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Revisiting tubercidin against kinetoplastid parasites: aromatic substitutions at position 7 improve activity and reduce toxicity

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Abstract

The nucleoside antibiotic tubercidin displays strong activity against different target organisms, but it is notoriously toxic to mammalian cells. However, the effects of tubercidin against *T. brucei* parasites inspired us to synthesize several C7 substituted analogs for *in vitro* evaluation, in order to find suitable hit compounds. C7 Deazaadenosines substituted with electron-poor phenyl groups were found to have micromolar activity against *T. brucei in vitro*. Replacement of the phenyl for a pyridine ring gave compound **13** with submicromolar potency and much-attenuated cytotoxicity compared to tubercidin. The veterinary pathogen *T. congolense* was equally affected by **13** *in vitro*. Transporter studies in *T. b. brucei* indicated that **13** is taken up efficiently by both the P1 and P2 adenosine transporters, making the occurrence of transporter-related resistance and cross-resistance with diamidine drugs such as diminazene aceturate and pentamidine as well as with melaminophenyl arsenicals unlikely. Evaluation of the *in vitro* metabolic stability of analog **13** indicated that this analog was significantly metabolized in mouse microsomal fractions, precluding further *in vivo* evaluation in mouse models of HAT.

Keywords

7-deazapurine nucleosides

Negishi cross coupling

Trypanosoma brucei brucei

Trypanosoma cruzi

Introduction

Human African Trypanosomiasis (HAT) is a deadly infectious disease that is prevalent in the African continent and caused by the parasites *Trypanosoma brucei rhodesiense* (East- and Southern Africa) and *Trypanosoma brucei gambiense* (West- and Central Africa) and transmitted by bites of infected tsetse flies. HAT exhibits two characteristic disease stages that are linked to parasite distribution in the body. Initially, parasites reside in the hemolymphatic system and cause rather non-specific symptoms (e.g. general malaise and fever) often leading to incorrect diagnosis. The second phase consists of parasites invading the central nervous system, causing severe neurological symptoms, including altered sleep/wake cycles, hence the name 'sleeping sickness'. If left untreated, HAT is almost invariably fatal.[1]

Treatment of HAT is cumbersome and extremely ineffective at present. Some of the approved drugs are only effective in stage I (suramin and pentamidine against *rhodesiense* and *gambiense* HAT, respectively), necessitating stage diagnosis via a risky lumbar puncture. For stage II disease, currently three drugs/drug combinations are approved: melarsoprol, eflornithine and nifurtimox/eflornithine, but eflonithine mono-therapy has been superseded by the nifurtimox combination. Melarsoprol suffers from a high toxicity burden and from high levels of drug resistance across Africa.[2] Recently, two novel entities, fexinidazole[3-4] and oxaborole SCYX-7158, have reached phase II/III clinical trials and could greatly improve treatment options if approved.[5-6] However, fexinidazole resistance is easily induced and shows cross-resistance with nifurtimox,[7] as both nitro drugs exhibit a similar mode-of-action.[6] These findings highlight the ever-pressing need for novel therapies, especially from other structural classes, of which a few examples were published in recent years.[8-12]

Nucleoside analogs have received considerable interest over the past six decades, with respect to many therapeutic areas but particularly as antiviral and anti-tumor agents.[13-14] Because most protozoan parasites lack the enzymes for *de novo* purine synthesis, they depend on purine salvage and may be especially vulnerable to the effects of purine nucleoside analogs. In this regard, potential inhibitors for enzymes of the salvage pathway (e.g. UAMC-00363),[15-17] as well as so-called 'subversive' substrates[18-21] (that are activated by the parasite's salvage pathway enzyme(s) before exerting their toxic effect), bearing a nucleoside structure have been conceived or discovered by screening efforts (Figure 1).[22] One

such subversive analog is the naturally occurring nucleoside antibiotic tubercidin (**1**).[22-24] Being a close mimic of adenosine, this analog was found to exert a plethora of biological effects,[25-27] but it is also overtly toxic to mammalian cells and hence of little practical value.

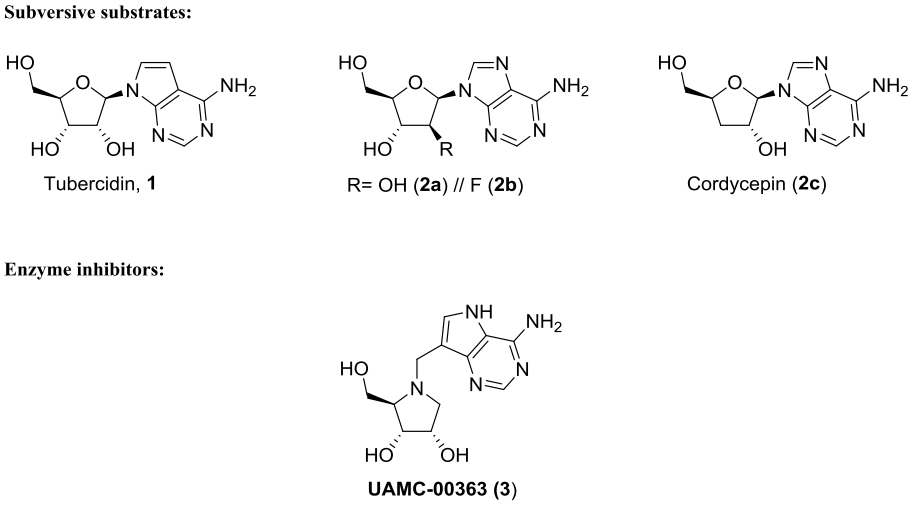


Figure 1: Examples of nucleoside analogs with activity against *T. brucei*.

Inspired by recent work of Hocek and colleagues,[28-29] in which it was found that selected C7 substituted 7-deazaadenosines [in the body of the text, purine numbering will be used; while in the experimental section IUPAC pyrrolo[2,3-*d*]pyrimidine numbering will be employed] are at most mildly cytotoxic to both tumor and fibroblast cells, we decided to prepare a small subset of C7 phenyl-substituted 7-deazaadenosines and evaluate their *in vitro* effect against *T. brucei*. In this work, we describe our efforts to optimize the *in vitro* potency from the initial hits to arrive at a new lead compound, **13**. An overview of all prepared nucleoside analogs in this study is presented in Figure 2.

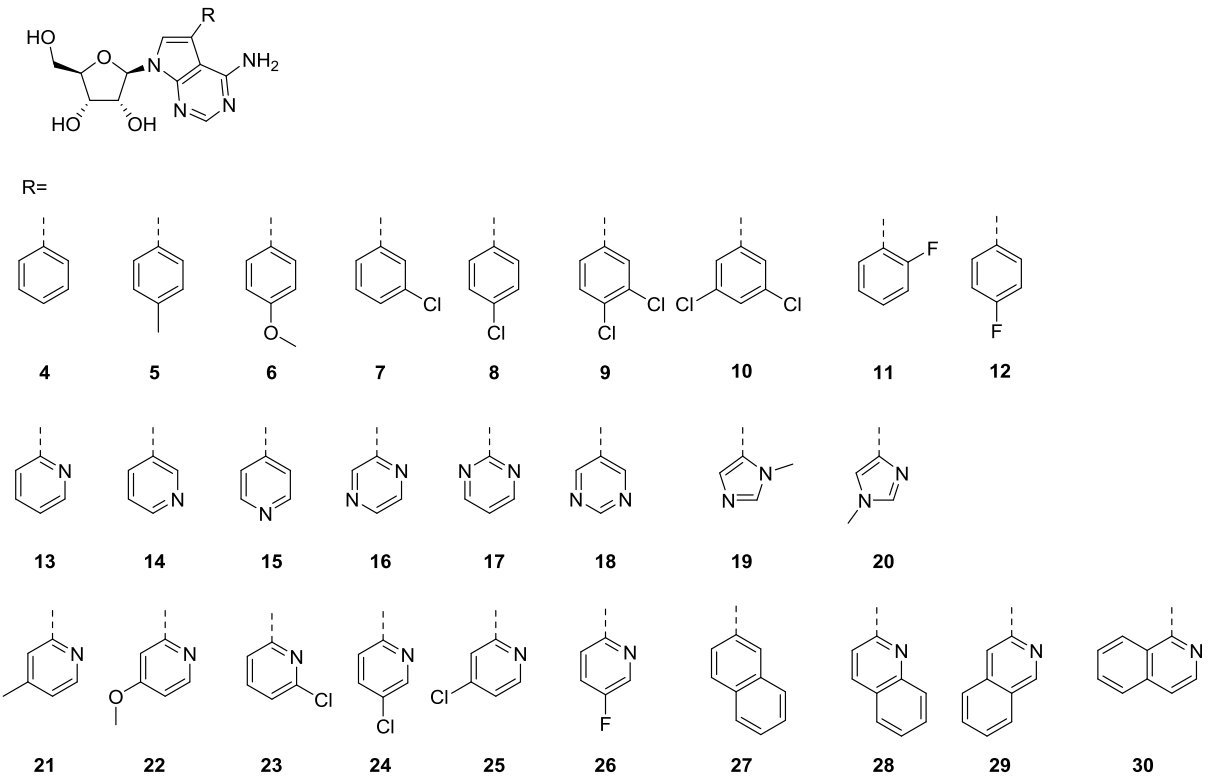
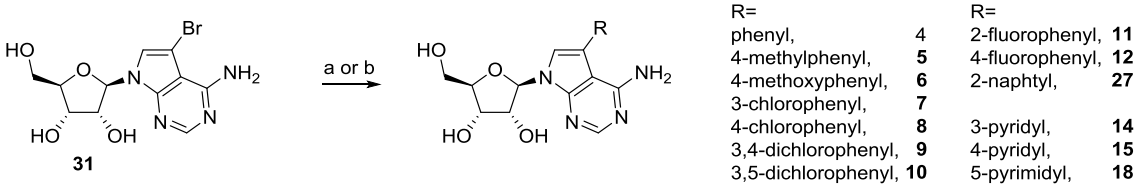


Figure 2: Overview of prepared substituted tubercidin nucleoside analogs.

Results and discussion

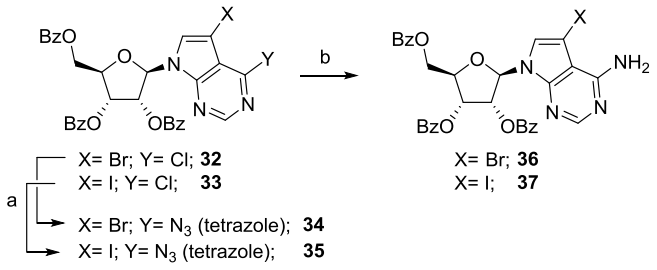
Chemistry

The synthesis is depicted in scheme 1-5. For the preparation of C7 substituted phenyl analogs **4**-**12** and **27** (Scheme 1), an aqueous Suzuki reaction with the known nucleoside bromide **31**,[30] was employed. Conditions reported previously[29] for the C7 iodo-nucleoside were perfectly translatable to the bromide **31**, as was recently reported by our group.[31] The synthesis of the 3-pyridyl analog **14**, which employed the corresponding pinacol boronic ester, required a prolonged reaction time. Application of these reaction conditions afforded the 4-pyridyl isomer **15** and 5-pyrimidyl derivative **18** in low yields only (< 5%), consistent with inherent pyridine/pyrimidine reactivity. Isolated yields could be improved by using the catalytic system reported by Fu,[32] affording **15** and **18** in moderate yields (Scheme 1).



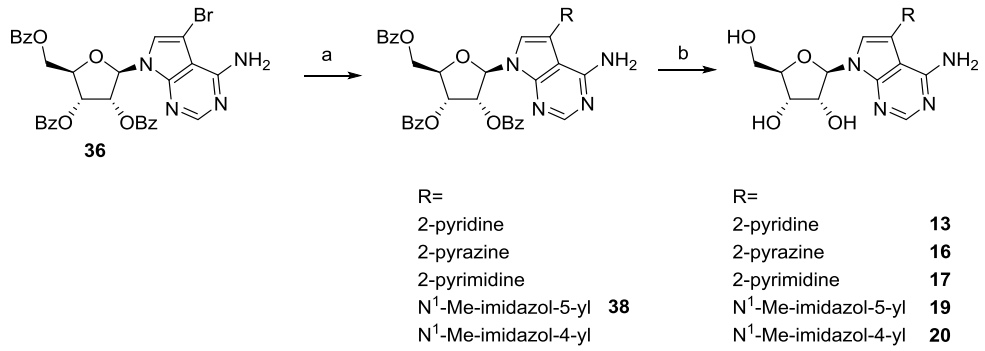
Scheme 1: Reagents and conditions: a) aryl-$B(OH)_2$ or aryl-B pinacol ester, Na_2CO_3, $Pd(OAc)_2$, TPPTS, $MeCN/H_2O$ (1/2 ratio), 100 °C, argon, 1–3 h (**4**–**12**, **27**), 20 h (**14**), 22–65 %; b) aryl-$B(OH)_2$, K_3PO_4, $Pd_2(dba)_3$, P(c-Hex)$_3$, H_2O/dioxane (1/2 ratio), 100 °C, argon, 20 h (**15**), 38 h (**18**), 26 % (**15**), 35 % (**18**).

For certain heterocycles for which the boronic acid or corresponding ester derivative are not commercially available or notoriously unstable (e.g. 2-subsituted pyridine),[33] a Stille coupling was envisioned (Scheme 3). The nucleoside coupling partner **36** (Scheme 2) could be obtained by nucleophilic aromatic displacement with sodium azide on the known nucleoside **32**[30], giving rise to the corresponding tetrazolo[1,5-*c*]pyrimidine **34**. The tetrazole tautomer of **34** predominates as was observed from the downfield shift of the H-2 proton in ^{1}H-NMR. In $CDCl_3$, the presence of both tautomers gave rise to a 'mixed' spectrum (data not shown), whereas in DMSO-d_6, the tetrazole form is found exclusively, which is in line with literature findings.[34] Next, **36** could be obtained by means of Staudinger reaction on **34** and subsequent iminophosphorane hydrolysis.[35-36] The iodonucleoside **37** was obtained analogously.



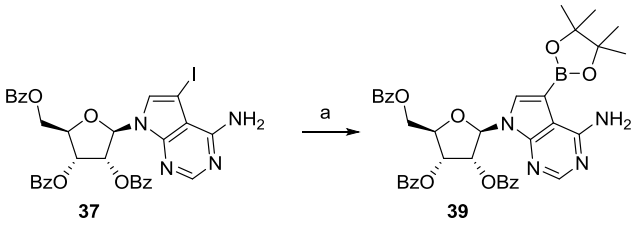
Scheme 2: Reagents and conditions: a) NaN_3, DMF, 65 °C, 30 min, 90% (**34**), 86% (**35**); b) i. PMe_3 (1M in THF), THF, rt., 30 min; ii. aq. HOAc (1M), MeCN, 65 °C, 1 h, 81% (**36**), 93% (**37**).

With protected nucleoside **36** in hand, Stille couplings were performed using either commercial (2-pyridyl, 2-pyrazinyl and 2-pyrimidinyl) or prepared (methyl-imidazolyl)[37-39] organostannanes, employing literature conditions.[29] In all cases except one (**38**), protected intermediates were immediately deprotected after Stille coupling, using 7N NH_3 in MeOH (Scheme 3).



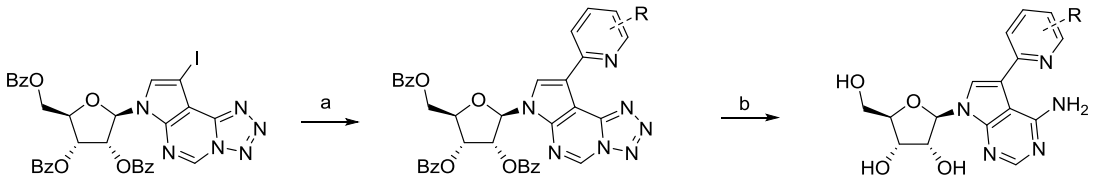
Scheme 3: Reagents and conditions: a) Ar-Sn(nBu)$_3$, Pd(Ph$_3$P)$_2$Cl$_2$, DMF, 100 °C, argon, overnight, 82 % (**38**); b) 7N NH_3 in MeOH, rt, overnight, 36 % (**16**, 2 steps), 29 % (**17**, 2 steps), 84 % (**19**), 25 % (**20**, 2 steps).

To obtain substituted pyridine analogs **21**–**26** and quinoline derivatives **28**–**30**, a different synthetic strategy was devised. Initially, we intended to use a Suzuki reaction with altered coupling partner polarity. Therefore, the synthesis of pinacol boronic ester[40-41] nucleoside **39** was envisioned (Scheme 4), starting from the corresponding iodo-nucleoside **37** (Scheme 2). However, only after multiple failed attempts (catalyst source, temperature and solvent exploration), it was found that with $PdCl_2$dppf·DCM as the catalyst, DMSO as the solvent and a reaction temperature of 100 °C, the desired product **39** could be obtained, albeit in moderate yield. All other tested conditions resulted in either no reaction at all or dehydrohalogenation (data no shown).



Scheme 4: Reagents and conditions: a) B_2pin_2, KOAc, $PdCl_2dppf{\cdot}DCM$, DMSO, 100 °C, argon, 3 h, 37%.

Given the rather disappointing yield of the borylation reaction, we changed to a Negishi coupling strategy (Scheme 5).[42] The nucleoside zinc derivative was prepared by magnesium-iodine exchange on **35** with Knochel's Turbo Grignard reagent,[43-44] followed by transmetallation with $ZnCl_2$ and subsequent Negishi coupling using $Pd_2(dba)_3$ and RuPhos.[45] It was found that for 2-bromopyridine and 2-bromo-4-chloropyridine, Negishi coupling was successful at ambient temperature, whereas with other 4-substituted-2-pyridine-bromides, virtually no product was formed. Elevating the temperature to 60 °C allowed to obtain all the desired coupling products. Control experiments, performed with 2-bromopyridine showed the importance of the Pd-source, the ligand and Pd and ligand combination (data not shown). Interestingly, the tetrazole tautomer of the C6 azide protected this functionality against the iodine/Mg exchange conditions, as no reduction products could be observed. Final compounds **13**, **21**-**26** and **28**-**30** were obtained by employing the above-mentioned sequence of Staudinger reduction, iminophosphorane hydrolysis and immediate deprotection using NaOMe/MeOH.



R= H; **40**	R= 4-Cl; **45**	R= H; **13**	R= 4-Cl; **25**
R= 4-Me; **41**	R= 5-F; **46**	R= 4-Me; **21**	R= 5-F; **26**
R= 4-OMe; **42**	2-quinoline; **47**	R= 4-OMe; **22**	2-quinoline; **28**
R= 6-Cl; **43**	3-isoquinoline; **48**	R= 6-Cl; **23**	3-isoquinoline; **29**
R= 5-Cl; **44**	1-isoquinoline; **49**	R= 5-Cl; **24**	1-isoquinoline; **30**

Scheme 5: Reagents and conditions: a) i. i-PrMgCl·LiCl (1.3 M in THF), THF, -65 °C, argon, 30 min; ii. $ZnCl_2$ (0.5 M in THF), -65 °C to rt, 25 min; iii. appropriate bromopyridine or bromo(iso)quinoline,

$Pd_2(dba)_3$, RuPhos, THF, 60 °C, overnight, 16–51 %; b) i. PMe_3 (1M in THF), THF, rt, overnight; ii. aq. HOAc (1M), MeCN, 65 °C, 1 h; iii. NaOMe, MeOH, rt, 30 min–1 h, 22–66 %.

Biological evaluation

In vitro evaluation

All synthesized nucleosides were tested *in vitro* against *T. b. brucei* Squib 427 and *T. b. rhodesiense* STIB-900 parasites. Cytotoxicity was assayed against MRC-5 fibroblasts. Results are depicted in Table 1.

Cpd.	*T. b. brucei* EC_{50} (µM)	*T. b. rhod.* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI *T.b. brucei*	SI *T.b. rhod.*	Cpd.	*T. b. brucei* EC_{50} (µM)	*T. b. rhod.* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI *T.b. brucei*	SI *T.b. rhod.*
1	0.48 ± 0.1	N.D.	2.23 ± 0.68	4.6	N.D.	**17**	2.26 ± 0.17	N.D.	14.3 ± 6.3	6.3	N.D.
4	32.3 ± 1.7	4.10 ± 0.39	56.2 ± 5.1	1.7	14	**18**	52.4 ± 11.6	N.D.	>64.0	>1.2	N.D.
5	29.1 ± 2.9	N.D.	26.2 ± 1.4	0.9	N.D.	**19**	33.3 ± 0.13	N.D.	>64.0	>1.9	N.D.
6	41.6 ± 7.3	N.D.	28.0 ± 3.4	0.67	N.D.	**20**	4.76 ± 0.39	N.D.	23.4 ± 1.4	4.9	N.D.
7	9.90 ± 1.18	6.88 ± 0.13	32.8 ± 1.7	3.3	4.8	**21**	38.2 ± 1.7	25.1 ± 2.6	>64.0	>1.7	>2.5
8	7.58 ± 0.51	N.D.	27.0 ± 2.5	3.6	N.D.	**22**	25.5 ± 7.7	8.15 ± 0.02	7.59 ± 2.08	0.3	0.9
9	3.07 ± 1.08	0.32 ± 0.18	14.7 ± 3.7	4.8	46	**23**	1.75 ± 0.32	0.62 ± 0.28	>64.0	>37	>103
10	8.17 ± 0.06	3.66 ± 0.46	>64.0	>7.8	>17	**24**	8.97 ± 0.40	4.60 ± 2.51	>64.0	>7.1	>13
11	33.3 ± 0.4	26.3 ± 1.4	61.0 ± 3.0	1.8	2.3	**25**	8.92 ± 0.49	6.88 ± 0.40	27.3 ± 3.4	3.1	4.0
12	8.81 ± 0.32	14.7 ± 0.42	>64.0	>7.3	>4.4	**26**	4.77 ± 1.91	1.69 ± 0.87	>64.0	>13	>38
13	0.31 ± 0.06	0.031 ± 0.005	15.1 ± 4.1	49	487	**27**	7.83 ± 0.60	6.79 ± 0.25	7.53 ± 0.57	0.96	1.2
14	>64.0	N.D.	>64.0	N.D.	N.D.	**28**	6.45 ± 1.20	3.72 ± 1.46	>64.0	>9.9	>17
15	>64.0	N.D.	55.8 ± 8.2	N.D.	N.D.	**29**	14.7 ± 7.2	6.16 ± 2.16	>64.0	>4.4	>10
16	1.95 ± 0.35	N.D.	0.55 ± 0.15	0.28	N.D.	**30**	>64.0	55.6 ± 8.4	>64.0	N.D.	>1.2
Suramin	0.05	0.04	>64.0	>1280	>1600						

Table 1: *In vitro* anti-trypanosomal activity of prepared nucleosides analogs. EC_{50} values are given in µM and are average and SEM of 2–6 independent determinations. N.D.: not determined. SI: Selectivity Index: (EC_{50} MRC-5) / (EC_{50} *T. b. brucei)* or (EC_{50} MRC-5) / (EC_{50} *T. b. rhodesiense*).

Our initial subset, comprising of tubercidin **1** and the C7 phenyl-substituted derivatives **4**–**6** and **8**, delivered weakly active analogs, with **8** being the most active one (low μM EC_{50}). Further derivatization focused on electron-poor and/or lipophilic phenyl analogs (**7**, **9**, **10**, **12** and **27**), which all displayed EC_{50} values <10 μM against *T. b. brucei*. The 3,4-dichloro analog **9** exerted the most potent anti-trypanosomal activity (EC_{50} = 3.07 ± 1.08 μM), with about tenfold better *in vitro* activity against *T. b. rhodesiense* (EC_{50} = 0.32 ± 0.18 μM). However, these modifications resulted only in moderately active analogs for which cytotoxicity was often equally increased (**9** has a SI of less than 5), therefore a bio-isosteric replacement for the phenyl group was considered. The observed preference for electron-poor substituents led us to explore pyridine substituents.[46] Of the three possible isomers, the 2-substituted pyridine **13** emerged as the most potent anti-trypanosomal agent with reasonable selectivity indices, *i.e.* 50 for *T. b. brucei* and ~500 for *T. b. rhodesiense*, whereas the 3- and 4-pyridines **14** and **15** displayed no discernible trypanocidal activity. Continued investigation into electron deficient 6-membered heteroaromatics led to the preparation of pyrazine (**16**) and pyrimidine (**17** and **18**) derivatives, which failed to show improved activity. A switch to *N*-methyl imidazole analogs (**19** and **20**) learned that only *N*-methyl-imidazol-4-yl derivative **20** exhibits low micromolar activity, about tenfold less potent than that of **13**. To mimic the position of the pyridine-N electron density, *ortho*-F phenyl analog **11** was synthetized, again with poor activity. Strikingly, from the present set of hetero-aromatic 7-substituted tubercidin analogs, only derivatives that feature an *ortho*-N atom proved to be active against the *T. b. brucei* parasite (compare pairs **13**, **14** & **15**; **17** & **18**; **19** & **20**). Examination of the ^{1}H-NMR spectrum of **13** clearly showed the splitting of the C6 NH_2 signal into two broad signals (one at δ = 7.29 ppm and one at δ = 9.88 ppm). This splitting pattern, as well as the significant downfield shift of one of the NH_2-protons, are indicative of the formation of an intramolecular H-bond.[47] Crystallization from water and subsequent single crystal X-ray analysis confirmed the presence of this H-bond (Figure 3 & Supporting Information). Recently, researchers from Merck used this H-bonding idea as a key design strategy in search of HCV NS5B polymerase inhibitors.[48]

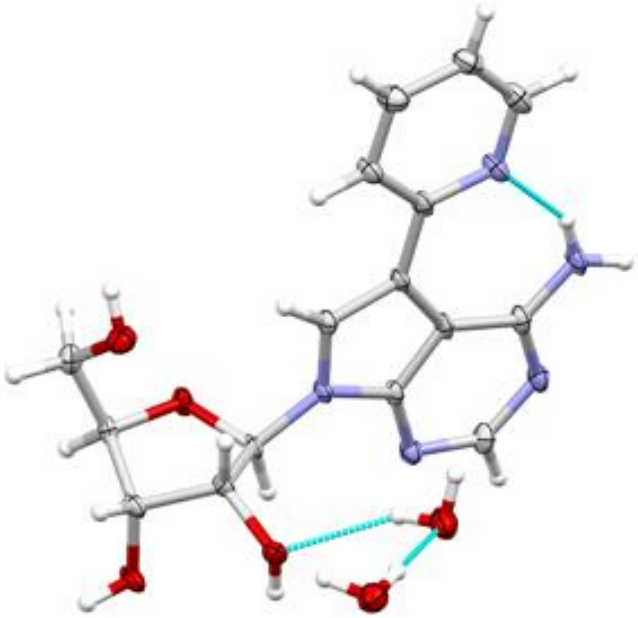


Figure 3: Asymmetric unit of the crystal structure of **13**, showing thermal displacement ellipsoids at the 50 % probability level. The presence of an intramolecular H-bond is highlighted, leading to the formation of a 7-membered pseudoring.

Further substitution of the pyridine ring (**21–26, 28-30**), failed to improve the anti-trypanosomal activity of **13**. The virtual inactivity of the 1-isoquinolyl derivative **30**, which is unable to form the H-bond because of steric clashing between H8 of the purine and H8 of the isoquinoline, confirms the importance of the H-bond for activity. However, the better activity observed with analogs having electron withdrawing rather than donating substituents makes a clear conclusion regarding the importance of the H-bond difficult.

Subsequently, **13** was further evaluated in three drug resistant *T. b. brucei* cell lines (Table 2). Drug resistance in African trypanosomes has mostly been attributed to altered transport activities.[49-52] This is of particular importance for nucleoside analogs. Their polar nature generally precludes passive diffusion across the cell membrane and some have been shown to rely completely on uptake by the TbAT1/P2 transporter.[19, 53-55] This transporter is encoded by a single gene, which is non-essential,[56] and is therefore

likely to yield drug-resistant mutants. Indeed, resistance of *T. brucei* spp. to the veterinary trypanocide diminazene has been clearly linked to loss of TbAT1/P2,[57-59] as well as to reduced sensitivity to the HAT treatments pentamidine and melarsoprol.[56, 60-62] From the data presented in Table 2, it is clear that **13** is much less dependent on the P2 transporter than tubercidin (**1**). Deletion of the P2 transporter (TbAT1-KO strain) results in a modest (2.4-fold) loss of sensitivity to **13**, while a 17-fold increase in resistance is observed for **1**. The advantage of a nucleoside analog recognized by more than one transporter (see also next section) is even more evident when comparing sensitivities between the wild-type (Lister 427) strain and the multi-drug resistant strain B48, which displayed resistance factors (RF) of 28.7 and 1.6 for **1** and **13**, respectively. The trend further held for the isometamidium-resistant cell line ISMR1 (Table 2). Prompted by the results seen on the ISMR1 cell-line, we also investigated the effects of **13** on the veterinary parasite, *T. congolense*, which represents the main causative agent of animal trypanosomiasis (Table 2). As *T. congolense* drug resistance against the animal trypanocides diminazene and isometamidium is high,[63] we were pleased to find that not only does **13** exhibits statistically identical activity against *T. congolense* as to *T. b. brucei*, it also is at least as potent as the standard treatment for *T. congolense*, diminazene aceturate.

Compound	**Lister-427 EC_{50} (µM)**	**TbAT1-KO EC_{50} (µM)**	**RF**	**B48 EC_{50} (µM)**	**RF**	**ISMR1 EC_{50} (µM)**	**RF**	***T. congolense*[a] EC_{50} (µM)**
Tubercidin 1	0.15 ± 0.03	2.61 ± 0.7	17.2	4.3 ± 1.3	28.7	1.7 ± 0.5	11.1	N.D.
13	0.17 ± 0.04	0.40 ± 0.09	2.4	0.27 ± 0.04	1.6	0.43 ± 0.07	2.5	0.20 ± 0.01
Pentamidine	0.011 ± 0.001	0.018 ± 0.002	1.8	0.99 ± 0.16	94.6	0.14 ± 0.04	13.8	1.9 ± 0.20
Diminazene	0.42 ± 0.064	4.5 ± 0.9	10.6	7.2 ± 1.6	16.9	2.9 ± 0.4	6.9	0.22 ± 0.003
Isometamidium	0.65 ± 0.085	0.75 ± 0.14	1.2	0.56 ± 0.13	0.85	3.1 ± 0.5	4.8	N.D.

Table 2: *In vitro* anti-trypanosomal evaluation against three drug-resistant *T. b. brucei* cell lines; and *T. congolense*. EC_{50} values are given in µM; and are mean and SEM of three independent determinations.

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. [a] Trypomastigotes of *T. congolense* IL3000.

Transporter (P1 / P2) studies of 13

It has previously been reported that uptake of tubercidin (**1**) is mostly dependent on *T. brucei*'s P2/TbAT1 transporter and loses most of its activity against the TbAT1-KO strain,[53] as confirmed above. This is explained by the fact that, in contrast to adenosine, which is salvaged by both high-affinity nucleoside transporters in bloodstream trypanosomes (P1 and P2),[61] tubercidin is a poor substrate for P1, being transported with just 1% of the efficiency of adenosine (expressed as V_{max}/K_m).[64] This is because the translocation of purines by the P1 transporter partially depends on an interaction with the N7 of the purine ring.[55, 64] In contrast, N-7 is not part of the main recognition motif of the P2 transporter[65] as this moiety does not form a significant interactions with the P2 binding pocket.[62] Competition experiments with [^{3}H]-adenosine for both the P1 and the P2 transporter were performed to gain insights into the preferences of the regioisomeric pyridine analogs **13**–**15** for either transporter (Figure 4 and Table 3).

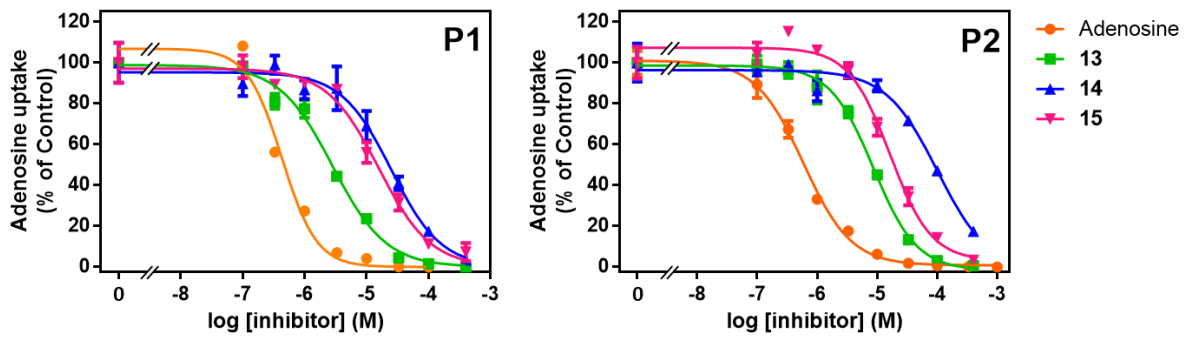


Figure 4: Transport of [^{3}H]-Adenosine via P1 and P2 transporters in the presence of increasing concentrations of nucleoside analogs **13**, **14** and **15**. Transport via P1 was measured in B48 cells (lacking P2), while transport via P2 was evaluated in the same cells but transfected with a construct

overexpressing the TbAT1/P2 transporter, in the presence of 100 µM inosine to block P1.[62] The graphs show one representative of three independent experiments in triplicate. Error bars are SEM, when not shown, they fall within the symbol.

None of the three pyridine derivatives show a pronounced P2 transporter-type preference, which could lead to uptake-based resistance. Note that P1 activity is encoded by multiple genes[54, 64, 66] and P1-based resistance has never been reported. Analogs **13** and **15** give a 10- to 20-fold increase in binding inhibition constant for P2, while this is a 100-fold increase for **14**, possibly due to a non-favorable interaction between the basic *meta* nitrogen and the transporter's binding pocket.

In comparison to tubercidin (**1**), all pyridine analogs but **13**, show increased binding affinity for the P1 transporter. Considering the vast amount of literature data[21, 53-55, 64] that point to a crucial interaction of the N7 nitrogen with the P1 binding pocket, this result is surprising. Although **13** is unable to form this H-bond (loss of 6.9 kJ/mol in binding), the loss in Gibbs free energy is less than half that of **1**, indicating that this additional ring is well-accommodated in the binding pocket and that its restricted rotation enables an energetically favorable binding orientation. Similar to P2, the P1 transporter appears to disfavor a *meta*-pyridine ring. These results corroborate the drug sensitivity profile of **13** against the TbAT1-KO and B48 strains, in that reasonable binding to the P1 transporter allows efficient delivery of this analog inside the parasite cell, showing that these analogs do not just inhibit binding of adenosine to the transporter but are substrates themselves.

Taken together, it seems that uptake of these three pyridine-substituted tubercidin analogs is mediated by both the P1 and P2 transporters and greatly dependent on the N-position (*ortho>para>meta*) in the pyridine ring and accounts, in part, for the observed difference in activity against the parasite *in vitro*. Nevertheless, other factors, such as engagement with the (to be elucidated) intracellular target are also important and that transporter studies alone are unlikely to completely explain the substantial difference in trypanocidal activity.

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	**0.16 ± 0.03**	-38.8		**0.53 ± 0.02**	-35.8	
Tubercidin, 1	78 ± 6.4[1]	-23.4	15.4	3.8 ± 0.7[1]	-30.9	4.9
13	2.54 ± 0.3	-31.9	6.9	4.57 ± 0.25	-30.4	5.4
14	21.7 ± 1.9	-26.6	12.2	49.3 ± 3.4	-24.6	11.2
15	12.5 ± 0.9	-28	10.8	9.34 ± 1.0	-28.7	7.1

Table 3: Kinetic parameters of adenosine and C7 modified analogs for the nucleoside transporters of bloodstream *T. brucei*. K_m (highlighted in **bold**) and K_i values are in μM and ΔG^0 is in kJ/mol. $\delta(\Delta G^0)$ was calculated in comparison to adenosine. [1]The K_i for tubercidin was taken from reference [55].

Metabolic stability of 13 in mouse, rat and human microsomes

In order to assess *in vitro* metabolic stability, compound **13** was exposed to mouse, rat and pooled human liver S9 microsomal fractions. The percentages of parent compound **13** remaining after incubation with the various microsomes with NADPH-dependent and UGT enzymes are presented in Table 4. The results indicate that compound **13** is susceptible to extensive Phase-I metabolism in mouse liver microsomes with only about 12.6% of parent drug remaining after 30 minutes, with an acceptable cut-off being set at >50%. After 60 min, metabolic degradation in mouse liver microsomes was almost complete. Analysis of the LS/MS chromatograms showed increasing amounts of a metabolite with a mass corresponding to [M+16], consistent with pyridine-*N*-oxide / pyridone formation (data not shown). Some Phase-I decay was observed in rat liver microsomes. Phase I metabolism in human microsomes was absent and Phase II metabolism could not be demonstrated in any of the three species. The data obtained from the mouse microsomal stability study preclude the further *in vivo* evaluation of **13** in a mouse model of *T. brucei*.

Phase I / II	Time	MOUSE % 13 remaining		RAT % 13 remaining		HUMAN % 13 remaining	
		Mean	STDEV	Mean	STDEV	Mean	STDEV
CYP - NADPH	0	100	-	100	-	100	-
	15	36.5	6.4	89	20.4	96	1.8
	30	12.6	1.7	77	10.9	102	2.9
	60	1.9	0.3	31	3.2	82	20.3
UGT Enzymes	0	100	-	100	-	100	-
	15	112	14.7	115	3.5	123	5.4
	30	102	28.1	125	6.3	121	14.4
	60	122	3.9	122	1.3	92	8.2

Table 4: Assessment of *in vitro* Phase I and Phase II metabolic stability of **13** using mouse, rat and human S9 microsomal fractions. Indicated are the percentage parent remaining compound in two replicates at various time points of incubation (0-15-30-60 min). Proper functioning of the *in vitro* assay was confirmed with the reference drug diclofenac (susceptible to Phase-I and Phase-II metabolism) and fluconazole (metabolically stable through Phase-I) (data not shown).

Evaluation against other protozoa

Trypanosoma cruzi, the etiological agent of Chagas disease and *Leishmania* spp., which causes leishmaniasis, are kinetoplastid parasites related to *T. brucei*. Therefore, the nucleosides synthesized in this study were also assayed against these pathogens. *In vitro* evaluation against *Leishmania infantum* intracellular amastigotes failed to deliver any suitable hit compounds, although tubercidin **1** displayed an EC_{50} value of 0.13 µM, in close agreement with previous reports of the antileishmanial potency. As expected, there was no selectivity compared to the host cell (primary mouse macrophage) cytotoxicity (data not shown). Tubercidin is transported by the *Leishmania* NT1 transporters and resistance was associated with reduced purine uptake in several *Leishmania* species, including *L. donovani* and *L. mexicana*.[67-68] Yet, the 7-substituted tubercidin analogs displayed very low activity against intra-macrophage *L. infantum* amastigotes, with only analog **9** displaying a low micromolar EC_{50} (1.67 ± 0.35 µM), with only modest selectivity towards the host cell (EC_{50} = 20.0 ± 12.0 µM). We therefore speculate

that the aromatic substitutions at position 7 of tubercidin are poorly tolerated by the *L. infantum* NT1 adenosine/pyrimidine transporters, which are highly conserved between *Leishmania spp*, and their only mechanism for uptake of adenosine and its analogs.[69] The well-documented substrate-selectivity difference between the *T. brucei* and the *Leishmania* nucleoside transporters[54, 64-65, 69] probably drives major differences in anti-trypanosomal and anti-leishmanial activity of the nucleoside analogs.

In contrast, *in vitro* evaluation against *T. cruzi* revealed three analogs (**8**, **9** and **12**) with an up to 10-fold higher level of activity as compared to the reference drug benznidazole (table 5). All other nucleoside analogs did not display significant anti-*T. cruzi* activity or were non-selective (data not shown). Similar to *Leishmania spp.*, *T. cruzi* has previously been shown to be susceptible to tubercidin and resistance was linked to reduced uptake of the drug.[70] Candidate genes of the Equilibrative Nucleoside Transporter family can be readily identified in the *T. cruzi* genome,[71] and a recent study[72] uncovered the substrate profile of several of them, but adenosine transporter activity has yet to be demonstrated.

Compound	*T. cruzi* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI
Tubercidin, 1	0.34 ± 0.17	2.23 ± 0.68	6.5
8	0.47 ± 0.25	27.0 ± 2.5	57.4
9	0.19 ± 0.07	14.7 ± 3.7	77.4
12	0.49 ± 0.10	>64.0	>130
Benznidazole	2.73 ± 0.09	>64.0	>23

Table 5: *In vitro* activity of selected analogs against *Trypanosoma cruzi*. EC_{50} values are given in µM as average and SEM of 2–4 independent replicates. SI = (EC_{50} MRC-5 / EC_{50} *T. cruzi*).

Conclusion

In the present publication, we have described the reinvestigation of 7-deazaadenosine (tubercidin) against African trypanosomes, which led to the discovery of low micromolar *in vitro* active C7 phenyl analogs, with a preference for electron-poor rings. Bio-isosteric replacement with a pyridine led to

analog **13**, with nM potency against the *T. brucei* parasite. Only the 2-pyridine isomer, engaging in an intramolecular H-bond with the C6 NH_2, resulted in submicromolar *in vitro* activity and additional substitution of the ring was found not to be tolerated. **13** was found to be equally potent *in vitro* against the veterinary parasite *T. congolense*. Further investigation showed a mixed P1/P2 transporter profile for the compounds assayed, which is highly advantageous considering uptake-related resistance as shown in the absence of cross-resistance with first-line trypanocidal drugs. The advantage is also neatly illustrated by the susceptibility of *T. congolense*, which lacks the P2 transporter altogether, but does express a P1-type adenosine transporter.[58, 71] Unfortunately, analog **13** displayed poor metabolic stability when assayed in the presence of mouse microsomal fractions, precluding further *in vivo* evaluation in relevant (mouse) animal models of HAT. Additionally, several substituted phenyl 7-deazapurine nucleoside analogs displayed potent activity against intracellular *T. cruzi* amastigotes, which warrants further investigation.

Experimental section

Chemistry

All reagents and solvents were obtained from standard commercial sources and were of analytical grade. Unless otherwise specified, they were used as received. Compounds **31**[30], **32**[30], **33**[30], **1**[73], 1-methyl-5-(tributylstannyl)-1*H*-imidazole[37], 1-methyl-4-(tributylstannyl)-1*H*-imidazole[38-39] were prepared as described in literature. All moisture sensitive reactions were carried out under argon atmosphere. Reactions were carried out at ambient temperature, unless otherwise indicated. Analytical TLC was performed on Machery-Nagel® precoated F254 aluminum plates and were visualized by UV followed by staining with basic aq. $KMnO_4$, Cerium-Molybdate, or sulfuric acid-anisaldehyde spray. Column chromatography was performed using Davisil® (40-63 μm) or on a Reveleris X2 (Grace/Büchi) automated Flash unit employing pre-packed silica columns. Exact mass measurements were performed on a Waters LCT Premier XE™ Time of Flight (ToF) mass spectrometer equipped 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. 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. In ^{19}F-NMR, signals were referenced to $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm. Melting points were determined on a Büchi-545 apparatus and are uncorrected. Purity was assessed by means of analytical LC-MS employing either

(1) Waters Alliance 2695 XE separation Module using a Phenomenex Luna® reversed-phase C18 (2) column (3 µm, 100x2.00 mm) and a gradient system of HCOOH in H_2O (0.1 %, v/v)/HCOOH in MeCN (0.1 %, v/v) at a flow rate of 0.4 mL/min, 10:90 to 0:100 in 9 minutes. High-resolution MS spectra were recorded on a Waters LCT Premier XE Mass spectrometer.

(2) Waters AutoPurification system (equipped with ACQUITY QDa (mass; 100–1000 amu)) and 2998 Photodiode Array (220 – 400 nm)) using a Waters Cortecs® C18 (2.7 µm 100x4.6mm) column and a gradient system 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.

All obtained final compounds had purity > 95 %, as assayed by analytical HPLC (UV); unless otherwise indicated.

General procedure A (Suzuki coupling; adapted from ref. [29]):

31 (1 eq.), boronic acid (1.5 eq.) or pinacol ester [for compound **14** (1.5 eq.)], Na_2CO_3 (9 eq.), $Pd(OAc)_2$ (0.05 eq.) and TPPTS (0.15 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 (2 mL/mmol SM) and H_2O (4 mL/mmol SM) were added to the solids under argon. After 5 min of stirring, the mixture was heated to 100 °C in a pre-heated oil bath. When the starting material was fully consumed (usually 1–3 hours), the mixture was cooled to ambient temperature, and neutralized (pH ~ 7) with 0.5 M aq. HCl. The mixture was evaporated till dryness, resuspended in MeOH and evaporated (three times). Next, the mixture was adsorbed onto Celite® (from

MeOH) and eluted over a short silica pad (~ 5 cm) with 20 % MeOH/DCM. The liquid was evaporated *in vacuo* and purified by column chromatography.

General procedure B (Stille coupling; adapted from ref. [29]):

36 (1 eq.) and $Pd(Ph_3P)_2Cl_2$ (0.10 eq.) were added to a flame-dried 5 mL round bottom flask, equipped with a stir bar, under argon. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, degassed anhydrous DMF (4 mL/mmol SM) was added under argon. The resulting solution was stirred at for ~5 min after which the organostannane (2 eq.) was added *via* syringe. The mixture was then heated to 100 °C in a pre-heated oil bath overnight. Next, the mixture was cooled to ambient temperature and evaporated to dryness. The resulting oil was partitioned between MeCN/hexane. The MeCN-layer was extracted twice more with hexane, and then evaporated. The resulting mixture was purified by column chromatography. In most cases, the obtained product was immediately used in the next step (deprotection).

General procedure C (Negishi Coupling):

35 was dissolved in anhydrous toluene (10 mL) and evaporated till dryness. This procedure was repeated 3 times. Next, the residue was dissolved in anhydrous THF (8.5 mL/mmol SM) under argon. The solution was cooled to -65 °C. i-PrMgCl·LiCl solution (1.3M in THF; 1.1 eq.) was added in one portion. The resulting solution was stirred at -65 °C for 30 min, after which a small sample was quenched with sat. NH_4Cl solution and used for TLC analysis. Generally, full conversion was then observed. Next, $ZnCl_2$ solution (0.5M in THF, 1.2 eq.) was added in one portion, and the mixture stirred for another 5 – 10 min at -65 °C. Then, the cooling was removed, and the mixture stirred at ambient temperature for 20 min. Next, to a flame-dried Schlenk-tube (5 mL) containing a stir bar, were added $Pd_2(dba)_3$ (0.02 eq.), RuPhos (0.08 eq.) and the appropriate bromopyridine or bromo(iso-)quinoline (1.4 eq.) (when solid) under argon. The tube was evacuated and refilled with argon three times. Then, anhydrous THF (3 mL/mmol SM) was added as well as the pyridine-Br or (iso-)quinoline-Br (1.4 eq.) (when liquid). The mixture was stirred for approximately 5 min and the resulting solution was then transferred *via* syringe to the flask containing the nucleoside-zinc reagent. An additional 0.5 – 1 mL of anhydrous THF was

used to rinse the Schlenk tube and added to the mixture as well. The resulting solution was stirred at 60 °C overnight. After cooling to ambient temperature, the mixture was then quenched by adding water (~ 5 mL) and transferred to a separatory funnel. EA and aq. 1M EDTA (pH=8) solution were added. The layers were separated, and the water layer was extracted with EA two more times. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The resulting mixture was purified by column chromatography.

General procedure D (Staudinger reduction / iminophosphorane hydrolysis and subsequent deprotection of 7-deaza-7-pyridinyl / (iso-)quinolinyl derivatives):

The appropriate 6-azido-nucleoside (1 eq.) was dissolved in THF (10 mL/mmol). Then, PMe_3 solution (1M in THF; 2 eq.) was added and the mixture stirred at ambient temperature until TLC analysis showed full conversion of starting material (generally overnight). Next, the solution was evaporated till dryness, and subsequently re-dissolved in MeCN (10 mL/mmol). To this solution was added a 1M aq. HOAc solution (3.33 eq.), and the mixture heated in a pre-heated oil bath at 65 °C for 1 h. Next, the mixture was cooled to ambient temperature and poured into sat. aq. $NaHCO_3$ solution. DCM was added, layers were separated, and the water layer extracted two more times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography gave rise to the intermediate purine-amine derivative, which was used directly (deprotection). To a solution of the purine-amine derivative in MeOH (15 mL/mmol) was added NaOMe/MeOH solution (5.4 M, 0.2 eq.), and the mixture was stirred at ambient temperature until TLC analysis showed full conversion (generally between 30 min to 1 h). Next, the mixture was neutralized (pH ~ 7) with 0.5 M aq. HCl and evaporated till dryness. The residue was taken up in MeOH, and co-evaporated with Celite®, and subjected to column chromatography.

4-amino-5-phenyl-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (4) 4 was prepared according to General Procedure A (reaction time: 2 h). **31** (0.17 g, 0.50 mmol) gave rise to **4** as a white solid (0.11 g, 0.31 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 62 %. ^{1}H NMR (300 MHz,

DMSO-d_6) δ: 3.53 (ddd, *J* = 12.0, 6.3, 3.9 Hz, 1H, H-5''), 3.63 (dt, *J* = 12.0, 4.5 Hz, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.08 – 4.13 (m, 1H, H-3'), 4.46 (q, *J* = 6.0 Hz, 1H, H-2'), 5.11 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.18 (dd, *J* = 6.0, 5.1 Hz, 1H, OH-5'), 5.32 (d, *J* = 6.6 Hz, 1H, OH-2'), 6.10 (br. s, 2H, NH_2), 6.12 (d, *J* = 6.0 Hz, 1H, H-1'), 7.35 – 7.52 (m, 5H, H_{Phe}), 7.54 (s, 1H, H-6), 8.15 (s, 1H, H-2). HRMS (ESI): calculated for $C_{17}H_{19}N_4O_4$ ($[M+H]^+$): 343.1401, found: 343.1418. Spectral data are in accordance with literature values.[29]

4-amino-5-(4-methylphenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (5) 5 was prepared according to General Procedure A (reaction time: 2 h). **31** (0.17 g, 0.50 mmol) gave rise to **5** as a white solid (0.080 g, 0.22 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 45 %. Melting point: 165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 3.50 – 3.57 (m, 1H, H-5''), 3.61 – 3.66 (m, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.10 (br. s, 1H, H-3'), 4.46 (br. s, 1H, H-2'), 5.12 (br. s, 1H, OH-3'), 5.18 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.31 (br. s, 1H, OH-2'), 6.10 (br. s, 2H, NH_2), 6.11 (d, *J* = 6.3 Hz, 1H, H-1'), 7.28 – 7.31 (m, 2H, H_{Phe}), 7.35 – 7.38 (m, 2H, H_{Phe}), 7.49 (s, 1H, H-6), 8.14 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.7 (CH_3), 61.7 (C-5'), 70.6 (C-3'), 73.8 (C-2'), 85.1 (C-4'), 87.0 (C-1'), 100.6 (C-4a), 116.2 (C-5), 120.8 (C-6), 128.4 ($2C_{Phe}$), 129.6 ($2C_{Phe}$), 131.5 ($C\text{-}1_{Phe}$), 136.1 ($C\text{-}4_{Phe}$), 150.7 (C-7a), 151.6 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{21}N_4O_4$ ($[M+H]^+$): 357.1557, found: 357.1575.

4-amino-5-(4-methoxyphenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (6) 6 was prepared according to General Procedure A (reaction time: 1 h). **31** (0.17 g, 0.50 mmol) gave rise to **6** as a white solid (0.087 g, 0.23 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 47 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 11.7, 6.0, 3.6 Hz, 1H, H-5''), 3.60 – 3.66 (m, 1H, H-5'), 3.80 (s, 3H, OCH_3), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.08 – 4.25 (m, 1H, H-3'), 4.42 – 4.48 (m, 1H, H-2'), 5.10 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.18 (dd, *J* = 6.0, 5.4 Hz, 1H, OH-5'), 5.30 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.08 (br. s, 2H, NH_2), 6.10 (d, *J* = 6.3 Hz, 1H, H-1'), 7.03 – 7.08 (m, 2H, H_{Phe}), 7.36 – 7.41 (m, 2H, H_{Phe}), 7.45 (s, 1H, H-6), 8.13 (s, 1H, H-2). HRMS (ESI): calculated for $C_{18}H_{21}N_4O_5$ ($[M+H]^+$): 373.1506, found: 373.1525. Spectral data are in accordance with literature values.[29]

4-amino-5-(3-chlorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (7) 7 was prepared according to General Procedure A (reaction time: 1 h). **31** (0.24 g, 0.70 mmol) gave rise to **7** as a white solid (0.056 g, 0.15 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 22 %. Melting point: 135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 12.0, 6.0, 4.2 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 4.8, 4.2 Hz, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.09 – 4.13 (m, 1H, H-3'), 4.45 (q, *J* = 6.0 Hz, 1H, H-2'), 5.11 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.15 (dd, *J* = 6.0, 5.1 Hz, 1H, OH-5'), 5.32 (d, *J* = 6.6 Hz, 1H, OH-2'), 6.12 (d, *J* = 6.3 Hz, 1H, H-1'), 6.24 (br. s, 2H, NH_2), 7.39 – 7.45 (m, 2H, H-5_{Phe}, H-6_{Phe}), 7.49 (d, *J* = 7.8 Hz, 1H, H-4_{Phe}), 7.52 (t, *J* = 1.8 Hz, 1H, H-2_{Phe}), 7.64 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.5 (C-3'), 73.8 (C-2'), 85.1 (C-4'), 87.0 (C-1'), 100.2 (C-4a), 115.0 (C-5), 121.9 (C-6), 126.5 (C_{Phe}), 126.9 (C_{Phe}), 127.9 (C-2_{Phe}), 130.7 (C-4_{Phe}), 133.5 (C-3_{Phe}), 136.6 (C-1_{Phe}), 151.1 (C-7a), 151.8 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}ClN_4O_4$ ($[M+H]^+$): 377.1011, found: 377.0993.

4-amino-5-(4-chlorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (8) 8 was prepared according to the General Procedure A (reaction time: 3 h). **31** (0.17 g, 0.50 mmol) gave rise to **8** as a white solid (0.084 g, 0.22 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 45 %. Melting point: 130 – 132 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 11.7, 6.0, 3.9 Hz, 1H, H-5''), 3.60 – 3.67 (m, 1H, H-5'), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.08 – 4.13 (m, 1H, H-4'), 4.42 – 4.48 (m, 1H, H-2'), 5.11 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.17 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.32 (d, *J* = 6.6 Hz, 1H, OH-2'), 6.12 (d, *J* = 6.0 Hz, 1H, H-1'), 6.21 (br. s, 2H, NH_2), 7.46 – 7.49 (m, 2H, H_{Phe}), 7.52 – 7.55 (m, 2H, H_{Phe}), 7.58 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.6 (C-3'), 73.8 (C-2'), 85.1 (C-4'), 87.0 (C-1'), 100.3 (C-4a), 115.1 (C-5), 121.5 (C-6), 128.9 ($2C_{Phe}$), 130.1 ($2C_{Phe}$), 131.5 (C_{Phe}), 133.3 (C_{Phe}), 151.0 (C-7a), 151.7 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}ClN_4O_4$ ($[M+H]^+$): 377.1011, found: 377.1028.

4-amino-5-(3,4-dichlorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (9) 9 was prepared according to General Procedure A (reaction time: 3 h). **31** (0.17 g, 0.50 mmol) gave rise to **9** as a white solid (0.060 g, 0.15 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 29 %. Melting point: 221 – 223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (dd, *J* = 11.7, 3.3 Hz, 1H, H-

5’’), 3.64 (dd, *J* = 11.7, 3.6 Hz, 1H, H-5’), 3.90 (q, *J* = 3.6 Hz, 1H, H-4’), 4.42 – 4.47 (m, 1H, H-2’), 5.13 (br. s, 2H, OH-3’, OH-5’), 5.33 (d, *J* = 6.0 Hz, 1H, OH-2’), 6.12 (d, *J* = 6.3 Hz, 1H, H-1’), 6.41 (br. s, 2H, NH_2), 7.43 (dd, *J* = 8.4, 2.1 Hz, 1H, H-6_{Phe}), 7.68 – 7.72 (m, 3H, H-6, H-2_{Phe}, H-5_{Phe}), 8.17 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5’), 70.5 (C-3’), 73.8 (C-2’), 85.1 (C-4’), 87.0 (C-1’), 100.0 (C-4a), 114.2 (C-5), 122.2 (C-6), 128.5 (C_{Phe}), 129.2 (C_{Phe}), 129.9 (C_{Phe}), 130.8 (C_{Phe}), 131.3 (C_{Phe}), 135.0 (C_{Phe}), 151.1 (C-7a), 151.6 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}Cl_2N_4O_4$ ($[M+H]^+$): 411.0621, found: 411.0627.

4-amino-5-(3,5-dichlorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10) 10 was prepared according to General Procedure A (reaction time: 3 h). **31** (0.24 g, 0.70 mmol) gave rise to **10** as a white solid (0.080 g, 0.20 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 28 %. Melting point: 219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 12.0, 6.0, 4.2 Hz, 1H, H-5’’), 3.64 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5’), 3.90 (q, *J* = 3.6 Hz, 1H, H-4’), 4.09 – 4.13 (m, 1H, H-3’), 4.44 (q, *J* = 5.7 Hz, 1 H, H-2’), 5.12 – 5.16 (m, 2H, OH-5’, OH-3’), 5.33 (d, *J* = 6.3 Hz, 1H, OH-2’), 6.12 (d, *J* = 6.3 Hz, 1H, H-1’), 6.40 (br. s, 2H, NH_2), 7.48 (d, *J* = 2.1 Hz, 2H, H-2_{Phe}, H-6_{Phe}), 7.54 (t, *J* = 2.1 Hz, 1H, H-4_{Phe}), 7.73 (s, 1H, H-6), 8.17 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5’), 70.5 (C-3’), 73.8 (C-2’), 85.1 (C-4’), 86.9 (C-1’), 99.9 (C-4a), 113.9 (C-5), 122.6 (C-6), 125.9 (C-4_{Phe}), 126.8 (2C, C-2_{Phe}, C-6_{Phe}), 134.2 (2C, C-3_{Phe}, C-5_{PHe}), 137.9 (C-1_{Phe}), 151.3 (C-7a), 151.9 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}Cl_2N_4O_4$ ($[M+H]^+$): 411.0621, found: 411.0625.

4-amino-5-(2-fluorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11) 11 was prepared according to General Procedure A (reaction time: 1.5 h). **31** (0.24 g, 0.70 mmol) gave rise to **11** as a white solid (0.095 g, 0.27 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 40 %. Melting point: 130 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.32 – 3.66 (m, 2H, H-5’, H-5’’), 3.91 (dd, *J* = 6.9, 3.6 Hz, 1H, H-4’), 4.10 – 4.12 (m, 1H, H-3’), 4.46 (q, *J* = 6.3 Hz, 1H, H-2’), 5.12 (d, *J* = 4.8 Hz, 1H, OH-3’), 5.19 (dd, *J* = 6.0, 5.1 Hz, 1H, OH-5’), 5.33 (d, *J* = 6.6 Hz, 1H, OH-2’), 6.07 (br. s, 2H, NH_2), 6.12 (d, *J* = 6.3 Hz, 1H, H-1’), 7.29 – 7.37 (m, 2H, H_{Phe}), 7.41 – 7.47 (m, 2H, H_{Phe}), 7.57 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -115.56 – -115.48 (m). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5’), 70.6 (C-3’), 73.8 (C-2’), 85.2 (C-4’), 87.2 (C-1’), 101.4 (C-4a), 108.5

(C-5), 116.1 (d, *J* = 21.8 Hz, 1C, C_{Phe}), 121.8 (d, *J* = 14.9 Hz, 1C, C_{Phe}), 122.4 (C-6), 124.8 (d, *J* = 3.5 Hz, 1C, C_{Phe}), 129.4 (d, *J* = 8.0 Hz, 1C, C_{Phe}), 131.9 (d, *J* = 2.2 Hz, 1C, C_{Phe}), 150.6 (C-7a), 151.7 (C-2), 157.3 (C-4), 159.4 (d, *J* = 242.8 Hz, 1C, C-F). HRMS (ESI): calculated for $C_{17}H_{18}FN_4O_4$ ($[M+H]^+$): 361.1307, found: 361.1311.

4-amino-5-(4-fluorophenyl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (12) 12 was prepared according to General Procedure A (reaction time: 1.5 h). **31** (0.24 g, 0.70 mmol) gave rise to **12** as a white solid (0.075 g, 0.21 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 30 %. Melting point: 145 – 148 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (ddd, *J* = 12.0, 6.0, 3.9 Hz, 1H, H-5’’), 3.63 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5’), 3.90 (q, *J* = 3.6 Hz, 1H, H-4’), 4.11 (dd, *J* = 3.9, 5.1 Hz, 1H, H-3’), 4.42 – 4.48 (m, 1H, H-2’), 5.11 (d, *J* = 4.8 Hz, 1H, OH-3’), 5.17 (t, *J* = 5.7 Hz, 1H, OH-5’), 5.31 (d, *J* = 6.3 Hz, 1H, OH-2’), 6.11 (d, *J* = 6.3 Hz, 1H, H-1’), 6.15 (br. s, 2H, NH_2), 7.27 – 7.35 (m, 2H, H_{Phe}), 7.46 – 7.51 (m, 2H, H_{Phe}), 7.53 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -116.01 (ddd, *J* = 13.8, 9.0, 5.9 Hz, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5’), 70.6 (C-3’), 73.8 (C-2’), 85.1 (C-4’), 87.1 (C-1’), 100.5 (C-4a), 115.5 (d, *J* = 26.3 Hz, 2C, C-3_{Phe}, C-5_{Phe}), 116.0 (C-5), 121.2 (C-6), 130,4 (d, *J* = 8.0 Hz, 2C, C-2_{Phe}, C-6_{Phe}), 130.8 (d, *J* = 3.5 Hz, 1C, C-1_{Phe}), 150.9 (C-7a), 151.7 (C-2), 157.4 (C-4), 161.5 (d, *J* = 241.6 Hz, 1C, C-4_{Phe}). HRMS (ESI): calculated for $C_{17}H_{18}FN_4O_4$ ($[M+H]^+$): 361.1307, found: 361.1291.

4-amino-5-(pyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (13) 13 was prepared according to General Procedure D. **40** (0.585 g, 0.858 mmol) gave rise to **13** as a white solid (0.140 g, 0.408 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 48 %. Melting point: 238 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (ddd, *J* = 11.7, 6.6, 4.2 Hz, 1H, H-5’’), 3.69 (ddd, *J* = 12.0, 5.1, 4.2 Hz, 1H, H-5’), 3.92 (q, *J* = 3.9 Hz, 1H, H-4’), 4.14 (dd, *J* = 8.7, 5.1 Hz, 1H, H-3’), 4.48 (q, *J* = 5.7 Hz, 1H, H-2’), 5.28 (dd, *J* = 6.3, 5.4 Hz, 1H, OH-5’), 5.36 (d, *J* = 6.0 Hz, 1H, OH-2’), 6.09 (d, *J* = 5.7 Hz, 1H, H-1’), 7.25 (ddd, *J* = 7.2, 5.1, 0.9 Hz, 1H, H-5_{Pyr}), 7.29 (br. s, 1H, NH), 7.85 (ddd, *J* = 8.1, 7.5, 1.8 Hz, 1H, H-4_{Pyr}), 7.98 (dt, *J* = 8.1, 0.9 Hz, 1H, H-3_{Pyr}), 8.07 (s, 1H, H-2), 8.26 (s, 1H, H-6), 8.55 (ddd, *J* = 5.1, 1.8, 0.9 Hz, 1H, H-6_{Pyr}), 9.88 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5’), 70.4 (C-3’), 73.7 (C-2’), 85.1 (C-4’), 87.3 (C-1’), 100.6 (C-4a), 116.0 (C-5), 120.3 (C-3_{Pyr}), 121.1 (C-

5_{Pyr}), 123.3 (C-6), 137.7 (C-4_{Pyr}), 147.9 (C-6_{Pyr}), 151.5 (C-7a), 152.5 (C-2), 153.1 (C-2_{Pyr}), 158.7 (C-4). HRMS (ESI): calculated for $C_{16}H_{18}N_5O_4$ ($[M+H]^+$): 344.1353, found: 344.1370.

4-amino-5-(pyridin-3-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (14) 14 was prepared according to General Procedure A (reaction time: 20 h), with the use of 3-pyridinylboronic acid pinacol ester. **31** (0.17 g, 0.50 mmol) gave rise to **14** as a white solid (0.060 g, 0.18 mmol). Column chromatography: 1 → 20 % MeOH/DCM. Yield = 35 %. Melting point: 220 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 – 3.57 (m, 1H, H-5''), 3.60 – 3.67 (m, 1H, H-5'), 3.91 (dd, *J* = 7.2, 3.9 Hz, 1H, H-4'), 4.09 – 4.14 (m, 1H, H-3'), 4.46 (dd, *J* = 11.4, 6.3 Hz, 1H, H-2'), 5.13 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.17 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.33 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.13 (d, *J* = 6.0 Hz, 1H, H-1'), 6.26 (br. s, 2H, NH_2), 7.48 (ddd, *J* = 8.1, 5.1, 0.9 Hz, 1H, H-4_{Pyr}), 7.67 (s, 1H, H-6), 7.85 (dt, *J* = 8.1, 2.1 Hz, 1H, H-5_{Pyr}), 8.17 (s, 1H, H-2), 8.55 (dd, *J* = 4.8, 1.8 Hz, 1H, H-6_{Pyr}), 8.70 (dd, *J* = 2.4, 0.9 Hz, 1H, H-2_{Pyr}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.6 (C-3'), 73.8 (C-2'), 85.1 (C-4'), 87.1 (C-1'), 100.4 (C-4a), 112.8 (C-5), 122.0 (C-6), 123.8 (C-5_{Pyr}), 130.3 (C-3_{Pyr}), 135.6 (C-4_{Pyr}), 147.7 (C-6_{Pyr}), 148.8 (C-2_{Pyr}), 151.2 (C-7a), 151.8 (C-2), 157.5 (C-4). HRMS (ESI): calculated for $C_{16}H_{18}N_5O_4$: 344.1353 ($[M+H]^+$), found: 344.1353.

4-amino-5-(pyridin-4-yl)-N7-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (15) 31 (0.172 g, 0.5 mmol, 1 eq.), 4-pyridinylboronic acid (0.092 g, 0.75 mmol, 1.5 eq.), $Pd_2(dba)_3$ (0.023 g, 0.025 mmol, 0.05 eq.) and P(c-hexyl)$_3$ (0.017 g, 0.06 mmol, 0.12 eq.) were added to a 10 mL round-bottom flask, containing a stir bar. Next, the flask was evacuated and refilled with argon three times. Then, 1,4-dioxane was added (1.33 mL, 2.67 mL/mmol SM) together with 1.27 M aq. K_3PO_4 solution (0.67 mL, 1.33 mL/mmol SM). The flask was stirred at ambient temperature for ~ 5 min and then transferred to a pre-heated oil bath at 100 °C. Heating was continued for 20 h, after which the mixture was cooled to ambient temperature. The mixture was neutralized (pH ~ 7) with 0.5 M aq. HCl. The mixture was evaporated till dryness and re-suspended in MeOH and evaporated (three times). Next, the mixture was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~ 5 cm) with 20 % MeOH/DCM. The liquid was evaporated *in vacuo* and purified by column chromatography 5 → 20 % MeOH/EA. **15** was isolated as a yellow solid (0.06 g, 0.175 mmol). Yield = 35 %. Melting point: 260 – 261 °C. ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 3.51 – 3.58 (m, 1H, H-5''), 3.61 – 3.68 (m, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.12 (br. s, 1H, H-3'), 4.45 (br. s, 1H, H-2'), 5.13 – 5.20 (m, 2H, OH-3', OH-5'), 5.36 (br. s, 1H, OH-2'), 6.13 (d, *J* = 6.3 Hz, 1H, H-1'), 6.38 (br. s, 2H, NH_2), 7.47 (dd, *J* = 4.5, 1.8 Hz, 2H, H-3_{Pyr}, H-5_{Pyr}), 7.79 (s, 1H, H-6), 8.18 (s, 1H, H-2), 8.61 (dd, *J* = 4.5, 1.8 Hz, 2H, H-2_{Pyr}, H-6_{Pyr}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.5 (C-3'), 73.8 (C-2'), 85.2 (C-4'), 87.1 (C-1'), 99.9 (C-4a), 114.0 (C-5), 122.8 (2C, C-3_{Pyr}, C-5_{Pyr}), 123.0 (C-6), 141.9 (C-4_{Pyr}), 149.9 (2C, C-2_{Pyr}, C-6_{Pyr}), 151.5 (C-7a), 152.0 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{16}H_{18}N_5O_4$: 344.1353 ($[M+H]^+$), found: 344.1350.

4-amino-5-(pyrazin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (16) 16 was prepared according to General Procedure B. The mixture was purified by column chromatography 25 → 100 % EA / Hex. Product containing fractions were pooled and evaporated (still containing some impurities). The resulting solid was dissolved in 15 mL 7N NH_3 / MeOH and stirred at ambient temperature overnight. Next, the mixture was evaporated till dryness. Purification by column chromatography (8 → 15 % MeOH/DCM). Product containing fractions were pooled and evaporated till near-dryness, after which the product precipitated out of the solution. **36** (0.40 g, 0.60 mmol) gave rise to **16** (0.075 g, 0.22 mmol) as a white solid. Yield = 36 %. Melting point: 257 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 – 3.62 (m, 1H, H-5''), 3.67 – 3.74 (m, 1H, H-5'), 3.93 (dd, *J* = 7.5, 3.9 Hz, 1H, H-4'), 4.15 (dd, *J* = 9.0, 5.1 Hz, 1H, H-3'), 4.47 (q, *J* = 5.7 Hz, 1H, H-4'), 5.15 (d, *J* = 5.1 Hz, 1H, OH-3'), 5.25 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.39 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.11 (d, *J* = 6.0 Hz, 1H, H-1'), 7.43 (br. s, 1H, NH), 8.11 (s, 1H, H-2), 8.45 (d, *J* = 2.7 Hz, 1H, H-6_{Pyra}), 8.49 (s, 1H, H-6), 8.59 (dd, *J* = 2.7, 1.5 Hz, 1H, H-5_{Pyra}), 9.14 (br. s, 1H, NH), 9.30 (d, *J* = 1.2 Hz, 1H, H-3_{Pyra}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.5 (C-5'), 70.3 (C-3'), 73.8 (C-2'), 85.2 (C-4'), 87.3 (C-1'), 100.5 (C-4a), 113.1 (C-5), 124.2 (C-6), 141.1 (C-6_{Pyra}), 142.3 (C-5_{Pyra}), 142.9 (C-3_{Pyra}), 148.9 (C-2_{Pyra}), 151.8 (C-7a), 152.8 (C-2), 158.5 (C-4). HRMS (ESI): calculated for $C_{15}H_{17}N_6O_4$ ($[M+H]^+$): 345.1306, found: 345.1291.

4-amino-5-(pyrimidin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (17) 17 was prepared according to General Procedure B. The mixture was purified by column chromatography 25 → 100 % EA/Hex. Product containing fractions were pooled and evaporated (still containing some impurities).

The resulting solid was dissolved in MeOH (10 mL) to which was added 0.1 mL NaOMe solution (5.4 M in MeOH). The mixture was stirred at ambient temperature for 1 hour, and neutralized (pH ~ 7) with 0.5 M aq. HCl. Next, the mixture was evaporated till dryness. Purification by column chromatography (2 → 15 % MeOH/DCM). **36** (0.33 g, 0.50 mmol) gave rise to **17** (0.050 g, 0.15 mmol) as a white solid. Yield = 29 %. Melting point: 200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.54 – 3.69 (m, 2H, H-5', H-5''), 3.94 (dd, *J* = 6.0, 3.3 Hz, 1H, H-4'), 4.09 – 4.12 (m, 1H, H-3'), 4.46 (dd, *J* = 11.7, 6.0 Hz, 1H, H-2'), 5.16 (d, *J* = 4.2 Hz, 1H, OH-3'), 5.27 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.36 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.13 (d, *J* = 6.3 Hz, 1H, H-1'), 7.32 (t, *J* = 4.8 Hz, 1H, H-5$_{Pyrim}$), 7.39 (br. s, 1H, N-H), 8.10 (s, 1H, H-2), 8.37 (s, 1H, H-6), 8.80 (d, *J* = 4.8 Hz, 2H, H-4$_{Pyrim}$, H-6$_{Pyrim}$), 9.56 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.6 (C-3'), 74.0 (C-2'), 85.4 (C-4'), 87.1 (C-1'), 100.3 (C-4a), 115.4 (C-5), 118.2 (C-5$_{Pyrim}$), 126.9 (C-6), 151.7 (C-7a), 152.7 (C-2), 157.4 (C-4$_{Pyrim}$, C-6$_{Pyrim}$), 158.5 (C-4), 161.4 (C-2$_{Pyrim}$). HRMS (ESI): calculated for $C_{15}H_{17}N_6O_4$ ([M+H]$^+$): 345.1306, found: 345.1316.

4-amino-5-(pyrimidin-5-yl)-N7-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (18) 31 (0.17 g, 0.50 mmol, 1 eq.), pyrimidin-5-yl-boronic acid (0.092 g, 0.75 mmol, 1.5 eq.), $Pd_2(dba)_3$ (0.005 g, 0.005 mmol, 0.01 eq.) and P(c-hexyl)$_3$ (0.0035 g, 0.012 mmol, 0.024 eq.) were added to a 10 mL round-bottom flask containing a stir bar. Next, the flask was evacuated and refilled with argon three times. Then, 1,4-dioxane was added (1.33 mL, 2.67 mL/mmol SM) together with 1.27 M aq. K_3PO_4 solution (0.67 mL, 1.3 mL/mmol). The flask was stirred at ambient temperature for ~ 5 min and then transferred to a pre-heated oil bath at 100 °C. Heating was continued for 38 h, after which the mixture was cooled to ambient temperature. The mixture was neutralized (pH ~ 7) with 0.5 M aq. HCl. The mixture was evaporated till dryness re-suspended in MeOH and evaporated (three times). Next, the mixture was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~ 5 cm) with 20 % MeOH/DCM. The liquid was evaporated *in vacuo* and purified by column chromatography 5 → 20 % MeOH/EA. **18** was isolated as a yellow solid (0.045 g, 0.13 mmol). Yield = 26 %. Melting point: 275 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 – 3.57 (m, 1H, H-5''), 3.60 – 3.67 (m, 1H, H-5'), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.09 – 4.14 (m, 1H, H-3'), 4.45 (dd, *J* = 11.1, 5.7 Hz, 1H, H-2'), 5.14 – 5.18 (m, 2H, OH-3', OH-5'), 5.36 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.13 (d, *J* = 6.0 Hz, 1H, H-1'), 6.51 (br. s, 2H, NH_2), 7.77 (s, 1H,

H-6), 8.18 (s, 1H, H-2), 8.86 (s, 2H, H-4_{Pyrim}, H-6_{Pyrim}), 9.13 (s, 1H, H-2_{Pyrim}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.5 (C-3'), 73.8 (C-2'), 85.2 (C-4'), 87.1 (C-1'), 100.3 (C-4a), 109.3 (C-5), 122.8 (C-6), 128.5 (C-5_{Pyrim}), 151.4 (C-7a), 152.0 (C-2), 155.5 (2C, C-4_{Pyrim}, C-6_{Pyrim}), 156.2 (C-2_{Pyrim}), 157.6 (C-4). HRMS (ESI): calculated for $C_{15}H_{17}N_6O_4$ ([M+H]$^+$): 345.1306, found: 345.1329.

4-amino-5-(1-methyl-1*H*-imidazol-5-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (19) 38 (0.407 g, 0.618 mmol) was dissolved in 7N NH_3 / MeOH (15 mL) and stirred at ambient temperature overnight. The resulting mixture was evaporated till dryness and purified by column chromatography 10 → 17.5 % MeOH/DCM. **19** was obtained as a white solid (0.18 g, 0.52 mmol). Yield = 84 %. Melting point: 170 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 – 3.57 (m, 1H, H-5''), 3.53 (s, 3H, CH_3), 3.61 – 3.68 (m, 1H, H-5''), 3.91 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.14 (m, 1H, H-3'), 4.45 (dd, *J* = 11.4, 6.3 Hz, 1H, H-2'), 5.13 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.21 (dd, *J* = 6.3, 5.4 Hz, 1H, OH-5'), 5.36 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.10 (d, *J* = 6.0 Hz, 1H, H-1'), 6.16 (br. s, 2H, NH_2), 6.98 (s, 1H, H-4_{Imid}), 7.61 (s, 1H, H-6), 7.76 (s, 1H, H-2_{Imid}), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 31.5 (CH_3), 61.6 (C-5'), 70.5 (C-3'), 73.9 (C-2'), 85.1 (C-4'), 87.4 (C-1'), 101.7 (C-4a), 102.3 (C-5), 123.0 (C-6), 125.1 (C-5_{Imid}), 128.3 (C-4_{Imid}), 139.4 (C-2_{Imid}), 150.3 (C-7a), 152.0 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{15}H_{19}N_6O_4$ ([M+H]$^+$): 347.1462, found: 347.1481.

4-amino-5-(1-methyl-1*H*-imidazol-4-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (20) 20 was prepared according to General Procedure B, employing 1-methyl-4-(tributylstannyl)-*1H*-imidazole[38-39] as the coupling partner. The mixture was purified by column chromatography 0 → 3.5 % MeOH / DCM. Product containing fractions were pooled and evaporated (still containing some impurities). The resulting solid was dissolved in 20 mL 7N NH_3/MeOH and stirred at ambient temperature overnight. Next, the mixture was evaporated till dryness. Purification by column chromatography (10 % MeOH/DCM). **36** (0.40 g, 0.60 mmol) gave rise to **20** (0.025 g, 0.072 mmol) as a white solid. Yield = 25 %. Melting point: 162 – 164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 – 3.57 (m, 1H, H-5''), 3.61 – 3.69 (m, 1H, H-5'), 3.70 (s, 3H, CH_3), 3.90 (q, *J* = 3.6 Hz, 1H, H-4'), 4.07 – 4.12 (m, 1H, H-3'), 4.41 (dd, *J* = 11.1, 6.0 Hz, 1H, H-2'), 5.10 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.25 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.30 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.03 (d, *J* = 6.3 Hz, 1H, H-1'), 7.10 (br. s, 1H, NH),

7.47 (d, *J* = 1.2 Hz, 1H, H-5_{Imid}), 7.64 (s, 1H, H-6), 7.74 (d, *J* = 0.9 Hz, 1H, H-2_{Imid}), 8.01 (s, 1H, H-2). 9.81 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.2 (CH_3), 61.9 (C-5'), 70.7 (C-3'), 73.6 (C-2'), 85.0 (C-4'), 87.1 (C-1'), 100.5 (C-4a), 110.4 (C-5), 116.0 (C-5_{Imid}), 117.7 (C-6), 135.6 (C-4_{Imid}), 137.1 (C-2_{Imid}), 150.4 (C-7a), 151.9 (C-2), 158.3 (C-4). HRMS (ESI): calculated for $C_{15}H_{19}N_6O_4$ ($[M+H]^+$): 347.1462, found: 347.1462. **Purity: 92%**

4-amino-5-(4-methylpyridin-2-yl)-N7-β-(D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (21) 21 was prepared according to General Procedure D. Purification by column chromatography 5 → 15 % MeOH/DCM. **41** (0.13 g, 0.18 mmol) gave rise to **21** (0.023 g, 0.064 mmol) as a white solid. Yield = 36 %. Melting point: 230 – 234 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ: 2.40 (s, 3H, CH_3), 3.78 (dd, *J* = 12.6, 3.0 Hz, 1H, H-5''), 3.91 (dd, *J* = 12.6, 2.7 Hz, 1H, H-5'), 4.14 (q, *J* = 3.0 Hz, 1H, H-4'), 4.33 (dd, *J* = 5.4, 3.3 Hz, 1H, H-3'), 4.66 (dd, *J* = 6.0, 5.4 Hz, 1H, H-2'), 6.08 (d, *J* = 6.3 Hz, 1H, H-1'), 7.05 (dd, *J* = 5.4, 0.9 Hz, 1H, H-5_{Pyr}), 7.75 (br. s, 1H, H-3_{Pyr}), 8.04 (s, 1H, H-2), 8.10 (s, 1H, H-6), 8.35 (d, *J* = 3.9 Hz, 1H, H-6_{pyr}). ^{13}C NMR (75 MHz, MeOH-d_4) δ: 21.2 (CH_3), 63.3 (C-5'), 72.3 (C-3'), 75.6 (C-2'), 87.3 (C-4'), 91.3 (C-1'), 103.2 (C-4a), 118.4 (C-5), 122.0 (C-3_{Pyr}), 123.5 (C-5_{Pyr}), 124.9 (C-6), 148.6 (C-6_{Pyr}), 150.3 (C-4_{Pyr}), 151.7 (C-7a), 152.6 (C-2_{Pyr}), 154.2 (C-2), 160.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{20}N_5O_4$ ($[M+H]^+$): 358.1510, found: 358.1490.

4-amino-5-(4-methoxypyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (22) 22 was prepared according to General Procedure D. Purification by column chromatography 5 → 10 % MeOH/DCM. **42** (0.12 g, 0.17 mmol) gave rise to **22** (0.014 g, 0.037 mmol) as a white solid. Yield = 22 %. Melting point: 135 – 140 °C / 225 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ: 3.78 (dd, *J* = 12.6, 3.0 Hz, 1H, H-5''), 3.92 (dd, *J* = 12.6, 2.7 Hz, 1H, H-5'), 3.94 (s, 3H, OCH_3), 4.14 (q, *J* = 3.0 Hz, 1H, H-4'), 4.34 (dd, *J* = 5.1, 3.3 Hz, 1H, H-3'), 4.65 (t, *J* = 5.7 Hz, 1H, H-2'), 6.08 (d, *J* = 6.0 Hz, 1H, H-1'), 6.82 (dd, *J* = 6.0, 2.4 Hz, 1H, H-5_{Pyr}), 7.42 (d, *J* = 2.1 Hz, 1H, H-3_{Pyr}), 8.05 (s, 1H, H-2), 8.15 (s, 1H, H-6), 8.34 (dd, *J* = 6.0, 0.6 Hz, 1H, H-6_{Pyr}). ^{13}C NMR (75 MHz, MeOH-d_4) δ: 56.0 (OCH_3), 63.2 (C-5'), 72.2 (C-3'), 75.5 (C-2'), 87.3 (C-4'), 91.4 (C-1'), 103.2* (C-4a), 106.35 (C-3_{Pyr}), 109.5 (C-5_{Pyr}), 118.4 (C-5), 125.0 (C-6), 150.2 (C-6_{Pyr}), 151.6 (C-7a), 152.7 (C-2), 156.0 (C-2_{Pyr}), 160.4 (C-4), 168.4 (C-4_{Pyr}).

HRMS (ESI): calculated for $C_{17}H_{20}N_5O_5$ ($[M+H]^+$): 374.1459, found: 374.1437. *This value was obtained from the gHMBC spectrum; and could not be observed from the ^{13}C-NMR.

4-amino-5-(6-chloro-pyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (23) 23 was prepared according to General Procedure D. After evaporation, the resulting mixture was taken up in MeOH and the precipitate collected by filtration to yield pure **23**, which did not require column chromatography. **43** (0.14 g, 0.20 mmol) gave rise to **23** (0.045 g, 0.12 mmol) as a white solid. Yield = 59 %. Melting point: 214 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.51 – 3.60 (m, 1H, H-5''), 3.62 – 3.72 (m, 1H, H-5'), 3.92 (q, *J* = 3.9 Hz, 1H, H-4'), 4.14 (t, *J* = 4.2 Hz, 1H, H-3'), 4.46 (t, *J* = 5.4 Hz, 1H, H-2'), 5.17 (br. s, 1H, OH-3'), 5.27 (br. s, 1H, OH-5'), 5.40 (br. s, 1H, OH-2'), 6.10 (d, *J* = 6.0 Hz, 1H, H-1'), 7.36 (dd, *J* = 7.8, 0.9 Hz, 1H, H-5_{Pyr}), 7.47 (br. s, 1H, NH), 7.91 (t, *J* = 7.8 Hz, 1H, H-4_{Pyr}), 7.99 (d, *J* = 7.8 Hz, 1H, H-3_{Pyr}), 8.09 (s, 1H, H-2), 8.37 (s, 1H, H-6), 9.15 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.5 (C-5'), 70.3 (C-3'), 73.7 (C-2'), 85.2 (C-4'), 87.2 (C-1'), 100.2 (C-4a), 114.7 (C-5), 119.2 (C-3_{Pyr}), 120.8 (C-5_{Pyr}), 124.6 (C-6), 141.0 (C-4_{Pyr}), 148.4 (C-6_{Pyr}), 151.7 (C-7a), 152.6 (C-2), 154.1 (C-2_{Pyr}), 158.6 (C-4). HRMS (ESI): calculated for $C_{16}H_{17}ClN_5O_4$ ($[M+H]^+$): 378.0964, found: 378.0964.

4-amino-5-(5-chloro-pyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (24) 24 was prepared according to General Procedure D. After evaporation, the resulting mixture was taken up in MeOH and the precipitate collected by filtration to yield pure **24**, which did not require column chromatography. **44** (0.20 g, 0.28 mmol) gave rise to **24** (0.051 g, 0.14 mmol) as a white solid. Yield = 49 %. Melting point: 203 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 – 3.61 (m, 1H, H-5''), 3.69 (dt, *J* = 12.3, 4.5 Hz, 1H, H-5'), 3.92 (q, *J* = 3.6 Hz, 1H, H-4'), 4.14 (br. s, 1H, H-3'), 4.60 (br. s, 1H, H-2'), 5.14 (d, *J* = 3.0 Hz, 1H, OH-3'), 5.25 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.38 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.10 (d, *J* = 6.0 Hz, 1H, H-1'), 7.34 (br. s, 1H, NH), 7.98 (dd, *J* = 8.7, 2.4 Hz, 1H, H-4_{Pyr}), 8.02 (dd, *J* = 9.0, 0.9 Hz, 1H, H-3_{Pyr}), 8.08 (s, 1H, H-2), 8.30 (s, 1H, H-6), 8.63 (dd, *J* = 2.4, 0.9 Hz, 1H, H-6_{Pyr}), 9.38 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.4 (C-3'), 73.7 (C-2'), 85.2 (C-4'), 87.2 (C-1'), 100.3 (C-4a), 115.1 (C-5), 121.7 (C-3_{Pyr}), 124.0 (C-6), 127.9 (C-5_{Pyr}), 137.5 (C-4_{Pyr}), 146.5 (C-

6_{Pyr}), 151.6 (C-7a), 151.8 (C-2_{Pyr}), 152.6 (C-2), 158.6 (C-4). HRMS (ESI): calculated for $C_{16}H_{17}ClN_5O_4$ ($[M+H]^+$): 378.0964, found: 378.0961.

4-amino-5-(4-chloro-pyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (25) 25 was prepared according to General Procedure D. Purification by column chromatography 5 → 15 % MeOH/DCM. **45** (0.11 g, 0.16 mmol) gave rise to **25** (0.019 g, 0.050 mmol) as a white solid. Yield = 32 %. Melting point: 258 – 261 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.52 – 3.60 (m, 1H, H-5’’), 3.65 – 3.72 (m, 1H, H-5’), 3.96 (q, *J* = 3.9 Hz, 1H, H-4’), 4.12 – 4.16 (m, 1H, H-3’), 4.48 (q, *J* = 6.0 Hz, 1H, H-2’), 5.13 (d, *J* = 4.8 Hz, 1H, OH-3’), 5.20 (t, *J* = 5.7 Hz, 1H, OH-5’), 5.37 (d, *J* = 6.3 Hz, 1H, OH-2’), 6.10 (d, *J* = 6.0 Hz, 1H, H-1’), 7.33 (br. s, 1H, NH), 7.38 (dd, *J* = 5.7, 1.8 Hz, 1H, H-5_{Pyr}), 8.09 (s, 1H, H-6), 8.17 (d, *J* = 1.5 Hz, 1H, H-3_{Pyr}), 8.38 (s, 1H, H-2), 8.54 (d, *J* = 5.4 Hz, 1H, H-6_{Pyr}), 9.62 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5’), 70.3 (C-3’), 73.5 (C-2’), 85.1 (C-4’), 87.0 (C-1’), 100.4 (C-4a), 115.0 (C-5), 120.0 (C-3_{Pyr}), 121.0 (C-5_{Pyr}), 124.3 (C-6), 144.3 (C-4_{Pyr}), 149.5 (C-6_{Pyr}), 151.70 (C-7a), 152.7 (C-2), 154.8 (C-2_{Pyr}), 158.6 (C-4). HRMS (ESI): calculated for $C_{16}H_{17}ClN_5O_4$ ($[M+H]^+$): 378.0964, found: 378.0976.

4-amino-5-(5-fluoro-pyridin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (26) 26 was prepared according to General Procedure D. After evaporation, the resulting mixture was taken up in MeOH and the precipitate collected by filtration to yield **26**, which did not require column chromatography. **46** (0.15 g, 0.22 mmol) gave rise to **26** (0.046 g, 0.13 mmol) as a white solid. Yield = 59 %. Melting point: 220 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 – 3.60 (m, 1H, H-5’’), 3.62 – 3.72 (m, 1H, H-5’), 3.92 (q, *J* = 3.9 Hz, 1H, H-4’), 4.13 – 4.15 (m, 1H, H-3’), 4.47 (t, *J* = 5.7 Hz, 1H, H-2’), 5.19 (br. s, 1H, OH-3’), 5.27 (br. s, 1H, OH-5’), 5.39 (br. s, 1H, OH-2’), 6.09 (d, *J* = 6.0 Hz, 1H, H-1’), 7.28 (br. s, 1H, NH), 8.07 (dt, *J* = 8.7, 3.0 Hz, 1H, H-4_{Pyr}), 8.07 (s, 1H, H-2), 8.07 (dd, *J* = 8.7, 4.5 Hz, 1H, H-3P_{yr}), 8.24 (s, 1H, H-6), 8.59 (dd, *J* = 3.0 Hz, 1H, H-6_{Pyr}), 9.37 (br. s, 1H, NH). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -130.99 (dd, *J* = 8.5, 4.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5’), 70.4 (C-3’), 73.7 (C-2’), 85.1 (C-4’), 87.1 (C-1’), 100.4 (C-4a), 115.2 (C-5), 122.1 (C-3_{Pyr}), 123.3 (C-6), 125.35 (d, *J* = 19.43 Hz, 1C, C-4_{Pyr}), 135.56 (d, *J* = 24.0 Hz, 1C, C-6_{Pyr}), 150.1 (C-2_{Pyr}), 151.6 (C-7a),

152.5 (C-2), 157.4 (d, *J* = 250.7 Hz, 1C, C-5_{Pyr}), 158.6 (C-4). HRMS (ESI): calculated for $C_{16}H_{17}FN_5O_4$ ($[M+H]^+$): 362.1259, found: 362.1263.

4-amino-5-(naphthalen-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (27) 27 was prepared according to General Procedure A (reaction time: 1.5 h). **31** (0.24 g, 0.70 mmol) gave rise to **27** as a white solid (0.17 g, 0.43 mmol). Column chromatography: 1 → 10 % MeOH/DCM. Yield = 62 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (ddd, *J* = 12.0, 6.3, 3.9 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.1, 3.9 Hz, 1H, H-5'), 3.93 (q, *J* = 3.6 Hz, 1H, H-4'), 4.11 – 4.15 (m, 1H, H-3'), 4.49 (q, *J* = 6.0 Hz, 1H, H-2'), 5.13 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.19 (dd, *J* = 6.0, 5.1 Hz, 1H, OH-5'), 5.34 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.16 (d, *J* = 6.3 Hz, 1H, H-1'), 6.21 (br. s, 2H, NH_2), 7.50 – 7.59 (m, $2H_{Naf}$), 7.64 (dd, *J* = 8.1, 1.8 Hz, 1H, H-3_{Naf}), 7.67 (s, 1H, H-6), 7.96 – 7.99 (m, $3H_{Naf}$), 8.03 (d, *J* = 8.7 Hz, 1H, H-4_{Naf}), 8.18 (s, 1H, H-2). HRMS (ESI): calculated for $C_{21}H_{21}N_4O_4$ ($[M+H]^+$): 393.1557, found: 393.1557. Spectral data are in accordance to literature values.[29]

4-amino-5-(quinolin-2-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (28) 28 was prepared according to the General Procedure D. Column chromatography 0 → 20 % MeOH/DCM. **47** (0.15 g, 0.20 mmol) gave rise to **28** (0.025 g, 0.064 mmol) as a white solid. Yield = 31 %. Melting point: 231 – 234 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.58 – 3.62 (m, 1H, H-5''), 3.71 – 3.75 (m, 1H, H-5'), 3.95 (q, *J* = 3.6 Hz, 1H, H-4'), 4.17 (q, *J* = 4.2 Hz, 1H, H-3'), 4.52 (q, *J* = 5.4 Hz, 1H, H-2'), 5.16 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.30 (s, 1H, OH-5'), 5.41 (d, *J* = 5.7 Hz, 1H, OH-2'), 6.13 (d, *J* = 6.0 Hz, 1H, H-1'), 7.45 (br. s, 1H, NH), 7.58 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, H-6_{Quin}), 7.79 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H, H-7_{Quin}), 7.89 (d, *J* = 8.4 Hz, 1H, H-8_{Quin}), 7.97 (d, *J* = 8.1 Hz, 1H, H-5_{Quin}), 8.13 (s, 1H, H-2), 8.15 (*J* = 8.7 Hz, 1H, H-3_{Quin}), 8.41 (d, *J* = 8.7 Hz, 1H, H-4_{Quin}), 8.52 (s, 1H, H-6), 10.64 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5'), 70.4 (C-3'), 73.7 (C-2'), 85.2 (C-4'), 87.3 (C-1'), 100.7 (C-4a), 116.6 (C-5), 119.3 (C-3_{Quin}), 125.5 (C-6), 126.1 (C-6_{Quin} / C-$4a_{Quin}$), 126.8 (C-8_{Quin}), 127.9 (C-5_{Quin}), 130.3 (C-7_{Quin}), 137.2 (C-4_{Quin}), 146.1 (C-$8a_{Quin}$), 151.8 (C-7a), 152.6 (C-2), 153.2 (C-2_{Quin}), 158.7 (C-4). HRMS (ESI): calculated for $C_{20}H_{20}N_5O_4$ ($[M+H]^+$): 394.1510, found: 394.1497.

4-amino-5-(isoquinolin-3-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (29) 29 was prepared according to General Procedure D. After evaporation, the resulting mixture was taken up in

MeOH and the precipitate collected by filtration to yield **29**, which did not require column chromatography. **48** (0.17 g, 0.23 mmol) gave rise to **29** (0.060 g, 0.15 mmol) as a white solid. Yield = 66 %. Melting point: 258 – 264 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.60 (dd, *J* = 11.7, 4.2 Hz, 1H, H-5''), 3.72 (dd, *J* = 11.7, 3.9 Hz, 1H, H-5'), 3.95 (q, *J* = 3.6 Hz, 1H, H-4'), 4.18 (dd, *J* = 4.8, 3.9 Hz, 1H, H-3'), 4.51 (t, *J* = 5.7 Hz, 1H, H-2'), 5.18 – 5.50 (m, 3H, OH-2', OH-3', OH-5'), 6.14 (d, *J* = 6.0 Hz, 1H, H-1'), 7.34 (br. s, 1H, NH), 7.63 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, H-7_{Isoq}), 7.79 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, H-6_{Isoq}), 7.93 (dd, *J* = 8.4, 0.9 Hz, 1H, H-5_{Isoq}), 8.10 (s, 1H, H-2), 8.12 (dd, *J* = 8.4, 0.9 Hz, 1H, H-8_{Isoq}), 8.30 (s, 1H, H-6), 8.40 (s, 1H, H-4_{Isoq}), 9.38 (s, 1H, H-1_{Isoq}), 9.78 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5'), 70.4 (C-3'), 73.7 (C-2'), 85.1 (C-4'), 87.1 (C-1'), 100.8 (C-4a), 115.6 (C-4_{Isoq}), 116.2 (C-4a), 122.3 (C-6), 126.3 (C-5_{Isoq}), 126.5 (C-$8a_{Isoq}$), 126.9 (C-7_{Isoq}), 127.9 (C-8_{Isoq}), 131.4 (C-6_{Isoq}), 136.7 (C-$4a_{Isoq}$), 146.8 (C-3_{Isoq}), 151.2 (C-1_{Isoq}), 151.4 (C-7a), 152.2 (C-2), 158.6 (C-4). HRMS (ESI): calculated for $C_{20}H_{20}N_5O_4$ ($[M+H]^+$): 394.1510, found: 394.1503.

4-amino-5-(isoquinol-1-yl)-N7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (30) 30 was prepared according to General Procedure D. Column chromatography 5 → 10 % MeOH/DCM. **49** (0.13 g, 0.17 mmol) gave rise to **30** (0.035 g, 0.089 mmol) as a white solid. Yield = 51 %. Melting point: 130 – 135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (ddd, *J* = 11.7, 6.3, 3.3 Hz, 1H, H-5''), 3.68 (ddd, *J* = 12.0, 4.8, 3.6 Hz, 1H, H-5'), 3.96 (q, *J* = 3.6 Hz, 1H, H-4'), 4.16 – 4.20 (m, 1H, H-3'), 4.53 (q, *J* = 5.4 Hz, 1H, H-2'), 5.12 (d, *J* = 5.1 Hz, 1H, OH-3'), 5.25 (dd, *J* = 6.3, 4.8 Hz, 1H, OH-5'), 5.46 (d, *J* = 6.0 Hz, 1H, OH-2'), 6.20 (d, *J* = 5.4 Hz, 1H, H-1'), 7.54 (br. s, 2H, NH_2), 7.72 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H, H-7_{Isoq}), 7.78 (d, *J* = 5.4 Hz, 1H, H-4_{Isoq}), 7.84 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H, H-6_{Isoq}), 8.05 (d, *J* = 8.7 Hz, 1H, H-5_{Isoq}), 8.08 (s, 1H, H-6), 8.19 (s, 1H, H-2), 8.53 (d, *J* = 8.7 Hz, 1H, H-8_{Isoq}), 8.57 (d, *J* = 5.7 Hz, 1H, H-3_{Isoq}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.2 (C-5'), 70.4 (C-3'), 74.1 (C-2'), 85.1 (C-4'), 87.73 (C-1'), 101.6 (C-4a), 113.6 (C-5), 119.1 (C-4_{Isoq}), 126.3 (C-6), 126.4 (C-5_{Isoq}), 127.3 (C-8_{Isoq}), 127.3 (C-7_{Isoq}), 128.2 (C-$8a_{Isoq}$), 130.7 (C-6_{Isoq}), 137.0 (C$4a_{Isoq}$), 141.1 (C-3_{Isoq}), 150.9 (C-7a), 152.3 (C-2), 154.2 (C-1_{Isoq}), 158.6 (C-4). HRMS (ESI): calculated for $C_{20}H_{20}N_5O_4$ ($[M+H]^+$): 394.1510, found: 394.1506.

4-azido-5-bromo-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (34)

32[30] (1.0 g, 1.5 mmol) was dissolved in anhydrous DMF (15 mL, 10 mL/mmol SM). Next, NaN_3 (0.20 g, 3.1 mmol, 2.05 eq.) was added. The resulting mixture was heated in a pre-heated oil bath at 65 °C for 30 min. Next, the mixture was cooled to ambient temperature. Then, it was poured into half-saturated $NaHCO_3$ solution (75 mL) and EA (75 mL). The layers were separated, and the water layer extracted two more times with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography (30 % EA/Hex) to yield **34** as a white foam (0.93 g, 1.4 mmol). Yield = 90 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.72 (dd, *J* = 12.3, 5.1 Hz, 1H, H-5''), 4.83 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5'), 4.90 – 4.95 (m, 1H, H-4'), 6.10 – 6.14 (m, 1H, H-3'), 6.26 – 6.30 (m, 1H, H-2'), 6.85 (d, *J* = 5.1 Hz, 1H, H-1'), 7.41 – 7.53 (m, 6H, OBz), 7.61 – 7.70 (m, 3H, OBz), 7.86 – 7.89 (m, 2H, OBz), 7.93 – 7.97 (m, 2H, OBz), 7.98 – 8.01 (m, 2H, OBz), 8.30 (s, 1H, H-6), 9.94 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.5 (C-5'), 70.7 (C-3'), 74.0 (C-2'), 79.5 (C-4'), 88.9 (C-1'), 90.0, 103.9, 125.8 (C-6), 128.2, 128.5, 128.8, 129.2, 129.2, 129.4, 133.5, 133.9, 134.0, 135.1 (C-2), 140.6 (C-7a), 145.4 (C-4), 164.4 (C=O), 164.6 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{32}H_{24}BrN_6O_7$ ($[M+H]^+$): 683.0884, found: 683.0917.

4-azido-5-iodo-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (35) **35** was prepared according to the procedure as described for **34**. **33**[30] (3.56 g, 4.91 mmol) gave rise to **35** as a white foam (3.1 g, 4.2 mmol) in 86 % yield. Column chromatography 35 % EA/Hex. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.72 (dd, *J* = 12.0, 5.1 Hz, 1H, H-5''), 4.83 (dd, *J* = 12.0, 4.2 Hz, 1H, H-5'), 4.90 – 4.94 (m, 1H, H-4'), 6.12 (t, *J* = 6.0 Hz, 1H, H-3'), 6.27 (dd, *J* = 6.0, 5.1 Hz, 1H, H-2'), 6.83 (d, *J* = 5.1 Hz, 1H, H-1'), 7.43 – 7.53 (m, 6H, OBz), 7.60 – 7.70 (m, 3H, OBz), 7.85 – 7.88 (m, 2H, OBz), 7.93 – 8.01 (m, 4H, OBz), 8.27 (s, 1H, H-6), 9.90 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 55.9 (C-5), 63.6 (C-5'), 70.7 (C-3'), 74.0 (C-2'), 79.5 (C-4'), 86.7 (C-1'), 107.2 (C-4a), 128.2, 128.5, 128.8, 129.1, 129.2, 129.4, 130.6 (C-6), 133.6, 134.0, 134.02, 134.9 (C-2), 141.5 (C-7a), 146.0 (C-4), 164.4 (C=O), 164.7 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{32}H_{24}IN_6O_7$ ($[M+H]^+$): 731.0746, found: 731.0796.

4-amino-5-bromo-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl-pyrrolo[2,3-*d*]pyrimidine (36) 34 (0.52 g, 0.75 mmol) was dissolved in THF (7.5 mL, 10 mL / mmol). Then, PMe_3 solution (1M in THF; 1.5 mL, 1.5 mmol, 2 eq.) was added and the mixture stirred at ambient temperature for 30 min. Next, the solution was evaporated till dryness, and subsequently re-dissolved in MeCN (7.5 mL, 10 mL/mmol). To this solution was added a 1M aq. HOAc solution (2.5 mL, 3.33 eq.), and the mixture heated in a pre-heated oil bath at 65 °C for 1 h. Next, the mixture was cooled to ambient temperature and poured into sat. aq. $NaHCO_3$ solution. DCM was added, the layers were separated, and the water layer extracted two more times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography 62.5 % EA/Hex gave **36** as a white foam (0.40 g, 0.61 mmol). Yield = 81 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.67 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5''), 4.76 (q, *J* = 3.6 Hz, 1H, H-4'), 4.86 (dd, *J* = 12.3, 3.3 Hz, 1H, H-5'), 5.61 (br. s, 2H, NH_2), 6.07 – 6.13 (m, 2H, H-2', H-3'), 6.66 (d, *J* = 5.1 Hz, 1H, H-1'), 7.11 (s, 1H, H-6), 7.33 – 7.41 (m, 4H, OBz), 7.47 – 7.64 (m, 5H, OBz), 7.92 – 7.99 (m, 4H, OBz), 8.11 – 8.14 (m, 2H, OBz), 8.26 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.7 (C-5'), 71.6 (C-3'), 74.2 (C-2'), 80.4 (C-2'), 86.1 (C-1'), 89.3 (C-5), 102.8 (C-4a) 121.0 (C-6), 128.6, 128.7, 128.7, 128.9, 128.9, 129.6, 129.9, 129.99, 130.04, 133.6, 133.8, 150.7 (C-7a), 153.3 (C-2), 157.0 (C-4), 165.3 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{32}H_{26}BrN_4O_7$ ($[M+H]^+$): 657.0979, found: 657.0970.

4-amino-5-iodo-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (37) 37 was prepared according to the procedure as described for **36**. **35** (2.10 g, 2.87 mmol) gave rise to **37** as a slight yellow foam (1.89 g, 2.68 mmol) in 93 % yield. Column chromatography 62.5 % EA/Hex. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.67 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5''), 4.74 – 4.78 (m, 1H, H-4'), 4.87 (dd, *J* = 12.0, 3.3 Hz, 1H, H-5'), 5.77 (br. s, 2H, NH_2), 6.09 – 6.14 (m, 2H, H-3', H-2'), 6.66 (dd, *J* = 3.0, 1.8 Hz, 1H, H-1'), 7.20 (s, 1H, H-6), 7.33 – 7.40 (m, 4H, OBz), 7.47 – 7.64 (m, 5H, OBz), 7.92 – 7.99 (m, 4H, OBz), 8.14 – 8.15 (m, 2H, OBz), 8.26 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 52.4 (C-5), 63.9 (C-5'), 71.6 (C-3'), 74.2 (C-2'), 80.4 (C-4'), 86.2 (C-1'), 104.7 (C-4a), 126.3 (C-6), 128.6, 128.6, 128.7, 128.9, 129.5, 129.9, 129.95, 130.01, 133.6, 133.8, 150.8 (C-7a), 152.7 (C-2), 157.1 (C-4), 165.2

(C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{32}H_{26}IN_4O_7$: 705.0841 ($[M+H]^+$), found: 705.0822.

4-amino-5-(1-methyl-1*H*-imidazol-5-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (38) 38 was prepared according to General Procedure B. **36** (0.49 g, 0.75 mmol) and 1-methyl-5-(tributylstannyl)-*1H*-imidazole[37] (0.56 g, 0.46 mL, 1.5 mmol) gave rise to **38** as a white foam (0.41 g, 0.62 mmol). Purification by column chromatography: 7.5 % MeOH/DCM. Yield = 82 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.37 (s, 3H, CH_3), 4.65 (dd, *J* = 12.3, 3.6 Hz, 1H, H-5''), 4.78 (q, *J* = 3.6 Hz, 1H, H-4'), 4.92 (dd, *J* = 12.3, 3.3 Hz, 1H, H-5'), 5.27 (br. s, 2H, NH_2), 6.13 (dd, *J* = 6.0, 4.2 Hz, 1H, H-3'), 6.18 (t, *J* = 5.7 Hz, 1H, H-2'), 6.75 (d, *J* = 5.4 Hz, 1H, H-1'), 7.05 (s, 1H, H-4$_{Imid}$), 7.08 (s, 1H, H-6), 7.33 – 7.42 (m, 6H, OBz), 7.50 – 7.59 (4H (3+1), OBz (3H), H-2$_{Imid}$(1H)), 7.93 – 8.00 (m, 4H, OBz), 8.07 – 8.10 (m, 2H, OBz), 8.30 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 31.9 (CH_3), 63.8 (C-5'), 71.7 (C-3'), 74.1 (C-2'), 80.4 (C-4'), 86.1 (C-1'), 103.1, 104.0, 121.7 (C-6), 124.9 (C-5$_{Imid}$), 128.58, 128.62, 128.7, 128.9, 129.5, 129.60 (C-4$_{Imid}$), 129.67, 129.72, 129.9, 130.0, 133.5, 133.8, 139.3 (C-2$_{Imid}$), 151.3 (C-7a), 153.1 (C-2), 157.3 (C-4), 165.2 (C=O), 165.5 (C=O), 166.2 (C=O). HRMS (ESI): calculated for $C_{36}H_{31}N_6O_7$ ($[M+H]^+$): 659.2249, found: 659.2277.

4-amino-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl-pyrrolo[2,3-*d*]pyrimidine (39) To a flame-dried 5 mL Schlenk tube, equipped with a stir bar were added **37** (0.35 g, 0.50 mmol, 1 eq.), B_2pin_2 (0.19 g, 0.75 mmol, 1.5 eq.), KOAc (0.15 g, 1.5 mmol, 3 eq.) and $PdCl_2$dppf·DCM (0.021 g, 0.025 mmol, 0.05 eq.) under argon. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times, in total. Then, 2.5 mL (5 mL/mmol SM) anhydrous and degassed DMSO was added, under argon. The mixture was stirred at ambient temperature for ~ 5 min after which it was heated to 100 °C in a pre-heated oil bath. After three hours, the mixture was cooled to ambient temperature and poured into water/EA. The water layer was extracted two more times with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography 25 → 75 % EA/Hex gave **39** as an oil (0.12 g, 0.18 mmol). Yield = 37 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (s, 12H, CH_3), 4.70 (dd, *J* = 11.4, 3.9 Hz, 1H, H-5''), 4.74 – 4.78 (m, 1H, H-4'), 4.79 – 4.84 (dd, *J* = 11.1, 3.0 Hz, 1H, H-5'), 6.12 –

6.21 (m, 3H, H-2', H-3', NH), 6.65 (d, *J* = 5.4 Hz, 1H, H-1'), 7.32 – 7.48 (m, 6H, OBz), 7.50 – 7.60 (m, 3H, OBz), 7.63 (s, 1H, H-6), 7.92 – 7.98 (m, 4H, OBz), 8.09 – 8.13 (m, 2H, OBz), 8.28 (s, 1H, H-2). HRMS (ESI): calculated for $C_{38}H_{38}BN_4O_9$ ($[M+H]^+$): 705.2726, found: 705.2742. [note: the NH_2 signal did only integrate for 1H, instead of two]

4-azido-5-(pyridin-2-yl)-N7-(2'-3'-5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[3,2-*d*]pyrimidine (40) 40 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.73 g, 1.1 mmol) gave rise to **40** as a yellowish foam (0.35 g, 0.51 mmol). Column chromatography: 0 → 35 % EA/PET. Yield = 51 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.77 (dd, *J* = 12.0, 5.10 Hz, 1H, H-5''), 4.87 (dd, *J* = 12.3, 3.6 Hz, 1H, H-5'), 4.94 – 4.99 (m, 1H, H-4'), 6.14 – 6.18 (m, 1H, H-3'), 6.39 (t, *J* = 6.0 Hz, 1H, H-2'), 6.98 (d, *J* = 5.4 Hz, 1H, H-1'), 7.36 (ddd, *J* = 7.5, 4.8, 0.9 Hz, 1H, H-5_{Pyr}), 7.40 – 7.53 (m, 6H, OBz), 7.60 – 7.71 (m, 3H, OBz), 7.84 – 7.88 (m, 2H, OBz), 7.97 – 8.06 (m, 5H (4+1H), OBz, H-4_{Pyr}), 8.62 (ddd, *J* = 4.8, 2.1, 0.9 Hz, 1H, H-6_{Pyr}), 8.77 (s, 1H, H-6), 9.05 (dt, *J* = 7.8, 0.9 Hz, 1H, H-3_{Pyr}), 9.98 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.6 (C-5'), 70.9 (C-3'), 73.9 (C-2'), 79.6 (C-4'), 86.9 (C-1'), 100.7 (C-4a), 119.1 (C-5), 121.4 (C-3_{Pyr}), 122.3 (C-5_{Pyr}), 125.8 (C-6), 128.2, 128.6, 128.7, 128.77, 128.80, 129.1, 129.3, 129.3, 129.4, 133.5, 133.9, 134.0, 134.6 (C-2), 137.4 (C-4_{Pyr}), 141.9 (C-7a), 146.7 (C-4), 149.6 (C-6_{Pyr}), 150.6 (C-2_{Pyr}), 164.4 (C=O), 164.7 (C=O), 165.5 (C=O). HRMS (ESI): calculated for $C_{37}H_{28}N_7O_7$ ($[M+H]^+$): 682.2045, found: 682.2097.

4-azido-5-(4-methyl-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (41) 41 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **41** as a yellow foam (0.13 g, 0.19 mmol). Column chromatography: 0 → 10 % EA/Toluene. Yield = 27 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.48 (s, 1H, CH_3), 4.77 (dd, *J* = 12.3, 5.1 Hz, 1H, H-5''), 4.86 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5'), 4.94 – 4.98 (m, 1H, H-4'), 6.16 (t, *J* = 5.7 Hz, 1H, H-3'), 6.39 (t, *J* = 6.0 Hz, 1H, H-2'), 6.97 (d, *J* = 5.4 Hz, 1H, H-1'), 7.20 (d, *J* = 4.5 Hz, 1H, H-5_{Pyr}), 7.40 – 7.53 (m, 6H, OBz), 7.60 – 7.70 (m, 3H, OBz), 7.84 – 7.87 (m, 2H, OBz), 7.94 – 8.03 (m, 4H, OBz), 8.47 (d, *J* = 4.8 Hz, 1H, H-6_{Pyr}), 8.75 (s, 1H, H-6), 8.89 (br. s, 1H, H-3_{Pyr}), 9.98 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.9 (CH_3), 63.7 (C-5'), 70.9 (C-3'), 73.9 (C-2'), 79.6 (C-4'), 86.9 (C-1'), 100.7 (C-4a), 119.2 (C-5), 122.2 (C-5_{Pyr}), 123.1 (C-3_{Pyr}), 125.9 (C-6), 128.2, 128.6, 128.72,

127.78, 128.8, 129.16, 129.28, 129.36, 129.42, 133.5, 133.95, 134.03, 134.5 (C-2), 141.9 (C-7a), 146.8 (C-4), 147.9 (C-4_{Pyr}), 149.3 (C-6_{Pyr}), 150.5 (C-2_{Pyr}), 164.5 (C=O), 164.7 (C=O), 165.5 (C=O). HRMS (ESI): calculated for $C_{38}H_{30}N_7O_7$ ($[M+H]^+$): 696.2201, found: 696.2231.

4-azido-5-(4-methoxy-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-N7-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (42) 42 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **42** as a yellow foam (0.080 g, 0.11 mmol). Column chromatography: 0 → 10 % EA/Toluene. Yield = 16 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.01 (s, 3H, OCH_3), 4.77 (dd, *J* = 12.0, 5.1 Hz, 1H, H-5''), 4.86 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5'), 4.94 – 4.98 (m, 1H, H-4'), 6.15 (dd, *J* = 6.3, 5.1 Hz, 1H, H-3'), 6.38 (t, *J* = 6.0 Hz, 1H, H-2'), 6.96 (dd, *J* = 5.7, 2.4 Hz, 1H, H-5_{Pyr}), 6.97 (d, *J* = 5.1 Hz, 1H, H-1'), 7.40 – 7.53 (m, 6H, OBz), 7.60 – 7.71 (m, 3H, OBz), 7.84 – 7.95 (m, 2H, OBz), 7.95 – 8.07 (m, 4H, OBz), 8.43 (d, *J* = 5.7 Hz, 1H, H-6_{Pyr}), 8.77 (s, 1H, H-8), 8.80 (d, *J* = 2.7 Hz, 1H, H-3_{Pyr}), 9.98 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 55.6 (OCH_3), 63.7 (C-5'), 70.9 (C-3'), 73.9 (C-2'), 86.9 (C-1'), 100.6 (C-4a), 108.0 (C-3_{Pyr}), 108.2 (C-5_{Pyr}), 119.1 (C-5), 126.1 (C-6), 128.6, 128.72, 128.78, 128.81, 129.1, 129.27, 129.36, 129.40, 133.5, 133.9, 134.0, 134.6 (C-2), 141.9 (C-7a), 146.8 (C-4), 150.8 (C-6_{Pyr}), 152.0 (C-2_{Pyr}), 164.4 (C=O), 164.7 (C=O), 165.5 (C=O), 166.4 (C-4_{Pyr}). HRMS (ESI): calculated for $C_{38}H_{30}N_7O_8$ ($[M+H]^+$): 712.2150, found: 712.2186.

4-azido-5-(6-chloro-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (43) 43 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **43** as a yellow foam (0.14 g, 0.20 mmol). Column chromatography: 0 → 20 % EA/PET. Yield = 29 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.77 (dd, *J* = 12.0, 5.4 Hz, 1H, H-5''), 4.88 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5'), 4.93 – 4.98 (m, 1H, H-4'), 6.16 (t, *J* = 6.0 Hz, 1H, H-3'), 6.42 (t, *J* = 6.0 Hz, 1H, H-2'), 6.98 (d, *J* = 5.4 Hz, 1H, H-1'), 7.40 – 7.52 (m, 6H, OBz), 7.44 (d, *J* = 7.8 Hz, 1H, H-5_{Pyr}), 7.59 – 7.71 (m, 3H, OBz), 7.85 – 7.88 (m, 2H, OBz), 7.97 – 8.02 (m, 4H, OBz), 8.12 (t, *J* = 7.8 Hz, 1H, H-4_{Pyr}), 8.78 (s, 1H, H-6), 9.08 (dd, *J* = 7.8, 0.6 Hz, 1H, H-3_{Pyr}), 9.99 (s, 1H, H-2). HRMS (ESI): calculated for $C_{37}H_{27}ClN_7O_7$ ($[M+H]^+$): 716.1655, found: 716.1660.

4-azido-5-(5-chloro-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (44) 44 was prepared according to General Procedure C [reaction temperature = 60 °C].

35 (0.51 g, 0.70 mmol) gave rise to **44** as a yellow foam (0.15 g, 0.21 mmol). Column chromatography: 0 → 20 % EA/PET. Yield = 30 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.87 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5''), 4.76 (dd, *J* = 12.3, 5.1 Hz, 1H, H-5'), 4.94 – 4.99 (m, 1H, H-4'), 6.16 (dd, *J* = 6.0, 5.4 Hz, 1H, H-3'), 6.39 (dd, *J* = 6.3, 5.4 Hz, 1H, H-2'), 6.97 (d, *J* = 5.1 Hz, 1H, H-1'), 7.40 – 7.54 (m, 6H, OBz), 7.60 – 7.72 (m, 3H, OBz), 7.84 – 7.87 (m, 2H, OBz), 7.96 – 8.02 (m, 4H, OBz), 8.21 (dd, *J* = 8.4, 2.7 Hz, 1H, H-4$_{Pyr}$), 8.63 (dd, *J* = 2.7, 0.6 Hz, 1H, H-6$_{Pyr}$), 8.77 (s, 1H, H-6), 9.09 (dd, *J* = 8.4, 0.6 Hz, 1H, H-3$_{Pyr}$), 10.00 (s, 1H, H-2). HRMS (ESI): calculated for $C_{37}H_{27}ClN_7O_7$ ([M+H]$^+$): 716.1655, found: 716.1642.

4-azido-5-(4-chloro-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (45) 45 was prepared according to General Procedure C [reaction temperature = ambient temperature]. **35** (0.51 g, 0.70 mmol) gave rise to **45** as an orange foam (0.12 g, 0.16 mmol). Column chromatography: 0 → 35 % EA/PET. Yield = 23 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.76 (dd, *J* = 12.3, 5.1 Hz, 1H, H-5''), 4.87 (dd, *J* = 12.3, 3.6 Hz, 1H, H-5'), 4.94 – 4.99 (m, 1H, H-4'), 6.16 (dd, *J* = 6.3, 5.1 Hz, 1H, H-3'), 6.39 (t, *J* = 5.7 Hz, 1H, H-2'), 6.98 (d, *J* = 5.1 Hz, 1H, H-1'), 7.40 – 7.53 (m, 7H (6+1), OBz, H-5$_{Pyr}$), 7.60 – 7.70 (m, 3H, OBz), 7.71 – 7.88 (m, 2H, OBz), 7.96 – 8.03 (m, 4H, OBz), 8.59 (dd, *J* = 5.1, 0.6 Hz, 1H, H-6$_{Pyr}$), 8.82 (s, 1H, H-6), 9.23 (dd, *J* = 1.8, 0.6 Hz, 1H, H-3$_{Pyr}$), 10.01 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.6 (C-5'), 70.9 (C-3'), 73.9 (C-2'), 79.7 (C-4'), 87.0 (C-1'), 100.6, 117.8, 121.1 (C-3$_{Pyr}$), 122.2 (C-5$_{Pyr}$), 128.2 (C-6), 128.6, 128.70, 128.78, 128.81, 129.1, 129.27, 129.36, 129.40, 133.53, 133.94, 134.0, 134.8 (C-2), 142.0 (C-7a), 144.0 (C-4$_{Pyr}$), 146.6 (C-4), 151.0 (C-6$_{Pyr}$), 152.3 (C-2$_{Pyr}$), 164.4 (C=O), 164.7 (C=O), 165.5 (C=O). HRMS (ESI): calculated for $C_{37}H_{27}ClN_7O_7$ ([M+H]$^+$): 716.1655, found: 716.1661.

4-azido-5-(5-fluoro-pyridin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (46) 46 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **46** as a yellow foam (0.15 g, 0.21 mmol). Column chromatography: 0 → 20 % EA/PET. Yield = 31 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.76 (dd, *J* = 12.0, 5.1 Hz, 1H, H-5''), 4.87 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5'), 4.94 – 4.99 (m, 1H, H-4'), 6.16 (t, *J* = 5.7 Hz, 1H, H-3'), 6.39 (t, *J* = 5.7 Hz, 1H, H-2'), 6.97 (d, *J* = 5.4 Hz, 1H, H-1'), 7.70 – 7.53 (m, 6H, OBz), 7.60 – 7.71 (m,

3H, OBz), 7.84 – 7.88 (m, 2H, OBz), 7.97 – 8.03 (m, 4H, OBz), 8.03 (dd, *J* = 5.4, 3.3 Hz, 1H), 8.60 (dt, *J* = 3.3, 0.6 Hz, 1H), 8.70 (s, 1H, H-6), 9.10 (ddd, *J* = 9.0, 4.5, 0.6 Hz, 1H), 9.99 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -129.16 (dd, *J* = 8.5, 4.8 Hz). HRMS (ESI): calculated for $C_{37}H_{27}FN_7O_7$ ($[M+H]^+$): 700.1951, found: 700.1985.

4-azido-5-(quinolin-2-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (47) 47 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **47** as a pink foam (0.15 g, 0.20 mmol). Column chromatography: 0 → 10 % EA/Toluene. Yield = 29 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.80 (dd, *J* = 12.3, 5.4 Hz, 1H, H-5''), 4.90 (dd, *J* = 12.3, 3.6 Hz, 1H, H-5'), 4.97 – 5.02 (m, 1H, H-4'), 6.20 (t, *J* = 5.7 Hz, 1H, H-3'), 6.45 (t, *J* = 6.0 Hz, 1H, H-2'), 7.04 (d, *J* = 5.1 Hz, 1H, H-1'), 7.40 – 7.53 (m, 6H, OBz), 7.57 – 7.72 (m, 4H (3+1), OBz (3H), H_{Quin}), 7.75 – 7.80 (m, 1H, H_{Quin}), 7.86 – 7.89 (2H, m, OBz), 7.91 (d, *J* = 8.4 Hz, 1H, H_{Quin}), 7.98 – 8.06 (m, 5H (4+1), OBz (4H), H_{Quin}), 8.59 (d, *J* = 8.7 Hz, 1H, H-4$_{Quin}$), 8.92 (s, 1H, H-6), 9.13 (d, *J* = 8.7 Hz, 1H, H-3$_{quin}$), 10.00 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.8 (C-5'), 71.0 (C-3'), 74.0 (C-2'), 79.7 (C-4'), 87.1 (C-1'), 101.3 (C-4a), 119.2 (C-5), 120.3 (C-3$_{Quin}$), 125.3 (C_{Quin}), 126.2 (C_{Quin}), 126.7 (C_{Quin}), 126.9 (C-6), 128.2 (C_{Quin}), 128.6, 128.72, 128.76, 128.81, 128.89, 129.16, 129.28, 129.37, 129.4, 130.0 (C_{Quin}), 133.5, 133.95, 134.01, 134.6 (C-2), 137.2 (C-4$_{Quin}$), 141.9 (C-7a), 146.8 (C-4), 147.6 (C-8a$_{Quin}$), 151.0 (C-2$_{Quin}$), 164.5 (C=O), 164.7 (C=O), 165.5 (C=O). HRMS (ESI): calculated for $C_{41}H_{30}N_7O_7$ ($[M+H]^+$): 732.2201, found: 732.2239.

4-azido-5-(isoquinolin-3-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (48) 48 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **48** as a white foam (0.18 g, 0.24 mmol). Column chromatography: 0 → 5 % EA/DCM. Yield = 34 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.79 (dd, *J* = 12.0, 5.1 Hz, 1H, H-5''), 4.88 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5'), 4.96 – 5.00 (m, 1H, H-4'), 6.18 (dd, *J* = 6.0, 5.4 Hz, 1H, H-3'), 6.43 (t, *J* = 6.0 Hz, 1H, H-2'), 7.01 (d, *J* = 5.4 Hz, 1H, H-1'), 7.40 – 7.54 (m, 6H, OBz), 7.60 – 7.71 (m, 4H (3+1), OBz (3H), H-7$_{Isoq}$ (1H)), 7.72 – 7.88 (m, 3H (2+1), OBz (2H), H-6$_{Isoq}$ (1H)), 7.80 – 8.08 (m, 5H (4+1), OBz (4H), H-5$_{Isoq}$ (1H)), 8.18 (d, *J* = 8.4 Hz, 1H, H-8$_{Isoq}$), 8.87 (s, 1H, H-6), 9.36 (s, 1H, H-1$_{Isoq}$), 9.51 (s, 1H, H-4$_{Isoq}$), 10.01 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.7 (C-5'),

70.9 (C-3'), 73.9 (C-2'), 79.6 (C-4'), 86.9 (C-1'), 100.6 (C-4a), 116.7 (C-4_{Isoq}), 119.5 (C-5), 125.9 (C-6), 126.8 (C-5_{Isoq}), 127.2 (C-7_{Isoq} // C-$8a_{Isoq}$), 127.3 (C-7_{Isoq} // C-$8a_{Isoq}$), 127.9 (C-6_{Isoq}), 128.2, 128.6, 128.7, 128.76, 128.81, 129.18, 129.28, 129.36, 129.42, 131.2 (C-6_{Isoq}), 133.5, 133.95, 134.01, 134.6 (C-2), 136.1 (C-4_{Isoq}), 142.1 (C-7a), 144.4 (C-3_{Isoq}), 146.9 (C-4), 152.5 (C-1_{Isoq}), 164.5 (C=O), 164.7 (C=O), 165.5 (C=O). HRMS (ESI): calculated for $C_{41}H_{30}N_7O_7$ ($[M+H]^+$): 732.2201, found: 732.2200.

4-azido-5-(isoquinolin-1-yl)-N7-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (49) 49 was prepared according to General Procedure C [reaction temperature = 60 °C]. **35** (0.51 g, 0.70 mmol) gave rise to **49** as a yellow foam (0.14 g, 0.18 mmol). Column chromatography: 0 → 20 % EA/Toluene. Yield = 26 %. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.75 (dd, *J* = 12.3, 5.4 Hz, 1H, H-5''), 4.87 (dd, *J* = 12.3, 3.9 Hz, 1H, H-5'), 4.96 – 5.00 (m, 1H, H-4'), 6.21 (t, *J* = 5.7 Hz, 1H, H-3'), 6.45 (dd, *J* = 6.0, 5.1 Hz, 1H, H-2'), 7.01 (d, *J* = 5.1 Hz, 1H, H-1'), 7.33 – 7.38 (m, 2H, OBz), 7.43 – 7.56 (m, 6H (5+1), OBz (5H), H_{Isoq}), 7.62 – 7.71 (m, 2H, OBz), 7.82 (ddd, *J* = 8.4, 7.2, 1.2 Hz, 1H, H_{Isoq}), 7.90 – 7.99 (m, 7H (6+1), OBz (6H), H-4_{Isoq}), 8.05 (dd, *J* = 8.4, 0.6 Hz, 1H, H_{Isoq}), 8.10 (d, *J* = 8.4 Hz, 1H, H_{Isoq}), 8.45 (s, 1H, H-6), 8.63 (d, *J* = 5.7 Hz, 1H, H-3_{Isoq}), 9.99 (s, 1H, H-2). HRMS (ESI): calculated for $C_{41}H_{30}N_7O_7$ ($[M+H]^+$): 732.2201, found: 732.2220.

Biology

Strains and cultures of *Trypanosoma brucei brucei* and *T. brucei rhodesiense*

The following clonal strains of *T. b. brucei* were cultured at the University of Glasgow, all as long-slender bloodstream trypomastigotes only: Lister 427 (wild-type); TbAT1-KO, derived from 427WT by genetic deletion of the TbAT1 gene;[56] B48, derived from TbAT1-KO by *in vitro* exposure to increasing concentrations of pentamidine;[49] and ISMR1, derived from 427WT by increasing exposure to isometamidium *in vitro*.[74] Bloodstream forms of savannah-type *T. congolense* strain IL3000 were cultured exactly as described before.[75] At the University of Antwerp, *T. brucei* Squib 427 (suramin-sensitive) and *T. b. rhodesiense* STIB-900 were used for the *in vitro* susceptibility tests. All trypanosome strains were cultured in the standard HMI-9 medium supplemented with 10% fetal bovine serum (FBS) at 37 ºC in a 5% CO_2 atmosphere, exactly as described before.[11]

Drug susceptibility tests

Trypanosoma brucei

Drug susceptibility tests with Lister 427WT, TbAT1-KO, B48 and ISMR1 were performed exactly as described previously,[76] using an assay based on the viability indicator dye resazurin (Alamar blue) in 96-well plates, each well containing 2×10^4 cells. The plates were incubated for 48 h with a doubling dilution series of the test compounds in HMI-9/FBS at 37 ºC/5% CO_2 (23 dilutions starting at 100 µM, except for the pentamidine control (50 µM)), after which resazurin was added to each well and the plates incubated for another 24 h. Fluorescence was 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).

Susceptibility assays with *T. brucei* Squib 427 or *T. b. rhodesiense* STIB-900 were performed under similar conditions as above but using 10 concentrations of a 4-fold compound dilution series starting at 64 µM. *T. brucei* Squib 427 was seeded at 1.5×10^4 parasites/well and *T. b. rhodesiense* at 4×10^3 parasites/well, followed by addition of resazurin after 24 hours (*T. brucei*) or 6 hours (*T. b. rhodesiense*).

Trypanosoma congolense

The resazurin assay was performed exactly as for *T. b. brucei* at the University of Glasgow, the only difference being the use of 5×10^4 bloodstream forms for *T. congolense* instead of 2×10^4 for *T. brucei*, as described,[11] due to their slower growth rate in culture.

Trypanosoma cruzi

Drug activity against *T. cruzi* was tested with the nifurtimox-sensitive Tulahuen CL2 β galactosidase strain.[77] This strain was maintained on MRC-5_{SV2} (human lung fibroblast) cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C/5% CO_2. Assays were with 4×10^3 MRC-5 cells/well and 4×10^4 parasites/well. Impact of test compound dilution series (10 concentrations of a 4-fold compound dilution series starting at 64 µM) on parasite growth was analyzed after 7 days incubation by adding the substrate chlorophenolred β-D-galactopyranoside. The change in color was measured

spectrophotometrically at 540 nm after 4 hours incubation at 37 °C. The results were expressed as % reduction in parasite burdens compared to control wells from which an EC_{50} was calculated.

Leishmania infantum

L. infantum [MHOM/MA(BE)/67] was used for the drug susceptibility assays as described previously.[78] This strain was maintained in golden Syrian hamsters and amastigotes were collected from the spleen of an infected donor hamster. Splenic amastigotes were used for infection of primary peritoneal mouse macrophages from Swiss mice after a 2-day peritoneal stimulation with a 2 % potato starch suspension. The macrophages were infected after 48 hours of adherence. The compound dilution series (10 concentrations of a 4-fold compound dilution series starting at 64 µM) were added after 2 hours of infection. Assays were performed in 96-well microtiter plates with 3×10^4 macrophages and 4.5×10^5 parasites/well in RPMI-1640 medium supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C/5% CO_2. After 5 days incubation, parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed after staining the cells with a 10% Giemsa solution. The results were expressed as % reduction in parasite burden compared to untreated control wells from which an EC_{50} was calculated.

Cytotoxicity on MRC-5 fibroblasts

Drug cytotoxicity assays were performed in MRC-5_{SV2} human embryonic lung fibroblasts that 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. 10 µl of the compound dilutions in water were added to 190 µl of MRC-5_{SV2} (3×10^4 cells/ml). Cell growth was compared to untreated-control wells (100% cell growth) and medium-control wells (0% cell growth). After 3 days 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). The results were expressed as percentage reduction in cell growth / viability compared to control wells and an EC_{50} was determined. Tamoxifen was used as reference compound (data not shown).

Transport assays

Transport via P1 was measured using B48 cells, which lack the P2 transport system[49], whereas the transport via P2 was assessed in B48 cells transfected with TbAT1/P2 gene (B48 + TbAT1)[62] for a constant, high level of expression, in presence of 100 µM of inosine to block P1 transporter. The transport of [^{3}H]-Adenosine (40 Ci/mmol; American Radiolabeled Chemicals, St Louis, MO) was measured using a previously described uptake protocol.[21, 79] 1×10^7 cells were incubated with 100 nM [^{3}H]-Adenosine in assay buffer[79] for 60 seconds and rapid termination by addition of ice-cold 2 mM adenosine followed by immediate centrifugation through an oil layer for one minute at maximum speed. The incubation times used were well within the linear phase of uptake.[80] Inhibition constants (K_i) were calculated from 50% inhibition values (IC_{50}) calculated from non-linear regression (sigmoid curve with variable slope; GraphPad 5.0) and the Cheng-Prusoff equation, as described.[79] The Gibbs Free Energy was calculated from $\Delta G^0 = -RT\ln(K_i)$ as described,[55] in which R is the gas constant and T the absolute temperature.

Microsomal stability assays

Mouse, rat and pooled human liver microsomes were purchased from a commercial source (Corning) and stored at -80 °C. NADPH generating system solutions A and B and UGT reaction mix solutions A and B (Corning) were kept at -20 °C. The test compound and the reference compounds diclofenac (MW 296.15) and fluconazole (MW 306.27) were formulated in DMSO at 10 mM. The microsomal stability assay was carried out based on the BD Biosciences Guidelines for Use (TF000017 Rev1.0) with minor adaptations. The metabolic stability of the compounds was studied through the CYP_{450} superfamily (Phase I metabolism) by fortification with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and through uridine glucuronosyl-transferase (UGT) enzymes (Phase II metabolism) by fortification with uridine diphosphate glucuronic acid (UDPGA). For the CYP_{450} and other NADPH dependent enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in potassium phosphate buffer in a reaction started by the addition of 1 mM NADPH and

stopped at 0, 15, 30 and 60 minutes. At these time points, 20 µl was withdrawn from the reaction mixture and 80 µl cold acetonitrile (ACN), containing the internal standard tolbutamide, was added to inactivate the enzymes and precipitate the proteins. The mixture was vortexed for 30 s and centrifuged at 4 °C for 5 min at 15,000 rpm. The supernatant was stored at -80 °C until analysis. For the UGT enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in a reaction started by the addition of 2 mM UDPGA cofactor. The corresponding loss of parent compound was determined using liquid chromatography (UPLC) (Waters Aquity^{TM}) coupled with tandem quadrupole mass spectrometry (MS^2) (Waters Xevo^{TM}), equipped with an electrospray ionization (ESI) interface and operated in multiple reaction monitoring (MRM) mode.

Ancillary information

Supporting information

Copies of ^{1}H, ^{13}C and ^{19}F NMR spectra of compounds **5**, **7-26**, **28-30** can be found in the Supporting Information, as well as single crystal X-ray diffraction data of **13**.

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Abbreviations used:

B_2pin_2, bis(pinacolato)diboron; dba, dibenzylidene-acetone; EA, ethyl acetate, HAT, Human African Trypanosomiasis; SM, starting material, TPPTS, trisodium 3-bis(3-sulfonatophenyl)phosphanylbenzenesulfonate; RuPhos, 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl.

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