 g, 96.9 mmol, 2 eq.) was added to a flame-dried round bottom flask under argon. The flask was cooled to 0 °C in an ice bath. After stirring at that temperature for ~20 min, $SOCl_2$ (14.3 mL, 23.3 g, 196 mmol, 4 eq.) was added dropwise. After complete addition, the ice bath was removed, and the resulting mixture stirred at ambient temperature for 24 h. Next, aq. NH_2NH_2 (2.5 mL, 50 mmol, 1 eq.) as a solution in DMF (10 mL) was added carefully in a dropwise fashion. After complete addition, the mixture was stirred at ambient temperature for 2 days, after which the resulting precipitate was filtered. The solids were washed with DMF once and subsequently with Et_2O. The solid was dried under vacuum to give the intermediate dihydrochloride salt (8.50 g, 39.5 mmol) in 79 % yield. Next, this intermediate (4.33 g, 20.1 mmol) was stirred with 1M aq. NaOH sol. (70 mL) for ~20 min. Then, $CHCl_3$ (50 mL) was added, and the layers separated. The water layer was extracted twice more with $CHCl_3$ (50 mL), the organic layers combined, dried over Na_2SO_4, filtered and evaporated till dryness to give **61** (2.50 g, 17.6 mmol) as a white solid in 87 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.72 (s, 12H, CH_3), 7.69 (s, 2H, CH). Spectral data are in accordance with literature values.[52]

Biology

Trypanosoma brucei

Drug susceptibility tests with Lister 427WT, TbAT1-KO, B48 and ISMR1 were performed exactly as described previously,[63] with the viability indicator dye resazurin (Alamar blue) in 96-well plates as the assay endpoint read-out. Each well contained 2×10^4 cells. Plates were incubated for 48 h with a doubling dilution series of the nucleoside test compounds in HMI-9 medium supplemented with FBS at 37 ºC/5 % CO_2 (23 dilutions starting at 100 µM, except for the pentamidine control (50 µM)). After 48 h, resazurin was added to each well and the plates were further incubated for 24 h. Fluorescence was then determined using a FLUOstar Optima (BMG Labtech, Durham, NC) and the results fitted to a sigmoid curve with variable slope using Prism 5.0 (GraphPad, San Diego, Ca) to derive an EC_{50} value.

Susceptibility of *T. brucei* Squib 427 or *T. b. rhodesiense* STIB-900 parasites to the nucleoside analogues was assayed essentially as described above except for the use of 10 concentrations of a 4-fold compound dilution series starting at an initial concentration of 64 µM. *T. brucei* Squib 427 parasites were seeded at 1.5×10^4 parasites/well while *T. b. rhodesiense* parasites were seeded at 4×10^3 parasites/well. After incubation for 3 days, the viability indicator resazurin was added and read-out was performed after incubating for another 24 h (*T. brucei*) or 6 h (*T. b. rhodesiense*).

Cytotoxicity on MRC-5 fibroblasts[17]

Drug cytotoxicity assays were performed in MRC-5_{SV2} human embryonic lung fibroblasts, which were cultured in Minimum Essential Medium with Earle's salts-medium, supplemented with L-glutamine, $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C with 5% CO_2. To each well containing 190 µl of MRC-5 $_{SV2}$ (3 x 10^4 cells/mL) was added the appropriately diluted (with water) compound (10 µL). After 3 days of incubation, cell viability was assessed fluorimetrically after addition of 50 µL resazurin per well. After 4 h at 37 °C, fluorescence was measured (λ_{ex} 550 nm, λ_{em} 590 nm). Cell growth was determined and compared to untreated-control wells (100 % cell growth) and medium-control wells (0 % cell growth). The results were expressed as percentage reduction in cell growth / viability compared to control wells after which an EC_{50} was determined. Tamoxifen was used as reference compound (data not shown).

Transport studies

The inhibition of uptake of [^{3}H]-adenosine by different concentration of test compound was performed by the method reported previously.[64] Briefly, 50 nM 2,8-[^{3}H]-adenosine (American Radiolabelled Chemicals, 40 Ci/mmol) was added to 10^7 bloodstream form *T. brucei* cells, layered on top of an oil layer (di-n-butylphtalate (DBH)/light mineral oil (Sigma)). After the addition of the test compound, uptake was terminated after 60 s by the addition of 1 mL ice-cold 250 µM unlabelled adenosine solution followed by immediate centrifugation through the oil layer, separating the cells from the uptake medium. After flash freezing in liquid nitrogen, the cell pellet was collected by cutting of the tip of the microfuge tube into a scintillation vial. Then, radioactivity was determined in a Hidex 300SL scintillation counter (Lablogic, Sheffield, UK).

Ancillary information

Supporting information

Copies of ^{1}H, ^{13}C spectra of compounds **15**, **17**, **27**, **29-40**, **42**, **43**, **45** and **46** can be found in the Supporting Information.

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Abbreviations used

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; HAT, Human Africant Trypanosomiasis; TMSOTf, Trimethylsilyl trifluoromethanesulfonate; TPPTS, Trisodium 3-bis(3-sulfonatophenyl)phosphanylbenzenesulfonate.

Declaration of interest

The authors declare that they have no competing interests.

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