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6-Methyl-7-Aryl-7-Deazapurine Nucleosides as Anti-*Trypanosoma cruzi* Agents: Structure-Activity Relationship and *in vivo* Efficacy

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Chagas disease is a tropical infectious disease resulting in progressive organ-damage and currently lacks efficient treatment and vaccine options. The causative pathogen, *Trypanosoma cruzi*, requires uptake and processing of preformed purines from the host because it cannot synthesize these *de novo*, instigating the evaluation of modified purine nucleosides as potential trypanocides. By modifying the pyrimidine part of a previously identified 7-aryl-7-deazapurine nucleoside, we found that substitution of a 6-methyl for a 6-amino group allows retaining *T. cruzi* amastigote growth inhibitory activity but confers improved selectivity towards mammalian cells. By keeping the 6-methyl group unaltered, and introducing different 7-aryl groups, we identified several analogues with submicromolar antitrypanosomal activity. The 7-(4-chlorophenyl) analogue **14**, which was stable in microsomes, was evaluated in an acute mouse model. Oral administration of 25 mg/kg b.i.d. suppressed peak parasitemia and protected mice from infection-related mortality, gave similar reductions as the reference drug of blood parasite loads determined by qPCR, but as benznidazole failed to induce sterile cure in the short time period of drug exposure (5 days).

Introduction

Chagas disease (CD) is a neglected tropical disease that is endemic in the Latin American countries.[1–2] CD is caused by the kinetoplastid parasite *Trypanosoma cruzi* (*T. cruzi*) and is transmitted primarily in endemic regions via triatomine bugs. While taking a bloodmeal this vector defecates, releasing *T. cruzi* trypomastigotes which then establish an infection into the vertebrate mammalian host.[3] Notwithstanding the vector-mediated transmission route in endemic areas being most important, CD may also be contracted by ingesting contaminated food or drinks (oral route). Additionally, a diverse range of non-vector mediated transmission routes, such as congenital infection, blood transfusion or organ transplantation, plays a major role in non-endemic countries.[4] This is particularly worrisome in light of increased migration, often accompanied by an unawareness of infection status, rendering CD a global health concern.[5–6]

There are two stages in the progression of CD: the acute and chronic stage. The initial clinical manifestation in the acute phase is mild or even oligosymptomatic, but the parasite can typically be observed through blood examination. If no treatment is provided, the disease enters into a chronic phase and may remain asymptomatic for years and decades, displaying a subpatent parasitism due to an effective host's immune system. In the later chronic phase, for reasons not yet well known, some patients (30–40%) develop a progressive cardiomyopathy and/or digestive disorders besides neurological dysfunction.[7–9] The continuous damage of the heart muscle ultimately results in heart failure and sometimes sudden death.[10] Currently, no vaccine is available, making chemotherapy the only option.

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cmdc.202100144

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Benznidazole (BZ) and nifurtimox are two nitro-containing drugs used for the treatment of CD. However, both drugs suffer from several limitations such as low efficacy mainly at the later chronic stage, and significant side effects besides the occurrence of parasite strains that are naturally resistant to both nitro derivatives. Drug toxicity is particularly important as it contributes to poor patient compliance and premature treatment cessation, thus contributing to the persistence of the parasitism.[11–13] Therefore, the discovery and development of new treatment options are of critical importance.[14–15]

An attractive drug discovery paradigm is to exploit certain parasitic auxotrophies. Particularly, *T. cruzi* cannot synthesize purine rings from amino acids, resulting in their reliance on salvage[16–18] of preformed purine rings from the host for growth and proliferation, thereby increasing the likeliness for (modified) purine analogues as potential antiparasitic agents.[19–20]

Tubercidin, a natural antibiotic, displays attractive inhibitory potency against multiple pathogenic organisms, but its application is limited because of toxic effects on mammalian cells.[21–22] Selectivity improvement of 7-deazapurine nucleoside analogues has been achieved via modifications at C6[23] and/or C7.[22,24] Introduction of a 4-chlorophenyl substituent (analogue **2**, Figure 1) at C7 of tubercidin was found to confer potent anti-*T. cruzi* activity *in vitro*[22,25] and *in vivo*,[25] while decreasing cytotoxicity. Several other nucleoside analogues with modifications in the pyrimidine part of the purine were reported to exhibit potent activity against *T. cruzi*, including puromycin aminonucleoside (**3**),[26] 7-iodo-1,7-dideazaadenosine (**4**),[27] 3-deazaguanosine (**5**)[28] and 2-amino-6-chloro-7-deazaadenosine (**6**) (Figure 1a).[29]

In this study, we set out to explore the structure-activity-relationship (SAR) of analogue **2** with particular attention to modifications of the pyrimidine part of the 7-deazapurine system. After having identified a favourable 6-methyl modification, we combined this modification with different C7 modifications (Figure 1b). One promising analogue with favourable *in vitro* metabolic stability was selected for efficacy evaluation in an experimental mouse model of *T. cruzi* infection.

Results and Discussion

Biological evaluation

In vitro evaluation

All prepared compounds were evaluated *in vitro* for their antitrypanosomal activity against *T. cruzi*, employing different parasite strains belonging to distinct DTUs (Tulahuen expressing β-galactosidase and Y strains, DTU VI and II, respectively) and parasite forms relevant for mammalian host (intracellular amastigotes and bloodstream trypomastigotes) always comparing to the findings obtained with BZ as a reference drug. In parallel, the *in vitro* cytotoxicity of the compounds was assessed in different host cells including the MRC-5 human fibroblast cell line and primary cardiac cell cultures grown as 2 and 3-D matrices (Tables 1–3, 6).

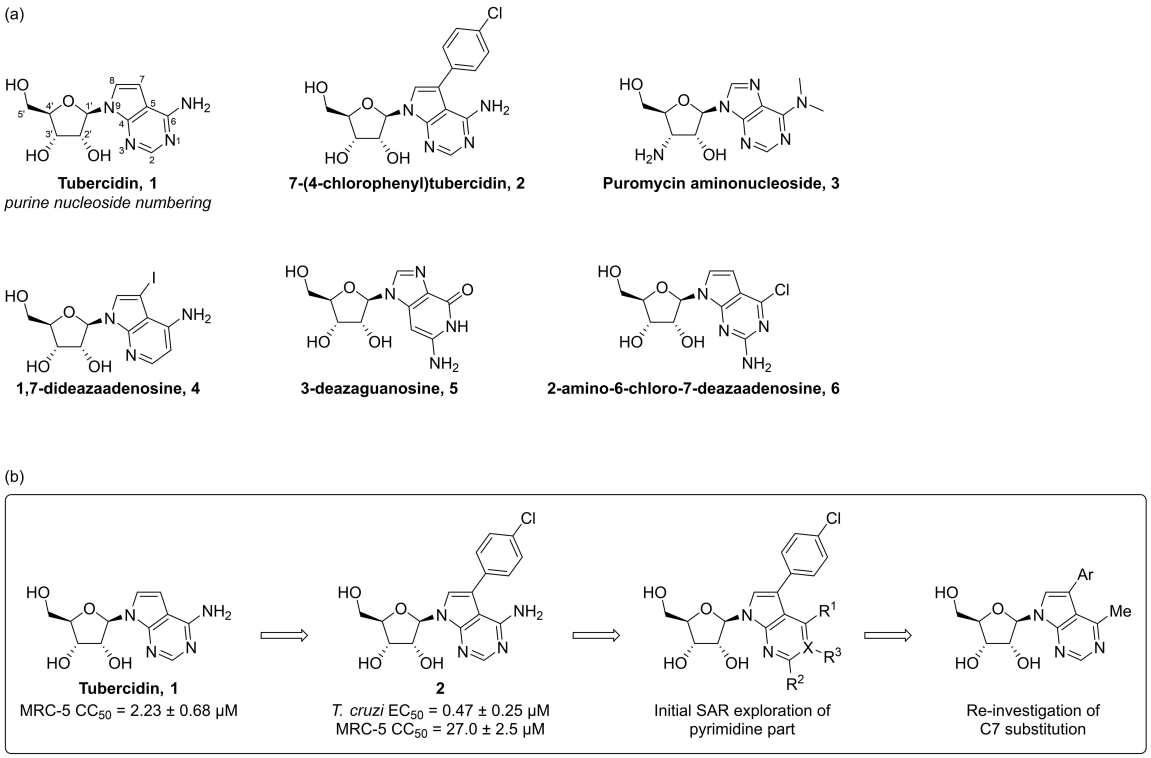
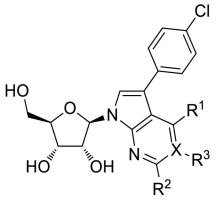


Figure 1. (a) Representative antitrypanosomal nucleoside analogues with C7 or pyrimidine ring modifications; (b) SAR exploration of the pyrimidine ring of **2** and exploration of C7-aryl modified 6-methyl-7-deazapurinecleoside.

Table 1. < Activity of 7-(*para*-chlorophenyl) nucleoside analogues with modifications in pyrimidine moieties against intracellular *T. cruzi* amastigotes.



Cpd.	X	R^1	R^2	R^3	*T. cruzi*[a] EC_{50} [μM]	MRC-5[b] EC_{50} [μM]	SI[c]
2[22,25]	N	NH_2	H	–	0.47 ± 0.25	27.0 ± 2.5	57
7	N	NHMe	H	–	41.6	> 64.0	> 1.54
8	N	NMe_2	H	–	33.4	> 64.0	> 1.92
9	N	OH	H	–	> 64.0	> 64.0	N.D.[d]
10[30]	N	OMe	H	–	43.1	61.1	1.42
11	N	OEt	H	–	26.3	50.4	1.92
12	N	SMe	H	–	20.3	> 64.0	> 3.15
13	N	H	H	–	11.9	> 64.0	> 5.38
14	N	Me	H	–	0.85 ± 0.21	> 64.0	> 75
15	N	Et	H	–	35.3	36.3	1.03
16	N	Ph	H	–	> 64.0	> 64.0	N.D.
17	N	OH	NH_2	–	> 64.0	> 64.0	N.D.
18	N	OMe	NH_2	–	30.6	22.5	0.74
19[31]	C	NH_2	H	H	> 64.0	> 64.0	N.D.
20	C	Me	H	H	9.76 ± 0.45	> 64.0; 20.2	N.D.
21	C	H	H	F	24.1	18.2	0.76
22	C	H	H	Cl	7.80	50.8	6.51
23	C	H	H	NO_2	8.45 ± 0.12	> 64.0	> 7.57
24	C	H	H	NH_2	> 64.0	> 64.0	N.D.
25	C	H	H	Me	9.98	23.4	2.34
BZ					2.28 ± 0.05	N.D.	N.D.

[a]Concentration of compound inhibiting growth by 50% (EC_{50}) of intracellular *T. cruzi* amastigotes (Tulahuen strain-expressing β-galactosidase). EC_{50} values are described as mean ± SE of 2 or 4 independent experiments. Where SE is not provided, values (EC_{50} > 10 μM) were determined in a single experiment; [b] Concentration of compound inhibiting growth by 50% (EC_{50}) of MRC-5 fibroblasts; [c] SI = selectivity index, EC_{50} (MRC-5)/EC_{50} (*T. cruzi*); [d] N.D.: not determined.

First, the activity against intracellular forms (Tulahuen strain) was investigated. Substitution of the 6-amino group of **2** by alternative groups (**7**–**12**) failed to give analogues with improved or comparable anti-*T. cruzi* activity. Likewise, complete removal of the 6-NH_2 (**13**) or its replacement by a phenyl (**16**) was not tolerated. Only the 6-methyl analogue **14** displayed sub-micromolar anti-*T. cruzi* activity, comparable to **2**. This analogue does not exhibit toxicity to the MCR-5 host cells at the highest concentration tested (> 64 μM), marking **14** as an interesting hit. Remarkably, homologation to a 6-ethyl analogue (**15**) resulted in complete loss of antiparasitic activity. Both deazaguanosine analogues **17** and **18** were devoid of anti-*T. cruzi* activity.

Then, we assessed the importance of N1, as we have previously shown that 1,7-dideazapurine analogues may display interesting anti-*T. cruzi* activity.[27] In this series, however, the combination of a 6-amino group with the 7-*para*-chlorophenyl (**19**) previously proved detrimental for activity.[27] Remarkably, replacing the 6-amino group of **19** by a methyl group (**20**) restored the anti-*T. cruzi* activity (EC_{50} ~10 μM). However, this 1,7-dideaza analogue remained 10-fold less active than its 7-deaza congener **14**. Removal of the N1 nitrogen atom allows modifications at this position which were hardly explored before. 1-Chloro or nitro analogues showed modest activities (EC_{50} < 10 μM), while the 1-amino analogue **24** was inactive.

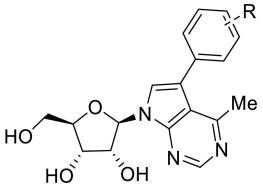
Having identified a 6-methyl group as a potential 6-amino isostere, we decided to further investigate the SAR of the 7-substituent of **14** (Table 2).

Removal of the chloro-substituent (**26**) led to a substantial activity drop, indicating the importance of *para* substitution. Several alternative substituents resulted in less potent analogues (**27**–**35**), including 4-F (**27**), 4-CF_3 (**28**) and 4-Me (**32**) that retained low micromolar anti-*T. cruzi* activity, similar to BZ. Shifting the halogen to the *meta* (**36**, **37**) or *ortho* (**38**) position reduced the anti-*T. cruzi* activity, in line with previous SAR observations with the 6-amino analogues.[22,25]

Generally, 3,4-disubstituted analogues (**39**, **40**) were more potent than 2,4-disubstituted (**44**, **45**) or 3,5-disubstituted analogues (**46**). Introduction of a fluoro (**40**) or a methoxy group (**43**) in the *meta* position of **14** gave a slight activity improvement. Selected bicyclic analogues of **26** (e.g. **49** and **53**) also showed reasonable activity. However, the effect of the aryl substituents on the activity did not show a clear trend. The fact that the observed SAR is in line with previous findings for 3′-deoxy-7-aryl tubercidin analogues,[25] suggests a similar target engagement.

Finally, we investigated the effect of 3′-deoxygenation (Table 3), previously shown to significantly improve antitrypanosomal activity.[25,32–33] However, the 3′-deoxy analogue **54**

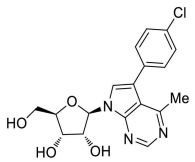
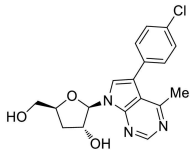
Table 2. Activity of 7-aromatic-deazapurine nucleoside analogues against intracellular *T. cruzi* amastigotes.[a]



Cpd.	R or structure	*T. cruzi*[a] EC_{50} [μM]	MRC-5[b] EC_{50} [μM]	SI[c]
14	4-Cl	0.85 ± 0.21	> 64.0	> 75
26	H	40.3	> 64.0	> 1.5
27	4-F	2.89 ± 0.35	> 64.0	> 22
28	4-CF_3	2.55 ± 0.11	> 64.0	> 25
29	4-OCF_3	31.5	> 64.0	> 2.0
30	4-CN	33.5	> 64.0	> 1.9
31	4-NO_2	13.4	> 64.0	> 4.7
32	4-Me	2.83 ± 0.03	> 64.0	> 22
33	4-Et	42.3	> 64.0	> 1.5
34	4-OMe	44.7	> 64.0	> 1.4
35	4-OH	7.47 ± 0.01	> 64.0	> 8.5
36	3-Cl	12.9 ± 0.78	> 64.0	> 4.9
37	3-F	12.7	> 64.0	> 5.0
38	2-Cl	9.27 ± 0.06	> 64.0	> 6.9
39	3,4-2Cl	1.58 ± 0.70	> 64.0	> 40
40	3-F-4-Cl	0.61 ± 0.04	> 64.0	> 104
41	3-CF_3-4-Cl	> 64.0	> 64.0	N.D.[d]
42	3-Me-4-Cl	1.61 ± 0.26	> 64.0	> 39
43	3-OMe-4-Cl	0.59 ± 0.02	> 64.0	> 108
44	2,4-2Cl	10.3 ± 0.09	> 64.0	> 6.2
45	2-F-4-Cl	1.51 ± 0.70	> 64.0	> 42
46	3,5-2Cl	10.9 ± 0.36	> 64.0	> 5.8
47	3,5-2F-4-Cl	2.49 ± 0.11	> 64.0	> 25
48	4-Ph	10.8 ± 2.52	> 64.0	> 5.9
49	2-naphthalene	3.06 ± 0.16	29.6; > 64.0	N.D.
50	5-(benzo[*d*][1,3]dioxole)	60.9	> 64	1
51	6-(2,3-dihydrobenzo[*b*][1,4]dioxine)	> 64	> 64	N.D.
52	6-1*H*-indole	> 64	> 64	N.D.
53	6-(1-methyl-1*H*-indole)	3.34	44.30	13
BZ		2.28 ± 0.05	N.D.	N.D.

[a] Concentration of compound inhibiting growth by 50% (EC_{50}) of intracellular *T. cruzi* amastigotes (Tulahuen strain-expressing β-galactosidase). EC_{50} values are described as mean ± SE of 2 or 4 independent experiments. Where SE is not provided, values (EC_{50} > 10 μM) were determined in a single experiment; [b] Concentration of compound inhibiting growth by 50% (EC_{50}) of MRC-5 fibroblasts; [c] SI = selectivity index, EC_{50} (MRC-5)/EC_{50} (*T. cruzi*); [d] N.D.: not determined.

Table 3. Comparison of activity of compound 14 and the corresponding 3′-deoxy- analogue against intracellular *T. cruzi* amastigotes.[a]

Cpd.	Structure	*T. cruzi*[a] EC_{50} [μM]	MRC-5[b] EC_{50} [μM]	SI[c]
14		0.85 ± 0.21	> 64.0	> 75
54		2.58 ± 0.67	> 64.0	> 25

[a] Concentration of compound inhibiting growth by 50% (EC_{50}) of intracellular *T. cruzi* amastigotes (Tulahuen strain-expressing β-galactosidase). EC_{50} values are described as mean ± SE of 2 or 4 independent experiments; [b] Concentration of compound inhibiting growth by 50% (EC_{50}) of MRC-5 fibroblasts; [c] SI = selectivity index, EC_{50} (MRC-5)/EC_{50} (*T. cruzi*).

elicited a 3-fold weaker anti-*T. cruzi* activity than its ribose congener **14**.

In summary, a new series of 6-methyl 7-aryl-7-deazapurine nucleosides with activity against *T. cruzi* was discovered. SAR efforts showed that the 6-methyl group can function as a suitable bioisostere for the amino functionality, typically present in purines. Such replacement was previously exploited for antiviral[23,31,34–35] and cytotoxic nucleoside analogues.[23] Noteworthy is the complete lack of cytotoxicity against MRC-5 fibroblasts observed for almost all of the 6-methyl-7-(substituted) phenyl nucleosides, which represents a benefit compared to our previously reported analogue **7**.[22,25] It is noteworthy that the most promising analogues of this study (**14**, **40** and **43**) all possess a (modified) 7-(4-chlorophenyl) substituent, as summarized in Figure 2. Given that these three analogues display comparable *in vitro* antitrypanosomal activity, the lowest molecular weight compound **14** was selected for further evaluation (Tables 4-6). Compound **14** was not cardiotoxic ($CC_{50} > 400$ μM) and presented a high potency against intracellular forms (Y strain) present within primary cardiac cell cultures ($EC_{50} = 0.77 \pm 0.05$ μM) (Table 6). Also, the analogue continued to exert a promising biological effect when assessed in cardiac spheroids. The low cardiotoxicity profile of **14** was confirmed in 3D matrices ($CC_{50} > 200$ μM) and it displayed similar trypanocidal effects at a 10 μM concentration as BZ, reducing the *T. cruzi* (Y strain) parasite loads in cardiac spheroids by 77.5 ± 6.4 and 87 ± 4.2% for **14** and BZ respectively, as evidenced by qPCR analysis.

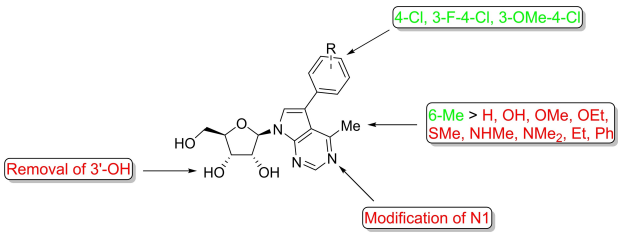


Figure 2. Summary of SAR trends for *T. cruzi*

In vitro metabolic stability of 14

The metabolic stability of **14** in the presence of mouse and human liver microsomal fractions was evaluated (Table 4). The percentage of remaining parent compound was monitored at three time points and compared to the start of the assay, indicating that **14** remained largely unaffected in the presence of either NADPH fortification (phase I) or UGT fortification (phase II). Hence, this analogue was further evaluated in a mouse model of acute *T. cruzi* infection.

In vivo evaluation of 14

The *in vivo* effect of **14** was evaluated in a stringent Y-strain *T. cruzi* acute male Swiss mouse model.[36]

Dose titration schedules of the different treatment groups are summarized Table 5. The nucleoside analogue **14** was administered at 0.25, 2.5, 25 mg/kg b.i.d. by oral gavage for 5 consecutive days, respectively, while BZ was administered orally at 10 or 100 mg/kg s.i.d. as suboptimal and optimal doses, for 5 consecutive days as a positive control. Two animal control groups (untreated and uninfected, as well as untreated and infected) were also included. To investigate the potential benefit of a combination treatment regimen, **14** (2.5 mg/kg) and BZ (10 mg/kg) were co-administrated in a separate group (G6, Table 5).

Treatment was initiated at the onset of parasitemia (5 days post-infection (dpi)) and the peak parasitemia readout was performed at 8 dpi. **14** showed a concentration-dependent effect (Figure 3). It exerted a peak parasitemia reduction of 47%, 60% and 92% at 8 dpi in treatment regimens of 0.25, 2.5, 25 mg/kg b.i.d., respectively. The efficacy of **14** at 25 mg/kg b.i.d. (92%) approached that of BZ at 100 mg/kg s.i.d. (>99%), when measured at 8 dpi and indistinguishable from BZ at 10 dpi and onwards. No toxic effects could be noticed in mice treated with **14**. Unexpectedly, co-administration of **14** (at 2.5 mg/kg) and BZ (at 10 mg/kg) led to a weaker parasitemia reduction than either compound separately. Treatment with **14** at 25 mg/kg gave 83.3% survival at 34 dpi, whereas lower doses (0.25, 2.5 mg/kg) did not protect against mortality (Figure 3 (E) and Table 5). All co-administrated animals succumbed to the

Table 4. Evaluation of *in vitro* metabolic stability of 14 in the presence of male mouse and pooled human S9 microsomal fractions.[a]

Phase I or II	Time [min]	Cpd. 14 remaining [%] MOUSE mean	SD	HUMAN mean	SD
CYP – NADPH	0	100	–	100	–
	15	93	2.7	104	5.8
	30	88	5.0	95	10.7
	60	86	0.7	96	5.4
UGT Enzymes	0	100	–	100	–
	15	98	0.6	97	1.8
	30	94	1.1	91	6.1
	60	97	0.8	93	3.0

[a] Values represent percentages of remaining parent compound. Four time points (0–15–30–60 min) of incubation were monitored. Values are reported as means and SD of two independent experiments. The reference drug was diclofenac, susceptible to phase I and phase II metabolism (data not shown).

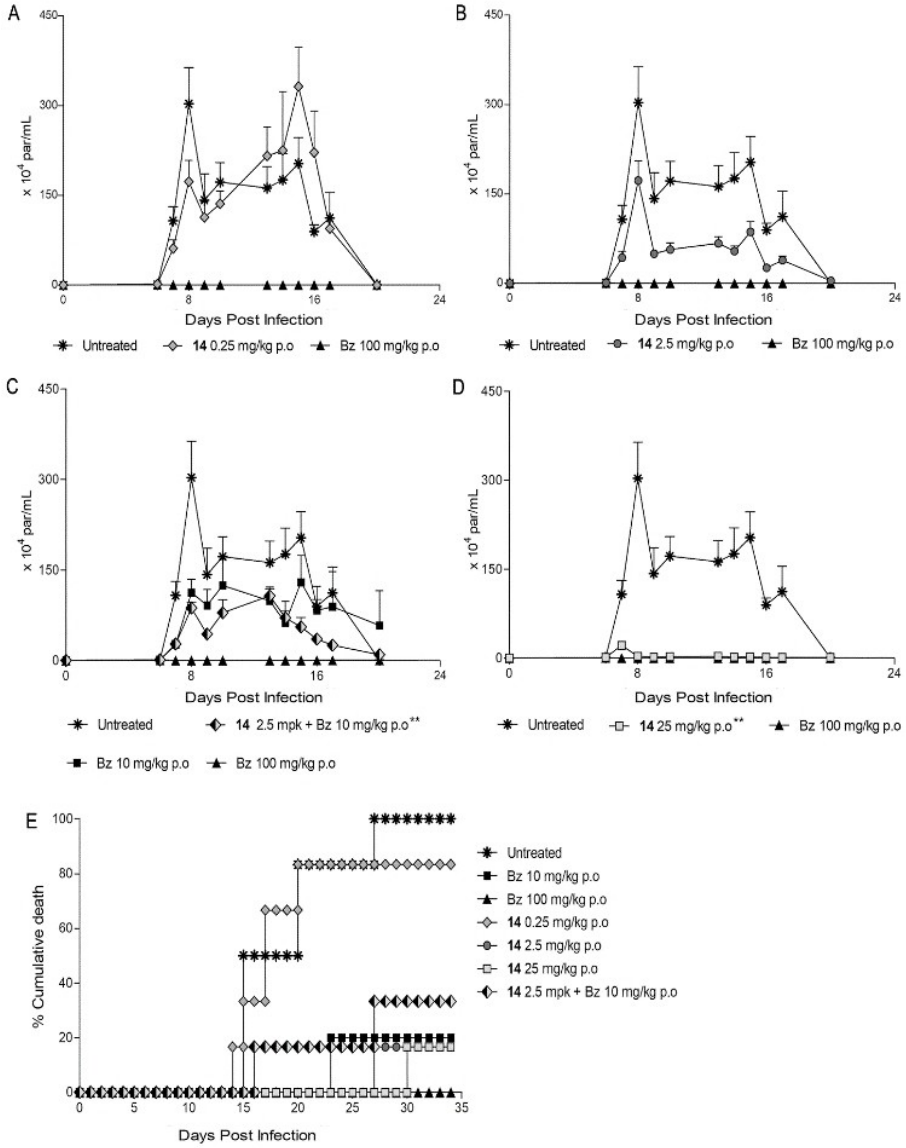


Figure 3. *In vivo* efficacy of **14** was evaluated in the mouse model of acute infection with Y-strain *T. cruzi.* (A–D) Blood parasitemia levels were measured via direct microscopic counting of the number of parasites in the blood (5 μL) from a tail vein puncture. (E) Cumulative mortality rate of the animals was recorded until 34 dpi. All treatments were for 5 consecutive days and were initiated at the onset of parasitemia (5 dpi).

Table 5. Administration and efficacy of 14 and BZ in acute CD mice model.[a]

Group	Compound	Dose [mg/kg]	Doses per day	Survival
G1	–	Uninfected	–	6/6
G2	–	Untreated (vehicle)	–	0/6
G3	**14**	0.25	2	1/6
G4	**14**	2.5	2	0/6
G5	**14**	25	2	5/6
G6	**14**+**BZ**	2.5+10 (**BZ**)	(2+1)	0/6
G7	**BZ**	10	1	4/5
G8	**BZ**	100	1	5/5

[a] All treatments were executed at the onset of parasitemia (5 dpi) and treated for 5 consecutive days (p.o.).

infection, in line with the parasitemia reduction trend. Unfortunately, none of treated animals had negative parasitemia at 34 dpi, indicating a lack of sterile cure at the used dosing regimens.

According to our previous work, the potential reason for *in vivo* failure may be the lack of activity against the bloodstream form (BF) trypomastigotes, since **14** was not active against BF trypomastigotes (Y strain) after 2 and 24 hours of incubation. Compound **14** displayed EC_{50} values >81 μM, while BZ gave 11.5 μM (Table 6). Also, the lack of cure may be due to the short period of drug exposure in this proof of concept study (only 5 days of drug therapy). Under this treatment scheme, BZ also failed to provide sterile cure: all surviving **14**- and BZ-treated animals displayed positive blood parasitism evaluated by qPCR. Accordingly, the qPCR findings demonstrated a similar blood parasite load when both groups were analysed, reaching mean values of 83.9 ± 129.4 and 84.1 ± 90.7 par. eq./mL for **14** and BZ, respectively.

Chemistry

The synthesis of several 6-modified 7-chlorophenyl-7-deaza nucleoside analogues is depicted in Schemes 1–3. First, **55**[37] was reacted with different nucleophiles to effect 6-substitution and concomitant deprotection[38] (Scheme 1), providing the 7-iodo nucleoside intermediates **56**–**60**. Then, aqueous Suzuki coupling[22,24–25] allowed to construct the target 7-(4-chlorophenyl) derivatives **7**–**8**, **10**–**12**. To access inosine analogue **9**, **10** was demethylated with in situ generated TMSI.[37–38] 7-(4-chlorophenyl)-7-deazaguanosine (**17**) was prepared from the known **61**[23,39] using the same sequence of Suzuki coupling and *O*-demethylation.

Catalytic dechlorination of **62**[38] afforded **63. 62** also served as a precursor for the introduction of a 6-methyl (**65**) or 6-ethyl group[40] (**66**) (Scheme 2). C7 halogenation was accomplished with the appropriate halosuccinimides. Subsequent ammonolysis and Suzuki coupling furnished the desired 4-chlorophenyl analogues **13**, **14** and **15**.

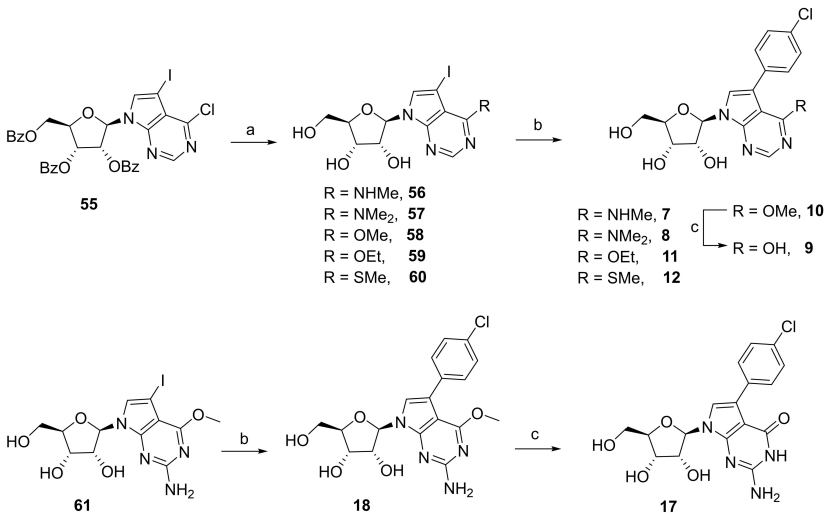
For the synthesis of the 6-phenyl analogue **16**, the phenyl group was first introduced on 6-chloro-7-deazapurine (Scheme 3). After iodination in DMF, heterocycle **70** was glycosylated under Vorbrüggen conditions[37] to give 7-iodo **71** after subsequent deprotection. Final introduction of the *para*-chlorophenyl at C7 via Pd-based cross coupling gave the desired **16**.

To probe the importance of the N1 nitrogen, several pyrrolo [2,3-*b*]pyridine (1,7-dideazapurine) nucleoside analogues were prepared (Scheme 4). First, 6-methyl analogue **72**[27] was subjected to standard aqueous Suzuki coupling to introduce the 4-chlorophenyl group. To obtain several 1-substituted nucleoside analogues, commercially available 5-substituted pyrrolo[2,3-*b*] pyridines were employed as the starting material. Bromination at C3 was effected by NBS/DMF to yield substituted heterocyclic analogues **73**–**76**.[41–42] Vorbrüggen glycosylation[27] afforded the desired analogues **77**–**80** after direct deprotection in saturated

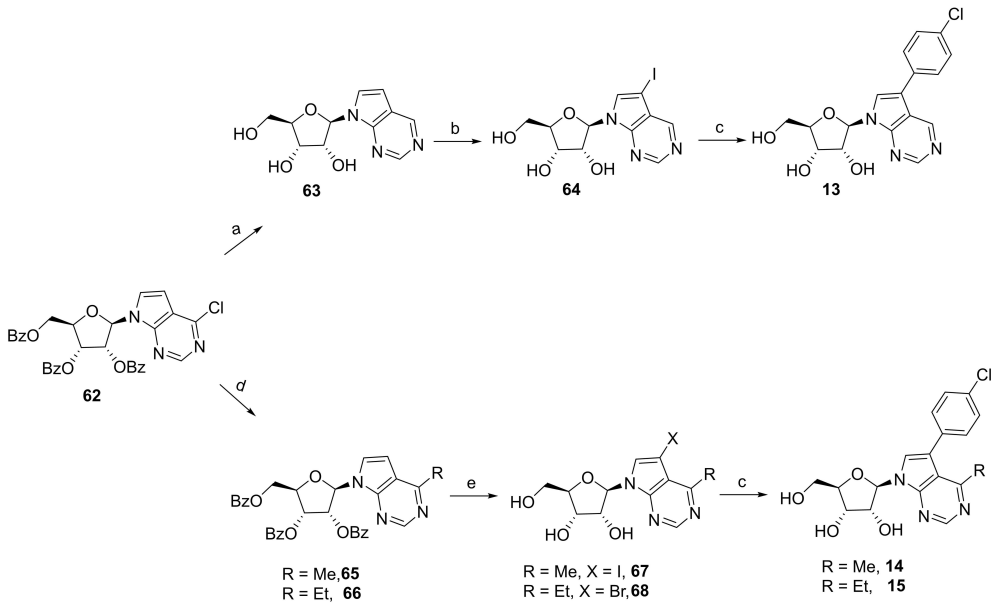
Table 6. Activity of 14 against *T. cruzi* (Y strain) bloodstream trypomastigotes and intracellular amastigotes in 2D primary cardiac cells.[a]

Cpd.	Bloodstream trypomastigotes EC_{50} [μM]	Intracellular Amastigotes[b] EC_{50} [μM]	2D primary cardiac cells CC_{50} [μM]
14	>81	0.77 ± 0.05	481.9
BZ	11.5 ± 1.1*	5.76 ± 0.09	>1000

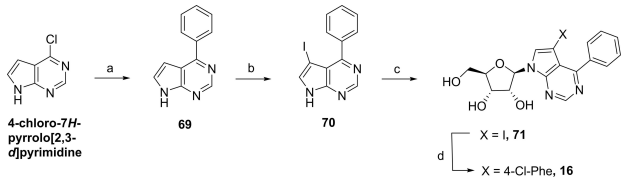
[a] EC_{50} values are described as means $\pm$ SD of 2 independent experiments; [b] The activities of analogues against intracellular amastigotes are estimated as the reduction of infection index (percentage of infected host cells × number of parasites per host cell); * Hulpia et al., 2018.[25]



Scheme 1. Reagents and conditions: (a) methylamine (40% W/V in water), 82% (**56**); dimethylamine (40% W/V in water), 72% (**57**); 0.5 M NaOMe/MeOH or NaOEt/EtOH, 50 °C, 68% (**58**); 51% (**59**); NaSMe, EtOH, r.t., 79% (**60**); (b) (4-chlorophenyl)boronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 39% (**7**), 13% (**8**), 36% (**10**), 33% (**11**), 34% (**12**), 18% (**18**); (c) NaI, TMSCl, MeCN, 74% (**9**), 68% (**17**).



Scheme 2. Reagents and conditions: (a) $Pd(OH)_2/C$, H_2, K_2CO_3, MeOH, 84%; (b) NIS, DMF, 38%; (c) (4-chlorophenyl)boronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 32% (**13**), 63% (**14**), 49% (**15**); (d) Me_3Al or Et_3Al, $Pd(PPh_3)_4$, THF, 100 °C, 93% (**65**), 65% (**66**); (e) (i) NIS for **65**, NBS for **66**, DMF; (ii) 7 N NH_3/MeOH, 61% (**67**), 64% (**68**) for two steps.



Scheme 3. Reagents and conditions: (a) phenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene, 100 °C, 95%; (b) NIS, DMF, 85%; (c) (i) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, BSA, TMSOTf, MeCN, 80 °C; (ii) 7 N NH_3/MeOH, 22% for two steps; (d) (4-chlorophenyl)boronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 23%.

methanolic ammonia. The desired *para*-chlorophenyl group was then introduced via Suzuki reaction to yield nucleoside analogues **21**, **23** and **25**. For the synthesis of **22**, a different Pd-catalyst system was used to reduce the formation the 7-dehalogenated product of **78**, which was difficult to remove from the desired **22**. To gain access to 1-amino derivative **24**, the 1-nitro group of **79** was reduced by catalytic hydrogenation (Pt/C) without concomitant dechlorination.

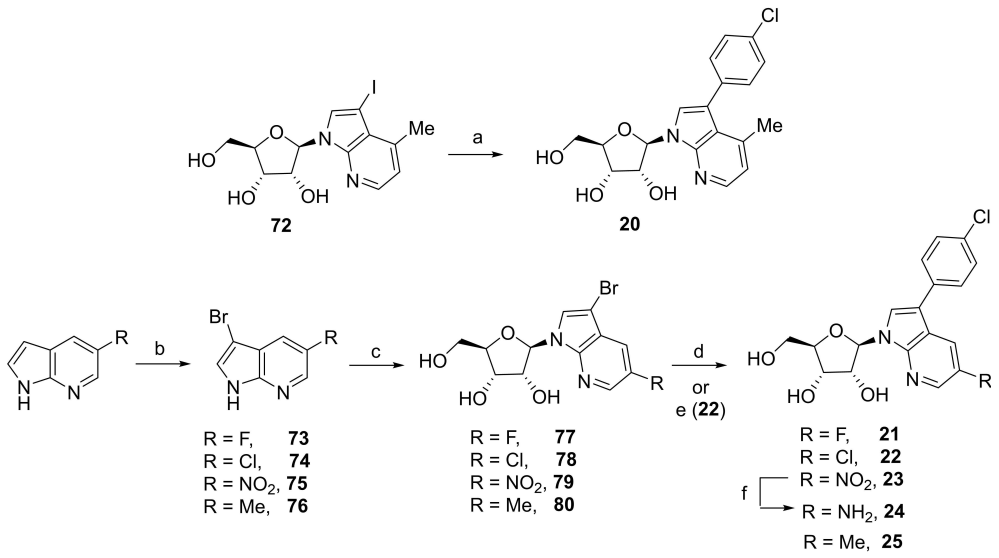
Encouraged by the observed SAR, various aromatic substituents were introduced at position 7 via Pd-based cross coupling of the 6-methyl-7-iode precursor **67** (Scheme 5).

To gain access to a 3′-deoxyribofuranose analogue, a Vorbrüggen reaction was performed involving 4-chloro-5-iodo-pyrrolo[2,3-*d*]pyrimidine[43] and 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-α/β-D- ribofuranose[25,32–33] to give **81**[33] (Scheme 6). A magnesium-iodine exchange with the Knochel reagent (iPrMgCl·LiCl)[23,32] and subsequent aqueous workup also removed the 7-iodo group. Palladium catalysed C6 methylation of the resulting intermediate with Al_3Me gave **82**. Subsequent removal of the benzoyl groups, introduction of a 7-bromo group with NBS/DMF afforded **84**, which was converted to the desired **54**.

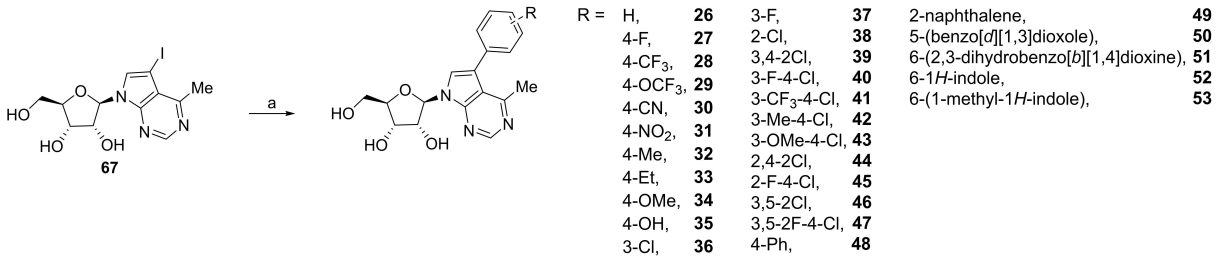
Conclusion

In a first set of *in vitro* assays of this study, we have investigated the influence of modifying the pyrimidine part of a previously reported 7-deazapurine nucleoside analogue on *T. cruzi* intracellular amastigotes. We found that a 6-methyl group acts as a suitable bioisostere and conferred a better selectivity. Next, keeping the 6-methyl group unaltered, we investigated alternative 7-substituents and found two 3,4-substituted phenyl analogues **40** and **43** that are equipotent to **14**. Nucleoside **14** showed a high potency against intracellular forms belonging to different strains and DTUs (Y and Tulahuen strains) and a low cardiotoxic profile in primary cardiac cell cultures. Our analyses employing a variety of *in vitro* models (fibroblast cell lines, 2D and 3D cardiac cell cultures and parasite strains belonging to distinct DTUs) is desirable since the metabolism of mammalian cells and parasite strains and host-parasite interactions have an impact on anti-*T. cruzi* compound activity.[44] In fact, using different *in vitro* assays in the drug discovery pipeline may better predict *in vivo* outcomes since the parasite invades different mammalian host cell types, and thus compounds must be effective in a range of host cell environments. The inclusion of cardiac organoids for anti-*T. cruzi* drug evaluation is recommended as the heart is one of the main target organs for infection and inflammation in CD and the drug responses may be influenced by the host cellular metabolism.[44–45]

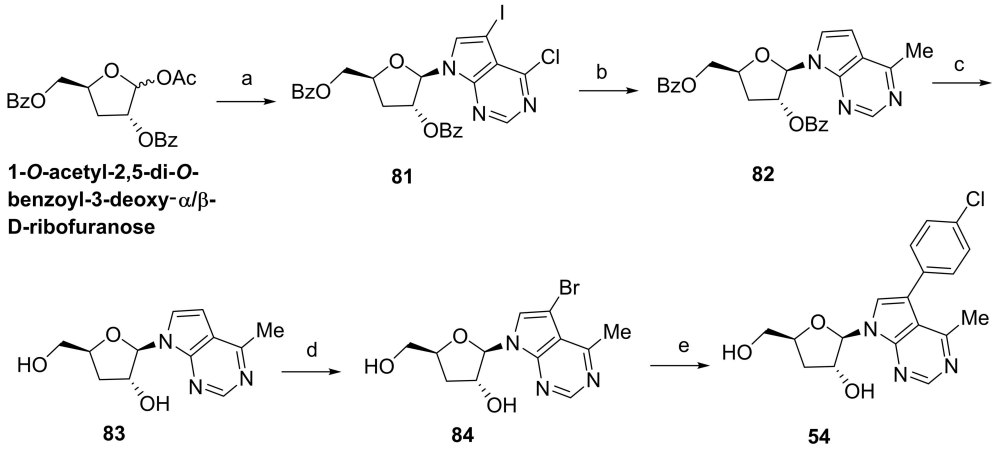
In addition, we also tested the toxicity and anti-*T. cruzi* activity of compound **14** using cardiac spheroids that corrobo-



Scheme 4. Reagents and conditions: (a) (4-chlorophenyl)boronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 61%; (b) NBS, DMF, 95% (**73**), 92% (**74**), 84% (**75**), 81% (**76**); (c) (i) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, BSA, TMSOTf, MeCN, 80 °C; (ii) 7 N NH_3/MeOH, r.t., 24% (**77**), 32% (**78**), 79% (**79**), 61% (**80**) for two steps; (d) (4-chlorophenyl)boronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 13% (**21**), 15% (**23**), 14% (**25**); (e) Pd $(PPh_3)_4$, K_2CO_3, 1,4-dioxane/water (1:1 ratio), 100 °C, 24% (**22**); (f) Pt/C, H_2, MeOH, 68%.



Scheme 5. Reagents and conditions: (a) arylboronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 21%–77%.



Scheme 6. Reagents and conditions: (a) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α/β-D-ribofuranose, BSA, TMSOTf, MeCN, 80 °C,74%; (b) (i) iPrMgCl·LiCl, THF, −10 °C; (ii) 0.5 M aq. HCl, 0 °C; (ii) Me_3Al, $Pd(PPh_3)_4$, THF, 100 °C, 66% for three steps; (c) 0.1 M NaOMe/MeOH, 83%; (d) NBS, DMF, 50 °C, 73%; (e) 4-chlorophenylboronic acid, $Pd(OAc)_2$, TPPTS, Na_2CO_3, MeCN/water (1:2 ratio), 100 °C, 36%.

rated the cardiac 2-D assays, demonstrating lack of cardiotoxicity and similar antiparasitic effect of **14** as compared to the reference drug. The use of cardiac spheroids represents a very promising approach since 3-D cultures more closely reproduce

the cellular microenvironments in *in vivo* systems and has gained great interest in drug discovery programs due to its cost-effectiveness.[46] Our *in vivo* data endorsed the use of 3-D cardiac cells as a sensitive *in vitro* model for drug evaluation of novel trypanosomicidal agents as reported.[45]

Following confirmation of metabolic stability *in vitro*, compound **14** was evaluated in a mouse model of acute experimental CD. At the highest dose (25 mg/kg p.o. b.i.d. for 5 days), it elicited comparable peak parasitemia reduction as the positive control BZ, and treated animals were protected from infection-caused mortality. Unfortunately, at 34 dpi, none of the surviving animals showed negative parasitemia evaluated by light microscopy. qPCR analysis at the endpoint showed that treatment with BZ at 100 mg/kg and **14** at 25 mg/kg resulted in similar blood parasite loads, demonstrating comparable efficacies under the tested treatment regimens. However, as already noticed using other nucleoside analogues,[25] a lack of activity was found when **14** was assayed against bloodstream trypomastigotes which may explain at least in part, the failure of sterile cure. As a new drug should be active against a heterogeneity of intracellular multiplicative amastigotes, non-replicative trypomastigotes and dormant forms,[44] we hoped that introducing additional modifications may have led to highly potent antitrypanosomal agents to be further evaluated in pre-clinical studies.

Experimental Section

Chemistry

All commercially available reagents and solvents were purchased in an analytical grade without further purification. All reactions were monitored under TLC analysis or HRMS or analytical LC–MS. Precoated F254 aluminium plates from Macherey-Nagel® were used and visualized by UC for TLC analysis. For analytical LC-MS, the conditions were that: a C18 column (2.7 mm, 100×4.6 mm) from Waters Cortecs; a gradient system consisting of 0.2% formic acid in H_2O (v/v)/MeCN; a gradient from 95:5 to 0:100 in 10 min with a flow rate of 1.44 mL/min. All purification was completed via silica gel column chromatography or preparative RP-HPLC. The 60 M silica gel (40–63 μm) from Macherey-Nagel® or an automated flash unit (Reveleris X2) from Grace/Büchi® with prepacked silica columns were used for silica gel column chromatography. For preparative RP-HPLC, the conditions were that: a Luna Omega Polar column (5 μm, 250×21 mm) from Phenomenex®; a gradient system consisting of 0.2% formic acid in H_2O (v/v)/ MeCN; a flow rate of 20 mL/min. a Waters AutoPurification system was applied in both of analytical LC–MS and preparative RP-HPLC, equipped with ACQUITY QDa (mass; 100–1000 amu) and 2998 Photodiode Array (220–400 nm). A LCT Premier XE time-of-flight (ToF) mass spectrometer from Waters was applied in exact mass measurements, equipped with a standard electrospray (ESI) and modular interface from Lockspray®. A 300 MHz spectrometer from Varian Mercury or a 400 MHz spectrometer from Bruker Avance Neo was used for NMR spectra. ^{1}H-^{1}H 2D NOESY and ^{1}H-^{13}C gHMBC were used respectively to ascertain the stereochemistry at C-1′. A Büchi-545 apparatus (uncorrected) was used for melting points measurements. Purity of final compounds was measured through integration of UV signal (total UV chromatogram and wavelength selected at 254 nm) in analytical LC-MS. Purity of all target compounds was at least 95%.

Nucleoside analogue **2**, **10** were prepared as described previously.[27,47]

In this section, the IUPAC nomenclature and numbering were applied in the pyrrolo[2,3-*d*]pyrimidine system, differing with the purine numbering in the body of the text.

General procedure A for Suzuki coupling reaction (as described in reference[24–25,48])

Iodo-derivative or **bromo-derivative** (1 eq.), boronic acid (1.5 eq.), $Pd(OAc)_2$ (0.05 eq.), TPPTS (0.15 eq.) and Na_2CO_3 (9 eq.) were added to a 10 mL round-bottom flask, equipped with a stir bar. Then, the flask was purged with argon thrice after removal of the air *in vacuo*. A mixture of MeCN/water (1/2 ratio, 6 mL/mmol SM) was added into the mixture via syringes. The reaction mixture was stirred at ambient temperature for 5 min, and then heated at 100 °C. When the consumption of SM was observed by HRMS (~1 to 2 h), the reaction was cooled to ambient temperature. Then, 0.5 M aq. HCl was added to neutralize the mixture. The solution was evaporated *in vacuo*, and the residue resuspended in MeOH and evaporated three times. Then the residue was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~5 cm) with 20% MeOH/DCM. The resulting solution was evaporated till dryness and purified by column chromatography (MeOH/DCM gradient).

4-Methylamino-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (56[23]). 55[23] (600 mg, 0.83 mmol) was dissolved in 1,4-dioxane (1 mL) and stirred in a 10 mL pressure tube. Methylamine solution (40% W/V solution in water, 5 mL) was added and then the reaction mixture was stirred at 100 °C overnight. After cooling to room temperature, the reaction mixture was evaporated till dryness, and purified by flash column chromatography (0→20% MeOH/DCM) to afford **56** (273 mg, 0.67 mmol) as a yellow foam in 82% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.03 (d, J=4.7 Hz, 3 H, $NHCH_3$), 3.49–3.56 (m, 1 H, H-5″), 3.58–3.65 (m, 1 H, H-5′), 3.88 (q, J=3.5 Hz, 1 H, H-4′), 4.06 (q, J=4.2 Hz, 1 H, H-3′), 4.35 (q, J=6.1 Hz, 1 H, H-2′), 5.10–5.16 (m, 2 H, OH-5′ and OH-3′), 5.30 (d, J=6.2 Hz, 1 H, OH-2′), 6.03 (d, J=6.2 Hz, 1 H, H-1′), 6.44 (q, J=4.4 Hz, 1 H, NH), 7.65 (s, 1 H, H-6), 8.19 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{12}H_{16}IN_4O_4$ ([M+H]$^+$): 407.0211, found: 407.0230.

4-Dimethylamino-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (57[23]). 55[23] (600 mg, 0.83 mmol) was dissolved in 1,4-dioxane (1 mL) and stirred in a 10 mL pressure tube. Dimethylamine solution (40% W/V solution in water, 5 mL) was added and then the reaction mixture was stirred at 100 °C overnight. After cooling to room temperature, the reaction mixture was evaporated till dryness, and purified by flash column chromatography (0→20% MeOH/DCM) to afford **57** (250 mg, 0.60 mmol) as a yellow foam in 72% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.16 (s, 6 H, $2CH_3$), 3.52–3.56 (m, 1 H, H-5″), 3.60–3.64 (m, 1 H, H-5′), 3.89 (q, J=3.5 Hz, 1 H, H-4′), 4.06–4.08 (m, 1 H, H-3′), 4.36 (t, J=5.3 Hz, 1 H, H-2′), 5.12 (br. s., 2 H, OH-5′ and OH-3′), 5.32 (br. s., 1 H, OH-2′), 6.11 (d, J=6.1 Hz, 1 H, H-1′), 7.86 (s, 1 H, H-6), 8.24 (s, 1 H, H-2).). HRMS (ESI): calculated for $C_{13}H_{18}IN_4O_4$ ([M+H]$^+$): 421.0367, found: 421.0346.

4-Methoxy-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (58[23]). 55[23] (600 mg, 0.83 mmol) was dissolved in 0.5 M NaOMe in MeOH (10 mL) and the reaction mixture was stirred at 50 °C for 3 h. Then, the reaction was cooled to room temperature. Then, 0.5 M aq. HCl solution was added to neutralize the reaction solution. The resulting mixture was evaporated till dryness, and purified by flash column chromatography (0→10% MeOH/DCM) to afford **58** (230 mg, 0.56 mmol) as a white solid in 68% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.53–3.57 (m, 1 H, H-5″), 3.62–3.66 (m, 1 H, H-5′), 3.91 (dd, J=3.0, 1.9 Hz, 1 H, H-4′), 4.06–4.09 (m, 4 H, H-3′

and OCH_3), 4.35–4.38 (m, 1 H, H-2′), 5.07–5.09 (m, 1 H, OH-5′), 5.15 (br. s., 1 H, OH-3′), 5.36 (d, J=5.4 Hz, 1 H, OH-2′), 6.13 (dd, J=6.2, 1.8 Hz, 1 H, H-1′), 7.88 (d, J=2.0 Hz, 1 H, H-6), 8.44 (d, J=2.1 Hz, 1 H, H-2). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_5$ ($[M+H]^+$): 408.0051, found: 408.0063.

4-Ethoxy-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (59). 55[23] (300 mg, 0.41 mmol) was dissolved in 0.5 M NaOEt in EtOH (10 mL) and the reaction mixture was stirred at 50 °C for 3 h. Then, the reaction was cooled to room temperature. Then, 0.5 M aq. HCl solution was added to neutralize the reaction solution. The resulting mixture was evaporated till dryness, and purified by flash column chromatography (0→10% MeOH/DCM) to afford **59** (88 mg, 0.21 mmol) as a white solid in 51% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.40 (t, J=7.2 Hz, 3 H, CH_3), 3.54 (ddd, J=12.0, 5.6, 4.0 Hz, 1 H, H-5″), 3.63 (ddd, J=12.0, 5.4, 4.0 Hz, 1 H, H-5′), 3.90 (q, J=3.5 Hz, 1 H, H-4′), 4.08 (td, J=4.8, 3.2 Hz, 1 H, H-3′), 4.34–4.40 (m, 1 H, H-2′), 4.53 (q, J=7.0 Hz, 2 H, CH_2), 5.08 (t, J=5.6 Hz, 1 H, OH-5′), 5.15 (d, J=4.7 Hz, 1 H, OH-3′), 5.35 (d, J=6.2 Hz, 1 H, OH-2′), 6.12 (d, J=6.2 Hz, 1 H, H-1′), 7.86 (s, 1 H, H-6), 8.41 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{13}H_{17}IN_3O_5$ ($[M+H]^+$): 422.0207, found: 422.0199.

4-Methylsulfanyl-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (60[23]**). 55**[23] (600 mg, 0.83 mmol) and sodium thiomethoxide (126 mg, 1.83 mmol, 2.2 eq.) was added in EtOH (10 mL) and the reaction mixture was stirred at ambient temperature for 4 h. Then, the resulting mixture was evaporated till dryness, and purified by flash column chromatography (0→10% MeOH/DCM) to afford **60** (273 mg, 0.66 mmol) as a white solid in 79% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.64 (s, 3 H, CH_3), 3.51–3.58 (m, 1 H, H-5″), 3.60–3.67 (m, 1 H, H-5′), 3.91 (q, J=3.5 Hz, 1 H, H-4′), 4.36 (q, J=5.9 Hz, 1 H, H-3′), 5.07 (t, J=5.4 Hz, 1 H, H-2′), 5.16 (d, J=5.0 Hz, 1 H, OH-5′), 5.37 (d, J=6.4 Hz, 1 H, OH-3′), 6.15 (d, J=6.2 Hz, 1 H, OH-2′), 7.99 (s, 1 H), 8.62 (s, 1 H). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_4S$ ($[M+H]^+$): 423.9822, found: 423.9809.

4-Methylamino-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (7). 7 was prepared according to General procedure A. **56** (210 mg, 0.52 mmol) gave rise to **7** (80 mg, 0.077 mmol) as a white solid in 13% yield. Melting point: 122 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.91 (d, J=4.7 Hz, 3 H, CH_3), 3.43–3.57 (m, 1 H, H-5″), 3.58–3.71 (m, 1 H, H-5′), 3.90 (q, J=3.5 Hz, 1 H, H-4′), 4.03–4.19 (m, 1 H, H-3′), 4.45 (t, J=5.6 Hz, 1 H, H-2′), 5.17 (br. s., 2 H, OH-5′ and OH-3′), 5.32 (br. s., 1 H, OH-2′), 5.74 (q, J=4.6 Hz, 1 H, HN), 6.12 (d, J=6.2 Hz, 1 H, H-1′), 7.38–7.49 (m, 2 H, H–Ph, 7.49–7.60 (m, 3 H, H–Ph and H-6), 8.24 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.0 (CH_3), 61.6 (C-5′), 70.6 (C-3′), 73.8 (C-2′), 85.1 (C-4′), 87.0 (C-1′), 100.5 (C-4a), 115.0 (C-5), 121.3 (C-6), 128.9 (2 C, C-3_{Ph} and C-5_{Ph}), 130.0 (2 C, C-2_{Ph} and C-6_{Ph}), 131.4 (C-4_{Ph}), 133.3 (C-1_{Ph}), 150.2 (C-7a), 151.6 (C-2), 156.8 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_4$ ($[M+H]^+$): 391.1168, found: 391.1159.

4-Dimethylamino-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (8). 8 was prepared according to General procedure A. **57** (250 mg, 0.59 mmol) gave rise to **8** (30 mg, 0.20 mmol) as a white solid in 39% yield. Melting point: 75 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.75 (s, 6 H, $2CH_3$), 3.48–3.57 (m, 1 H, H-5″), 3.58–3.70 (m, 1 H, H-5′), 3.90 (q, J=3.5 Hz, 1 H, H-4′), 4.06–4.15 (m, 1 H, H-3′), 4.46 (q, J=6.0″ Hz, 1 H, H-2′), 5.04–5.19 (m, 2 H, OH-5′ and OH-3′), 5.33 (d, J=6.4 Hz, 1 H, OH-2′), 6.18 (d, J=6.2 Hz, 1 H, H-1′), 7.36–7.56 (m, 4 H, H–Ph), 7.71 (s, 1 H, H-6), 8.29 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 40.7 (2 C, CH_3), 61.6 (C-5′), 70.5 (C-3′), 73.8 (C-2′), 85.1 (C-4′), 86.8 (C-1′), 102.0 (C-4a), 115.7 (C-5), 122.1 (C-6), 128.5 (2 C, C–Ph), 129.7 (2 C, C–Ph), 131.1 (C-4_{Ph}), 134.6 (C-1_{Ph}), 150.3 (C-2), 152.3 (C-7a), 160.0 (C-4). HRMS (ESI): calculated for $C_{19}H_{22}ClN_4O_4$ ($[M+H]^+$): 405.1324, found: 405.1323.

4-Ethoxy-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (11). 11 was prepared according to General procedure A. **59** (110 mg, 0.26 mmol) gave rise to **11** (35 mg, 0.086 mmol) as a white solid in 33% yield. Melting point: 138 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.36 (t, J=7.0 Hz, 3 H, CH_3), 3.56 (ddd, J=11.9, 5.8, 4.2 Hz, 1 H, H-5″), 3.66 (ddd, J=12.0, 5.2, 4.2 Hz, 1 H, H-5′), 3.93 (q, J=4.0 Hz, 1 H, H-4′), 4.08–4.20 (m, 1 H, H-3′), 4.41–4.57 (m, 3 H, H-2′ and CH_2), 5.08 (t, J=5.6 Hz, 1 H, OH-5′), 5.16 (d, J=5.0 Hz, 1 H, OH-3′), 5.37 (d, J=6.4 Hz, 1 H, OH-2′), 6.22 (d, J=6.2 Hz, 1 H, H-1′), 7.40–7.52 (m, 2 H, H–Ph), 7.67–7.79 (m, 2 H, H–Ph), 7.93 (s, 1 H, H-6), 8.45 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 14.2 (CH_3), 61.5 (C-5′), 62.2 (CH_2), 70.4 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.9 (C-1′), 102.6 (C-4a), 115.1 (C-5), 122.9 (C-6), 128.0 (2 C, C-3_{Ph} and C-5_{Ph}), 130.2 (2 C, C-2_{Ph} and C-6_{Ph}), 131.1 (C-4_{Ph}), 132.5 (C-1_{Ph}), 150.9 (C-2), 152.7 (C-7a), 162.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_5$ ($[M+H]^+$): 406.1144, found: 406.1156.

4-Oxo-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (9). 10 (70 mg, 0.18 mmol) and NaI (135 mg, 0.90 mmol, 5 eq.) were placed in anhydrous MeCN (5 mL). Next, TMSCl (114 µL, 0.90 mmol, 5 eq.) was slowly added into the reaction mixture, and the mixture was stirred at ambient temperature for 2 h. The precipitate was filtered, washed with MeCN, and dissolved in water. Then, aq. sat. $NaHCO_3$ was added to neutralize the solution. The mixture was evaporated till dryness, and purified by flash column chromatography (0→20% MeOH/DCM) to afford **9** (50 mg, 0.13 mmol) as a white solid in 74% yield. Melting point: 75 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (ddd, J=11.8, 5.6, 4.2 Hz, 1 H, H-5″), 3.65 (ddd, J=12.0, 5.4, 4.0 Hz, 1 H, H-5′), 3.90 (q, J=3.8 Hz, 1 H, H-4′), 4.07–4.16 (m, 1 H, H-3′), 4.38 (q, J=6.0 Hz, 1 H, H-2′), 5.04 (t, J=5.6 Hz, 1 H, OH-5′), 5.14 (d, J=5.0 Hz, 1 H, OH-3′), 5.36 (d, J=6.2 Hz, 1 H, OH-2′), 6.10 (d, J=6.2 Hz, 1 H, H-1′), 7.38–7.41 (m, 1 H, H–Ph), 7.41–7.45 (m, 1 H, H–Ph), 7.78 (s, 1 H, H-6), 7.95–7.98 (m, 2 H, H–Ph and H-2), 7.98–8.02 (m, 1 H, H–Ph), 12.10 (br. s., 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.4 (C-5′), 70.4 (C-3′), 74.1 (C-2′), 85.1 (C-4′), 86.8 (C-1′), 105.0 (C-4a), 119.0 (C-5), 119.2 (C-6), 127.9 (2 C, C-3_{Ph} and C-5_{Ph}), 129.6 (2 C, C-2_{Ph} and C-6_{Ph}), 130.7 (C-4_{Ph}), 132.5 (C-1_{Ph}), 144.3 (C-2), 149.0 (C-7a), 158.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}ClN_3O_5$ ($[M+H]^+$): 378.0851, found: 378.0840.

4-Methylsulfanyl-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (12). 12 was prepared according to General procedure A. **60** (260 mg, 0.61 mmol) gave rise to **12** (85 mg, 0.21 mmol) as a white solid in 34% yield. Melting point: 209 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.55 (s, 3 H, CH_3), 3.55 (ddd, J=12.0, 5.6, 4.0 Hz, 1 H, H-5″), 3.64 (ddd, J=12.0, 5.2, 4.2 Hz, 1 H, H-5′), 3.93 (q, J=3.8 Hz, 1 H, H-4′), 4.05–4.21 (m, 1 H, H-3′), 4.45 (q, J=5.8 Hz, 1 H, H-2′), 5.04 (t, J=5.6 Hz, 1 H, OH-5′), 5.17 (d, J=5.0 Hz, 1 H, OH-3′), 5.39 (d, J=6.2 Hz, 1 H, OH-2′), 6.25 (d, J=6.2 Hz, 1 H, H-1′), 7.40–7.57 (m, 4 H, H–Ph), 7.85 (s, 1 H, H-6), 8.68 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 11.9 (CH_3), 61.4 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.2 (C-4′), 86.7 (C-1′), 114.0 (C-4a), 115.4 (C-5), 124.5 (C-6), 127.9 (2 C, C–Ph), 131.7 (2 C, C–Ph), 132.1 (C-4_{Ph}), 132.4 (C-1_{Ph}), 148.7 (C-7a), 150.4 (C-2), 161.1 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4S$ ($[M+H]^+$): 408.0779, found: 408.0795.

2-Amino-4-methoxy-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (18). 18 was prepared according to General procedure A, except for the use of 90 °C instead of 100 °C. **61** (300 mg, 0.71 mmol) gave rise to **18** (53 mg, 0.13 mmol) as a white solid in 18% yield. Melting point: 202 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.44–3.56 (m, 1 H, H-5″), 3.56–3.69 (m, 1 H, H-5′), 3.84 (q, J=4.0 Hz, 1 H, H-4′), 3.91 (s, 3 H, OCH_3), 4.02–4.15 (m, 1 H, H-3′), 4.37 (q, J=6.0 Hz, 1 H, H-2′), 5.00 (t, J=5.6 Hz, 1 H, OH-5′), 5.05 (d, J=4.4 Hz, 1 H, OH-3′), 5.26 (d, J=6.2 Hz, 1 H, OH-2′), 6.05 (d, J=6.2 Hz, 1 H, H-1′), 6.32 (br. s., 2 H, NH_2), 7.36–7.41 (m, 2 H, H–Ph and H-6), 7.42–7.47 (m, 1 H, H–Ph), 7.59–7.63 (m, 1 H, H–Ph), 7.64–7.68 (m, 1 H, H–Ph). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 53.0 (OCH_3), 61.6 (C-

5′), 70.5 (C-3′), 73.4 (C-2′), 84.7 (C-4′), 85.8 (C-1′), 95.0 (C-4a), 115.5 (C-5), 118.4 (C-6), 128.0 (2 C, C-3_{Ph} and C-5_{Ph}), 129.5 (2 C, C-2_{Ph} and C-6_{Ph}), 130.5 (C-4_{Ph}), 133.5 (C-1_{Ph}), 155.7 (C-7a), 159.4 (C-2), 163.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_5$ ($[M+H]^+$): 407.1117, found: 407.1134.

2-Amino-4-oxo-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (17). 18 (53 mg, 0.13 mmol) and NaI (98 mg, 0.65 mmol, 5 eq.) were placed in anhydrous MeCN (5 mL). Next, TMSCl (82 μL, 0.65 mmol, 5 eq.) was slowly added into the reaction mixture, and the mixture was stirred at ambient temperature for 2 h. The precipitate was filtered, washed with MeCN, and dissolved in water. Then, aq. sat. $NaHCO_3$ was added to neutralize the solution. The mixture was evaporated till dryness, and purified by flash column chromatography (0→20% MeOH/DCM) to afford **17** (35 mg, 0.089 mmol) as a white solid in 68% yield. Melting point: 249 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.45–3.55 (m, 1 H, H-5″), 3.56–3.68 (m, 1 H, H-5′), 3.82 (q, J=4.1 Hz, 1 H, H-4′), 4.00–4.12 (m, 1 H, H-3′), 4.31 (q, J=6.2 Hz, 1 H, H-2′), 4.97 (t, J=5.6 Hz, 1 H, OH-5′), 5.04 (d, J=4.4 Hz, 1 H, OH-3′), 5.26 (d, J=6.2 Hz, 1 H, OH-2′), 5.94 (d, J=6.2 Hz, 1 H, H-1′), 6.32 (br. s, 2 H, NH_2), 7.32–7.35 (m, 1 H, H–Ph), 7.35–7.37 (m, 1 H, H–Ph), 7.38 (s, 1 H, H-6), 7.96–8.00 (m, 1 H, H–Ph), 8.00–8.03 (m, 1 H, H–Ph), 10.48 (s, 1 H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.6 (C-5′), 70.4 (C-3′), 73.5 (C-2′), 84.6 (C-4′), 85.8 (C-1′), 97.2 (C-4a), 115.8 (C-6), 118.7 (C-5), 127.8 (2 C, C-3_{Ph} and C-5_{Ph}), 129.1 (2 C, C-2_{Ph} and C-6_{Ph}), 130.1 (C-4_{Ph}), 133.2 (C-1_{Ph}), 152.6 (C-7a), 152.7 (C-2), 158.8 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}ClN_4O_5$ ($[M+H]^+$): 393.0960, found: 393.0976.

N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (63). 62[23,49] (200 mg, 0.33 mmol) was dissolved in MeOH (5 mL), and solid K_2CO_3 (91 mg, 0.66 mmol, 2 eq.) was added. The flask was purged with N_2, and a cat. amount of $Pd(OH)_2$/C was added. Then, the N_2 gas was exchanged with H_2 (balloon; bubbling). The reaction mixture was stirred at room temperature until HRMS showed full consumption of the SM (2 h). Then, the H_2 balloon was removed and K_2CO_3 (136 mg, 0.99 mmol, 3 eq.) was added, and the resulting mixture stirred at ambient temperature until all benzoyl groups of the SM were removed (3 h). 0.5 M aq. HCl solution was added to neutralize the mixture, after which the solution was filtered over Celite®. The filtrate was evaporated till dryness and purified by column chromatography (0→10% MeOH/DCM) to afford **63** (70 mg, 0.28 mmol) as a white solid in 84% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (ddd, J=12.0, 5.6, 4.0 Hz, 1 H, H-5″), 3.64 (ddd, J=12.0, 5.4, 4.2 Hz, 1 H, H-5′), 3.93 (q, J=3.7 Hz, 1 H, H-4′), 4.10–4.15 (m, 1 H, H-3′), 4.41–4.47 (m, 1 H, H-2′), 5.06 (t, J=5.4 Hz, 1 H, OH-5′), 5.17 (d, J=5.0 Hz, 1 H, OH-3′), 5.36 (d, J=6.4 Hz, 1 H, OH-2′), 6.22 (d, J=6.2 Hz, 1 H, H-1′), 6.71 (d, J=3.8 Hz, 1 H, H-5), 7.87 (d, J=3.8 Hz, 1 H, H-6), 8.80 (s, 1 H, H-2), 9.03 (s, 1 H, H-4). HRMS (ESI): calculated for $C_{11}H_{14}N_3O_4$ ($[M+H]^+$): 252.0979, found: 252.0970.

5-Iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (64). 63 (470 mg, 1.87 mmol) was dissolved in DMF (5 mL), and NIS (631 mg, 2.81 mmol, 1.5 eq.) was added. The resulting mixture was stirred at 50 °C for 2 h. Then, the mixture was evaporated till dryness and purified by column chromatography (0→10% MeOH/DCM) to afford **64** (270 mg, 0.71 mmol) as a white solid in 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.53–3.58 (m, 1 H, H-5″), 3.62–3.67 (m, 1 H, H-5′), 3.93 (q, J=3.6 Hz, 1 H, H-4′), 4.11 (q, J=4.2 Hz, 1 H, H-3′), 4.42 (q, J=5.8 Hz, 1 H, H-2′), 5.08 (t, J=5.4 Hz, 1 H, OH-5′), 5.18 (d, J=4.8 Hz, 1 H, OH-3′), 5.40 (d, J=6.4 Hz, 1 H, OH-2′), 6.21 (d, J=6.3 Hz, 1 H, H-1′), 8.12 (s, 1 H, H-6), 8.77 (s, 1 H, H-2), 8.87 (s, 1 H, H-4). HRMS (ESI): calculated for $C_{11}H_{13}IN_3O_4$ ($[M+H]^+$): 377.9945, found: 377.9954.

5-(4-Chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (13). 13 was prepared according to General procedure A. **64** (260 mg, 0.69 mmol) gave rise to **13** (80 mg, 0.22 mmol) as a grey solid in 32% yield. Melting point: 65 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.58 (ddd, J=11.8, 5.8, 4.4 Hz, 1 H, H-5″), 3.68 (ddd, J=12.0, 5.4, 4.2 Hz, 1 H, H-5′), 3.95 (q, J=4.0 Hz, 1 H, H-4′), 4.09–4.22 (m, 1 H, H-3′), 4.51 (q, J=6.0 Hz, 1 H, H-2′), 5.08 (t, J=5.6 Hz, 1 H, OH-5′), 5.20 (d, J=5.0 Hz, 1 H, OH-3′), 5.42 (d, J=6.2 Hz, 1 H, OH-2′), 6.28 (d, J=5.9 Hz, 1 H, H-1′), 7.41–7.58 (m, 2 H, H–Ph), 7.74–7.92 (m, 2 H, H–Ph), 8.31 (s, 1 H, H-6), 8.89 (s, 1 H, H-2), 9.38 (s, 1 H, H-4). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.5 (C-5′), 70.5 (C-3′), 73.9 (C-2′), 85.3 (C-4′), 86.5 (C-1′), 114.0 (C-5), 116.7 (C-4a), 124.9 (C-6), 128.2 (2 C, C-2_{Ph} and C-6_{Ph}), 129.0 (2 C, C-3_{Ph} and C-5_{Ph}), 131.2 (C-4_{Ph}), 132.0 (C-1_{Ph}), 149.1 (C-4), 151.3 (C-2), 151.5 (C-7a). HRMS (ESI): calculated for $C_{17}H_{17}ClN_3O_4$ ($[M+H]^+$): 362.0902, found: 362.0922.

4-Methyl-N7-(2′,3′,5′-tri-*O*-benzoyl-β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (65). 62[23,49] (9.27 g, 15.5 mmol) and $Pd(PPh_3)_4$ (358 mg, 0.31 mmol, 0.02 eq.) were placed into a 250 mL round bottom flask. The flask was purged with argon thrice after removal of the air *in vacuo*. Next, anhydrous THF (62 mL, 4 mL/mmol) was added via syringe, and $AlMe_3$ solution (2 M in toluene, 9.3 mL, 18.6 mmol, 1.2 eq.) was added dropwise. After the mixture was stirred for 15 min at ambient temperature, the reaction was refluxed at 100 °C until TLC analysis showed the SM was consumed completely (1 h). After cooling in an ice-water bath, 0.5 M aq. HCl (10 mL) was added dropwise and slowly. [**Caution**: methane gas was generated, with potential excessive foaming]. Next, EA (50 mL) was added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (10→50% EA/PET) to give **65** (7.7 g, 13.3 mmol) as a yellow foam in 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.71 (s, 3 H, CH_3), 4.65–4.71 (m, 1 H, H-5″), 4.76–4.80 (m, 1 H, H-4′), 4.84–4.89 (m, 1 H, H-5′), 6.16 (dd, J=5.9, 4.4 Hz, 1 H, H-3′), 6.22–6.26 (m, 1 H, H-2′), 6.57 (d, J=3.8 Hz, 1 H, H-1′), 6.73 (d, J=5.9 Hz, 1 H, H-5), 7.31–7.63 (m, 10 H, H-6 and H-OBz), 7.91–7.94 (m, 2 H, H-OBz), 7.98–8.02 (m, 2 H, H-OBz), 8.11–8.14 (m, 2 H, H-OBz), 8.76 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{33}H_{28}N_3O_7$ ($[M+H]^+$): 578.1922, found: 578.1924.

4-Methyl-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (67).[23] **65** (10 g, 17.3 mmol) was dissolved in anhydrous DMF (120 mL), and NIS (4.67 g, 20.8 mmol, 1.2 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. EA (200 mL) and water (200 mL) were added. The layers were separated and the water layer extracted once more with EA. The organic layers were combined and washed with brine (200 mL), dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a yellow oil (10.0 g, 14.2 mmol) in 82% yield. This obtained product (4 g, 5.68 mmol) was charged in a 250 mL round bottom flask, and NH_3 in MeOH (7 N, 20 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated till dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **67** (1.95 g, 4.98 mmol) as a slight yellow solid in 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.89 (s, 3 H, CH_3), 3.52–3.58 (m, 1 H, H-5″), 3.61–3.66 (m, 1 H, H-5′), 3.91 (q, J=3.5 Hz, 1 H, H-4′), 4.09 (q, J=4.1 Hz, 1 H, H-3′), 4.38 (q, J=6.0 Hz, 1 H, H-2′), 5.08 (t, J=5.38 Hz, 1 H, OH-5′), 5.16 (d, J=4.75 Hz, 1 H, OH-3′), 5.36 (d, J=6.38 Hz, 1 H, OH-2′), 6.18 (d, J=6.25 Hz, 1 H, H-1′), 8.06 (s, 1 H, H-6), 8.67 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.5 (CH_3), 53.8 (C-5), 61.4 (C-5′),70.4 (C-3′), 74.1 (C-2′), 85.3 (C-4′), 86.5 (C-1′), 117.8 (C-4a), 131.4 (C-6), 150.0 (C-7a), 150.9 (C-2), 159.5 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_4$ ($[M+H]^+$): 392.0102, found: 392.0105.

4-Methyl-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (14). 14 was prepared according to General procedure A. **67** (150 mg, 0.38 mmol) gave rise to **14** (90 mg, 0.24 mmol) as a white solid in 63% yield. Melting point: 166 °C. ^{1}H

NMR (400 MHz, DMSO-d_6) δ: 2.46 (s, 3 H, CH_3), 3.49–3.59 (m, 1 H, H-5''), 3.59–3.73 (m, 1 H, H-5'), 3.93 (q, J = 3.8 Hz, 1 H, H-4'), 4.13 (d, J = 3.8 Hz, 1 H, H-3'), 4.46 (dd, J = 11.2, 6.0 Hz, 1 H, H-2'), 5.04 (t, J = 5.0 Hz, 1 H, OH-5'), 5.17 (d, J = 4.4 Hz, 1 H, OH-3'), 5.37 (d, J = 6.4 Hz, 1 H, OH-2'), 6.27 (d, J = 6.2 Hz, 1 H, H-1'), 7.45–7.58 (m, 4 H, H–Ph), 7.88 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.9 (CH_3), 61.5 (C-5'), 70.5 (C-3'), 74.0 (C-2'), 85.2 (C-4'), 86.6 (C-1'), 115.7 (C-4a), 115.7 (C-5), 125.0 (C-6), 128.2 (2 C, C–Ph), 131.5 (2 C, C–Ph), 132.0 (C-4_{Ph}), 133.2 (C-1_{Ph}), 150.7 (C-7a), 150.9 (C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4$ ([M + H]$^+$): 376.1059, found: 376.1068.

4-Ethyl-N7-(2',3',5'-tri-O-benzoyl-β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (66).[50] **62**[23,49] (700 mg, 1.17 mmol) and Pd(PPh_3)$_4$ (135 mg, 0.12 mmol, 0.1 eq.) was placed into a 50 mL round bottom flask. The flask was purged with argon thrice after removal of the air *in vacuo*. Next, anhydrous THF (5 mL, 4 mL/mmol) was added via syringe, and $AlEt_3$ solution (1 M in hexanes, 1.4 mL, 1.4 mmol, 1.2 eq.) was added dropwise. After the mixture was stirred for 15 min at ambient temperature, the reaction was refluxed at 100 °C until TLC analysis showed the SM was consumed completely (1 h). After cooling in an ice-water bath, 0.5 M aq. HCl (5 mL) was added dropwise and slowly. [**Caution**: methane gas was generated, with potential excessive foaming]. Next, EA (10 mL) was added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (10→50% EA/PET) to give **66** (450 mg, 0.76 mmol) as a colorless foam in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (t, J = 7.6 Hz, 3 H, CH_3), 3.02 (q, J = 7.6 Hz, 2 H, CH_2), 4.68 (dd, J = 12.1, 3.9 Hz, 1 H, H-5''), 4.78 (q, J = 3.8 Hz, 1 H, H-4'), 4.87 (dd, J = 12.1, 3.2 Hz, 1 H, H-5'), 6.14–6.17 (m, 1 H, H-3'), 6.25 (t, J = 5.8 Hz, 1 H, H-2'), 6.59 (d, J = 3.8 Hz, 1 H, H-1'), 6.75 (d, J = 5.9 Hz, 1 H, H-5), 7.26–7.42 (m, 5 H, H-6 and H-OBz), 7.46–7.62 (m, 5 H, H-OBz), 7.93 (d, J = 7.4 Hz, 2 H, H-OBz), 8.00 (d, J = 7.3 Hz, 2 H, H-OBz), 8.13 (d, J = 7.4 Hz, 2 H, H-OBz), 8.79 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{34}H_{30}N_3O_7$ ([M + H]$^+$): 592.2078, found: 592.2067.

4-Ethyl-5-bromo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (68). 66 (430 mg, 0.73 mmol) was dissolved in anhydrous DMF (5 mL), and NBS (168 mg, 0.94 mmol, 1.3 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. EA (10 mL) and water (10 mL) were added. The layers were separated and the water layer extracted once more with EA. The organic layers were combined and washed with brine (10 mL), dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a colorless foam (356 mg, 0.53 mmol) in 73% yield. This obtained product (350 mg, 0.52 mmol) was charged in a 50 mL round bottom flask, and NH_3 in MeOH (7 N, 5 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated till dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **68** (165 mg, 0.46 mmol) as a white solid in 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.31 (t, J = 7.6 Hz, 3 H, CH_3), 3.23 (qd, J = 7.5, 1.1 Hz, 2 H, CH_2), 3.55 (ddd, J = 11.8, 5.6, 4.0 Hz, 1 H, H-5''), 3.64 (ddd, J = 12.0, 5.2, 4.2 Hz, 1 H, H-5'), 3.92 (q, J = 3.8 Hz, 1 H, H-4'), 4.10 (td, J = 4.8, 3.3 Hz, 1 H, H-3'), 4.39 (q, J = 5.8 Hz, 1 H, H-2'), 5.08 (t, J = 5.4 Hz, 1 H, OH-5'), 5.17 (d, J = 4.8 Hz, 1 H, OH-3'), 5.39 (d, J = 6.4 Hz, 1 H, OH-2'), 6.22 (d, J = 6.1 Hz, 1 H, H-1'), 8.06 (s, 1 H, H-6), 8.75 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 13.4 (CH_3), 26.7 (CH_2), 61.4 (C-5'), 70.5 (C-3'), 74.1 (C-2'), 85.4 (C-4'), 86.4 (C-1'), 87.6 (C-5), 114.6 (C-4a), 126.4 (C-6), 149.8 (C-7a), 151.6 (C-2), 164.0 (C-4). HRMS (ESI): calculated for $C_{13}H_{17}BrN_3O_4$ ([M + H]$^+$): 358.0397, found: 358.0390.

4-Ethyl-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (15). 15 was prepared according to General procedure A. **68** (100 mg, 0.28 mmol) gave rise to **15** (53 mg, 0.14 mmol) as a white solid in 49% yield. Melting point: 100 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.07 (t, J = 7.5 Hz, 3 H, CH_3), 2.77 (q, J = 7.5 Hz, 2 H, CH_2), 3.48–3.58 (m, 1 H, H-5''), 3.59–3.70 (m, 1 H, H-5'), 3.93 (q, J = 3.8 Hz, 1 H, H-4'), 4.07–4.20 (m, 1 H, H-3'), 4.47 (q, J = 6.1 Hz, 1 H, H-2'), 5.04 (t, J = 5.4 Hz, 1 H, OH-5'), 5.17 (d, J = 4.8 Hz, 1 H, OH-3'), 5.37 (d, J = 6.4 Hz, 1 H, OH-2'), 6.27 (d, J = 6.3 Hz, 1 H, H-1'), 7.41–7.60 (m, 4 H, H–Ph), 7.86 (s, 1 H, H-6), 8.75 (s, 1 H, H-2).). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 12.3 (CH_3), 28.0 (CH_2), 61.5 (C-5'), 70.5 (C-3'), 74.0 (C-2'), 85.2 (C-4'), 86.6 (C-1'), 115.0 (C-4a), 115.5 (C-5), 125.2 (C-6), 128.3 (2 C, C–Ph), 131.4 (2 C, C–Ph), 132.1 (C-4_{Ph}), 133.5 (C-1_{Ph}), 150.8 (C-7a), 151.0 (C-2), 163.8 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_4$ ([M + H]$^+$): 390.1215, found: 390.1217.

4-Phenyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine (69).[50] 4-Chloro-7*H*-pyrrolo [2,3-*d*]pyrimidine (300 mg, 1.95 mmol), phenylboronic acid (480 mg, 3.91 mmol, 2 eq.), K_2CO_3 (808 mg, 5.85 mmol, 3 eq.) and Pd(PPh_3)$_4$ (225 mg, 0.20 mmol, 0.1 eq.) were charged to a 25 mL round-bottom flask, equipped with a stir bar. Next, the flask was purged with argon thrice after removal of the air *in vacuo*. Then, toluene (8 ml, 4 mL/mmol SM) was added into the mixture via syringe. The mixture was stirred at ambient temperature for 5 min, and then heated at 100 °C. After the consumption of SM was observed by analytical LC/MS (1 h), the reaction was cooled to ambient temperature. Then, 0.5 M aq. HCl was added to neutralize the mixture. Next, the solution was evaporated *in vacuo*, and the residue was evaporated till dryness and purified by column chromatography (10→50% EA/PET) to give **69** (360 mg, 1.84 mmol) as an orange solid in 95% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.89 (dd, J = 3.5, 1.8 Hz, 1 H, H-5), 7.54–7.61 (m, 3 H, H–Ph), 7.66 (dd, J = 3.5, 2.5 Hz, 1 H, H-6), 8.16–8.19 (m, 2 H, H–Ph), 8.84 (s, 1 H, H-2), 12.26 (br. s., 1 H, NH). HRMS (ESI): calculated for $C_{12}H_{10}N_3$ ([M + H]$^+$): 196.0869, found: 196.0874.

4-Phenyl-5-iodo-7*H*-pyrrolo[2,3-*d*]-pyrimidine (70). 69 (380 mg, 1.95 mmol) was dissolved in anhydrous DMF (5 mL), and NIS (461 mg, 2.05 mmol, 1.05 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. Then, water (cooled in the ice-water bath, 15 mL) was added and the precipitate generated and was filtered. The solids were washed with ice-cold water. The solid was collected and dried under high vacuum to give **70** (530 mg, 1.65 mmol) as a brown solid in 85% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.51–7.55 (m, 3 H, H–Ph), 7.65–7.69 (m, 2 H, H–Ph), 7.86 (s, 1 H, H-6), 8.82 (s, 1 H, H-2), 12.69 (br. s., 1 H, NH). HRMS (ESI): calculated for $C_{12}H_9IN_3$ ([M + H]$^+$): 321.9836, found: 321.9841.

4-Phenyl-5-iodo-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (71). 70 (200 mg, 0.62 mmol) was placed in a 25 mL two-neck round bottom flask, after which the flask was purged with N_2 gas. Anhydrous MeCN (5 mL, 7.5 mL/mmol of SM) was added. BSA (183 μL, 0.75 mmol, 1.2 eq.) was added to the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (378 mg, 0.75 mmol, 1.2 eq.) was added, and immediately followed by TMSOTf (147 μL, 0.81 mmol, 1.3 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When full conversion of **70** was observed via HRMS, the mixture was cooled to room temperature. Then, EA (10 mL) and aq. sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a yellow foam (140 mg, 0.18 mmol) in 29% yield. This obtained product (330 mg, 0.43 mmol) was charged in a 50 mL round bottom flask, and NH_3 in MeOH (7 N, 5 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated until dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **71**

(150 mg, 0.33 mmol) as a white solid in 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.54–3.60 (m, 1 H, H-5″), 3.63–3.68 (m, 1 H, H-5′), 3.95 (q, J = 3.5 Hz, 1 H, H-4′), 4.12 (q, J = 4.2 Hz, 1 H, H-3′), 4.45 (q, J = 6.0 Hz, 1 H, H-2′), 5.11 (t, J = 5.3 Hz, 1 H, OH-5′), 5.20 (d, J = 4.6 Hz, 1 H, OH-3′), 5.42 (d, J = 6.4 Hz, 1 H, OH-2′), 6.28 (d, J = 6.3 Hz, 1 H, H-1′), 7.52–7.56 (m, 3 H, H–Ph), 7.66–7.68 (m, 2 H, H–Ph), 8.17 (s, 1 H, H-6), 8.89 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 54.5 (C-5), 61.4 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.4 (C-4′), 86.6 (C-1′), 116.5 (C-4a), 127.6 (2 C, C–Ph), 129.6 (C–Ph), 130.8 (2 C, C–Ph), 133.5 (C-6), 135.6 (C–Ph), 150.9 (C-2), 151.3 (C-7a), 159.7 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}IN_3O_4$ ([M + H]$^+$): 454.0258, found: 454.0255.

4-Phenyl-5-(4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (16). **16** was prepared according to General procedure A. **71** (140 mg, 0.31 mmol) gave rise to a slightly impure **16** (65 mg, 0.15 mmol) as a white solid. **16** was purified by preparative RP-HPLC gradient: 0.2% formic acid in water:MeCN at a flow rate of 20 mL/min; The initial gradient composition (95% A/ 05% B) was held for 2.0 min, increased to 80% B in 12 min, then increased to 98% B in 3 min. After RP-HPLC purification, **16** was obtained as a white solid (31 mg, 0.071 mmol) in 23% yield. Melting point: 234 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.51–3.62 (m, 1 H, H-5″), 3.63–3.74 (m, 1 H, H-5′), 3.97 (q, J = 3.5 Hz, 1 H, H-4′), 4.09–4.24 (m, 1 H, H-3′), 4.54 (q, J = 6.1 Hz, 1 H, H-2′), 5.07 (t, J = 5.6 Hz, 1 H, OH-5′), 5.21 (d, J = 5.0 Hz, 1 H, OH-3′), 5.43 (d, J = 6.4 Hz, 1 H, OH-2′), 6.38 (d, J = 6.2 Hz, 1 H, H-1′), 6.98 (d, J = 8.5 Hz, 2 H, H–Ph), 7.08–7.27 (m, 4 H, H–Ph), 7.28–7.46 (m, 3 H, H–Ph), 8.09 (s, 1 H, H-6), 8.95 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.3 (C-4′), 86.6 (C-1′), 113.3 (C-4a), 115.5 (C-5), 126.4 (C-6), 127.5 (4 C, C-3$_{Ph}$ and C-5$_{Ph}$ and C-3′$_{Ph}$ and C-5′$_{Ph}$), 129.2 (C-4′$_{Ph}$), 129.5 (2 C, C-2′$_{Ph}$ and C-6′$_{Ph}$), 130.5 (2 C, C-2$_{Ph}$ and C-6$_{Ph}$), 131.0 (C-4$_{Ph}$), 132.6 (C-1$_{Ph}$), 137.3 (C-1′$_{Ph}$), 151.0 (C-2), 152.2 (C-7a), 158.7 (C-4). HRMS (ESI): calculated for $C_{23}H_{21}ClN_3O_4$ ([M + H]$^+$): 438.1215, found: 438.1213.

3-(4-Chloro-phenyl)-4-methyl-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (20). 20 was prepared according to General procedure A. **72**[27] (85 mg, 0.22 mmol) gave rise to **20** (50 mg, 0.13 mmol) as a white solid in 61% yield. Melting point: 96 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.29 (s, 3 H, CH_3), 3.51–3.56 (m, 1 H, H-5″), 3.61–3.66 (m, 1 H, H-5′), 3.91 (q, J = 3.4 Hz, 1 H, H-4′), 4.10–4.14 (m, 1 H, H-3′), 4.48 (q, J = 6.0 Hz, 1 H, H-2′), 5.11 (d, J = 4.8 Hz, 1 H, OH-5′), 5.14 (t, J = 5.6 Hz, 1 H, OH-3′), 5.30 (d, J = 6.4 Hz, 1 H, OH-2′), 6.29 (d, J = 6.1 Hz, 1 H, H-1′), 6.97 (d, J = 4.9 Hz, 1 H, H-5), 7.48 (s, 4 H, H–Ph), 7.76 (s, 1 H, H-2), 8.16 (d, J = 4.8 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.8 (CH_3), 61.7 (C-5′), 70.6 (C-3′), 73.7 (C-2′), 84.9 (C-4′), 87.0 (C-1′), 115.5 (C-3), 118.3 (C-3a), 118.6 (C-5), 124.9 (C-2), 128.0 (2 C, C–Ph), 131.5 (C-4$_{Ph}$), 131.7 (2 C, C–Ph), 134.5 (C-1$_{Ph}$), 140.1 (C-4), 142.7 (C-6), 147.6 (C-7a). HRMS (ESI): calculated for $C_{19}H_{20}ClN_2O_4$ ([M + H]$^+$): 375.1106, found: 375.1110.

3-Bromo-5-fluoro-1*H*-pyrrolo[2,3-*b*]pyridine (73).[41–42] 5-Fluoro-1*H*-pyrrolo[2,3-*b*]pyridine (500 mg, 3.67 mmol) was dissolved in anhydrous DMF (11 mL, 3 mL/mmol of SM), and NBS (686 mg, 3.86 mmol, 1.05 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. Then, water (cooled in an ice-water bath, 30 mL) was added and the precipitate generated and was filtered. The solids were washed with ice-cold water. The solid was collected and dried under high vacuum to give **73** (753 mg, 3.50 mmol) as a white solid in 95% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.71 (dd, J = 8.9, 2.6 Hz, 1 H, H-2), 7.82 (d, J = 2.8 Hz, 1 H, H-6), 8.28 (t, J = 2.2 Hz, 1 H, H-4), 12.24 (br. s., 1 H, NH). ^{19}F NMR (376 MHz, DMSO-d_6) δ: −138.03. HRMS (ESI): calculated for $C_7H_5BrFN_2$ ([M + H]$^+$): 214.9615, found: 214.9623.

3-Bromo-5-fluoro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (77). 73 (640 mg, 2.98 mmol) was placed in a 100 mL two-neck round bottom flask, after which the flask was purged with N_2 gas. Anhydrous MeCN (22 mL, 7.5 mL/mmol of SM) was added. BSA (801 μL, 3.28 mmol, 1.1 eq.) was added into the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.80 g, 3.57 mmol, 1.2 eq.) was added, and immediately followed by TMSOTf (646 μL, 3.57 mmol, 1.2 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When the full conversion of **73** was observed via HRMS, the mixture was cooled to ambient temperature. Then, EA (10 mL) and aq. sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a colorless foam (1.17 g, 1.78 mmol). This obtained product (1.15 g, 1.74 mmol) was charged in a 50 mL round bottom flask, and NH_3 in MeOH (7 N, 10 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated till dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **77** (285 mg, 0.82 mmol) as a white solid in 24% yield for two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.45–3.58 (m, 1 H, H-5″), 3.59–3.70 (m, 1 H, H-5′), 3.90 (q, J = 3.8 Hz, 1 H, H-4′), 4.04–4.16 (m, 1 H, H-3′), 4.40 (q, J = 6.1 Hz, 1 H, H-2′), 5.04 (t, J = 5.4 Hz, 1 H, OH-5′), 5.15 (d, J = 4.9 Hz, 1 H, OH-3′), 5.36 (d, J = 6.3 Hz, 1 H, OH-2′), 6.22 (d, J = 6.1 Hz, 1 H, H-1′), 7.82 (dd, J = 8.8, 2.7 Hz, 1 H, H-4), 8.14 (s, 1 H, H-2), 8.32–8.41 (m, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.4 (C-5′), 70.4 (C-3′), 74.0 (C-2′), 85.1 (C-4′), 86.8 (C-1′), 88.2 (C-3), 113.1 (d, J = 22.5 Hz, 1 C, C-4), 119.8 (d, J = 7.3 Hz, 1 C, C-3a), 128.0 (C-2), 132.6 (d, J = 29.1 Hz, 1 C, C-6), 143.3 (C-7a), 155.8 (d, J = 242.0 Hz, 1 C, C-5). ^{19}F NMR (376 MHz, DMSO-d_6)δ: −136.76. HRMS (ESI): calculated for $C_{12}H_{13}BrFN_2O_4$ ([M + H]$^+$): 347.0037, found: 347.0035.

3-(4-Chloro-phenyl)-5-fluoro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (21). 21 was prepared according to General procedure A. **77** (100 mg, 0.29 mmol) gave rise to **21** (14 mg, 0.037 mmol) as a white solid in 13% yield. Melting point: 131 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.44–3.62 (m, 1 H, H-5″), 3.62–3.75 (m, 1 H, H-5′), 3.92 (q, J = 3.9 Hz, 1 H, H-4′), 4.12–4.19 (m, 1 H, H-3′), 4.49 (q, J = 6.0 Hz, 1 H, H-2′), 5.05 (t, J = 5.6 Hz, 1 H, OH-5′), 5.15 (d, J = 4.9 Hz, 1 H, OH-3′), 5.37 (d, J = 6.3 Hz, 1 H, OH-2′), 6.27 (d, J = 6.0 Hz, 1 H, H-1′), 7.44–7.57 (m, 2 H, H–Ph), 7.70–7.83 (m, 2 H, H–Ph), 8.22 (dd, J = 9.7, 2.7 Hz, 1 H, H-4), 8.32 (s, 1 H, H-2), 8.35 (dd, J = 2.4, 1.2 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.5 (C-5′), 70.4 (C-3′), 73.8 (C-2′), 84.9 (C-4′), 86.9 (C-1′), 113.7 (d, J = 4.4 Hz, 1 C, C-3), 114.1 (d, J = 21.1 Hz, 1 C, C-4), 118.1 (d, J = 6.5 Hz, 1 C, C-3a), 126.5 (C-2), 128.0 (2 C, C-2$_{Ph}$ and C-6$_{Ph}$), 128.9 (2 C, C-3$_{Ph}$ and C-5$_{Ph}$), 130.7 (C-4$_{Ph}$), 131.3 (d, J = 29.1 Hz, 1 C, C-6), 132.7 (C-1$_{Ph}$), 144.9 (C-7a), 155.9 (d, J = 241.2 Hz, 1 C, C-5). ^{19}F NMR (376 MHz, DMSO-d_6) δ: −137.45. HRMS (ESI): calculated for $C_{18}H_{17}BrFN_2O_4$ ([M + H]$^+$): 379.0855, found: 379.0848.

3-Bromo-5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (74).[41–42] 5-Chloro-1*H*-pyrrolo[2,3-*b*]pyridine (500 mg, 3.28 mmol) was dissolved in anhydrous DMF (10 mL, 3 mL/mmol of SM), and NBS (642 mg, 3.61 mmol, 1.1 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. The water (cooled in an ice-water bath, 30 mL) was added and the precipitate generated and was filtered. The solids were washed with ice-cold water. The solid was collected and dried under high vacuum to give **74** (700 mg, 3.02 mmol) as a white solid in 92% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 (d, J = 2.3 Hz, 1 H, H-2), 7.92 (d, J = 2.1 Hz, 1 H, H-6), 8.29 (dd, J = 1.9, 1.1 Hz, 1 H, H-4), 12.33 (br. s., 1 H, NH). HRMS (ESI): calculated for $C_7H_5BrClN_2$ ([M + H]$^+$): 230.9319, found: 230.9313.

3-Bromo-5-chloro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (78). 74 (640 mg, 2.98 mmol) was placed in a 100 mL two-neck round bottom flask, after which the flask was purged with N_2 gas.

Anhydrous MeCN (22 mL, 7.5 mL/mmol of SM) was added. BSA (801 μL, 3.28 mmol, 1.1 eq.) was added into the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.80 g, 3.57 mmol, 1.2 eq.) was added, and immediately followed by TMSOTf (646 μL, 3.57 mmol, 1.2 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When full conversion of **74** was observed via HRMS, the mixture was cooled to room temperature. Then, EA (10 mL) and aq. sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a colorless foam (850 mg, 1.26 mmol). This obtained product (850 mg, 1.26 mmol) was charged in a 50 mL round bottom flask, and NH_3 in MeOH (7 N, 10 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated till dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **78** (410 mg, 1.13 mmol) as a white solid in 32% yield for two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.47–3.58 (m, 1 H, H-5''), 3.59–3.71 (m, 1 H, H-5'), 3.91 (q, J = 3.5 Hz, 1 H, H-4'), 4.04–4.18 (m, 1 H, H-3'), 4.39 (q, J = 5.9 Hz, 1 H, H-2'), 5.06 (t, J = 5.4 Hz, 1 H, OH-5'), 5.17 (d, J = 4.9 Hz, 1 H, OH-3'), 5.39 (d, J = 6.3 Hz, 1 H, OH-2'), 6.23 (d, J = 6.1 Hz, 1 H, H-1'), 8.01 (d, J = 2.3 Hz, 1 H, H-4), 8.15 (s, 1 H, H-2), 8.37 (d, J = 2.3 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.4 (C-5'), 70.4 (C-3'), 74.1 (C-2'), 85.1 (C-4'), 86.6 (C-1'), 88.1 (C-3a), 120.5 (C-3), 124.2 (C-5), 126.4 (C-4), 127.6 (C-2), 142.3 (C-6), 145.0 (C-7a). HRMS (ESI): calculated for $C_{12}H_{13}BrClN_2O_4$ ($[M+H]^+$): 362.9742, found: 362.9738.

3-(4-Chloro-phenyl)-5-chloro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (22). 78 (100 mg, 0.28 mmol), phenylboronic acid (52 mg, 0.33 mmol, 1.2 eq.), K_2CO_3 (116 mg, 0.84 mmol, 3 eq.) and $Pd(PPh_3)_4$ (16 mg, 0.014 mmol, 0.05 eq.) were charged to a 25 mL round-bottom flask, equipped with a stir bar. Next, the air was removed and backfilled with argon. This was repeated thrice. Then, a mixture solution of 1,4-dioxane and H_2O (1:1 ratio, 2 mL) was added into the reaction mixture via syringes. The mixture was then stirred at ambient temperature (5 min), and then was refluxed at 100 °C. The reaction was monitored by HRMS for consumption of SM (1 h), after which it was allowed to cool to ambient temperature. Then, 0.5 M aq. HCl was added to neutralize the mixture. Then, the volatiles were removed *in vacuo*, and the residue purified by column chromatography (0→10% MeOH/DCM) to give **22** (27 mg, 0.068 mmol) as a light yellow solid in 24% yield. Melting point: 102 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.48–3.61 (m, 1 H, H-5''), 3.64–3.70 (m, 1 H, H-5'), 3.93 (q, J = 3.9 Hz, 1 H, H-4'), 4.09–4.22 (m, 1 H, H-3'), 4.48 (q, J = 6.0 Hz, 1 H, H-2'), 5.05 (t, J = 5.6 Hz, 1 H, OH-5'), 5.16 (d, J = 4.9 Hz, 1 H, OH-3'), 5.38 (d, J = 6.4 Hz, 1 H, OH-2'), 6.28 (d, J = 6.0 Hz, 1 H, H-1'), 7.45–7.58 (m, 2 H, H–Ph), 7.71–7.82 (m, 2 H, H–Ph), 8.31 (s, 1 H, H-2), 8.35 (d, J = 2.3 Hz, 1 H, H-6), 8.40 (d, J = 2.3 Hz, 1 H, H-4). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.5 (C-5'), 70.4 (C-3'), 73.9 (C-2'), 85.0 (C-4'), 86.7 (C-1'), 113.5 (C-3), 118.8 (C-3a), 124.0 (C-5), 126.1 (C-2), 127.3 (C-4), 128.1 (2 C, C-2_{Ph} and C-6_{Ph}), 128.9 (2 C, C-3_{Ph} and C-5_{Ph}), 130.8 (C-4_{Ph}), 132.5 (C-1_{Ph}), 141.3 (C-6), 146.5 (C-7a). HRMS (ESI): calculated for $C_{18}H_{17}Cl_2N_2O_4$ ($[M+H]^+$): 395.0560, found: 395.0572.

3-Bromo-5-nitro-1*H*-pyrrolo[2,3-*b*]pyridine (75). 5-Nitro-1*H*-pyrrolo [2,3-*b*]pyridine (500 mg, 3.06 mmol) was dissolved in dry DMF (15 mL, 5 mL/mmol of SM), and NBS (600 mg, 3.37 mmol, 1.1 eq.) was added. Then, the reaction mixture was stirred at ambient temperature overnight. Then, water (cooled in an ice-water bath, 30 mL) was added and the precipitate generated and was filtered. The solids were washed with ice-cold water. The solid was collected and dried under high vacuum to give **75** (625 mg, 3.02 mmol) as a yellow solid in 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.06 (s, 1 H, H-2), 8.59 (s, 1 H, H-6), 9.13–9.14 (m, 1 H, H-4), 12.92 (br. s., 1 H, NH). HRMS (ESI): calculated for $C_7H_5BrN_3O_2$ ($[M+H]^+$): 241.9560, found: 241.9564.

3-Bromo-5-nitro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (79). 75 (620 mg, 2.56 mmol) was placed in a 100 mL two-neck round bottom flask, after which the flask was purged with N_2 gas. Anhydrous MeCN (19 mL, 7.5 mL/mmol of SM) was added. BSA (0.7 mL, 2.82 mmol, 1.1 eq.) was added into the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.55 g, 3.07 mmol, 1.2 eq.) was added, and immediately followed by TMSOTf (0.6 mL, 3.07 mmol, 1.2 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When the full conversion of **75** was observed via HRMS, the mixture was cooled to ambient temperature. Then, EA (10 mL) and aq. sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a colorless foam (1.70 g, 2.48 mmol). This obtained product (200 mg, 0.29 mmol) was charged in a 25 mL round bottom flask, and NH_3 in MeOH (7 N, 5 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated until dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **79** (88 mg, 0.24 mmol) as a light yellow solid in 79% yield for two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.58 (ddd, J = 12.0, 5.4, 4.0 Hz, 1 H, H-5''), 3.66 (ddd, J = 12.0, 5.2, 4.2 Hz, 1 H, H-5'), 3.95 (q, J = 3.7 Hz, 1 H, H-4'), 4.08–4.19 (m, 1 H, H-3'), 4.40 (q, J = 5.8 Hz, 1 H, H-2'), 5.10 (t, J = 5.4 Hz, 1 H, OH-5'), 5.22 (d, J = 5.0 Hz, 1 H, OH-3'), 5.46 (d, J = 6.3 Hz, 1 H, OH-2'), 6.33 (d, J = 6.0 Hz, 1 H, H-1'), 8.36 (s, 1 H, H-2), 8.65 (d, J = 2.4 Hz, 1 H, H-4), 9.21 (d, J = 2.4 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.3 (C-5'), 70.4 (C-3'), 74.4 (C-2'), 85.5 (C-4'), 86.9 (C-1'), 90.9 (C-3a), 118.8 (C-3), 123.6 (C-4), 129.4 (C-2), 139.8 (C-5), 140.1 (C-6), 148.3 (C-7a). HRMS (ESI): calculated for $C_{12}H_{13}BrN_3O_6$ ($[M+H]^+$): 373.9982, found: 373.9974.

3-(4-Chloro-phenyl)-5-nitro-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (22). 22 was prepared according to General procedure A. **79** (150 mg, 0.40 mmol) gave rise to **22** (24 mg, 0.059 mmol) as a white solid in 15% yield. Melting point: 112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.52–3.64 (m, 1 H, H-5''), 3.65–3.77 (m, 1 H, H-5'), 3.97 (q, J = 3.8 Hz, 1 H, H-4'), 4.11–4.27 (m, 1 H, H-3'), 4.49 (q, J = 6.0 Hz, 1 H, H-2'), 5.09 (t, J = 5.5 Hz, 1 H, OH-5'), 5.22 (d, J = 5.0 Hz, 1 H, OH-3'), 5.46 (d, J = 6.4 Hz, 1 H, OH-2'), 6.39 (d, J = 6.0 Hz, 1 H, H-1'), 7.51–7.65 (m, 2 H, H–Ph), 7.75–7.91 (m, 2 H, H–Ph), 8.48 (s, 1 H, H-2), 9.01 (d, J = 2.4 Hz, 1 H, H-4), 9.22 (d, J = 2.4 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.3 (C-5'), 70.4 (C-3'), 74.2 (C-2'), 85.4 (C-4'), 86.9 (C-1'), 116.1 (C-3), 117.1 (C-3a), 124.5 (C-4), 127.6 (C-2), 128.5 (2 C, C-2_{Ph} and C-6_{Ph}), 129.2 (2 C, C-3_{Ph} and C-5_{Ph}), 131.6 (C–Ph), 131.7 (C–Ph), 139.4 (C-5), 139.7 (C-6), 149.7 (C-7a). HRMS (ESI): calculated for $C_{18}H_{17}ClN_3O_6$ ($[M+H]^+$): 406.0800, found: 406.0795.

3-(4-Chloro-phenyl)-5-amino-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (24). 22 (30 mg, 0.074 mmol) was dissolved in MeOH (5 mL). The flask was purged with N_2, and a catalytic amount of Pt/C was added. Then, the N_2 gas was exchanged with H_2 (balloon; bubbling). The reaction mixture was stirred until the HRMS showed full consumption of the SM (2 h). The solution was filtered over Celite® and washed with MeOH. The filtrate was evaporated to dryness and purified by column chromatography (0→10% MeOH/DCM) to afford **24** (19 mg, 0.051 mmol) as a white solid in 68% yield. Melting point: 104 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.47–3.58 (m, 1 H, H-5''), 3.62 −3.67 (m, 1 H, H-5'), 3.89 (q, J = 3.6 Hz, 1 H, H-4'), 4.09–4.16 (m, 1 H, H-3'), 4.51 (q, J = 6.2 Hz, 1 H, H-2'), 4.95 (br. s., 2 H, NH_2), 5.08 (d, J = 4.8 Hz, 1 H, OH-5'), 5.21 (t, J = 5.8 Hz, 1 H, OH-3'), 5.28 (d, J = 6.4 Hz, 1 H, OH-2'), 6.10 (d, J = 6.3 Hz, 1 H, H-1'),

7.43–7.53 (m, 3 H, H–Ph and H-4), 7.61–7.70 (m, 2 H, H–Ph), 7.80 (d, J = 2.4 Hz, 1 H, H-6), 7.97 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 61.8 (C-5′), 70.6 (C-3′), 73.3 (C-2′), 84.8 (C-4′), 87.3 (C-1′), 110.9 (C-4), 111.9 (C-3), 118.6 (C-3a), 124.5 (C-2), 127.5 (2 C, C-2_{Ph} and C-6_{Ph}), 128.8 (2 C, C-3_{Ph} and C-5_{Ph}), 129.9 (C-4_{Ph}), 132.4 (C-6), 134.1 (C-1_{Ph}), 140.3 (C-5), 141.9 (C-7a). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4$([M + H]$^+$): 376.1059, found: 376.1065.

3-Bromo-5-methyl-1*H*-pyrrolo[2,3-*b*]pyridine (76). 5-Methyl-1*H*-pyrrolo[2,3-*b*]pyridine (500 mg, 3.06 mmol) was dissolved in dry DMF (11 mL, 3 mL/mmol of SM), and NBS (741 mg, 4.16 mmol, 1.1 eq.) was added. Next, the reaction mixture was stirred at ambient temperature overnight. Then, water (cooled in an ice-water bath, 30 mL) was added and the precipitate generated and was filtered. The solids were washed with ice-cold water. The solid was collected and dried under high vacuum to give **76** (560 mg) as a yellow solid. Since part of **76** was dissolved in the filtrate, the water was collected and extracted twice with EA (2×20 mL). Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a yellow solid (90 mg). Total amount of **76** was 650 mg (3.07 mmol) in 81% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3 H, CH_3), 7.63–7.64 (m, 2 H, H-6 and H-2), 8.13 (d, J = 1.9 Hz, 1 H, H-4), 11.91 (br. s., 1 H, NH). HRMS (ESI): calculated for $C_8H_8BrN_2$ ([M + H]$^+$): 210.9865, found: 210.9870.

3-Bromo-5-methyl-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (80). 76 (650 mg, 3.08 mmol) was placed in a 100 mL two-neck round bottom flask, after which the flask was purged with N_2 gas. Anhydrous MeCN (23 mL, 7.5 mL/mmol of SM) was added. BSA (828 μL, 3.39 mmol, 1.1 eq.) was added into the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.86 g, 3.70 mmol, 1.2 eq.) was added, and immediately followed by TMSOTf (670 μL, 3.70 mmol, 1.2 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When the full conversion of **76** was observed via HRMS, the mixture was cooled to ambient temperature. Then, EA (20 mL) and aq. sat. $NaHCO_3$ (10 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a yellow foam (1.70 g, 2.59 mmol). This obtained product (1.70 g, 2.59 mmol) was charged in a 25 mL round bottom flask, and NH_3 in MeOH (7 N, 10 mL) was added. The mixture was stirred at ambient temperature overnight. Then, the reaction solution was evaporated till dryness, purified by column chromatography (0→10% MeOH/DCM) to afford **80** (650 mg, 1.89 mmol) as a white solid in 61% yield for two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.42 (s, 3 H, CH_3), 3.52–3.56 (m, 1 H, H-5″), 3.60–3.65 (m, 1 H, H-5′), 3.89 (q, J = 3.8 Hz, 1 H, H-4′), 4.10 (q, J = 4.2 Hz, 1 H, H-3′), 4.42 (q, J = 6.1 Hz, 1 H, H-2′), 5.09–5.12 (m, 2 H, OH-5′ and OH-3′), 5.31 (d, J = 6.4 Hz, 1 H, OH-2′), 6.20 (d, J = 6.3 Hz, 1 H, H-1′), 7.69 (d, J = 1.2 Hz,1 H, H-4), 7.96 (s, 1 H, H-2), 8.19 (d, J = 1.8 Hz, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 17.9 (CH_3), 61.6 (C-5′), 70.5 (C-3′), 73.8 (C-2′), 85.0 (C-4′), 86.7 (C-1′), 88.0 (C-3a), 119.5 (C-3), 125.8 (C-2), 126.3 (C-5), 126.8 (C-4), 144.7 (C-6), 145.2 (C-7a). HRMS (ESI): calculated for $C_{13}H_{16}BrN_2O_4$([M + H]$^+$): 343.0288, found: 343.0292.

3-(4-Chloro-phenyl)-5-methyl-N1-(β-D–ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (25). 25 was prepared according to General procedure A. **79** (150 mg, 0.44 mmol) gave rise to **25** (23 mg, 0.061 mmol) as a white solid in 14% yield. Melting point: 112 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3 H, CH_3), 3.53–3.58 (m, 1 H, H-5″), 3.64–3.69 (m, 1 H, H-5′), 3.92 (d, J = 3.4 Hz, 1 H, H-4′), 4.15 (q, J = 4.0 Hz, 1 H, H-3′), 4.52 (q, J = 5.8 Hz, 1 H, H-2′), 5.12–5.17 (m, 2 H, OH-5′ and OH-3′), 5.32 (d, J = 6.3 Hz, 1 H, OH-2′), 6.25 (d, J = 6.0 Hz, 1 H, H-1′), 7.50 (d, J = 8.5 Hz, 2 H, H–Ph), 7.75 (d, J = 8.5 Hz, 2 H, H–Ph), 8.14 (s, 2 H, H-4 and H-2), 8.17 (s, 1 H, H-6). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18.1 (CH_3), 61.7 (C-5′), 70.6 (C-3′), 73.6 (C-2′), 84.9 (C-4′), 87.0 (C-1′), 113.0 (C-3), 118.1 (C-3a), 124.7 (C-2), 125.9 (C-5), 127.9 (3 C, C-2_{Ph} and C-6_{Ph} and C-4), 128.8 (2 C, C-3_{Ph} and C-5_{Ph}), 130.3 (C-4_{Ph}), 133.4 (C-1_{Ph}), 143.7 (C-6), 146.8 (C-7a). HRMS (ESI): calculated for $C_{19}H_{20}ClN_2O_4$ ([M + H]$^+$): 375.1106, found: 375.1092.

4-Methyl-5-phenyl-N7-(β-D–ribofuranosyl)-7H-pyrrolo[2,3-*d*]-pyrimidine (26).[23] **26** was prepared according to General procedure A. **67** (156 mg, 0.40 mmol) gave rise to **26** (105 mg, 0.31 mmol) as a white solid in 77% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.46 (s, 3H, CH_3), 3.48–3.59 (m, 1 H, H-5″), 3.60–3.72 (m, 1 H, H-5′), 3.93 (q, J = 3.5 Hz, 1 H, H-4′), 4.13 (dd, J = 8.2, 4.7 Hz, 1 H, H-3′), 4.47 (q, J = 6.0 Hz, 1 H, H-2′), 5.05 (t, J = 5.4 Hz, 1 H, OH-5′), 5.16 (d, J = 4.7 Hz, 1 H, OH-3′), 5.37 (d, J = 6.4 Hz, 1 H, OH-2′), 6.28 (d, J = 5.9 Hz, 1 H, H-1′), 7.34–7.62 (m, 5 H, H–Ph), 7.85 (s, 1 H, H-6), 8.69 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.9 (CH_3), 61.4 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.8 (C-4a), 117.0 (C-5), 124.7 (C-6), 127.1 (C–Ph), 128.2 (2 C, C–Ph), 129.8 (2 C, C–Ph), 134.2 (C–Ph), 150.6 (C-7a), 150.8 (C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}N_3O_4$ ([M + H]$^+$): 342.1448, found: 342.1443.

4-Methyl-5-(4-fluoro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (27). 27 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **27** (32 mg, 0.089 mmol) as a white solid in 34% yield. Melting point: 203 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.44 (s, 3 H, CH_3), 3.55 (ddd, J = 12.0, 5.7, 4.0 Hz, 1 H, H-5″), 3.64 (ddd, J = 12.0, 5.3, 4.1 Hz, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.06–4.19 (m, 1 H, H-3′), 4.47 (dd, J = 11.4, 6.4 Hz, 1 H, H-2′), 5.04 (t, J = 5.4 Hz, 1 H, OH-5′), 5.17 (d, J = 5.0 Hz, 1 H, OH-3′), 5.37 (d, J = 6.4 Hz, 1 H, OH-2′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.22–7.37 (m, 2 H, H–Ph), 7.47–7.61 (m, 2 H, H–Ph), 7.85 (s, 1 H, H-6), 8.69 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.8 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.0 (C-5), 115.2 (2 C, C-3_{Ph} and C-5_{Ph}), 115.9 (C-4a), 124.8 (C-6), 130.6 (C-1_{Ph}), 131.8 (d, J = 8.1 Hz, 2 C, C-2_{Ph} and C-6_{Ph}), 150.6 (C-7a), 150.8 (C-2), 159.2 (C-4), 161.6 (d, J = 244.2 Hz, 1 C, C-4_{Ph}). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −115.56–−115.45 (m, 1 F). HRMS (ESI): calculated for $C_{18}H_{19}FN_3O_4$ ([M + H]$^+$): 360.1354, found: 360.1364.

4-Methyl-5-(4-trifluoromethyl-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (28). 28 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **28** (33 mg, 0.081 mmol) as a white solid in 31% yield. Melting point: 196 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.48 (s, 3 H, CH_3), 3.48–3.59 (m, 1 H, H-5″), 3.60–3.73 (m, 1 H, H-5′), 3.94 (q, J = 3.6 Hz, 1 H, H-4′), 4.14 (dd, J = 8.4, 5.0 Hz, 1 H, H-3′), 4.48 (q, J = 6.1 Hz, 1 H, H-2′), 5.05 (t, J = 5.4 Hz, 1 H, OH-5′), 5.18 (d, J = 4.7 Hz, 1 H, OH-3′), 5.40 (d, J = 6.2 Hz, 1 H, OH-2′), 6.29 (d, J = 6.2 Hz, 1 H, H-1′), 7.67–7.79 (m, 2 H, H–Ph), 7.79–7.90 (m, 2 H, H–Ph), 7.99 (s, 1 H, H-6), 8.73 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.2 (CH_3), 61.4 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 115.6 (C-5 and C-4a), 124.4 (q, J = 271.8 Hz, 1 H, CF_3), 125.1 (q, J = 4.6 Hz, 1 H, 2 C, C-3_{Ph} and C-5_{Ph}), 125.7 (C-6), 127.5 (q, J = 31.1 Hz, 1 C, C-4_{Ph}), 130.4 (2 C, C-2_{Ph} and C-6_{Ph}), 138.6 (C-1_{Ph}), 150.8 (C-2), 151.0 (C-7a), 159.3 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −60.83. HRMS (ESI): calculated for $C_{19}H_{19}F_3N_3O_4$ ([M + H]$^+$): 410.1322, found: 410.1323.

4-Methyl-5-(4-trifluoromethoxy-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (29). 29 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **29** (40 mg, 0.094 mmol) as a white solid in 36% yield. Melting point: 188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.47 (s, 3 H, CH_3), 3.49–3.59 (m, 1 H, H-5″), 3.60–3.74 (m, 1 H, H-5′), 3.93 (q, J = 3.4 Hz, 1 H, H-4′), 4.13 (dd, J = 8.3, 4.8 Hz, 1 H, H-3′), 4.47 (q, J = 6.1 Hz, 1 H, H-2′), 5.04 (t, J = 5.4 Hz, 1 H, OH-5′), 5.17 (d, J = 4.7 Hz, 1 H, OH-3′), 5.38 (d, J =

6.4 Hz, 1 H, OH-2′), 6.28 (d, J = 6.2 Hz, 1 H, H-1′), 7.46 (d, J = 8.5 Hz, 2 H, H–Ph), 7.65 (d, J = 8.5 Hz, 2 H, H–Ph), 7.91 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.9 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 115.5 (C-5), 115.7 (C-4a), 120.1 (q, J = 255.7 Hz, 1 C, OCF_3), 120.9 (2 C, C-3_{Ph} and C-5_{Ph}), 125.2 (C-6), 131.6 (2 C, C-2_{Ph} and C-6_{Ph}), 133.7 (C-1_{Ph}), 147.6 (d, J = 2.3 Hz, 1 C, C-4_{Ph}), 150.7 (C-2), 150.9 (C-7a), 159.2 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −56.77. HRMS (ESI): calculated for $C_{19}H_{19}F_3N_3O_5$ ([M + H]$^+$): 426.1271, found: 426.1244.

4-Methyl-5-(4-cyano-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (30). 30 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **30** (30 mg, 0.082 mmol) as a white solid in 31% yield. Melting point: 119–120 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3 H, CH_3), 3.47–3.61 (m, 1 H, H-5′′), 3.61–3.73 (m, 1 H, H-5′), 3.94 (q, J = 3.7 Hz, 1 H, H-4′), 4.08–4.19 (m, 1 H, H-3′), 4.47 (dd, J = 11.5, 6.2 Hz, 1 H, H-2′), 5.05 (t, J = 5.6 Hz, 1 H, OH-5′), 5.18 (d, J = 5.0 Hz, 1 H, OH-3′), 5.39 (d, J = 6.4 Hz, 1 H, OH-2′), 6.28 (d, J = 6.2 Hz, 1 H, H-1′), 7.74 (d, J = 8.2 Hz, 2 H, H–Ph), 7.93 (d, J = 8.2 Hz, 2 H, H–Ph), 8.01 (s, 1 H, H-6), 8.73 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.3 (CH_3), 61.4 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.5 (C-4′), 86.7 (C-1′), 109.6 (C-4_{Ph}), 115.4 (C-4a), 115.6 (C-5), 118.9 (CN), 126.0 (C-6), 130.5 (2 C, C-2_{Ph} and C-6_{Ph}), 132.2 (2 C, C-3_{Ph} and C-5_{Ph}), 139.4 (C-1_{Ph}), 150.9 (C-7a), 151.1 (C-2), 159.4 (C-4). HRMS (ESI): calculated for $C_{19}H_{19}N_4O_4$ ([M + H]$^+$): 367.1401, found: 367.1394.

4-Methyl-5-(4-nitro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (31). 31 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **31** (31 mg, 0.080 mmol) as a yellow solid in 31% yield. Melting point: 172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.52 (s, 3 H, CH_3), 3.50–3.60 (m, 1 H, H-5′′), 3.61–3.75 (m, 1 H, H-5′), 3.95 (q, J = 3.8 Hz, 1 H, H-4′), 4.08–4.22 (m, 1 H, H-3′), 4.48 (dd, J = 11.8, 6.2 Hz, 1 H, H-2′), 5.06 (t, J = 5.4 Hz, 1 H, OH-5′), 5.19 (d, J = 5.0 Hz, 1 H, OH-3′), 5.41 (d, J = 6.2 Hz, 1 H, OH-2′), 6.29 (d, J = 5.9 Hz, 1 H, H-1′), 7.75–7.89 (m, 2 H, H–Ph), 8.08 (s, 1 H, H-6), 8.25–8.39 (m, 2 H, H–Ph), 8.75 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.4 (CH_3), 61.4 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.3 (C-4′), 86.7 (C-1′), 115.2 (C-5), 115.4 (C-4a), 123.5 (2 C, C-3_{Ph} and C-5_{Ph}), 126.3 (C-6), 130.6 (2 C, C-2_{Ph} and C-6_{Ph}), 141.4 (C-1_{Ph}), 146.3 (C-4_{Ph}), 151.0 (C-2), 151.2 (C-7a), 159.4 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}N_4O_6$ ([M + H]$^+$): 387.1299, found: 387.1315.

4-Methyl-5-(4-methyl-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (32). 32 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **32** (58 mg, 0.16 mmol) as a white solid in 63% yield. Melting point: 114 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.37 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3), 3.54 (ddd, J = 11.8, 5.7, 3.9 Hz, 1 H, H-5′′), 3.64 (ddd, J = 11.8, 5.3, 4.1 Hz, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.13 (td, J = 4.9, 3.4 Hz, 1 H, H-3′), 4.46 (dd, J = 11.8, 6.4 Hz, 1 H, H-2′), 5.05 (t, J = 5.6 Hz, 1 H, OH-5′), 5.16 (d, J = 5.0 Hz, 1 H, OH-3′), 5.36 (d, J = 6.4 Hz, 1 H, OH-2′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.26–7.28 (m, 2 H, H–Ph), 7.33–7.45 (m, 2 H, H–Ph), 7.79 (s, 1 H, H-6), 8.68 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.8 (CH_3), 22.8 (CH_3), 61.5 (C-5′), 70.6 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.9 (C-4a), 116.9 (C-5), 124.5 (C-6), 128.8 (2 C, C-3_{Ph} and C-5_{Ph}), 129.7 (2 C, C-2_{Ph} and C-6_{Ph}), 131.3 (C-1_{Ph}), 136.4 (C-4_{Ph}), 150.6 (C-2), 150.7 (C-7a), 159.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{22}N_3O_4$ ([M + H]$^+$): 356.1605, found: 356.1581.

4-Methyl-5-(4-ethyl-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (33). 33 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **33** (39 mg, 0.11 mmol) as a white solid in 41% yield. Melting point: 105 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.23 (t, J = 7.5 Hz, 3 H, CH_3), 2.46 (s, 3 H, CH_3), 2.67 (q, J = 7.6 Hz, 2 H, CH_2), 3.46–3.59 (m, 1 H, H-5′′), 3.59–3.70 (m, 1 H, H-5′), 3.93 (q, J = 3.5 Hz, 1 H, H-4′), 4.07–4.19 (m, 1 H, H-3′), 4.46 (dd, J = 11.4, 6.4 Hz, 1 H, H-2′), 5.05 (t, J = 5.4 Hz, 1 H, OH-5′), 5.16 (d, J = 5.0 Hz, 1 H, OH-3′), 5.36 (d, J = 6.2 Hz, 1 H, OH-2′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.21–7.35 (m, 2 H, H–Ph), 7.36–7.49 (m, 2 H, H–Ph), 7.80 (s, 1 H, H-6), 8.68 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.6 (CH_3), 22.8 (CH_3), 27.9 (CH_2), 61.5 (C-5′), 70.6 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.9 (C-4a), 116.9 (C-5), 124.5 (C-6), 127.6 (2 C, C-3_{Ph} and C-5_{Ph}), 129.7 (2 C, C-2_{Ph} and C-6_{Ph}), 131.5 (C-1_{Ph}), 142.7 (C-4_{Ph}), 150.6 (C-2), 150.7 (C-7a), 159.2 (C-4). HRMS (ESI): calculated for $C_{20}H_{24}N_3O_4$ ([M + H]$^+$): 370.1761, found: 370.1771.

4-Methyl-5-(4-methoxy-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (34). 34 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **34** (68 mg, 0.18 mmol) as a white solid in 70% yield. Melting point: 112 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.45 (s, 3 H, CH_3), 3.45–3.59 (m, 1 H, H-5′′), 3.59–3.72 (m, 1 H, H-5′), 3.81 (s, 3 H, OMe), 3.93 (q, J = 3.6 Hz, 1 H, H-4′), 4.08–4.19 (m, 1 H, H-3′), 4.46 (dd, J = 11.5, 6.3 Hz, 1 H, H-2′), 5.05 (t, J = 5.6 Hz, 1 H, OH-5′), 5.16 (d, J = 4.7 Hz, 1 H, OH-3′), 5.36 (d, J = 6.2 Hz, 1 H, OH-2′), 6.26 (d, J = 6.2 Hz, 1 H, H-1′), 6.94–7.10 (m, 2 H, H–Ph), 7.34–7.49 (m, 2 H, H–Ph), 7.76 (s, 1 H, H-6), 8.67 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 22.7 (CH_3), 55.1 (OMe), 61.5 (C-5′), 70.6 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 113.7 (2 C, C-3_{Ph} and C-5_{Ph}), 116.0 (C-4a), 116.6 (C-5), 124.3 (C-6), 126.4 (C-1_{Ph}), 131.0 (2 C, C-2_{Ph} and C-6_{Ph}), 150.6 (C-2), 150.7 (C-7a), 158.6 (C-4_{Ph}), 159.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{22}N_3O_5$ ([M + H]$^+$): 372.1554, found: 372.1550.

4-Methyl-5-(4-hydroxy-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (35). 35 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **35** (70 mg, 0.20 mmol) as a white solid in 75% yield. Melting point: 75 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.45 (s, 3 H, CH_3), 3.48–3.58 (m, 1 H, H-5′′), 3.59–3.73 (m, 1 H, H-5′), 3.92 (q, J = 3.5 Hz, 1 H, H-4′), 4.04–4.18 (m, 1 H, H-3′), 4.45 (t, J = 5.1 Hz, 1 H, H-2′), 5.04 (br. s., 1 H, OH-5′), 5.15 (br. s., 1 H, OH-3′), 5.34 (br. s., 1 H, OH-2′), 6.25 (d, J = 6.2 Hz, 1 H, H-1′), 6.77–6.90 (m, 2 H, H–Ph), 7.20–7.35 (m, 2 H, H–Ph), 7.72 (s, 1 H, H-6), 8.67 (s, 1 H, H-2), 9.52 (s, 1 H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.5 (CH_3), 61.5 (C-5′), 70.6 (C-3′), 73.9 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.1 (2 C, C-3_{Ph} and C-5_{Ph}), 116.1 (C-4a), 117.1 (C-5), 124.1 (C-6), 124.6 (C-1_{Ph}), 131.0 (2 C, C-2_{Ph} and C-6_{Ph}), 150.5, 150.5, 156.8 (C-4_{Ph}), 159.1 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}N_3O_5$ ([M + H]$^+$): 358.1397, found: 358.1412.

4-Methyl-5-(3-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (36). 36 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **36** (23 mg, 0.061 mmol) as a white solid in 24% yield. Melting point: 158 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.48 (s, 3H, CH_3), 3.55 (ddd, J = 12.0, 5.6, 4.2 Hz, 1 H, H-5′′), 3.65 (ddd, J = 12.0, 5.4, 4.2 Hz, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.13 (dd, J = 8.2, 5.0 Hz, 1 H, H-3′), 4.47 (dd, J = 11.5, 6.0 Hz, 1 H, H-2′), 5.04 (t, J = 5.6 Hz, 1 H, OH-5′), 5.17 (d, J = 5.0 Hz, 1 H, OH-3′), 5.38 (d, J = 6.4 Hz, 1 H, OH-2′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.38–7.54 (m, 3 H, H–Ph), 7.56–7.65 (m, 1 H, H–Ph), 7.93 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.0 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 115.5 (C-5), 115.6 (C-4a), 125.3 (C-6), 127.0 (C–Ph), 128.8 (C–Ph), 129.2 (C-2_{Ph}), 130.0 (C-5_{Ph}), 132.9 (C-3_{Ph}), 136.5 (C-1_{Ph}), 150.7 (C-7a), 150.9 (C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4$ ([M + H]$^+$): 376.1059, found: 376.1080.

4-Methyl-5-(3-fluoro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (37). 37 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **37** (62 mg, 0.17 mmol) as a white solid in 66% yield. Melting point: 201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3 H, CH_3), 3.49–3.60 (m, 1 H, H-5′′), 3.61–3.73 (m, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.13–4.14 (d, J = 3.5 Hz, 1 H, H-3′), 4.47 (q, J = 5.8 Hz, 1 H, H-2′), 5.05 (t, J = 4.8 Hz, 1 H, OH-5′), 5.17 (d, J = 4.7 Hz, 1 H, OH-4′), 5.38 (d, J = 6.2 Hz, 1 H, OH-3′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.13–7.30 (m, 1 H, H–Ph),

7.31–7.43 (m, 2 H, H–Ph), 7.51 (td, J = 8.0, 6.3 Hz, 1 H, H–Ph), 7.92 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.0 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 113.9 (d, J = 21.9 Hz, 1 C, C–Ph), 115.6 (C-4a), 116.1 (d, J = 59.9 Hz, 1 C, C–Ph), 116.2 (C-5), 125.3 (C-6), 126.1 (d, J = 2.3 Hz, 1 C, C–Ph), 130.2 (d, J = 8.1 Hz, 1 C, C–Ph), 136.7 (d, J = 9.2 Hz, 1 C, C–Ph), 150.6 (C-7a), 150.9 (C-2), 159.2 (C-4), 161.9 (d, J = 243.0 Hz, 1 C, C–Ph). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −113.42–−113.33 (m, 1F). HRMS (ESI): calculated for $C_{18}H_{19}FN_3O_4$ ($[M+H]^+$): 360.1354, found: 360.1354.

4-Methyl-5-(2-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo [2,3-*d*]-pyrimidine (38). 38 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **38** (30 mg, 0.080 mmol) as a white solid in 31% yield. Melting point: 119 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.28 (s, 3 H, CH_3), 3.55 (ddd, J = 11.8, 5.6, 4.2 Hz, 1 H, H-5′′), 3.65 (ddd, J = 11.8, 5.3, 4.0 Hz, 1 H, H-5′), 3.94 (q, J = 3.8 Hz, 1 H, H-4′), 4.07–4.18 (m, 1 H, H-3′), 4.47 (dd, J = 11.5, 6.1 Hz, 1 H, H-2′), 5.04 (t, J = 5.4 Hz, 1 H, OH-5′), 5.18 (d, J = 5.0 Hz, 1 H, OH-3′), 5.38 (d, J = 6.4 Hz, 1 H, OH-2′), 6.26 (d, J = 6.2 Hz, 1 H, H-1′), 7.39–7.55 (m, 3 H, H–Ph), 7.56–7.70 (m, 1 H, H–Ph), 7.86 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.1 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.2 (C-4′), 86.8 (C-1′), 113.4 (C-5), 116.7 (C-4a), 125.4 (C-6), 127.2 (C–Ph), 129.3 (C–Ph), 129.8 (C–Ph), 132.9 (C–Ph), 133.3 (C-1_{Ph}), 134.0 (C–Ph), 150.2 (C-7a), 150.9 (C-2), 159.0 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4$ ($[M+H]^+$): 376.1059, found: 367.1057.

4-Methyl-5-(3,4-dichloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (39). 39 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **39** (27 mg, 0.066 mmol) as a white solid in 25% yield. Melting point: 228 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 3.55 (ddd, J = 12.0, 5.8, 4.1 Hz, 1 H, H-5′′), 3.65 (ddd, J = 11.8, 5.3, 4.3 Hz, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.14 (dd, J = 8.8, 4.8 Hz, 1 H, H-3′), 4.46 (dd, J = 11.4, 6.0 Hz, 1 H, H-2′), 5.04 (t, J = 5.6 Hz, 1 H, OH-5′), 5.18 (d, J = 5.0 Hz, 1 H, OH-3′), 5.38 (d, J = 6.2 Hz, 1 H, OH-2′), 6.27 (d, J = 5.9 Hz, 1 H, H-1′), 7.53 (dd, J = 8.3, 2.2 Hz, 1 H, H–Ph), 7.72 (d, J = 8.5 Hz, 1 H, H–Ph), 7.81 (d, J = 2.1 Hz, 1 H, H–Ph), 7.97 (s, 1 H, H-6), 8.72 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.1 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 114.5 (C-5), 115.6 (C-4a), 125.6 (C-6), 129.9 (C-4_{Ph}), 130.1 (C-6_{Ph}), 130.3 (C-5_{Ph}), 131.0 (C-3_{Ph}), 131.3 (C-2_{Ph}), 135.1 (C-1_{Ph}), 150.7 (C-7a), 151.0 (C-2), 159.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}ClN_3O_4$ ($[M+H]^+$): 410.0669, found: 410.0682.

4-Methyl-5-(3-fluoro-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (40). 40 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **40** (40 mg, 0.10 mmol) as a white solid in 39% yield. Melting point: 205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 3.49–3.60 (m, 1 H, H-5′′), 3.60–3.71 (m, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.13 (dd, J = 8.4, 4.8 Hz, 1 H, H-3′), 4.46 (q, J = 5.9 Hz, 1 H, H-2′), 5.04 (t, J = 5.6 Hz, 1 H, OH-5′), 5.18 (d, J = 5.0 Hz, 1 H, OH-3′), 5.38 (d, J = 6.4 Hz, 1 H, OH-2′), 6.27 (d, J = 6.2 Hz, 1 H, H-1′), 7.39 (dd, J = 8.2, 1.5 Hz, 1 H, H–Ph), 7.61 (dd, J = 10.5, 2.1 Hz, 1 H, H–Ph), 7.64–7.72 (m, 1 H, H–Ph), 7.95 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.1 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 114.8 (C-5), 115.5 (C-4a), 117.9 (d, J = 20.7 Hz, 1 C, C–Ph), 118.3 (d, J = 17.3 Hz, 1 C, C–Ph), 125.6 (C-6), 127.1 (d, J = 3.5 Hz, 1 C, C–Ph), 130.4 (C–Ph), 135.6 (d, J = 8.1 Hz, 1 C, C–Ph), 150.7 (C-7a), 151.0 (C-2), 156.9 (d, J = 246.5 Hz, 1 C, C–Ph), 159.3 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −116.42–−116.36 (m, 1F). HRMS (ESI): calculated for $C_{18}H_{18}ClFN_3O_4$ ($[M+H]^+$): 394.0964, found: 394.0960.

4-Methyl-5-(3-trifluoromethyl-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (41). 41 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **41** (51 mg, 0.12 mmol) as a white solid in 45% yield. Melting point: 242 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.47 (m, 3 H, CH_3), 3.48–3.60 (m, 1 H, H-5′′), 3.60–3.73 (m, 1 H, H-5′), 3.93 (q, J = 3.8 Hz, 1 H, H-4′), 4.10–4.20 (m, 1 H, H-3′), 4.47 (q, J = 6.0 Hz, 1 H, H-2′), 5.03 (t, J = 5.6 Hz, 1 H, OH-5′), 5.19 (d, J = 5.0 Hz, 1 H, OH-3′), 5.39 (d, J = 6.2 Hz, 1 H, OH-2′), 6.28 (d, J = 5.9 Hz, 1 H, H-1′), 7.76–7.92 (m, 2 H, H–Ph), 7.97 (d, J = 1.8 Hz, 1 H, H–Ph), 8.03 (s, 1 H, H-6), 8.73 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.1 (CH_3), 61.4 (C-5′), 70.4 (C-3′), 73.9 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 114.3 (C-5), 115.5 (C-4a), 122.8 (q, J = 274.91 Hz, 1 C, CF_3), 125.9 (C-6), 126.5 (q, J = 31.1 Hz, 1 C, C-3_{Ph}), 128.5 (q, J = 5.8 Hz, 1 C, C-2_{Ph}), 129.4 (d, J = 2.3 Hz, 1 C, C-4_{Ph}), 131.5 (C-5_{Ph}), 134.0 (C-1_{Ph}), 135.3 (C-6_{Ph}), 150.8 (C-2), 151.0 (C-7a), 159.2 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −61.154394. HRMS (ESI): calculated for $C_{19}H_{18}ClF_3N_3O_4$ ($[M+H]^+$): 444.0932, found: 444.0912.

4-Methyl-5-(3-methyl-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (42). 42 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **42** (56 mg, 0.14 mmol) as a white solid in 55% yield. Melting point: 208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.40 (s, 3 H, CH_3), 2.47 (s, 3 H, CH_3), 3.55 (ddd, J = 11.8, 5.6, 4.0 Hz, 1 H, H-5′′), 3.64 (ddd, J = 12.0, 5.2, 4.0 Hz, 1 H, H-5′), 3.93 (q, J = 3.7 Hz, 1 H, H-4′), 4.07–4.19 (m, 1 H, H-3′), 4.46 (dd, J = 11.8, 6.2 Hz, 1 H, H-2′), 5.04 (t, J = 5.6 Hz, 1 H, OH-5′), 5.17 (d, J = 4.7 Hz, 1 H, OH-3′), 5.37 (d, J = 6.2 Hz, 1 H, OH-2′), 6.27 (d, J = 5.9 Hz, 1 H, H-1′), 7.35 (dd, J = 8.2, 1.8 Hz, 1 H, H–Ph), 7.45–7.55 (m, 2 H, H–Ph), 7.86 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.6 (CH_3), 23.0 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 115.7 (C-5), 115.8 (C-4a), 124.9 (C-6), 128.6 (C-5_{Ph}), 129.0 (C-6_{Ph}), 132.2 (C-3_{Ph}), 132.4 (C-2_{Ph}), 133.2 (C-1_{Ph}), 135.3 (C-4_{Ph}), 150.7 (C-7a), 150.9 (C-2), 159.3 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_4$ ($[M+H]^+$): 390.1215, found: 390.1198.

4-Methyl-5-(3-methoxy-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (43). 43 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **43** (36 mg, 0.089 mmol) as a light yellow solid in 34% yield. Melting point: 50 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.52–3.58 (m, 1 H, H-5′′), 3.62–3.67 (m, 1 H, H-5′), 3.91–3.95 (m, 4 H, H-4′ and CH_3), 4.13 (q, J = 4.2 Hz, 1 H, H-3′), 4.47 (q, J = 6.1 Hz, 1 H, H-2′), 5.05 (t, J = 5.4 Hz, 1 H, OH-5′), 5.18 (d, J = 4.9 Hz, 1 H, OH-3′), 5.37 (d, J = 6.5 Hz, 1 H, OH-2′), 6.27 (d, J = 6.1 Hz, 1 H, H-1′), 7.08 (dd, J = 8.0, 1.9 Hz, 1 H, H–Ph), 7.25 (d, J = 1.9 Hz, 1 H, H–Ph), 7.50 (d, J = 8.0 Hz, 1 H, H–Ph), 7.90 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.1 (CH_3), 56.2 (OCH_3), 61.4 (C-5′), 70.5 (C-3′), 74.0 (C-2′), 85.2 (C-4′), 86.6 (C-1′), 114.3 (C-2_{Ph}), 115.8 (C-4a), 116.1 (C-5), 120.0 (C-4_{Ph}), 122.7 (C-6_{Ph}), 125.1 (C-6), 129.6 (C-5_{Ph}), 134.6 (C-$_{Ph}$), 150.6 (C-7a), 150.9 (C-2), 154.1 (C-3_{Ph}), 159.3 (C-4). HRMS (ESI): calculated for $C_{19}H_{21}ClN_3O_5$ ($[M+H]^+$): 406.1164, found: 406.1187.

4-Methyl-5-(2-chloro-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (44). 44 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **44** (35 mg, 0.085 mmol) as a white solid in 33% yield. Melting point: 120–122 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.30 (s, 3 H, CH_3), 3.55 (ddd, J = 12.0, 5.5, 4.0 Hz, 1 H, H-5′′), 3.64 (ddd, J = 11.8, 5.2, 4.2 Hz, 1 H, H-5′), 3.94 (q, J = 3.8 Hz, 1 H, H-4′), 4.07–4.17 (m, 1 H, H-3′), 4.45 (dd, J = 11.5, 6.2 Hz, 1 H, H-2′), 5.04 (t, J = 5.4 Hz, 1 H, OH-5′), 5.18 (d, J = 5.0 Hz, 1 H, OH-3′), 5.39 (d, J = 6.2 Hz, 1 H, OH-2′), 6.25 (d, J = 5.9 Hz, 1 H, H-1′), 7.47–7.59 (m, 2 H, H–Ph), 7.80 (dd, J = 1.5, 0.9 Hz, 1 H, H–Ph), 7.89 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.2 (CH_3), 61.5 (C-5′), 70.5 (C-3′), 74.1 (C-2′), 85.2 (C-4′), 86.8 (C-1′), 112.2 (C-5), 116.6 (C-4a), 125.7 (C-6), 127.4 (C-3_{Ph}), 128.8 (C-5_{Ph}), 132.4 (C-1_{Ph}), 133.5 (C–Ph), 134.1 (C-2_{Ph}), 135.0 (C–Ph), 150.3 (C-2), 151.0 (C-7a), 159.0 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}Cl_2N_3O_4$ ($[M+H]^+$): 410.0669, found: 410.0664.

4-Methyl-5-(2-fluorol-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (45). 45 was prepared according to

General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **45** (33 mg, 0.084 mmol) as a white solid in 32% yield. Melting point: 223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.39 (s, 3 H, CH_3), 3.48–3.60 (m, 1 H, H-5''), 3.60–3.76 (m, 1 H, H-5'), 3.94 (q, J = 3.7 Hz, 1 H, H-4'), 4.09–4.18 (m, 1 H, H-3'), 4.47 (q, J = 6.2 Hz, 1 H, H-2'), 5.04 (t, J = 5.4 Hz, 1 H, OH-5'), 5.18 (d, J = 5.0 Hz, 1 H, OH-3'), 5.40 (d, J = 6.4 Hz, 1 H, OH-2'), 6.27 (d, J = 6.2 Hz, 1 H, H-1'), 7.42 (dd, J = 8.2, 2.1 Hz, 1 H, H–Ph), 7.55 (t, J = 6.2 Hz, 1 H, H–Ph), 7.60 (dd, J = 9.7, 2.1 Hz, 1 H, H–Ph), 7.94 (s, 1 H, H-6), 8.72 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.4 (CH_3), 61.5 (C-5'), 70.5 (C-3'), 74.0 (C-2'), 85.3 (C-4'), 86.7 (C-1'), 108.3 (C-5), 116.4 (d, J = 21.9 Hz, 1 C, C–Ph), 116.4 (C-4a), 121.2 (d, J = 16.1 Hz, 1 C, C–Ph), 124.8 (d, J = 3.5 Hz, 1 C, C–Ph), 126.1 (C-6), 133.3 (d, J = 10.4 Hz, 1 C, C–Ph), 133.7 (d, J = 3.5 Hz, 1 C, C–Ph), 150.5 (C-7a), 151.0 (C-2), 159.2 (C-4), 159.7 (d, J = 247.6 Hz, 1 C, C–Ph). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −111.36–−111.30 (m, 1 F). HRMS (ESI): calculated for $C_{18}H_{18}ClFN_3O_4$ ([M + H]$^+$): 394.0964, found: 394.0972.

4-Methyl-5-(3,5-dichloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (46). 46 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **46** (35 mg, 0.085 mmol) as a white solid in 33% yield. Melting point: 230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.51 (s, 3 H, CH_3), 3.55 (ddd, J = 12.0, 5.7, 4.3 Hz, 1 H, H-5''), 3.65 (ddd, J = 12.0, 5.3, 4.1 Hz, 1 H, H-5'), 3.93 (q, J = 4.1 Hz, 1 H, H-4'), 4.09–4.18 (m, 1 H, H-3'), 4.46 (dd, J = 11.5, 6.0 Hz, 1 H, H-2'), 5.03 (t, J = 5.6 Hz, 1 H, OH-5'), 5.18 (d, J = 5.0 Hz, 1 H, OH-3'), 5.38 (d, J = 6.4 Hz, 1 H, OH-2'), 6.27 (d, J = 5.9 Hz, 1 H, H-1'), 7.56–7.69 (m, 3 H, H–Ph), 8.01 (s, 1 H, H-6), 8.72 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.1 (CH_3), 61.5 (C-5'), 70.4 (C-3'), 74.0 (C-2'), 85.2 (C-4'), 86.6 (C-1'), 114.3 (C-5), 115.5 (C-4a), 126.0 (C-6), 126.6 (C–Ph), 128.3 (C–Ph), 133.9 (C–Ph), 137.9 (C-1$_{Ph}$), 150.7 (C-2), 151.0 (C-7a), 159.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}Cl_2N_3O_4$ ([M + H]$^+$): 410.0669, found: 410.0669.

4-Methyl-5-(4-chloro-3,5-difluoro-4-chloro-phenyl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (47). 47 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **47** (37 mg, 0.090 mmol) as a white solid in 35% yield. Melting point: 212 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.54 (s, 3 H, CH_3), 3.55 (ddd, J = 12.0, 5.6, 4.2 Hz, 1 H, H-5''), 3.65 (ddd, J = 12.0, 5.4, 4.2 Hz, 1 H, H-5'), 3.93 (q, J = 3.8 Hz, 1 H, H-4'), 4.08–4.20 (m, 1 H, H-3'), 4.45 (q, J = 5.8 Hz, 1 H, H-2'), 5.04 (t, J = 5.6 Hz, 1 H, OH-5'), 5.19 (d, J = 5.0 Hz, 1 H, OH-3'), 5.39 (d, J = 6.4 Hz, 1 H, OH-2'), 6.27 (d, J = 6.2 Hz, 1 H, H-1'), 7.45–7.61 (m, 2 H, H–Ph), 8.01 (s, 1 H, H-6), 8.73 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.3 (CH_3), 61.4 (C-5'), 70.4 (C-3'), 74.1 (C-2'), 85.2 (C-4'), 86.6 (C-1'), 106.8 (C–Ph), 113.9 (d, J = 45.8 Hz, 1 C, C–Ph), 114.0 (C-5), 116.4 (d, J = 21.9 Hz, 1 C, C–Ph), 115.4 (C-4a), 126.1 (C-6), 135.6 (t, J = 10.2 Hz, 1 C, C–Ph), 150.7 (C-7a), 151.1 (C-2), 156.5 (d, J = 3.6 Hz, 1 C, C-1$_{Ph}$), 158.9 (d, J = 4.4 Hz, 1 C, C–Ph), 159.5 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −114.34–−114.31. HRMS (ESI): calculated for $C_{18}H_{17}ClF_2N_3O_4$ ([M + H]$^+$): 412.0870, found: 412.0860.

4-Methyl-5-([1,1'-biphenyl]-4-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (48). 48 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **48** (60 mg, 0.14 mmol) as a white solid in 55% yield. Melting point: 134 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.53 (s, 3 H, CH_3), 3.56 (ddd, J = 12.0, 5.6, 4.2 Hz, 1 H, H-5''), 3.66 (ddd, J = 12.0, 5.2, 4.2 Hz, 1 H, H-5'), 3.94 (q, J = 3.7 Hz, 1 H, H-4'), 4.10–4.22 (m, 1 H, H-3'), 4.49 (dd, J = 11.6, 6.2 Hz, 1 H, H-2'), 5.07 (t, J = 5.6 Hz, 1 H, OH-5'), 5.17 (d, J = 4.7 Hz, 1 H, OH-3'), 5.39 (d, J = 6.4 Hz, 1 H, OH-2'), 6.30 (d, J = 6.2 Hz, 1 H, H-1'), 7.33–7.43 (m, 1 H, H–Ph), 7.44–7.54 (m, 2 H, H–Ph), 7.57–7.67 (m, 2 H, H–Ph), 7.70–7.83 (m, 4 H, H–Ph), 7.91 (s, 1 H, H-6), 8.71 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 23.0 (CH_3), 61.5 (C-5'), 70.6 (C-3'), 74.0 (C-2'), 85.2 (C-4'), 86.6 (C-1'), 115.8 (C-4a), 116.6 (C-5), 124.9 (C-6), 126.5 (C–Ph), 126.6 (C–Ph), 127.5 (C–Ph), 127.8 (C–Ph), 129.0 (C–Ph), 130.3 (C–Ph), 133.4 (C–Ph), 138.8 (C–Ph), 139.7 (C–Ph), 150.8 (C-2 and C-7a), 159.3 (C-4). HRMS (ESI): calculated for $C_{24}H_{24}N_3O_4$ ([M + H]$^+$): 418.1761, found: 418.1749.

4-Methyl-5-(naphthalen-2-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (49). 49 was prepared according to General procedure A. **67** (100 mg, 0.26 mmol) gave rise to **49** (25 mg, 0.064 mmol) as a white solid in 25% yield. Melting point: 135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.49 (s, 3 H, CH_3), 3.56 (ddd, J = 11.8, 5.6, 4.1 Hz, 1 H, H-5''), 3.66 (ddd, J = 11.8, 5.2, 4.2 Hz, 1 H, H-5'), 3.95 (q, J = 3.6 Hz, 1 H, H-4'), 4.10–4.22 (m, 1 H, H-3'), 4.51 (dd, J = 11.5, 6.0 Hz, 1 H, H-2'), 5.06 (t, J = 5.4 Hz, 1 H, OH-5'), 5.18 (d, J = 4.7 Hz, 1 H, OH-3'), 5.40 (d, J = 6.4 Hz, 1 H, OH-2'), 6.31 (d, J = 6.2 Hz, 1 H, H-1'), 7.48–7.62 (m, 2 H, H-Nap), 7.68 (dd, J = 8.5, 1.8 Hz, 1 H, H-Nap), 7.90–8.13 (m, 5 H, H-Nap and H-6), 8.72 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.0 (CH_3), 61.5 (C-5'), 70.5 (C-3'), 74.0 (C-2'), 85.2 (C-4'), 86.5 (C-1'), 116.0 (C-4a), 116.9 (C-5), 125.0 (C-6), 126.0 (C-Nap), 126.4 (C-Nap), 127.6 (C-Nap), 127.8 (C-Nap), 128.0 (C-Nap), 128.3 (C-Nap), 131.7 (C-Nap), 132.9 (C-Nap), 150.8 (C-2), 150.9 (C-7a), 159.3 (C-4). HRMS (ESI): calculated for $C_{22}H_{22}N_3O_4$([M + H]$^+$): 392.1605, found: 392.1600.

4-Methyl-5-(benzo[d][1,3]dioxol-5-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (50). 50 was prepared according to General procedure A. **67** (150 mg, 0.38 mmol) gave rise to **50** (95 mg, 0.25 mmol) as a white solid in 65% yield. Melting point: 232 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.48 (s, 3 H, CH_3), 3.52–3.57 (m, 1 H, H-5''), 3.61–3.67 (m, 1 H, H-5'), 3.92 (q, J = 3.4 Hz, 1 H, H-4'), 4.13 (q, J = 4.5 Hz, 1 H, H-3'), 4.45 (q, J = 6.0 Hz, 1 H, H-2'), 5.05 (t, J = 5.4 Hz, 1 H, OH-5'), 5.16 (d, J = 4.9 Hz, 1 H, OH-3'), 5.36 (d, J = 6.4 Hz, 1 H, OH-2'), 6.08 (s, 2 H, CH_2), 6.26 (d, J = 6.0 Hz, 1 H, H-1'), 6.94 (d, J = 7.9 Hz, 1 H, H–Ph), 7.00 (d, J = 7.9 Hz, 1 H, H–Ph), 7.07 (s, 1 H, H–Ph), 7.78 (s, 1 H, H-6), 8.67 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.8 (CH_3), 61.5 (C-5'), 70.5 (C-3'), 74.0 (C-2'), 85.1 (C-4'), 86.5 (C-1'), 101.1 (CH_2), 108.1 (C–Ph), 110.3 (C–Ph), 115.9 (C-4a), 116.7 (C-5), 123.3 (C–Ph), 124.6 (C-6), 127.9 (C–Ph), 146.5 (C–Ph), 147.1 (C–Ph), 150.5 (C-7a), 150.7 (C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{20}N_3O_6$ ([M + H]$^+$): 386.1347, found: 386.1350.

4-Methyl-5-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (51). 51 was prepared according to General procedure A. **67** (120 mg, 0.31 mmol) gave rise to **51** (55 mg, 0.14 mmol) as a white solid in 44% yield. Melting point: 127 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.48 (s, 3 H, CH_3), 3.52–3.57 (m, 1 H, H-5''), 3.61–3.66 (m, 1 H, H-5'), 3.92 (q, J = 3.5 Hz, 1 H, H-4'), 4.12 (q, J = 4.3 Hz, 1 H, H-3'), 4.29 (s, 4 H, $2CH_2$), 4.45 (q, J = 6.0 Hz, 1 H, H-2'), 5.05 (t, J = 5.4 Hz, 1 H, OH-5'), 5.15 (d, J = 4.8 Hz, 1 H, OH-3'), 5.35 (d, J = 6.4 Hz, 1 H, OH-2'), 6.25 (d, J = 6.1 Hz, 1 H, H-1'), 6.93 (s, 2 H, H–Ph), 6.98 (s, 1 H, H–Ph), 7.76 (s, 1 H, H-6), 8.67 (s, 1 H, H-2) . ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.8 (CH_3), 61.5 (C-5'), 64.1 ($2CH_2$), 70.5 (C-3'), 74.0 (C-2'), 85.1 (C-4'), 86.5 (C-1'), 115.9 (C-4a), 116.5 (C-5), 116.8 (C–Ph), 118.3 (C–Ph), 122.9 (C–Ph), 124.5 (C-6), 127.3 (C–Ph), 142.8 (C–Ph), 143.0 (C–Ph),150.5 (C-7a), 150.7 (C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{20}H_{22}N_3O_6$ ([M + H]$^+$): 400.1503, found: 400.1509.

4-Methyl-5-(1H-indol-6-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (52). 52 was prepared according to General procedure A. **67** (120 mg, 0.31 mmol) gave rise to slightly impure **52** (35 mg, 0.092 mmol). **52** was purified by preparative RP-HPLC gradient: 0.2% formic acid in water:MeCN at a flow rate of 20 mL/min; The initial gradient composition (95% A/05% B) was held for 2.0 min, increased to 60% B in 13 min, then increased to 100% B in 1 min. After preparative RP-HPLC, **52** was isolated as a white solid (25 mg, 0.066 mmol) in 21% yield. Melting point: 153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.45 (s, 3 H, CH_3), 3.53–3.57 (m, 1 H, H-5''), 3.62–3.66 (m, 1 H, H-5'), 3.94 (q, J = 3.7 Hz, 1 H, H-4'), 4.13 (dd, J = 4.9, 3.3 Hz, 1 H, H-3'), 4.50 (t, J = 5.7 Hz, 1 H, H-2'), 5.22 (br.s. 3 H, OH-2', OH-3'and OH-5') 6.29 (d, J = 6.3 Hz, 1 H, H-1'), 6.47 (br.s., 1 H,

H-indole), 7.12 (d, $J=8.1$ Hz, 1 H, H-indole), 7.39 (t, $J=2.7$ Hz, 1 H, H-indole), 7.47 (s, 1 H, H-indole), 7.61 (d, $J=8.1$ Hz, 1 H, H-indole), 7.79 (s, 1 H, H-6), 8.68 (s, 1 H, H-2), 11.16 (br. s., 1 H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.8 (CH_3), 61.6 (C-5′), 70.6 (C-3′), 73.9 (C-2′), 85.2 (C-4′), 86.5 (C-1′), 101.0 (C-indole), 112.5 (C-indole), 116.2 (C-4a), 118.3 (C-5), 119.6 (C-indole), 121.5 (C-indole), 124.2 (C-6), 125.8 (C-indole), 126.8 (C-indole), 126.8 (C-indole), 135.8 (C-indole), 150.6 (C-7a and C-2), 159.2 (C-4). HRMS (ESI): calculated for $C_{20}H_{21}N_4O_4$ ($[M+H]^+$): 381.1557, found: 381.1565.

4-Methyl-5-(1-methyl-1H-indol-6-yl)-N7-(β-D–ribofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine (53). 53 was prepared according to General procedure A. **67** (150 mg, 0.38 mmol) gave rise to **53** (85 mg, 0.22 mmol) as a white solid in 57% yield. Melting point: 135 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.45 (s, 3 H, CH_3), 3.53–3.58 (m, 1 H, H-5′′), 3.62–3.67 (m, 1 H, H-5′), 3.82 (s, 3 H, CH_3), 3.94 (q, $J=3.2$ Hz, 1 H, H-4′), 4.14 (q, $J=4.2$ Hz, 1 H, H-3′), 4.49 (q, $J=5.7$ Hz, 1 H, H-2′), 5.06 (t, $J=5.4$ Hz, 1 H, OH-5′), 5.17 (dd, $J=4.8$, 1.2 Hz, 1 H, OH-3′), 5.37 (dd, $J=6.4$, 1.4 Hz, 1 H, OH-2′), 6.30 (d, $J=6.1$ Hz, 1 H, H-1′), 6.47 (dd, $J=3.1$, 0.6 Hz, 1 H, H-indole), 7.16 (dd, $J=8.1$, 1.2 Hz, 1 H, H-indole), 7.37 (d, $J=3.1$ Hz, 1 H, H-indole), 7.53 (s, 1 H, H-indole), 7.61 (d, $J=8.0$ Hz, 1 H, H-indole), 7.81 (s, 1 H, H-6), 8.69 (s, 1 H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.9 (CH_3), 32.5 (CH_3), 61.6 (C-5′), 70.6 (C-3′), 74.0 (C-2′), 85.1 (C-4′), 86.5 (C-1′), 100.3 (C-indole), 110.9 (C-indole), 116.2 (C-4a), 118.3 (C-5), 119.9 (C-indole), 121.5 (C-indole), 124.3 (C-6), 127.0 (C-indole), 127.1 (C-indole), 130.1 (C-indole), 136.3 (C-indole), 150.6 (C-7a), 150.7 (C-2), 159.3 (C-4). HRMS (ESI): calculated for $C_{21}H_{23}N_4O_4$ ($[M+H]^+$): 395.1714, found: 395.1711.

4-Chloro-5-iodo-N7-(2′,5′-di-*O*-benzoyl-3′-deoxy-β-D–ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (81).[33] 4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (500 mg, 1.79 mmol) was placed in a 100 mL two-neck round bottom flask, after which the flask was purged with N_2 gas. Anhydrous MeCN (15 mL, 7.5 mL/mmol of SM) was added. BSA (482 μL, 1.97 mmol, 1.1 eq.) was added into the suspension via syringe. The resulting mixture was stirred until the solid was completely dissolved. Next, 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-α/β-D-ribofuranose (760 mg, 1.97 mmol, 1.1 eq.) was added, and immediately followed by TMSOTf (357 μL, 1.97 mmol, 1.1 eq.). The reaction mixture was stirred at ambient temperature for 15 min, and was heated at 80 °C. When full conversion of 4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine was observed via HRMS, the mixture was cooled to room temperature. Then, EA (15 mL) and aq. sat. $NaHCO_3$ (15 mL) were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (5→30% EA/PET) to give a colorless foam (800 mg, 1.32 mmol) in 74% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (ddd, $J=14.1$, 5.6, 1.8 Hz, 1 H, CH_2), 2.76 (ddd, $J=14.1$, 10.4, 6.0 Hz, 1 H, CH_2), 4.56–4.61 (m, 1 H, H-5′′), 4.73–4.78 (m, 1 H, H-5′), 4.80–4.88 (m, 1 H, H-4′), 5.90–5.92 (m, 1 H, H-2′), 6.45 (d, $J=1.8$ Hz, 1 H, H-1′), 7.45–7.51 (m, 4 H, H-OBz), 7.57–7.65 (m, 3 H, H-6 and H-OBz), 8.01–8.09 (m, 4 H, H-OBz), 8.59 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{20}H_{25}ClIN_3O_5$ ($[M+H]^+$): 604.0131, found: 604.0126.

4-Methyl-N7-(2′,5′-di-*O*-benzoyl-3′-deoxy-β-D–ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (82). 81 (800 mg, 1.32 mmol) was placed in a 25 mL round bottom flask. Then, the flask was purged with argon. Next, anhydrous THF (5 mL) was added, and the reaction solution was stirred at −10 °C for 15 min. i-PrMgCl·LiCl (1.3 M in THF, 1.52 mL, 1.98 mmol, 1.1 eq.) was added dropwise slowly. When full conversion of the SM was observed by TLC (1 h), 0.5 M aq. HCl solution (5 mL) and EA (10 mL) were added. The layers were separated, and the water layer was extracted twice more with EA (2×10 mL). Then, organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was used directly for the next step. This crude product, $Pd(PPh_3)_4$ (116 mg, 0.10 mmol, 0.1 eq.) were added in a 25 mL two-neck round bottom flask. The flask was purged with argon three times. Next, anhydrous THF (4 mL, 4 mL/mmol) was added via syringe, and $AlMe_3$ solution (2 M in toluene, 0.6 mL, 1.2 mmol, 1.2 eq.) was added dropwise. After the mixture was stirred for 15 min at ambient temperature, the reaction was refluxed at 100 °C until the TLC showed the SM was consumed completely (1 h). After cooling at ice-water bath, 0.5 M aq. HCl (10 mL) was added dropwise. [**Caution**: methane gas was generated, with potential excessive foaming]. Next, EA (50 mL) was added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (10→50% EA/PET) to give **82** (400 mg, 0.87 mmol) as a yellow foam in 66% yield for two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (ddd, $J=14.1$, 5.6, 1.8 Hz, 1 H, CH_2), 2.71 (s, 3 H, CH_3), 2.80–2.90 (m, 1 H, CH_2), 4.53–4.59 (m, 1 H, H-5′′), 4.68–4.73 (m, 1 H, H-5′), 4.79–4.87 (m, 1 H, H-4′), 5.99 (dt, $J=6.2$, 1.8 Hz, 1 H, H-2′), 6.49 (d, $J=2.1$ Hz, 1 H, H-1′), 6.55 (d, $J=3.8$ Hz, 1 H, H-5), 7.33 (d, $J=3.8$ Hz, 1 H, H-6), 7.41–7.50 (m, 4 H, H-OBz), 7.52–7.71 (m, 2 H, H-OBz), 8.01–8.10 (m, 4 H, H-OBz), 8.74 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{26}H_{24}N_3O_5$ ($[M+H]^+$): 458.1710, found: 458.1718.

4-Methyl-N7-(3′-deoxy-β-D–ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (83). 82 (350 mg, 0.77 mmol) was placed in a 25 mL round bottom flask. Next, 0.5 M NaOMe in MeOH (5 mL) was added, and the reaction mixture was stirred for 2 h. Then, 0.5 M aq. HCl was added to neutralize the reaction solution. The resulting mixture was evaporated till dryness, and purified by flash column chromatography (0→10% MeOH/DCM) to afford **83** (160 mg, 0.64 mmol) as a white solid in 83% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.93 (ddd, $J=13.0$, 6.3, 3.2 Hz, 1 H, CH_2), 2.22 (ddd, $J=13.1$, 8.7, 6.0 Hz, 1 H, CH_2), 2.65 (s, 3 H, CH_3), 3.50–3.56 (m, 1 H, H-5′′), 3.62–3.67 (m, 1 H, H-5′), 4.28–4.36 (m, 1 H, H-4′), 4.42–4.45 (m, 1 H, H-2′), 4.98 (t, $J=5.6$ Hz, 1 H, OH-5′), 5.60 (d, $J=4.4$ Hz, 1 H, OH-2′), 6.16 (d, $J=2.6$ Hz, 1 H, H-1′), 6.72 (d, $J=3.8$ Hz, 1 H, H-5), 7.78 (d, $J=3.5$ Hz, 1 H, H-6), 8.65 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{12}H_{16}N_3O_3$ ($[M+H]^+$): 259.1186, found: 259.1180.

4-Methyl-5-bromo-N7-(3′-deoxy-β-D–ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (84). 83 (100 mg, 0.40 mmol) was dissolved in DMF (2 mL), and NBS (107 mg, 0.60 mmol, 1.5 eq.) was added. The resulting mixture was stirred at 50 °C for 2 h. Then, the mixture was evaporated till dryness and purified by column chromatography (0→10% MeOH/DCM) to afford **84** (96 mg, 0.29 mmol) as a white solid in 73% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.89 (ddd, $J=13.2$, 6.3, 3.1 Hz, 1 H, CH_2), 2.22 (ddd, $J=13.2$, 8.8, 5.9 Hz, 1 H, CH_2), 2.84 (s, 3 H, CH_3), 3.53 (ddd, $J=12.0$, 5.3, 4.1 Hz, 1 H), H-5′′, 3.70 (ddd, $J=12.0$, 5.4, 3.4 Hz, 1 H, H-5′), 4.29–4.37 (m, 1 H, H-4′), 4.39–4.44 (m, 1 H, H-4′), 5.05 (t, $J=5.4$ Hz, 1 H, OH-5′), 5.63 (d, $J=4.1$ Hz, 1 H, OH-2′), 6.18 (d, $J=2.3$ Hz, 1 H, H-1′), 8.07 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{12}H_{15}BrN_3O_3$ ($[M+H]^+$): 328.0291, found 328.0295.

4-Methyl-5-(4-chloro-phenyl)-N7-(3′-deoxy-β-D–ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (54). 54 was prepared according to General procedure A. **84** (90 mg, 0.27 mmol) gave rise to **54** (35 mg, 0.10 mmol) as a white solid in 36% yield. Melting point: 181 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.92 (ddd, $J=13.0$, 6.3, 2.9 Hz, 1 H, CH_2), 2.27 (ddd, $J=13.0$, 8.8, 6.0 Hz, 1 H, CH_2), 2.46 (s, 3 H, CH_3), 3.52 (ddd, $J=11.9$, 5.4, 4.1 Hz, 1 H, H-5′′), 3.67 (ddd, $J=11.9$, 5.6, 3.4 Hz, 1 H, H-5′), 4.27–4.40 (m, 1 H, H-4′), 4.42–4.55 (m, 1 H, H-2′), 4.98 (t, $J=5.4$ Hz, 1 H, OH-5′), 5.63 (d, $J=4.4$ Hz, 1 H, OH-2′), 6.25 (d, $J=2.3$ Hz, 1 H, H-1′), 7.44–7.58 (m, 4 H, H–Ph), 7.89 (s, 1 H, H-6), 8.70 (s, 1 H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.0 (CH_3), 34.5 (CH_2), 62.5 (C-5′), 75.0 (C-2′), 80.2 (C-4′), 90.0 (C-1′), 115.3 (C-4a), 115.5 (C-5), 124.8 (C-6), 128.2 (2 C, C–Ph), 131.5 (2 C, C–Ph), 131.9 (C-4_{Ph}), 133.3

($C\text{-}1_{Ph}$), 150.0 (C-7a), 150.8 (C-2), 159.1 (C-4). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_3$ ($[M+H]^+$): 360.1109, found: 360.1112.

Biology

In vitro *evaluation*

All *in vitro* compound evaluations were performed as described previously, including drug sensitivity assays on intracellular amastigotes of the Tulahuen (β-galactosidase expressing) *T. cruzi* strain in MRC-5 fibroblasts, Y-strain *T. cruzi* in primary mouse cardiac cells and Y-strain bloodstream trypomastigote forms (BF).[25] Cytotoxicity, as evaluated on MRC-5 fibroblasts and 2D primary mouse cardiac cells, was performed as described.[25]

Three-dimensional cardiac cell cultures: cardiac spheroids were obtained as reported.[45] For cardiotoxicity analyses, non-infected 3-D cultures were incubated for 48 h at 37 °C with increasing concentrations of BZ and compound **14** (up to 200 μM) and cellular viability determined using PrestoBlue® (CC) tests.[45] For determination of anti-*T. cruzi* activity, 3-D cultures were infected with BF (strain Y), using 20:1 parasite:host cell ratios. After 48 h, the cultures were rinsed and either or not incubated with 10 μM compound **14** or BZ (corresponding to the EC_{90} value of the reference drug upon intracellular forms) for 96 h at 37 °C.[51] To determine the parasite load, infected spheroids were transferred to a tube for extraction of total DNA using High Pure PCR Template Preparation Kit, according to manufacturer's instructions (Roche, USA). At the final step of the protocol, DNA was eluted in 100 μL of elution buffer. The absolute quantification of the parasite's DNA was performed in a ABI 7500 Fast Real Time PCR instrument (Applied Biosystems), using the Cruzi 1 (5′-ASTCGGCTGATCGTTTTCGA-3′) and Cruzi 2 (5′-AATTCCTCCAAG-CAGCGGATA-3′) primers and Cruzi 3 probe (5′-FAM-CACACACTG GACACCAANFQ-MGB-3′) targeting *T. cruzi* nuclear satellite DNA, as reported[52] with minor modifications. qPCR were carried out with 5 μL DNA, using 10 μL FastStart Universal Probe Master Mix [2×] (Roche), 750 nM primer Cruzi 1, 750 nM primer Cruzi 2 and 50 nM probe Cruzi 3 (FAM/NFQ-MGB), in a final volume of 20 μL. In parallel, quantification of cardiac cell DNA was performed using a TaqMan assay targeting mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cat. No. Mm99999915–g1, Applied Biosystems). Cycling conditions were a first step of 10 minutes at 95 °C followed by 40 cycles at 95 °C for 15 sec. and 58 °C for 1 min. Standard curves were constructed with 1:10 serial dilutions of total DNA obtained from a pool of 7.5×10^4 spheroids spiked with 10^5 epimastigotes. Thus, *T. cruzi* and cardiac cell quantities were quantified and expressed as parasite equivalents/cardiac cell.[45]

Microsomal stability assays

The *in vitro* metabolic stability assays were performed exactly as described elsewhere.[51]

In vivo evaluation

For the *in vivo* evaluation, male Swiss Webster mice were obtained from the Fundação Oswaldo Cruz (FIOCRUZ) animal facilities (ICTB /FIOCRUZ, Rio de Janeiro, Brazil). Mice were housed at a maximum of 6 per cage and kept in a standard room at 20 to 24 °C under a 12 h/12 h light/dark cycle. The animals were provided with sterilized water and chow *ad libitum*. Mice (18 to 23 g) were infected by intraperitoneal (i.p.) route with 10^4 bloodstream trypomastigotes of the Y strain of *T. cruzi*. Age-matched non-infected mice were maintained under identical conditions.[25] These mice were divided into eight experimental groups: non-infected (mice were neither infected nor treated), non-treated (mice were infected but treated with vehicle −10% (v/v) Tween 80 in sterile deionized water), and treatment (mice were infected and treated with **14** and/or the reference BZ). When a detectable parasitemia was observed at the onset of parasitemia (*i.e.*, 5 dpi), the corresponding treatments were initiated through oral administration. Compound **14** was administrated twice per day in 3 mice groups, using 10-fold dose titration schedules with 3 concentrations, namely 25, 2.5, 0.25 mg/kg, respectively. The reference drug BZ was administered orally at 10 (suboptimal) and 100 mg/kg (optimal dose) once per day, respectively. **14** was co-administered at 2.5 mg/kg (b.i.d) with BZ (once a day) at 10 mg/kg for consecutive 5 days, starting at parasitemia onset (5 dpi). All compounds were formulated freshly before every administration. 5 μL of blood of each individual experimental mouse was obtained via tail vein puncture and the number of parasites was counted under a light microscope to determine the blood parasitemia until 15 dpi. The mortality was monitored daily up to 24 days after the consecutive 5 days treatment (34 dpi) and presented as a percentage of cumulative mortality (CM). For qPCR analysis, at 136 dpi, after euthanasia, 500 μL of animal blood was diluted in 1:2 volume of guanidine solution (guanidine-HCl 6 M/EDTA 0.2 M), and heated for 90 sec. in boiling water. Guanidine-EDTA blood (GEB) samples were processed using the High Pure PCR Template Preparation kit, according to manufacturer's instructions (Roche, USA). At the final step of the protocol, DNA was eluted in 100 μL of elution buffer. Quantitative Real-Time PCR Multiplex assays were performed (40 cycles, threshold set at 0.01) for parasite detection of the *T. cruzi* satellite nuclear DNA and the internal amplification control-IAC (pZErO-2 plasmid containing an insert from the *Arabidopsis thaliana* aquaporin gene), as described.[53] The standard curves for absolute quantification were constructed with 1:10 serial dilutions of total DNA obtained from a negative GEB sample spiked with 10^5 parasite equivalents per milliliter of blood (par. eq./mL).

Ethics statement

All animal studies were carried out in strict accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA L038-2017).

Supporting Information

Copies of ^1H, ^{13}C and ^{19}F NMR spectra of compounds **77**, **21**, **27**, **28**, **29**, **37**, **40**, **41**, **45**, **47** and ^1H-^{13}C gHMBC and 2D NOESY spectra of compounds **67**, **14**, **16**, **20**, **77**, **78**, **79**, **25** and **54** can be found in the Supporting Information.

Abbreviations

BSA	*N,O*-bis(trimethylsilyl)acetamide
CD	Chagas disease
NTD	neglected tropical disease
T. cruzi	*Trypanosoma cruzi*
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPPTS	trisodium 3-bis(3-sulfonatophenyl) phosphanylbenzenesulfonate

Acknowledgements

C.L. thanks the China Scholarship Council (CSC) for a PhD scholarship (grant number 201708440366). F.H. thanks the Flanders Research Foundation (FWO) for a postdoctoral fellowship (1226921 N). The present work received funding from FWO (S.V.C., G.C., L.M.; project number G013118N). S.V.C. thanks the Hercules Foundation (project AUGE/17/22 "Pharm-NMR"). The present study was supported by grants from Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional Desenvolvimento científico e Tecnológico (CNPq), Fundação Oswaldo Cruz, PAEF/CNPq/Fiocruz, CAPES. M.N.C.S. and O.C.M. are research fellows of CNPq and CNE and JCNE researcher. The authors thank the excellent technical assistance of Marcos Meuser Batista, Roberson Donola Girão, Dr Cristian França da Silva, Dr Dara Denise da Gama Jaen Batista, Dr Gabriel M de Oliveira, Dr Ana Lia Mazzetti, Natascha Van Pelt, An Matheeussen and Margot Vleminckx.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: 7-deazapurine nucleosides · structure-activity relationships · *Trypanosoma cruzi* · in vivo efficacy

[1] B. Y. Lee, K. M. Bacon, M. E. Bottazzi, P. J. Hotez, *Lancet Infect. Dis.* **2013**, *13*, 342–348.
[2] J. M. Kratz, *Acta Trop.* **2019**, *198*, 105107.
[3] C. Bern, *N. Engl. J. Med.* **2015**, *373*, 456–466.
[4] C. Franco-Paredes, W. E. Villamil-Gomez, J. Schultz, A. F. Henao-Martinez, G. Parra-Henao, A. Rassi, A. J. Rodriguez-Morales, J. A. Suarez, *Travel. Med. Infect. Dis.* **2020**, *36*, 101565.
[5] S. Antinori, M. Corbellino, *Eur. J. Intern. Med.* **2018**, *48*, E29–E30.
[6] B. Monge-Maillo, R. Lopez-Velez, *Clin. Microbiol. Infect.* **2017**, *23*, 290–295.
[7] Drugs for Neglected Diseases initiative, *https://dndi.org/diseases/chagas/facts/* **2020**.
[8] US Centers for Disease Control and Prevention, *https://www.cdc.gov/parasites/chagas/biology.html* **2019**.
[9] World Health Organization, *https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)* **2020**.
[10] E. Bocchi, *Lancet* **2018**, *391*, 2209–2209.
[11] I. L. Galvan, J. Alonso-Padilla, N. Cortes-Serra, C. Alonso-Vega, J. Gascon, M. J. Pinazo, *Expert Rev. Anti-Infect. Ther.* **2020**, 1–10.
[12] I. Beltran-Hortelano, V. Alcolea, M. Font, S. Perez-Silanes, *Eur. J. Med. Chem.* **2020**, *206*, 112692.
[13] J. A. Urbina, *Lancet Infect. Dis.* **2018**, *18*, 419–430.
[14] M. De Rycker, B. Baragana, S. L. Duce, I. H. Gilbert, *Nature* **2018**, *559*, 498–506.
[15] M. C. Field, D. Horn, A. H. Fairlamb, M. A. Ferguson, D. W. Gray, K. D. Read, M. De Rycker, L. S. Torrie, P. G. Wyatt, S. Wyllie, I. H. Gilbert, *Nat. Rev. Microbiol.* **2017**, *15*, 217–231.
[16] M. Berg, P. Van der Veken, A. Goeminne, A. Haemers, K. Augustyns, *Curr. Med. Chem.* **2010**, *17*, 2456–2481.
[17] G. D. Campagnaro, H. P. de Koning, *Med. Res. Rev.* **2020**, *40*, 1679–1714.
[18] H. P. de Koning, D. J. Bridges, R. J. Burchmore, *FEMS Microbiol. Rev.* **2005**, *29*, 987–1020.
[19] A. K. Datta, R. Datta, B. Sen, *Adv. Exp. Med. Biol.* **2008**, *625*, 116–132.
[20] M. Berg, P. Van der Veken, A. Goeminne, A. Haemers, K. Augustyns, *Curr. Med. Chem.* **2010**, *17*, 2456–2481.
[21] P. Perlikova, M. Hocek, *Med. Res. Rev.* **2017**, *37*, 1429–1460.
[22] F. Hulpia, G. D. Campagnaro, M. Scortichini, K. Van Hecke, L. Maes, H. P. de Koning, G. Caljon, S. Van Calenbergh, *Eur. J. Med. Chem.* **2019**, *164*, 689–705.
[23] P. Naus, O. Caletkova, P. Konecny, P. Dzubak, K. Bogdanova, M. Kolar, J. Vrbkova, L. Slavetinska, E. Tloust'ova, P. Perlikova, M. Hajduch, M. Hocek, *J. Med. Chem.* **2014**, *57*, 1097–1110.
[24] A. Bourderioux, P. Naus, P. Perlikova, R. Pohl, I. Pichova, I. Votruba, P. Dzubak, P. Konecny, M. Hajduch, K. M. Stray, T. Wang, A. S. Ray, J. Y. Feng, G. Birkus, T. Cihlar, M. Hocek, *J. Med. Chem.* **2011**, *54*, 5498–5507.
[25] F. Hulpia, K. Van Hecke, C. Franca da Silva, D. da Gama Jaen Batista, L. Maes, G. Caljon, C. S. M. de Nazare, S. Van Calenbergh, *J. Med. Chem.* **2018**, *61*, 9287–9300.
[26] W. E. Gutteridge, J. Knowler, J. D. Coombes, *J. Protozool.* **1969**, *16*, 521–525.
[27] C. Lin, F. Hulpia, C. F. da Silva, D. Batista, K. Van Hecke, L. Maes, G. Caljon, M. N. C. Soeiro, S. Van Calenbergh, *J. Med. Chem.* **2019**, *62*, 8847–8865.
[28] J. L. Avila, T. Rojas, A. Avila, M. A. Polegre, R. K. Robins, *Antimicrob. Agents Chemother.* **1987**, *31*, 447–451.
[29] H. A. Tran, Z. Y. Zheng, X. H. Wen, S. Manivannan, A. Pastor, M. Kaiser, R. Brun, F. F. Snyder, T. G. Back, *Bioorg. Med. Chem.* **2017**, *25*, 2091–2104.
[30] F. Hulpia, J. Bouton, G. D. Campagnaro, I. A. Alfayez, D. Mabille, L. Maes, H. P. de Koning, G. Caljon, S. Van Calenbergh, *Eur. J. Med. Chem.* **2020**, *188*, 112018.
[31] C. Lin, C. Sun, X. Liu, Y. Zhou, M. Hussain, J. Wan, M. Li, X. Li, R. Jin, Z. Tu, J. Zhang, *Antiviral Res.* **2016**, *129*, 13–20.
[32] F. Hulpia, D. Mabille, G. D. Campagnaro, G. Schumann, L. Maes, I. Roditi, A. Hofer, H. P. de Koning, G. Caljon, S. Van Calenbergh, *Nat. Commun.* **2019**, *10*, 5564.
[33] F. Hulpia, G. D. Campagnaro, K. J. Alzahrani, I. A. Alfayez, M. A. Ungogo, D. Mabille, L. Maes, H. P. de Koning, G. Caljon, S. Van Calenbergh, *ACS Infect. Dis.* **2020**, *6*, 2045–2056.
[34] R. Wu, E. D. Smidansky, H. S. Oh, R. Takhampunya, R. Padmanabhan, C. E. Cameron, B. R. Peterson, *J. Med. Chem.* **2010**, *53*, 7958–7966.
[35] C. Lin, J. Yu, M. Hussain, Y. Zhou, A. Duan, W. Pan, J. Yuan, J. Zhang, *Antiviral Res.* **2018**, *149*, 95–105.
[36] E. M. de Souza, M. T. Rivera, T. C. Araujo-Jorge, S. L. de Castro, *Parasitol. Res.* **2001**, *87*, 513–520.
[37] F. Seela, X. Ming, *Tetrahedron* **2007**, *63*, 9850–9861.
[38] P. Naus, O. Caletkova, P. Konecny, P. Dzubak, K. Bogdanova, M. Kolar, J. Vrbkova, L. Slavetinska, E. Tloust'ova, P. Perlikova, M. Hajduch, M. Hocek, *J. Med. Chem.* **2014**, *57*, 1097–1110.
[39] F. Seela, X. Peng, *J. Org. Chem.* **2006**, *71*, 81–90.
[40] P. Perlikova, P. Konecny, P. Naus, J. Snasel, I. Votruba, P. Dzubak, I. Pichova, M. Hajduch, M. Hocek, *MedChemComm* **2013**, *4*, 1497–1500.
[41] M. P. Clark, M. W. Ledeboer, I. Davies, R. A. Byrn, S. M. Jones, E. Perola, A. Tsai, M. Jacobs, K. Nti-Addae, U. K. Bandarage, M. J. Boyd, R. S. Bethiel, J. J. Court, H. Deng, J. P. Duffy, W. A. Dorsch, L. J. Farmer, H. Gao, W. Gu, K. Jackson, D. H. Jacobs, J. M. Kennedy, B. Ledford, J. Liang, F. Maltais, M. Murcko, T. Wang, M. W. Wannamaker, H. B. Bennett, J. R. Leeman, C. McNeil, W. P. Taylor, C. Memmott, M. Jiang, R. Rijnbrand, C. Bral, U. Germann, A. Nezami, Y. Zhang, F. G. Salituro, Y. L. Bennani, P. S. Charifson, *J. Med. Chem.* **2014**, *57*, 6668–6678.
[42] L. J. Farmer, M. P. Clark, M. J. Boyd, E. Perola, S. M. Jones, A. Tsai, M. D. Jacobs, U. K. Bandarage, M. W. Ledeboer, T. Wang, H. Deng, B. Ledford, W. Gu, J. P. Duffy, R. S. Bethiel, D. Shannon, R. A. Byrn, J. R. Leeman, R. Rijnbrand, H. B. Bennett, C. O'Brien, C. Memmott, K. Nti-Addae, Y. L. Bennani, P. S. Charifson, *ACS Med. Chem. Lett.* **2017**, *8*, 256–260.
[43] C. Han, K. Green, E. Pfeifer, F. Gosselin, *Org. Process Res. Dev.* **2017**, *21*, 664–668.
[44] C. H. Franco, L. M. Alcantara, E. Chatelain, L. Freitas-Junior, C. B. Moraes, *Trop. Med. Infect. Dis.* **2019**, *4*, 82.
[45] L. F. de Almeida Fiuza, D. Batista, D. F. Nunes, O. C. Moreira, C. Cascabulho, M. N. C. Soeiro, *Exp. Parasitol.* **2021**, *221*, 108061.
[46] R. Edmondson, J. J. Broglie, A. F. Adcock, L. J. Yang, *Assay Drug Dev. Technol.* **2014**, *12*, 207–218.
[47] F. Hulpia, J. Bouton, G. D. Campagnaro, I. A. Alfayez, D. Mabille, L. Maes, H. P. de Koning, G. Caljon, S. Van Calenbergh, *Eur. J. Med. Chem.* **2020**, *188*, 112018.
[48] F. Hulpia, G. D. Campagnaro, M. Scortichini, K. Van Hecke, L. Maes, H. P. de Koning, G. Caljon, S. Van Calenbergh, *Eur. J. Med. Chem.* **2019**, *164*, 689–705.
[49] S. A. Ingale, P. Leonard, F. Seela, *J. Org. Chem.* **2018**, *83*, 8589–8595.
[50] Q. Li, L. Persoons, D. Daelemans, P. Herdewijn, *J. Org. Chem.* **2020**, *85*, 403–418.

[51] A. J. Romanha, S. L. Castro, N. Soeiro Mde, J. Lannes-Vieira, I. Ribeiro, A. Talvani, B. Bourdin, B. Blum, B. Olivieri, C. Zani, C. Spadafora, E. Chiari, E. Chatelain, G. Chaves, J. E. Calzada, J. M. Bustamante, L. H. Freitas-Junior, L. I. Romero, M. T. Bahia, M. Lotrowska, M. Soares, S. G. Andrade, T. Armstrong, W. Degrave, A. Andrade Zde, *Mem. Inst. Oswaldo Cruz* **2010**, *105*, 233–238.
[52] L. M. Nisimura, P. M. Ferrao, A. D. R. Nogueira, M. C. Waghabi, M. Meuser-Batista, O. C. Moreira, J. A. Urbina, L. R. Garzoni, *Mol. Biochem. Parasitol.* **2020**, *238*, 111283.
[53] T. Duffy, C. I. Cura, J. C. Ramirez, T. Abate, N. M. Cayo, R. Parrado, Z. D. Bello, E. Velazquez, A. Munoz-Calderon, N. A. Juiz, J. Basile, L. Garcia, A. Riarte, J. R. Nasser, S. B. Ocampo, Z. E. Yadon, F. Torrico, B. A. de Noya, I. Ribeiro, A. G. Schijman, *PLoS Neglected Trop. Dis.* **2013**, *7*, e2000.

Manuscript received: February 27, 2021
Accepted manuscript online: April 15, 2021
Version of record online: ■■, ■■■

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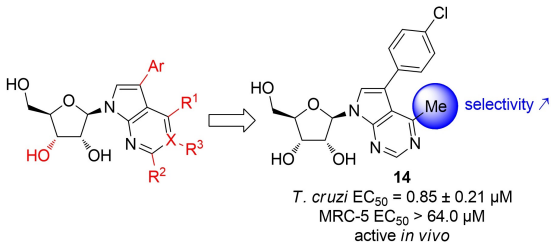

14

T. cruzi EC_{50} = 0.85 ± 0.21 μM
MRC-5 EC_{50} > 64.0 μM
active *in vivo*

C. Lin, L. Ferreira de Almeida Fiuza, C. Cardoso Santos, Dr. D. Ferreira Nunes, Dr. O. Cruz Moreira, J. Bouton, I. Karalic, Prof. L. Maes, Prof. G. Caljon, Dr. F. Hulpia, Prof. M. de Nazaré C. Soeiro*, Prof. S. Van Calenbergh**

A SAR investigation on the pyrimidine part of a previously identified 7-aryl-7-deazapurine nucleoside provided the most promising 6-methyl analogue against *T. cruzi*. A series of 7-aryl-6-methyl derivatives was prepared and evaluated, giving rise to several analogues with sub-micromolar antitrypanosomal activity. Compound **14** was found to be metabolically stable and active against *T. cruzi* after oral dose of 25 mg/kg b.i.d in an acute mouse model.