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Discovery of Novel 7-Aryl 7-Deazapurine 3′-Deoxy-ribofuranosyl Nucleosides with Potent Activity against Trypanosoma cruzi — Source link

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[Title page]

Discovery of novel 7-aryl 7-deazapurine 3'-deoxy-ribofuranosyl nucleosides with potent activity against *Trypanosoma cruzi*

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Abstract

Chagas disease, the leading cause of cardiac-related mortality in endemic Latin American countries. *Trypanosoma cruzi*, the disease-causing pathogen, is unable to synthesize purines *de novo*, necessitating salvage of pre-formed host purines. Therefore, purine and purine nucleoside analogs might constitute an attractive source to identify antitrypanosomal hits. In this study, structural elements of two purine nucleoside analogs, *i.e.* cordycepin and a recently discovered 7-substituted 7-deazaadenosine led to the identification of novel nucleoside analogs with potent *in vitro* activity. The structure-activity relationship of substituents at C7 was investigated, ultimately leading to the selection of compound **5** having a C7 *para*-chlorophenyl group for *in vivo* evaluation. This derivative showed complete suppression of *T. cruzi* Y-strain blood parasitemia when orally administered twice daily for 5 days at 25 mg/kg and was able to protect infected mice from parasite-induced mortality. However, sterile cure by immunosuppression could not be demonstrated.

Introduction

Trypanosoma cruzi, the etiological agent of Chagas disease, is a protozoan parasite that is endemic in Latin-American countries. Parasites are primarily transmitted via the faeces of the infected triatomine (or "kissing bug") vector. However, other routes for contracting the disease such as oral, congenital and iatrogenic (i.e. via blood transfusion or organ transplants) have been documented.[1-2] Although originally restricted to Latin-American countries, *T. cruzi* infections have also become an increasing concern in North-America, Japan, Europe and Australia as a result of increased migration flows. Clinical manifestations are dependent on two distinct disease stages. A first, acute disease stage generally lasts four to eight weeks, and is usually asymptomatic or patients develop rather vague symptoms such as fever and general malaise. After activation of the immune system, parasitemia fades (and becomes undetectable upon microscopic examination of blood smears), but the infection is never fully cleared. A second, chronic disease stage then develops that can remain unnoticed for decades. In around 30 to 40 percent of infected individuals, organ involvement will occur, most commonly cardiomyopathy and gastrointestinal disease leading to a so-called 'chronic symptomatic disease'. As a result, these organs will suffer irreversible damage, leading to major disability or death.[1-2]

At present, treatment of *T. cruzi* infections relies on benznidazole or nifurtimox,[3-4] of which only benznidazole has recently been approved by the FDA[5] (for pediatric use). Both drugs, in use since the early 1970s, suffer from several drawbacks: they have limited efficacy in the chronic stage of the disease and they cause significant side-effects.[1] Therefore, investigations into new chemical entities with improved efficacy and safety profiles and preferentially with a new mode-of-action should be pursued.[4] Recently, several reports on new lead molecules have been published, marking a renewed hope in finding drugs to treat Chagas disease.[6-10]

Trypanosoma cruzi, like their related *T. brucei* counterparts, are purine auxotrophs, *i.e.* they rely on the salvage of pre-formed purine analogs from the host as they are unable to synthesize the purine ring themselves.[11-14] Hence, focused purine (nucleoside) libraries are a promising source for discovering new antitrypanosomal agents. Natural antibiotics such as tubercidin,[15] Formycin A[16] and Formycin B,[17-19] cordycepin,[19-23] stylomycin aminonucleoside (also known as puromycin aminonucleoside) [24-25] as well

as allopurinol[26-27] and certain other inosine analogs[20, 28-29] have been found to possess activity against *T. cruzi* (Figure 1). Allopurinol, although not a nucleoside analog *sensu stricto* (its active metabolite is generated by phosphoribosylation in the parasite[26]), has been evaluated in clinical trials.[30]

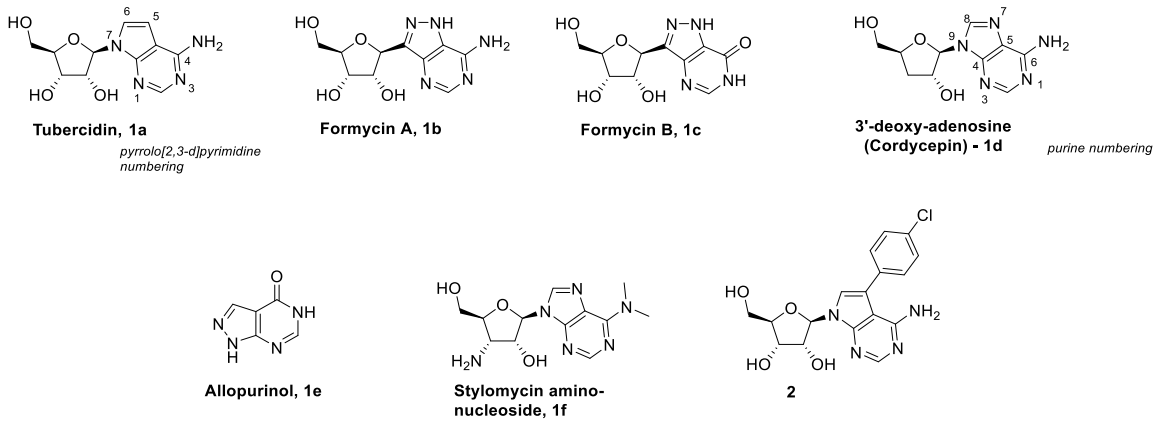


Figure 1: Examples of purine (nucleoside) analogs active against *T. cruzi*.

Our group recently revisited the natural nucleoside antibiotic tubercidin and a series of 7-substituted analogs [in the body of the text, purine numbering will be used for nucleoside analogs; however, in the experimental section, IUPAC nomenclature and numbering of the pyrrolo[2,3-*d*]pyrimidine system will be applied] in search of novel hits active against *T. brucei* spp. parasites.[31] This study indicated that certain phenyl-substituted analogs (*e.g.* **2**) also showed promising *in vitro* activity against intracellular *T. cruzi* amastigotes, which motivated us to explore related 7-substituted 7-deazapurine moieties with the carbohydrate group of cordycepin, *i.e.* a 3'-deoxy-D-ribofuranose for their activity against *T. cruzi*. Related sugar-modified 7-deazapurine nucleosides[32-34] have previously been assayed mainly for their antiviral and/or antiproliferative activity.[35]

An overview of the synthesized analogs is given in Figure 2. Optimization efforts of the *in vitro* potency against, as well as results from *in vivo* studies in a mouse model of *T. cruzi* infection will be discussed.

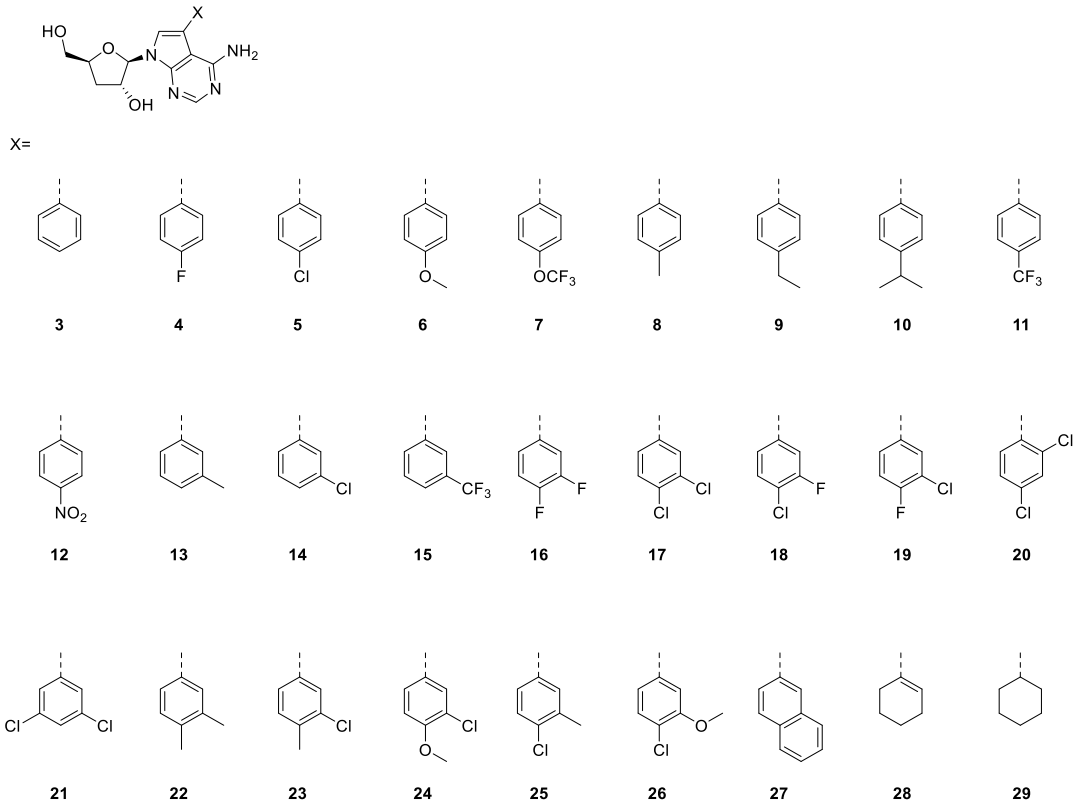
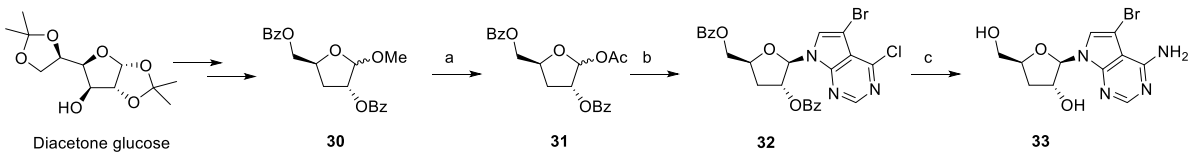


Figure 2: Overview of the 3'-deoxy-7-deazaadenosine analogs prepared in this study.

Results and discussion

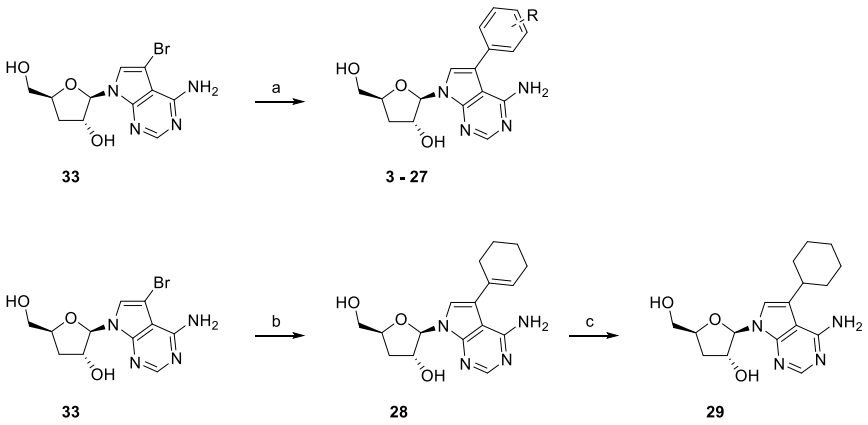
Chemistry

Synthesis of the target analogs was based on coupling of the ester-protected 3'-deoxyribofuranose **31** with 4-bromo-5-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine[36] under Vorbrüggen conditions (Scheme 1).[37] The glycosyl donor was prepared employing literature procedures starting from commercially available diacetone-D-glucose.[38-40] Initially, the 2-OH was protected as an acetate ester, but this was found to give vastly inferior glycosylation yields compared to the 2-*O*-benzoyl-protected donor (data not shown). After glycosylation, both the correct stereochemistry at C-1' (β) and the correct regio-chemistry (N9; purine numbering) of **32** was ascertained by means of 2D NMR techniques (2D NOESY, ^{1}H-^{13}C gHMBC respectively; see Supporting Information). Next, deprotection and C6 amination were accomplished upon treatment of **32** with 7N NH_3 in MeOH.[37]



Scheme 1: Reagents and conditions: a) Ac_2O, H_2SO_4, HOAc, 97 %; b) BSA, 4-chloro-5-bromo-7*H*-pyrrolo[2,3-*d*]pyrimidine,[36] TMSOTf, MeCN, 80 °C, 1H, 80 %; c) 7N NH_3/MeOH, 130 °C, overnight, 60 %.

Modification at the C7 position of the deazapurine ring was accomplished via aqueous Suzuki coupling with the corresponding arylboronic acids or, for the synthesis of analog **28**, with cyclohexenyl potassium trifluoroborate salt (Scheme 2).[31, 41] Cyclohexenyl derivative **28** was reduced to the corresponding cyclohexyl analog **29** by means of Pd-catalyzed hydrogenation.



Scheme 2: Reagents and conditions: a) arylboronic acid, Na_2CO_3, $Pd(OAc)_2$, TPPTS, MeCN/water (1:2 ratio), 100 °C, 30-82 %; b) potassium 1-cyclohexen-1-yltrifluoroborate, Cs_2CO_3, $Pd(OAc)_2$, TPPTS, MeCN/water (1 : 2 ratio), 100 °C, 88 %; c) Pd/C, H_2 (balloon), MeOH, 83 %.

Single crystal X-ray analysis of **5** was performed after crystallization from MeCN/water, and formally proved the structure. The ribose ring of the nucleoside analog **5** is found to adopt the 3'-endo conformation (Figure 3), consistent with literature.[42] In the crystal structure, dimers were formed. Additionally, one molecule of **5** displayed positional disorder, which is presented in the Supporting Information (Supplementary Figure S1-S4).

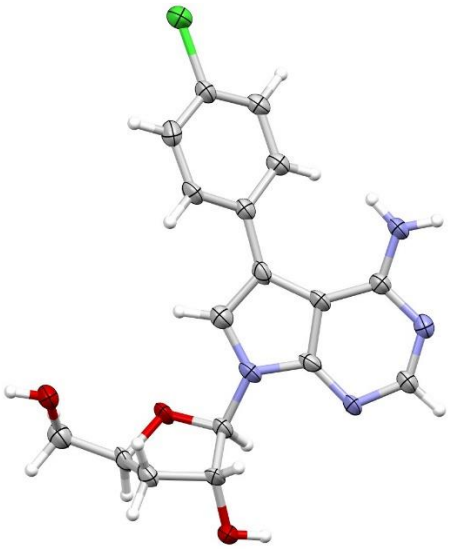


Figure 3: Molecular structure of **5**, showing thermal displacement ellipsoids at the 50% probability level. Only one (non-disordered) molecule of the asymmetric unit is shown.

Biological evaluation

In vitro evaluation

All synthesized compounds were evaluated against intracellular *T. cruzi* amastigotes (Tulahuen strain expressing β-galactosidase), as well as for cytotoxicity against MRC-5 fibroblasts (Table 1).

Cpd.	*T. cruzi* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI	Cpd.	*T. cruzi* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI
1d	2.51 ± 0.22	>64.0	>25	**16**	0.33 ± 0.02	>64.0	>190
2	0.47 ± 0.25[a]	27.0 ± 2.5[a]	57	**17**	0.059 ± 0.003	13.6 ± 4.5	230
3	2.57 ± 0.19	61.8 ± 2.3	24	**18**	0.071 ± 0.008	18.6 ± 3.5	260
4	0.64 ± 0.007	>64.0	>100	**19**	0.30 ± 0.04	45.5 ± 0.8	150
5	0.047 ± 0.006	19.1 ± 4.5	410	**20**	0.45 ± 0.06	19.6 ± 10.5	44
6	2.38 ± 0.33	24.3 ± 3.5	10	**21**	0.55 ± 0.03	30.2 ± 2.0	55
7	1.39 ± 0.27	40.4 ± 6.0	29	**22**	1.37 ± 0.37	22.3 ± 0.4	16
8	0.46 ± 0.13	21.0 ± 0.3	46	**23**	0.27 ± 0.13	17.8 ± 3.0	66
9	1.04 ± 0.25	18.4 ± 7.4	18	**24**	3.30 ± 0.24	>64.0	>19
10	8.48 ± 0.65	24.9 ± 0.5	2.9	**25**	0.21 ± 0.07	16.7 ± 0.2	80
11	0.53 ± 0.12	40.0 ± 10.5	76	**26**	0.059 ± 0.017	17.2 ± 2.7	29
12	1.21 ± 0.29	>64.0	>53	**27**	0.72 ± 0.03	5.0 ± 1.4	7.0
13	5.15 ± 1.47	>64.0	>12	**28**	31.8 ± 0.7	>64.0	>2.0
14	0.97 ± 0.18	36.1 ± 1.7	37	**29**	20.90 ± 0.98	>64.0	>3.0
15	11.2 ± 1.3	49.6 ± 10.7	4.4	**BZ**	2.28 ± 0.22	N.D.	N.D.

Table 1: *In vitro* activity of all final analogs against *T. cruzi* (Tulahuen strain expressing β-galactosidase) intracellular amastigotes. MRC-5 fibroblasts were used as host cell as wells as for cytotoxicity evaluation. EC_{50} values are presented in µM and are depicted as mean and SE of 2-3 independent determinations. BZ: Benznidazole. N.D.: Not determined. [a]Values taken from reference [31]. SI: selectivity index: (EC_{50} MRC-5) / (EC_{50} *T. cruzi*).

The activity of cordycepin (3'-deoxy-adenosine, **1f**, *TCMDC-143080*[19]) was confirmed in the intracellular amastigote assay. The EC_{50} of 2.51 ± 0.22 μM was similar to that reported by GSK (EC_{50} = 1 μM in the β-Gal assay).[19] Introduction of a phenyl group at the C7 position (**3**) hardly influenced the activity (EC_{50} = 2.57 ± 0.19 μM). Next, the influence of the substitution pattern of the phenyl ring of **3** was investigated. First, *para*-substituted **5** (Cl), **6** (OMe) and **8** (Me) were prepared, with the activity order being Cl > Me > H > OMe, showing a preference for both electron-withdrawing as well as lipophilic substituents. Pleasingly, **5** already displayed double-digit nM potency with an encouraging selectivity index (SI ~400). Next, investigation continued with electron-withdrawing (**7**, **11**, **12**, and *meta*-substituted analogs **14** & **15**), and lipophilic (alkyl) substituents (**9**, **10**, and *meta*-substituted **13**). A visual analysis tracking the activity (expressed as pEC_{50}) as a function of either electronic (σ) or lipophilic (π) parameter[43] is presented in Figure 5. It became apparent that sterically more demanding groups such as –isopropyl (**10**) are disfavored. The analysis of the different electron-withdrawing substituents presented a rather complicated story. The *para*-CF_3 analog (**11**), that features preferred +π and +σ descriptors, gave an EC_{50} value of 0.53 ± 0.12 μM; slightly weaker than the corresponding methyl analog **8** (EC_{50} = 0.46 ± 0.13 μM) which might be due to its bigger steric requirement.[44] Next, comparison of *para*- versus *meta*- substituted phenyl pairs (**5** & **14**, **8** & **13** and **11** & **15**) indicated that *para*-substituted analogs are preferred (equal or higher than 10-fold activity difference). Continuing, evaluation of the saturated cyclohexyl **29** and its cyclo-hexenyl precursor **28**, points to the importance of a flat aromatic system, and led us to discontinue further investigation into saturated analogs.

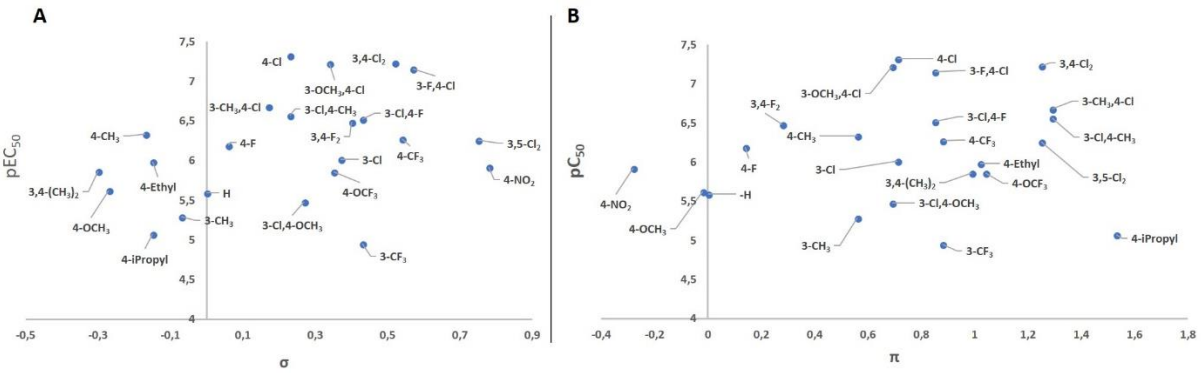


Figure 4: SAR analysis; pEC_{50} values (*T. cruzi;* Table 1) of selected nucleoside analogs are plotted as a function of either the electronic (σ, Panel A) or lipophilicity (π, Panel B) parameter of the substituent(s) present on the phenyl ring. Each dot represents a nucleoside analog with the phenyl ring substituent(s) indicated. σ- and π-values are taken from reference [43]

Next, different di-substituted analogs, **17**, **20**, **21** and **22**, as well as the 2-naphtyl derivative **27** were prepared and assayed. Four of these analogs gave EC_{50} values surpassing 1 µM, with **17** being equipotent to **5**. Chlorinated analogs performed better, especially those with a chlorine substituent in the *para*-position (compare **17**, **20** & **21**). Next, a final set of disubstituted derivatives **18**, **25**, **26**, all bearing a *para*-chlorine substituent, and their respective constitutional isomers **19**, **23**, **24** were prepared in an attempt to further improve the *in vitro* activity. Additionally, fluorinated derivatives **4** and **16** were synthesized as well. Remarkably, the 3-OMe-4-Cl phenyl analog **26**, but not the 3-Me-4-Cl phenyl analog **25**, displayed similar activity as did the mono-substituted **5**. As expected, **18** showed excellent activity ($EC_{50} = 0.071 \pm 0.008$), albeit not better than the parent *para*-chloro analog. Fluorinated analogs **4** and **16** gave slightly weaker antitrypanosomal activity (roughly 13- and 7-fold) with respect to **5**, but on the other hand were devoid of cytotoxicity (up to the highest assay concentration) against MRC-5 fibroblasts ($EC_{50} > 64.0$ µM).

Investigation into different mono- and di-substituted phenyl analogs attached to the C7 position of 3'-deoxy tubercidin gave rise to several analogs with sub-micromolar to double-digit nanomolar potency against intracellular amastigotes. Comparing the *in vitro* activity of **2**[31] and **5** revealed a 10-fold gain in

cellular activity that was accompanied by the removal of the 3'-OH, while cytotoxicity was comparable. Generally, all 3'-deoxy analogs showed a better *in vitro* profile,[31] except for analog **4**.

From this *in vitro* optimization, **5** emerged as the most active lead, with other analogs displaying similar activity (**17**, **18** and **26**), but they all have higher molecular weight. Therefore, **5** was chosen for further investigation in an *in vivo* mouse model of *T. cruzi*.

Evaluation of metabolic stability of compounds 2 & 5

Next, we assessed the *in vitro* metabolic stability of analog **5** by exposing it to mouse, rat, dog and human S9 microsomal fractions. The percentages of analog **5** remaining at different time points after incubation with the various microsomes in the presence of either NADPH (phase-I) and UGT-enzymes (phase-II) are presented in Table 2. Previously discovered analog **2** was investigated as well to explore possible differences of this ribofuranose nucleoside analog with its 3'-deoxy analog **5**.

Phase I / II	Time	MOUSE % comp. 2 remaining		RAT % comp. 2 remaining		DOG % comp. 2 remaining		HUMAN % comp. 2 remaining	
		Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
CYP - NADPH	0	100	-	100	-	100	-	100	-
	15	110	17.7	122	10.3	93	2.1	113	30.7
	30	95	1.2	104	14.4	96	0.6	93	16.5
	60	102	18.8	106	11.2	98	3.9	86	0.1
UGT Enzymes	0	100	-	100	-	100	-	100	-
	15	124	34.4	100	2.5	84	9.8	99	8.6
	30	110	3.3	93	10.0	70	5.5	96	0.6
	60	106	14.0	93	6.2	55	7.7	91	18.2

Phase I / II	Time	MOUSE % comp. 5 remaining		RAT % comp. 5 remaining		DOG % comp. 5 remaining		HUMAN % comp. 5 remaining	
		Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
CYP - NADPH	0	100	-	100	-	100	-	100	-
	15	86	3.0	101	6.8	98	4.7	102	0.1
	30	75	2.9	101	3.8	96	2.6	95	6.6
	60	68	6.6	91	1.5	90	4.3	93	4.6
UGT Enzymes	0	100	-	100	-	100	-	100	-
	15	101	8.2	101	1.0	96	3.0	100	5.0
	30	95	9.6	96	8.3	96	14.7	106	4.6
	60	89	0.2	96	5.4	95	8.8	100	5.7

Table 2: Assessment of *in vitro* phase I and phase II metabolic stability of analogs **2** and **5** using mouse, rat, dog and pooled human S9 microsomal fractions. Indicated is the percentage of parent compound remaining at various time points of incubation (0-15-30-60 min). Mean values were calculated from two replicates of two independent experiments. Proper functioning of the *in vitro* assay was confirmed with the reference drug diclofenac (susceptible to phase-I and phase-II metabolism) and fluconazole (metabolically stable through phase-I) (data not shown).

The data depicted in Table 2 show that neither analog is severely affected by either phase-I or phase-II metabolism (>50% parent remaining at 30 min as cut-off), irrespective of the species used. This is re-assuring as we previously noted extensive oxidative metabolism of a related analog.[31] We therefore conclude that the metabolic stability of both analogs warranted *in vivo* evaluation.

In vivo evaluation

Analogs **2** and **5** were dosed in an acute infection murine model of *T. cruzi* (Y-strain) at 25 mg/kg b.i.d. by oral gavage for five consecutive days. BZ was included as a reference control (100 mg/kg, s.i.d., p.o., five consecutive days). Treatment started at the onset of parasitemia, which corresponds to 5 days post infection (dpi). Compound **5** (Figure 5) completely suppressed parasitemia in treated animals and showed a 94.6 % decrease in the circulating parasitemia, measured at 8dpi (Figure 5, Panel C). The efficacy of **5** at 8dpi was similar to BZ (100 mg/kg, sid, p.o.). All vehicle-treated (control) animals succumbed to the infection by 15 dpi (Figure 5, Panel D), while administration of **5** was able to protect against mortality, as did benznidazole.

Compound **2** which was also evaluated in this acute infection model (Figure 5) and elicited more than 99.8 % parasitemia reduction as assayed at 8 dpi, presenting equal parasite reduction levels as BZ. All animals in the vehicle control group succumbed to the infection between 14 dpi and 22 dpi (Figure 5, Panel B), while all treated animals survived (Figure 5, Panel D).

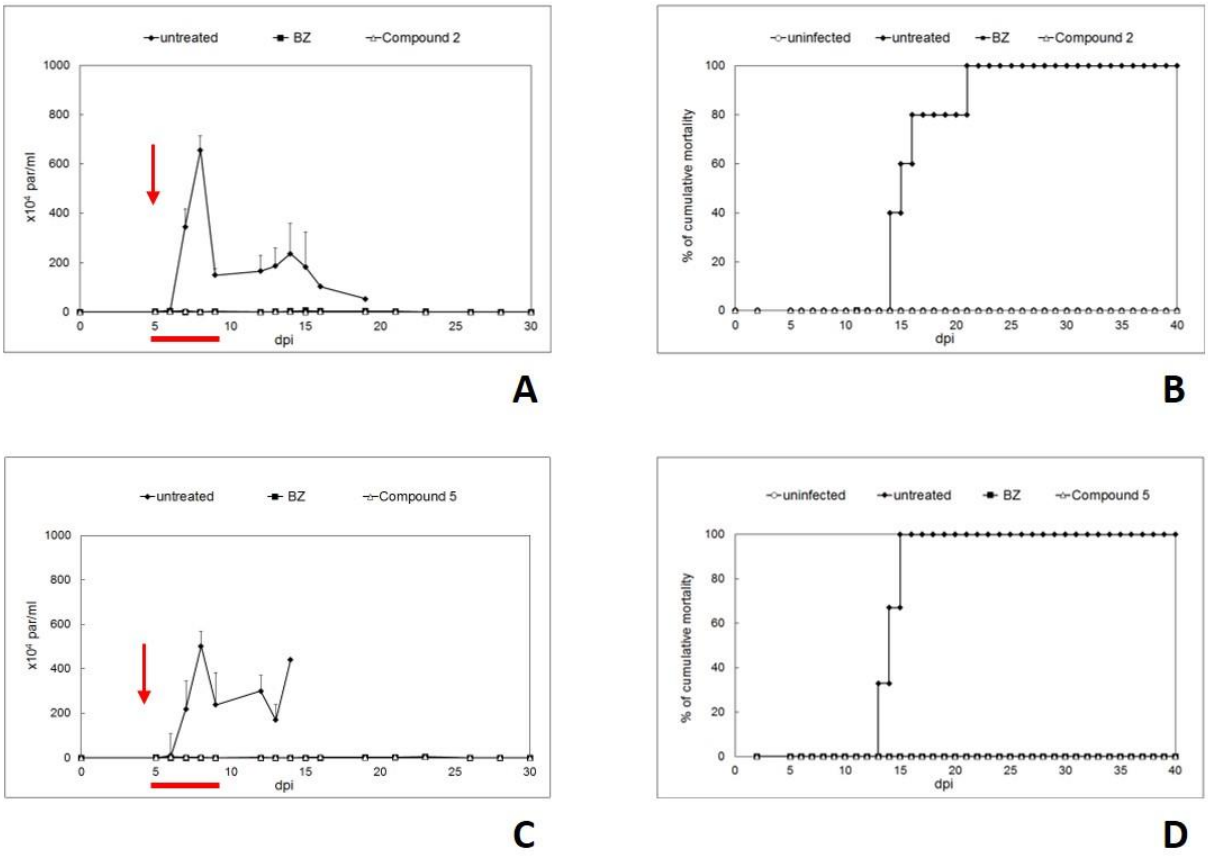


Figure 5: *In vivo* results of compound **2** and compound **5** in an acute Y-strain *T. cruzi* mouse infection model. Animals were infected with 10^4 Y-strain bloodstream trypomastigotes (i.p.) at day 0. Treatment with compound **2** (panel A & B) or compound **5** (panel C & D) at 25 mg/kg b.i.d. (oral gavage) for five consecutive days (red line) was initiated at 5dpi (red arrow). Benznidazole (BZ) given at 100 mg/kg s.i.d. (oral gavage) for five consecutive days was included as reference. Panel A & C: blood parasitemia as determined microscopically from tail vain blood (5 µL). Panel B & D: cumulative mortality.

Treatment with either **2** or **5** resulted in 100 % survival up to 30 days post-administration of the final dose (corresponding with 40 dpi), without relapses as determined by blood parasitemia.[45] Additionally, no adverse events nor weight loss were noted, indicating that these derivatives were well tolerated by the test animals. Encouraged by these results, surviving mice (from treatment with **2**, **5** as well as BZ) were immunosuppressed by three successive cycles of cyclophosphamide treatment (50 mg/kg, s.i.d., i.p.) in order to probe for sterile cure.[45] Unfortunately, all immunocompromised mice from the

nucleoside **2** and **5** treatment groups relapsed (data not shown). However, the same outcome was found for benznidazole, which is in accordance with recent studies.[46-48] In fact, the use of this highly stringent experimental model (Swiss male mice[49] infected with an inoculum of 10^4 bloodstream trypomastigotes of Y-strain *T. cruzi*) requires a longer treatment period with BZ to establish sterile cure.[46, 48]

Differences in drug potency between intracellular and bloodstream forms might explain the lack of sterile cure. This has been reported for CYP51 inhibitors,[50] including posaconazole[51] which was investigated in clinical trials.[52-53] In order to gain insight into this possibility, both **2** and **5** were assayed *in vitro* against Y-strain bloodstream form trypomastigotes and intracellular amastigotes (Table 3) as well as for cytotoxicity towards primary mouse cardiac host cells. Both nucleoside analogs were non-toxic towards the cardiac cells (EC_{50} >200 µM). Next, **2** as well as **5** displayed weaker activity against bloodstream trypomastigotes, while retaining potent activity against intracellular amastigotes. Y-strain amastigotes were equally sensitive to both **2** and **5**, which were at least 10-fold more potent than benznidazole.

Cpd.	Y-strain trypomastigotes EC_{50} (µM)	Y-strain intracellular amastigotes[a] EC_{50} (µM)	Cardiac cells EC_{50} (µM)
2	>20	0.029 ± 0.0	274 ± 48[b]
5	>20	0.029 ± 0.0	284 ± 38
Benznidazole	11.5 ± 1.1	0.354 ± 0.2	>400

Table 3: *In vitro* efficacy of selected nucleoside analogs against *T. cruzi* (Y-strain) bloodstream trypomastigotes, intracellular amastigotes (cardiac cells as host cell), as well as toxicity on cardiac cells. EC_{50} values are given in µM and are depicted as mean and SE of two independent determinations. [a]Activity against intracellular amastigotes is expressed as the reduction of infection index (*i.e.* percentage of infected host cells x number of parasites per host cell. [b]Cardiac cells incubated with compound **2** showed a lack of cardiac cell contractibility (*in vitro*) at 15 µM.

A stage-specific effect has previously been described for the nucleoside analog Formycin A (**1b**, Figure 1).[54] In this case, this could be linked – in part – to altered drug uptake between the different life-stage forms. It was found that the uptake of adenosine was affected as well, but not that of the corresponding inosine analog Formycin B (**1c**), which could possibly point to a specific amino-nucleoside recognition effect.[54] At present though, the details of nucleoside transport in *T. cruzi* are yet to be uncovered, and it is unknown to what extent – if any – compounds **2** and **5** share the same mechanism-of-action with Formycin A (**1b**) and/or B (**1c**). Additional studies regarding transporter-mediated uptake in *T. cruzi* as well as into the mechanism-of-action of these nucleoside analogs are of significant interest.

Conclusion

In the present paper, we have combined structural elements of both cordycepin (3'-deoxyribofuranose part) and the substituted purine ring of a previously discovered nucleoside analog **2**, with the goal to discover potent and novel leads for the treatment of *T. cruzi* infections. Structure-activity relationship investigation indicated that the C7 phenyl ring can have both lipophilic and electron withdrawing substituents in the *para*-position with a chlorine (compound **5**) representing the optimal substituent. 3'-deoxygenation generally conferred a ten-fold gain in *in vitro* potency (Tulahuen strain). Analog **5** was selected for further *in vivo* investigation along with its ribofuranose congener **2**. Both derivatives were able to fully suppress parasitemia in a 5-day b.i.d. oral dosing schedule, without apparent toxicity. Unfortunately, upon cyclophosphamide-induced immunosuppression recrudescence appeared. This may be related to the lower activity of the compounds against bloodstream forms as opposed to intracellular amastigote life-stage forms. Both nucleoside analog **2** and **5** represent promising lead compounds and are worthy of further investigation.

Experimental section

Chemistry

All reagents and solvents were obtained from standard commercial sources and were of analytical grade. Unless otherwise specified, they were used as received. **30** was prepared from commercially available diacetone-D-glucose according to literature procedures.[38-40] Cordycepin (**1d**) was prepared from adenosine, as described in literature.[55] All moisture sensitive reactions were carried out under argon atmosphere. Reactions were carried out at ambient temperature, unless otherwise indicated. Analytical TLC was performed on Machery-Nagel® pre-coated F254 aluminum plates and were visualized by UV followed by staining with basic aq. $KMnO_4$ or sulfuric acid-anisaldehyde spray. Column chromatography was performed using Davisil® (40-63 µm) or on a Reveleris X2 (Grace/Büchi) automated Flash unit employing pre-packed silica columns. Exact mass measurements were performed on a Waters LCT Premier XE™ Time of Flight (ToF) mass spectrometer equipped with a standard electrospray (ESI) and modular Lockspray™ interface. Samples were infused in a MeCN / water (1:1) + 0.1 % formic acid mixture at 100 µL / min. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts (δ) are given in ppm and spectra are referenced to the residual solvent peak signal. Coupling constants are given in Hz. In ^{19}F-NMR, signals were referenced to $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm. Melting points were determined on a Büchi-545 apparatus and are uncorrected. Purity of final compounds was assessed by means of analytical LC-MS employing a Waters AutoPurification system (equipped with ACQUITY QDa (mass; 100 – 1000 amu)) and 2998 Photodiode Array (220 – 400 nm)) using a Waters Cortecs® C18 (2.7 µm 100x4.6mm) column and a gradient system of formic acid in H_2O (0.2 %, v/v) / MeCN at a flow rate of 1.44 mL / min, 95:05 to 00:100 in 6.5 minutes. All obtained final compounds had purity > 95 %, as assayed by analytical HPLC (integration of UV signal: total UV as well as at 254 nm); unless otherwise indicated. Several compounds were found to possess residual MeOH, of which integration of the CH_3 signal in the ^{1}H NMR spectrum never exceeded 3H.

General procedure A (Suzuki coupling):

33 (1 eq.), boronic acid (1.5 eq.) or c-hexylpotassiumtrifluoroborate salt (1.5 eq.) [for compound **28**], Na_2CO_3 (9 eq.) or Cs_2CO_3 (9 eq.) [for compound **28**], $Pd(OAc)_2$ (0.05 eq.) and TPPTS (0.15 eq.) were added to a 10 mL round-bottom flask, equipped with a stir bar. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, degassed MeCN (2 mL / mmol SM) and H_2O (4 mL / mmol SM) were added to the solids under argon. After 5 min of stirring, the mixture was heated to reflux. When the starting material was fully consumed (usually 1 – 3 hours; as monitored by LC-MS analysis), the mixture was cooled to ambient temperature, and neutralized (pH ~ 7) with 0.5 M aq. HCl. The mixture was evaporated till dryness, resuspended in MeOH and evaporated (three times). Next, the mixture was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~ 5 cm) with 20 % MeOH / DCM. The liquid was evaporated *in vacuo* and purified by column chromatography 1 → 10 % MeOH / DCM.

4-amino-5-phenyl-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (3) 3 was prepared according to general procedure A. **33** (0.17 g, 0.50 mmol) gave rise to **3** (0.10 g, 0.29 mmol) as a white solid. Yield: 58 %. Melting point: 109 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.25 (ddd, *J* = 13.2, 8.7, 6.0 Hz, 1H, H-3''), 3.51 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.27 – 4.34 (m, 1H, H-4'), 4.45 – 4.50 (m, 1H, H-2'), 5.02 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.10 (br. s, 2H, NH_2), 6.13 (d, *J* = 2.4 Hz, 1H, H-1'), 7.34 – 7.41 (m, 1H, H_{Phe}), 7.46 – 7.52 (m, 4H, H_{Phe}), 7.55 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.2 (C-4a), 116.1 (C-5), 120.7 (C-6), 126.8 (C-1_{Phe}), 128.4 (2C, C_{Phe}), 129.0 (2C, C_{Phe}), 134.6 (C-4_{Phe}), 150.4 (C-7a), 151.7 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{17}H_{19}N_4O_3$ ($[M+H]^+$): 327.1452, found: 327.1448.

4-amino-5-(4-fluorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (4) 4 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **4** (0.096 g, 0.27 mmol)

as a white solid. Yield = 69 %. Melting point: 186 – 187 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.7, 4.2 Hz, 1H, H-3'), 3.50 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.43 – 4.49 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.11 (d, *J* = 2.7 Hz, 1H, H-1'), 6.14 (br. s, 2H, NH_2), 7.26 – 7.34 (m, 2H, H-3_{Phe}, H-5_{Phe}), 7.45 – 7.51 (m, 2H, H-2_{Phe}, H-6_{Phe}), 7.53 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -116.18 – -116.08 (m, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.2 (C-4a), 115.0 (C-5), 115.7 (d, *J* = 21.8 Hz, 2C, C-3_{Phe}, C-5_{Phe}), 120.7 (C-6), 130.3 (d, *J* = 8.3 Hz, 2C, C-2_{Phe}, C-6_{Phe}), 130.9 (C-1_{Phe}), 150.3 (C-7a), 151.7 (C-2), 157.3 (C-4), 161.4 (d, *J* = 241.5 Hz, 1C, C-4_{Phe}). HRMS (ESI): calculated for $C_{17}H_{18}FN_4O_3$ ($[M+H]^+$): 345.1357, found: 345.1363.

4-amino-5-(4-chlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (5) 5 was prepared according to general procedure A. **33** (0.16 g, 0.49 mmol) gave rise to **5** (0.062 g, 0.17 mmol) as a white solid. Yield = 35 %. Melting point: 200 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 12.9, 8.4, 6.0 Hz, 1H, H-3'), 3.50 (ddd, *J* = 11.7, 5.7, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 11.7, 5.4, 3.6 Hz, 1H, H-5'), 4.27 – 4.34 (m, 1H, H-4'), 4.44 – 4.49 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 6.19 (br. s, 2H, NH_2), 7.45 – 7.49 (m, 2H, H_{Phe}), 7.50 – 7.54 (m, 2H, H_{Phe}), 7.58 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.9 (C-5), 121.0 (C-6), 128.8 (2C, C_{Phe}), 130.1 (2C, C_{Phe}), 131.4 (C_{Phe}), 133.4 (C_{Phe}), 150.5 (C-7a), 151.7 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}ClN_4O_3$ ($[M+H]^+$): 361.1062, found: 361.1066.

4-amino-5-(4-methoxyphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (6) 6 was prepared according to general procedure A. **33** (0.16 g, 0.49 mmol) gave rise to **6** (0.11 g, 0.30 mmol) as a white solid. Yield = 60 %. Melting point: 163 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 3.50 (ddd, *J* = 12.0, 5.1, 4.5 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.4, 3.9 Hz, 1H, H-5'), 3.80 (s, 3H, OCH_3), 4.25 – 4.33 (m, 1H, H-4'), 4.43 – 4.49 (m, 1H, H-2'), 5.01 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.56 (d, *J* = 4.5 Hz, 1H, OH-2'),

6.06 (br. s, 2H, NH_2), 6.11 (d, *J* = 2.7 Hz, 1H, H-1'), 7.02 – 7.07 (m, 2H, H_{Phe}), 7.36 – 7.41 (m, 2H, H_{Phe}), 7.45 (s, 1H, H-6), 8.14 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 55.2 (OCH_3), 62.8 (C-5'), 74.8 (C-2'), 79.8 (C-4'), 90.1 (C-1'), 100.4 (C-4a), 114.4 (2C, C_{Phe}), 115.7 (C-5), 120.1 (C-6), 126.7 (C-1_{Phe}), 129.7 (2C, C_{Phe}), 150.2 (C-7a), 151.6 (C-2), 157.3 (C-4), 158.4 (C-4_{Phe}). HRMS (ESI): calculated for $C_{18}H_{21}N_4O_4$ ($[M+H]^+$): 357.1557, found: 357.1550.

4-amino-5-(4-trifluoromethoxyphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (7) 7 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **7** (0.15 g, 0.37 mmol) as a white solid. Yield = 82 %. Melting point: 194 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.7, 5.7 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 11.7, 5.4, 3.6 Hz, 1H, H-5'), 4.27 – 4.34 (m, 1H, H-4'), 4.43 – 4.49 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.13 (d, *J* = 2.4 Hz, 1H, H-1'), 6.20 (br. s, 2H, NH_2), 7.44 – 7.47 (m, 2H, H_{Phe}), 7.55 – 7.59 (m, 2H, H_{Phe}), 7.60 (s, 1H, H-6), 8.17 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -56.7. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 75.0 (C-2'), 80.0 (C-4'), 90.2 (C-1'), 100.1 (C-4a), 114.8 (C-5), 120.19 (q, *J* = 254.2 Hz, 1C, OCF_3), 121.3 (C-6), 121.5 (2C, C_{Phe}), 130.1 (2C, C_{Phe}), 134.0 (C-1_{Phe}), 147.2 (d, *J* = 2.3 Hz, 1C, C-4_{Phe}), 150.5 (C-7a), 151.8 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}F_3N_4O_4$ ($[M+H]^+$): 411.1275, found: 411.1262.

4-amino-5-(4-methylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (8) 8 was prepared according to general procedure A. **33** (0.17 g, 0.50 mmol) gave rise to **8** (0.066 g, 0.19 mmol) as a white solid. Yield = 38 %. Melting point: 116 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 2.36 (s, 3H, CH_3), 3.47 – 3.54 (m, 1H, H-5''), 3.62 – 3.68 (m, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.44 – 4.49 (m, 1H, H-2'), 5.02 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.09 (br. s, 2H, NH_2), 6.11 (d, *J* = 2.7 Hz, 1H, H-1'), 7.28 – 7.30 (m, 2H, H_{Phe}), 7.35 – 7.37 (m, 2H, H_{Phe}), 7.49 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 20.7 (CH_3), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.3 (C-4a), 116.0 (C-5), 120.3 (C-6), 128.4 (2C, C_{Phe}), 129.5 (2C, C_{Phe}), 131.6 (C-1_{Phe}), 136.1

(C-4_{Phe}), 150.3 (C-7a), 151.6 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{21}N_4O_3$ ([M+H]$^+$): 341.1608, found: 341.1602.

4-amino-5-(4-ethylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl-pyrrolo[2,3-*d*]pyrimidine (9) 9 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **9** (0.10 g, 0.28 mmol) as a white solid. Yield = 71 %. Melting point: 114 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.22 (t, *J* = 7.8 Hz, 3H, CH_3), 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 12.9, 8.7, 6.0 Hz, 1H, H-3'), 2.66 (q, *J* = 7.8 Hz, 2H, CH_2), 3.50 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.4, 3.3 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.44 – 4.49 (m, 1H, H-2'), 5.02 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.08 (br. s, 2H, NH_2), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 7.30 – 7.33 (m, 2H, H_{Phe}), 7.38 – 7.40 (m, 2H, H_{Phe}), 7.50 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.6 (CH_3), 27.8 (CH_2), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.3 (C-4a), 116.0 (C-5), 120.4 (C-6), 128.35 (2C_{Phe}), 128.40 (2C_{Phe}), 131.9 (C_{Phe}), 142.4 (C_{Phe}), 150.3 (C-7a), 151.6 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{23}N_4O_3$ ([M+H]$^+$): 355.1765, found: 355.1772.

4-amino-5-(4-isopropylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (10) 10 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **10** (0.13 g, 0.34 mmol) as a white solid. Yield = 76 %. Melting point: 111 – 115 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.25 (d, *J* = 7.2 Hz, 6H, CH_3), 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.19 – 2.27 (m, 1H, H-3'), 2.94 (sept., *J* = 6.9 Hz, 1H, CH), 3.50 (ddd, *J* = 11.7, 5.4, 4.2 Hz, 1H, H-5''), 3.65 (ddd, *J* = 11.7, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.43 – 4.49 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.56 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.08 (br. s, 2H, NH_2), 6.11 (d, *J* = 2.4 Hz, 1H, H-1'), 7.34 – 7.36 (m, 2H, H_{Phe}), 7.38 – 7.50 (m, 2H, , H_{Phe}), 7.50 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.9 (2C, CH_3), 33.2 (CH), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.3 (C-4a), 116.0 (C-5), 120.5 (C-6), 126.9 (2C, C_{Phe}), 128.4 (2C, C_{Phe}), 132.0 (C-1_{Phe}), 147.0 (C-4_{Phe}), 150.3 (C-7a), 151.6 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{20}H_{25}N_4O_3$ ([M+H]$^+$): 369.1921, found: 369.1923.

4-amino-5-(4-trifluoromethylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (11) 11 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **11** (0.13 g, 0.34 mmol) as a white solid. Yield = 76 %. Melting point: 223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.25 (ddd, *J* = 13.2, 8.7, 6.0 Hz, 1H, H-3'), 3.52 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.67 (ddd, *J* = 11.7, 5.4, 3.6 Hz, 1H, H-5'), 4.28 – 4.35 (m, 1H, H-4'), 4.43 – 4.52 (m, 1H, H-2'), 5.03 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.59 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.13 (d, *J* = 3.0 Hz, 1H, H-1'), 6.28 (br. s, 2H, NH_2), 7.66 – 7.69 (m, 2H, H_{Phe}), 7.70 (s, 1H, H-6), 7.80 – 7.82 (m, 2H, H_{Phe}), 8.18 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -60.7. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (C-3'), 62.7 (C-5'), 75.0 (C-2'), 80.1 (C-4'), 90.2 (C-1'), 99.9 (C-4a), 114.9 (C-5), 121.9 (C-6), 124.54 (q, *J* = 270.2 Hz, 1C, CF_3), 125.74 (q, *J* = 3.5, 2C, C-3_{Phe}, C-5_{Phe}), 126.81 (q, *J* = 32.1 Hz, 1C, C-4_{Phe}), 128.79 (2C, C-2_{Phe}, C-6_{Phe}), 138.76 (C-1_{Phe}), 150.76 (C-7a), 151.87 (C-2), 157.35 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}F_3N_4O_3$ ($[M+H]^+$): 395.1326, found: 395.1309.

4-amino-5-(4-nitrophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (12) 12 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **12** (0.11 g, 0.29 mmol) as a yellow solid. Yield = 64 %. Melting point: 254 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.26 (ddd, *J* = 13.2, 8.7, 6.0 Hz, 1H, H-3'), 3.52 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.68 (ddd, *J* = 12.0, 5.7, 3.6 Hz, 1H, H-5'), 4.29 – 4.36 (m, 1H, H-4'), 4.45 – 4.50 (m, 1H, H-2'), 5.04 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.61 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.14 (d, *J* = 2.4 Hz, 1H, H-1'), 6.39 (br. s, 2H, NH_2), 7.70 – 7.73 (m, 2H, H-2_{Phe}, H-6_{Phe}), 7.81 (s, 1H, H-6), 8.20 (s, 1H, H-2), 8.29 – 8.32 (m, 2H, H-3_{Phe}, H-5_{Phe}). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.5 (C-3'), 62.6 (C-5'), 75.0 (C-2'), 80.2 (C-4'), 90.2 (C-1'), 99.7 (C-4a), 114.5 (C-5), 122.7 (C-6), 124.1 (2C, C-3_{Phe}, C-5_{Phe}), 128.9 (2C, C-2_{Phe}, C-6_{Phe}), 141.7 (C-1_{Phe}), 145.7 (C-4_{Phe}), 151.0 (C-7a), 152.0 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}N_5O_5$ ($[M+H]^+$): 372.1302, found: 372.1299.

4-amino-5-(3-methylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (13) 13 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **13** (0.10 g, 0.29 mmol) as a white solid. Yield = 73 %. Melting point: 123 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 2.37 (s, 3H, CH_3),

3.50 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.40 – 4.49 (m, 1H, H-2'), 5.02 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.09 (br. s, 2H, NH_2), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 7.18 (d, *J* = 7.5 Hz, 1H, H_{Phe}), 7.25 (d, *J* = 7.5 Hz, 1H, H_{Phe}), 7.29 (s, 1H, H-2_{Phe}), 7.37 (t, *J* = 7.5 Hz, 1H, H-5_{Phe}), 7.52 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.1 (CH_3), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.2 (C-4a), 116.2 (C-5), 120.5 (C-6), 125.5 (C_{Phe}), 127.5 (C_{Phe}), 128.8 (C-5_{Phe}), 129.1 (C-2_{Phe}), 134.5 (C_{Phe}), 138.1 (C_{Phe}), 150.3 (C-7a), 151.6 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{21}N_4O_3$ ($[M+H]^+$): 341.1608, found: 341.1619.

4-amino-5-(3-chlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (14) 14 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **14** (0.081 g, 0.22 mmol) as a white solid. Yield = 54 %. Melting point: 130 °C / 190 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.89 – 1.94 (br. s., 1H, H-3''), 2.21 – 2.30 (br. s., 1H, H-3'), 3.47 – 3.54 (br. s., 1H, H-5''), 3.63 – 3.69 (br. s, 1H, H-5'), 4.26 – 4.36 (m, 1H, H-4'), 4.42 – 4.51 (m, 1H, H-2'), 5.02 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 6.22 (br. s, 2H, NH_2), 7.38 – 7.44 (m, 2H, H_{Phe}), 7.48 (d, *J* = 7.5 Hz, 1H, H_{Phe}), 7.52 (t, *J* = 1.5 Hz, 1H, H_{Phe}), 7.64 (s, 1H, H-6), 8.17 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.7 (C-5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.7 (C-5), 121.5 (C-6), 126.5 (C_{Phe}), 126.9 (C_{Phe}), 127.9 (C_{Phe}), 130.6 (C_{Phe}), 133.5 (C_{Phe}), 136.7 (C_{Phe}), 150.6 (C-7a), 151.8 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{18}ClN_4O_3$ ($[M+H]^+$): 361.1062, found: 361.1063.

4-amino-5-(3-trifluoromethylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (15) 15 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **15** (0.14 g, 0.33 mmol) as a white solid. Yield = 82 %. Melting point: 125 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.26 (ddd, *J* = 12.9, 8.7, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5'), 3.66 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.34 (br. s, 1H, H-4'), 4.44 – 4.52 (br. s, 1H, H-2'), 5.02 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.14 (d, *J* = 2.7 Hz, 1H, H-1'), 6.24 (br. s, 2H, NH_2), 7.70 – 7.77 (m, 4H, H_{Phe}), 7.70 (s, 1H, H-6), 8.18 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -61.1. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (C-3'), 62.7 (C-

5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.7 (C-5), 121.7 (C-6), 123.10 (q, *J* = 3.5 Hz, 1C, C_{Phe}), 124.25 (d, *J* = 271.3 Hz, 1C, CF_3), 124.62 (q, *J* = 3.5 Hz, 1C, C_{Phe}), 129.52 (q, *J* = 32.0 Hz, 1C, C-3_{Phe}), 129.9 (C_{Phe}), 132.2 (C_{Phe}), 135.5 (C-1_{Phe}), 150.7 (C-7a), 151.8 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{18}H_{18}F_3N_4O_3$ ($[M+H]^+$): 395.1326, found: 395.1322.

4-amino-5-(3,4-difluorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (16) **16** was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **16** (0.11 g, 0.30 mmol) as a white solid. Yield = 76 %. Melting point: 218 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 9.0, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 6.0, 4.5 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.34 (m, 1H, H-4'), 4.43 – 4.48 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 6.27 (br. s, 2H, NH_2), 7.25 – 7.30 (m, 1H, H_{Phe}), 7.43 – 7.56 (m, 2H, H_{Phe}, H_{Phe}), 7.59 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -142.14 – -141.97 (m, 1F), -138.31 – -138.16 (m, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.2 (C-5), 117.2 – 117.9 (m, 2C, C_{Phe}), 121.3 (C-6), 125.0 – 125.2 (m, 1C, C_{Phe}), 132.1 (m, 1C, C-1_{Phe}), 147.4 (dd, *J* = 65.3, 12.8 Hz, 1C, C_{Phe}), 150.4 (C-7a), 150.7 (dd, *J* = 66.8, 12.8 Hz, 1C, C_{Phe}), 151.8 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}F_2N_4O_3$ ($[M+H]^+$): 363.1263, found: 363.1277.

4-amino-5-(3,4-dichlorophenyl)-N7-(3'-deoxy-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (17) **17** was prepared according to general procedure A. **33** (0.16 g, 0.49 mmol) gave rise to **17** (0.10 g, 0.25 mmol) as a white solid. Yield = 50 %. Melting point: 169 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.25 (ddd, *J* = 13.2, 8.7, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.7, 3.6 Hz, 1H, H-5'), 4.26 – 4.34 (m, 1H, H-4'), 4.43 – 4.48 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 6.32 (br. s, 2H, NH_2), 7.42 (dd, *J* = 8.1, 2.1 Hz, 1H, H-6_{Phe}), 7.66 (s, 1H, H-6), 7.68 (d, *J* = 1.8 Hz, 1H, H-2_{Phe}), 7.69 (d, *J* = 8.1 Hz, 1H, H-5_{Phe}), 8.17 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 80.1 (C-4'), 90.1 (C-1'), 99.9 (C-4a), 113.9 (C-5), 121.7 (C-6), 128.5 (C-6_{Phe}), 129.1 (C_{Phe}), 130.0 (C-2_{Phe}), 130.8 (C-5_{Phe}), 131.3 (C_{Phe}), 135.2 (C_{Phe}), 150.7 (C-7a),

151.9 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}Cl_2N_4O_3$ ([M+H]$^+$): 395.0672, found: 395.0663.

4-amino-5-(3-fluoro-4-chlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (18) 18 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **18** (0.068 g, 0.18 mmol) as a white solid. Yield = 43 %. Melting point: 206 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.7, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.66 (ddd, *J* = 11.7, 5.4, 3.6 Hz, 1H, H-5'), 4.25 – 4.35 (br. s, 1H, H-4'), 4.41 – 4.49 (br. s, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 6.32 (br. s, 2H, NH_2), 7.30 (dd, *J* = 8.4, 2.1 Hz, 1H, H-5$_{Phe}$), 7.45 (dd, *J* = 10.5, 2.1 Hz, 1H, H-2$_{Phe}$), 7.65 (s, 1H, H-6), 7.65 (app. d, *J* = 16.2 Hz, 1H, H-6$_{Phe}$), 8.16 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -115.99 – -115.93 (m, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (C-3'), 62.7 (C-5'), 74.9 (C-2'), 80.1 (C-1'), 90.1 (C-1'), 99.8 (C-4a), 114.2 (C-5), 116.5 (d, *J* = 20.3 Hz, 1C, C-2$_{Phe}$), 117.47 (d, 1C, *J* = 16.5 Hz, 1C, C-4$_{Phe}$), 121.6 (C-6), 125.4 (d, *J* = 2.3 Hz, 1C, C-5$_{Phe}$), 130.82 (C-6$_{Phe}$), 135.7 (d, *J* = 7.5 Hz, 1C, C-1$_{Phe}$), 150.6 (C-7a), 151.8 (C-2), 157.29 (d, *J* = 244.5 Hz, 1C, C-3$_{Phe}$), 157.30 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}ClFN_4O_3$ ([M+H]$^+$): 379.0968, found: 379.0982.

4-amino-5-(3-chloro-4-fluorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (19) 19 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **19** (0.098 g, 0.25 mmol) as a white solid. Yield = 62 %. Melting point: 178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.25 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 11.7, 5.7, 4.8 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.4, 3.9 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.41 – 4.49 (m, 1H, H-2'), 5.00 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 6.26 (br. s, 2H, NH_2), 7.42 (ddd, *J* = 8.4, 5.1, 2.1 Hz, 1H, H-6$_{Phe}$), 7.49 (q, *J* = 9.0 Hz, 1H, H-5$_{Phe}$), 7.60 (s, 1H, H-6), 7.63 (dd, *J* = 7.2, 2.1 Hz, 1H, H-2$_{Phe}$), 8.16 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -119.95 – -119.87 (m, 1F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.0 (C-5), 117.1 (d, *J* = 21.8 Hz, 1C, C-5$_{Phe}$), 119.7 (d, *J* = 18.0 Hz, 1C, C-3$_{Phe}$), 121.3 (C-6), 128.9 (d, *J* = 6.8 Hz, 1C, C-6$_{Phe}$), 130.2 (C-2$_{Phe}$),

132.3 (d, *J* = 3.8 Hz, 1C, C-1_{Phe}), 150.5 (C-7a), 151.8 (C-2), 156.3 (d, *J* = 245.3 Hz, 1C, C-4_{Phe}), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}ClFN_4O_3$ ($[M+H]^+$): 379.0968, found: 379.0974.

4-amino-5-(2,4-dichlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (20)

20 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **20** (0.056 g, 0.14 mmol) as a white solid. Yield = 30 %. Melting point: 136 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.90 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.21 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 3.49 (ddd, *J* = 12.3, 5.1, 4.5 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.1, 3.6 Hz, 1H, H-5'), 4.27 – 4.34 (m, 1H, H-4'), 4.43 – 4.47 (m, 1H, H-2'), 5.00 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.59 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.07 (br. s, 2H, NH_2), 6.10 (d, *J* = 2.4 Hz, 1H, H-1'), 7.42 (d, *J* = 8.1 Hz, 1H, H-6_{Phe}), 7.49 (dd, *J* = 8.4, 2.1 Hz, 1H, H-5_{Phe}), 7.53 (s, 1H, H-6), 7.72 (d, *J* = 2.1 Hz, 1H, H-3_{Phe}), 8.14 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 75.0 (C-4'), 80.1 (C-2'), 90.4 (C-1'), 101.4 (C-4a), 111.2 (C-5), 121.8 (C-6), 127.5 (C-5_{Phe}), 129.3 (C-3_{Phe}), 132.0 (C_{Phe}), 132.8 (C_{Phe}), 133.5 (C-6_{Phe}), 134.1 (C_{Phe}), 149.8 (C-7a), 151.8 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}Cl_2N_4O_3$ ($[M+H]^+$): 395.0672, found: 395.0673.

4-amino-5-(3,5-dichlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (21)

21 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **21** (0.062 g, 0.16 mmol) as a white solid. Yield = 39 % yield. Melting point: 211 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.88 – 1.94 (br. s, 1H, H-3''), 2.21 – 2.30 (br. s, 1H, H-3'), 3.51 (ddd, *J* = 11.7, 5.7, 4.5 Hz, 1H, H-5''), 3.66 (ddd, *J* = 11.7, 5.7, 3.6 Hz, 1H, H-5'), 4.25 – 4.35 (br. s, 1H, H-4'), 4.42 – 4.50 (br. s, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.4 Hz, 1H, H-1'), 6.37 (br. s, 2H, NH_2), 7.47 (d, *J* = 1.8 Hz, 2H, H-2_{Phe}, H-6_{Phe}), 7.53 (t, *J* = 2.1 Hz, 1H, H-4_{Phe}), 7.71 (s, 1H, H-6), 8.18 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 75.0 (C-2'), 80.1 (C-4'), 90.1 (C-1'), 99.7 (C-4a), 113.7 (C-5), 122.2 (C-6), 125.8 (C-4_{Phe}), 126.8 (2C, C-2_{Phe}, C-6_{Phe}), 134.2 (2C, C-3_{Phe}, C-5_{Phe}), 138.0 (C-1_{Phe}), 150.8 (C-7a), 151.9 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}Cl_2N_4O_3$ ($[M+H]^+$): 395.0672, found: 395.0663.

4-amino-5-(3,4-dimethylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (22)

22 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **22** (0.10 g, 0.27

mmol) as a white solid. Yield = 68 %. Melting point: 125 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.86 – 1.96 (br. s, 1H, H-3''), 2.19 – 2.28 (br. s, 1H, H-3'), 2.27 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.45 – 3.55 (br. s, 1H, H-5''), 3.59 – 3.70 (br. s, 1H, H-5'), 4.24 – 4.34 (br. s, 1H, H-4'), 4.42 – 4.50 (br. s, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.56 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.06 (br. s, 2H, NH_2), 6.11 (d, *J* = 2.7 Hz, 1H, H-1'), 7.17 (dd, *J* = 7.5, 1.5 Hz, 1H, H-2_{Phe}), 7.24 (d, *J* = 6.9 Hz, 2H, H-5_{Phe}, H-6_{Phe}), 7.46 (s, 1H, H-6), 8.14 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.0 (CH_3), 19.5 (CH_3), 34.7 (C-3'), 62.8 (C-5'), 74.8 (C-2'), 79.8 (C-4'), 90.1 (C-1'), 100.3 (C-4a), 116.1 (C-5), 120.2 (C-6), 125.8 (C_{Phe}), 129.6 (C_{Phe}), 130.0 (C_{Phe}), 132.0 (C_{Phe}), 134.9 (C_{Phe}), 136.8 (C_{Phe}), 150.2 (C-7a), 151.6 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{19}H_{23}N_4O_3$ ([M+H]$^+$): 355.1765, found: 355.1767.

4-amino-5-(3-chloro-4-methylphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (23) 23 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **23** (0.11 g, 0.27 mmol) as a white solid. Yield = 75 %. Melting point: 135 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.25 (ddd, *J* = 12.9, 8.7, 6.0 Hz, 1H, H-3'), 2.37 (s, 3H, CH_3), 3.51 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 4.26 – 4.33 (m, 1H, H-4'), 4.42 – 4.50 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 6.18 (br. s, 2H, NH_2), 7.32 (dd, *J* = 7.8, 1.8 Hz, 1H, H-6_{Phe}), 7.44 (d, *J* = 8.1 Hz, 1H, H-5_{Phe}), 7.50 (d, *J* = 1.8 Hz, 1H, H-2_{Phe}), 7.59 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.3 (CH_3), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 114.6 (C-5), 121.1 (C-6), 127.0 (C_{Phe}), 128.4 (C_{Phe}), 131.6 ($2C_{Phe}$), 133.6 (C_{Phe}), 134.1 (C_{Phe}), 150.5 (C-7a), 151.7 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_3$ ([M+H]$^+$): 375.1218, found: 375.1213.

4-amino-5-(3-chloro-4-methoxyphenyl)-N7-(3'-deoxy-β-D-ribofuranosyl-pyrrolo[2,3-*d*]pyrimidine (24) 24 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **24** (0.089 g, 0.23 mmol) as a white solid. Yield = 57 %. Melting point: 249 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.6, 3.3 Hz, 1H, H-3''), 2.25 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 3.50 (ddd, *J* = 11.7, 5.7, 4.2 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.7, 3.6 Hz, 1H, H-5'), 3.90 (s, 3H, OCH_3), 4.25 – 4.33 (m, 1H, H-4'), 4.43 – 4.48 (m, 1H, H-2'), 5.01 (t, *J* = 5.7 Hz, 1H, OH-

5'), 5.56 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.11 (d, *J* = 2.7 Hz, 1H, H-1'), 6.16 (br. s, 2H, NH_2), 7.25 (d, *J* = 8.4 Hz, 1H, H-5_{Phe}), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1H, H-6_{Phe}), 7.50 (d, *J* = 2.1 Hz, 1H, H-2_{Phe}), 7.52 (s, 1H, H-6), 8.15 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 56.2 (OCH_3), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.2 (C-4a), 113.2 (C-5_{Phe}), 114.6 (C-5), 120.7 (C_{Phe}), 121.3 (C-6), 127.8 (C_{Phe}), 128.3 (C-6_{Phe}), 129.7 (C-2_{Phe}), 150.3 (C-7a), 151.7 (C-2), 153.5 (OCH_3), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_4$ ($[M+H]^+$): 391.1168, found: 391.1163.

4-amino-5-(3-methyl-4-chlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (25) 25 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **25** (0.092 g, 0.25 mmol) as a white solid. Yield = 61 % yield. Melting point: 125 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.24 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 2.39 (s, 3H, CH_3), 3.51 (ddd, *J* = 12.0, 5.7, 4.5 Hz, 1H, H-5''), 3.65 (ddd, *J* = 12.0, 5.7, 3.6 Hz, 1H, H-5'), 4.26 – 4.34 (m, 1H, H-4'), 4.43 – 4.49 (m, 1H, H-2'), 5.04 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 6.18 (br. s, 2H, NH_2), 7.29 (dd, *J* = 8.1, 2.4 Hz, 1H, H-6_{Phe}), 7.44 (d, *J* = 1.8 Hz, 1H, H-2_{Phe}), 7.49 (d, *J* = 8.1 Hz, 1H, H-5_{Phe}), 7.55 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.7 (CH_3), 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.1 (C-1'), 100.1 (C-4a), 115.0 (C-5), 120.9 (C-6), 127.5 (C_{Phe}), 129.2 (C_{Phe}), 131.1 (C_{Phe}), 131.8 (C_{Phe}), 133.5 (C_{Phe}), 135.9 (C_{Phe}), 150.4 (C-7a), 151.7 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_3$ ($[M+H]^+$): 375.1218, found: 375.1212.

4-amino-5-(3-methoxy-4-chlorophenyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (26) 26 was prepared according to general procedure A. **33** (0.14 g, 0.41 mmol) gave rise to **26** (0.12 g, 0.31 mmol) as a white solid. Yield = 78 % yield. Melting point: 208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (ddd, *J* = 12.9, 6.3, 3.0 Hz, 1H, H-3''), 2.25 (ddd, *J* = 12.9, 8.4, 6.0 Hz, 1H, H-3'), 3.51 (ddd, *J* = 12.0, 5.7, 4.2 Hz, 1H, H-5''), 3.67 (ddd, *J* = 12.0, 5.4, 3.6 Hz, 1H, H-5'), 3.91 (s, 3H, OCH_3), 4.27 – 4.34 (m, 1H, H-4'), 4.44 – 4.49 (m, 1H, H-2'), 5.03 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.58 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.12 (d, *J* = 2.7 Hz, 1H, H-1'), 6.25 (br. s, 2H, NH_2), 7.04 (dd, *J* = 8.1, 1.8 Hz, 1H, H-6_{Phe}), 7.17 (d, *J* = 1.8 Hz, 1H, H-2_{Phe}), 7.49 (d, *J* = 8.1 Hz, 1H, H-5_{Phe}), 7.62 (s, 1H, H-6), 8.16 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.6 (C-3'), 56.0 (OCH_3), 62.7 (C-5'), 74.9 (C-2'),

80.0 (C-4'), 90.1 (C-1'), 100.0 (C-4a), 112.9, 115.3, 119.4, 121.0, 121.1, 130.1, 134.8, 150.5 (C-7a), 151.7 (C-2), 154.6 (C-3_{Phe}), 157.3 (C-4). HRMS (ESI): calculated for $C_{18}H_{20}ClN_4O_4$ ($[M+H]^+$): 391.1168, found: 391.1163.

4-amino-5-(2-naphtyl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (27) 27 was prepared according to general procedure A. **33** (0.15 g, 0.46 mmol) gave rise to **27** (0.11 g, 0.29 mmol) as a white solid. Yield = 64 % yield. Melting point: 132 / 185 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.93 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.27 (ddd, *J* = 13.2, 8.4, 6.0 Hz, 1H, H-3'), 3.49 – 3.56 (br. s, 1H, H-5''), 3.64 – 3.71 (br. s, 1H, H-5'), 4.27 – 4.37 (br. s, 1H, H-4'), 4.46 – 4.55 (br. s, 1H, H-2'), 5.04 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.60 (d, *J* = 4.2 Hz, 1H, OH-2'), 6.16 (d, *J* = 2.7 Hz, 1H, H-1'), 6.20 (br. s, 2H, NH_2), 7.49 – 7.58 (m, 2H, H-6_{Naph}, H-7_{Naph}), 7.65 (dd, *J* = 8.7, 2.1 Hz, 1H, H-3_{Naph}), 7.66 (s, 1H, H-6), 7.95 – 7.98 (m, 3H, H-1_{Naph}, H-5_{Naph}, H-8_{Naph}), 8.02 (d, *J* = 8.4 Hz, 1H, H-4_{Naph}), 8.19 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.7 (C-3'), 62.8 (C-5'), 74.9 (C-2'), 79.9 (C-4'), 90.2 (C-1'), 100.4 (C-4a), 116.1 (C-5), 121.1 (C-6), 125.8 (C-6_{Naph}), 126.5 (C-7_{Naph}), 126.6 (C-1_{Naph}), 127.0 (C-3_{Naph}), 127.6 (C_{Naph}), 127.8 (C_{Naph}), 128.4 (C-4_{Naph}), 131.8 (C_{Naph}), 132.1 (C_{Naph}), 133.2 (C-$8a_{Naph}$), 150.5 (C-7a), 151.7 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{21}H_{21}N_4O_3$ ($[M+H]^+$): 377.1608, found: 377.1614.

4-amino-5-(cyclohex-1-en-1-yl)-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (28) 28 was prepared according to general procedure A, with the use of Cs_2CO_3 as the base and potassium 1-cyclohexen-1-yltrifluoroborate as the coupling partner. **33** (0.14 g, 0.43 mmol) gave rise to **28** (0.13 g, 0.38 mmol) as a white solid. Yield = 88 % yield. Melting point: 75 / 95 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.60 – 1.65 (m, 2H, CH_2), 1.69 – 1.77 (m, 2H, CH_2), 1.89 (ddd, *J* = 12.9, 6.3, 3.3 Hz, 1H, H-3''), 2.15 – 2.24 (m, 3H, CH_2, H-3'), 2.28 – 2.36 (br. s, 2H, CH_2), 3.49 (ddd, *J* = 11.7, 5.1, 4.8 Hz, 1H, H-5''), 3.63 (ddd, *J* = 12.0, 5.1, 3.9 Hz, 1H, H-5'), 4.22 – 4.30 (m, 1H, H-4'), 4.37 – 4.42 (m, 1H, H-2'), 5.02 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.51 (d, *J* = 4.5 Hz, 1H, OH-2'), 5.72 (t, *J* = 3.6 Hz, 1H, H-$2_{cyclohex}$), 6.04 (d, *J* = 2.7 Hz, 1H, H-1'), 6.31 (br. s, 2H, NH_2), 7.36 (s, 1H, H-6), 8.09 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.6 (CH_2), 22.5 (CH_2), 25.0 (CH_2), 29.6 (CH_2), 34.8 (C-3'), 62.9 (C-5'), 74.7 (C-2'), 79.7 (C-4'), 90.0 (C-1'), 100.0 (C-4a), 118.2 (C-5), 119.4 (C-6), 125.7 (C_{olefin}), 131.6 (C_{olefin}), 149.8

(C-7a), 151.4 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{23}N_4O_3$ ([M+H]$^+$): 331.1765, found: 331.1765.

4-amino-5-cyclohexyl-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (29) 29 (0.084 g, 0.25 mmol) was dissolved in EtOH (10 mL). Next, the flask was purged with N_2, after which a cat. amount of Pd / C was added. Then, the N_2-atmosphere was exchanged for H_2 (balloon; bubbling), and the mixture stirred until TLC showed full conversion of the SM (approx. 8H). Then, the H_2-balloon was removed, the mixture purged with N_2 and filtered over Celite®. The filtrate was evaporated till dryness and purified by column chromatography 1 → 8 % MeOH / DCM to give **29** (0.072 g, 0.21 mmol) as a white solid in 83 % yield. Melting point: 117 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.11 – 1.35 (br. s, 3H, CH_2), 1.42 – 1.59 (br. s, 2H, CH_2), 1.64 – 1.79 (br. s, 3H, CH_2), 1.88 (ddd, *J* = 12.9, 6.6, 3.6 Hz, 1H, H-3'), 1.92 – 2.02 (br. s, 2H, CH_2), 2.18 (ddd, *J* = 12.9, 8.1, 6.0 Hz, 1H, H-3'), 2.87 – 2.96 (m, 1H, CH), 3.43 – 3.53 (br. s, 1H, H-5''), 3.58 – 3.65 (br. s, 1H, H-5'), 4.20 – 4.29 (br. s, 1H, H-4'), 4.34 – 4.43 (br. s, 1H, H-2'), 5.04 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.47 (d, *J* = 4.5 Hz, 1H, OH-2'), 5.99 (d, *J* = 3.0 Hz, 1H, H-1'), 6.44 (br. s, 2H, NH_2), 7.09 (s, 1H, H-6), 8.02 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 25.8, 25.9, 34.1, 34.3, 35.0, 63.0 (C-5'), 74.5 (C-2'), 79.4 (C-4'), 90.1 (C-1'), 101.5 (C-4a), 116.9 (C-5), 121.5 (C-6), 150.1 (C-7a), 151.1 (C-2), 157.4 (C-4). HRMS (ESI): calculated for $C_{17}H_{25}N_4O_3$ ([M+H]$^+$): 333.1921, found: 333.1931.

1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-α/β-D-ribofuranose (31) 30[38-40] (7.5 g, 21 mmol, 1 eq.) was dissolved in glacial AcOH (60 mL, 3.0 mL / mmol SM). Next, Ac_2O (7.9 mL, 84 mmol, 4.4 eq.) was added and the mixture cooled in an ice bath. As soon as solidification occurred, c. H_2SO_4 (4.0 mL, 80 mmol, 3.8 eq.) was added slowly. After complete addition, the ice bath was removed, and the mixture stirred until TLC showed full conversion of the starting material (~30 min – 1H). Then the mixture was transferred to a separatory funnel containing DCM. Slowly, an aq. sat. solution of Na_2CO_3 was added to neutralize the excess acid. After neutralization, the layers were separated, the water layer extracted once more with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography 0 → 15 % EA / PET, gave **31** (7.9 g, 20 mmol) as a colourless oil, in 97 % yield. Ratio (NMR-based): α/β = 1 / 5. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01 (s,

3H, OAc-β), 2.07 (s, 3H, OAc-α), 3.36 – 3.40 (m, 2H, H-3-β, H-3'-β), 2.41 – 2.54 (m, 2H, H-3-α, H-3'-α), 4.38 (dd, *J* = 12.0, 5.4 Hz, 1H, H-5'-β // H-5'-α), 4.51 (dd, *J* = 12.0, 3.3 Hz, 1H, H-5-α), 4.59 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5-β), 4.78 – 4.86 (m, 1H, H-4-β / H-4-α), 5.49 (dd, *J* = 3.9, 2.4 Hz, 1H, H-2-β), 5.53 (td, *J* = 8.4, 4.5 Hz, 1H, H-2-α), 6.37 (s, 1H, H-1-β), 6.58 (d, *J* = 4.5 Hz, 1H, H-1-α), 7.42 – 7.49 (m, 2H, OBz), 7.55 – 7.62 (m, 1H, OBz), 8.00 – 8.11 (m, 2H, OBz). HRMS (ESI): calculated for $C_{19}H_{17}O_5$ ([M-OAc]$^+$): 325.1071, found: 325.1068.

4-chloro-5-bromo-N7-(2',5'-di-*O*-benzoyl-3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (32) In a flame-dried two-neck round bottom flask under argon was added 7*H*-4-chloro-5-bromo-pyrrolo[2,3-*d*]pyrimidine[36] (1.53 g, 6.65 mmol, 1 eq.). Next, MeCN (50 mL, 7.5 mL / mmol SM) was added. To the stirring suspension was added BSA (1.8 mL, 7.3 mmol, 1.1 eq.) in one portion. The resulting mixture was stirred at room temperature for ~10 min, after which the glycosyl donor **31** (2.8 g, 7.3 mmol, 1.1 eq.) was added in one portion, immediately followed by TMSOTf (1.40 mL, 7.75 mmol, 1.165 eq.). The resulting solution was stirred at ambient temperature for another 15 min, and then transferred to a pre-heated oil bath at 80 °C. Heating was continued until full consumption of the glycosyl donor was observed by TLC (generally ~1H). Then, the mixture was cooled to ambient temperature. Next, EA and aq. sat. $NaHCO_3$ were added. The layers were separated, and the water layer extracted twice more with EA. Organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was purified by column chromatography (15% EA / PET) to give **32** (3.0 g, 5.3 mmol) as a slight yellow foam in 80 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (ddd, *J* = 14.1, 5.7, 1.8 Hz, 1H, H-3''), 2.74 (ddd, *J* = 14.1, 10.2, 6.0 Hz, 1H, H-3'), 4.60 (dd, *J* = 12.6, 4.5 Hz, 1H, H-5''), 4.75 (dd, *J* = 12.6, 3.0 Hz, 1H, H-5'), 4.81 – 4.89 (m, 1H, H-4'), 5.91 (dt, *J* = 6.0, 1.5 Hz, 1H, H-2'), 6.46 (d, *J* = 1.5 Hz, 1H, H-1'), 7.45 – 7.52 (m, 4H, H-OBz$_{meta}$), 7.52 (s, 1H, H-6), 7.56 – 7.65 (m, 2H, H-OBz$_{para}$), 8.01 – 8.09 (m, 4H, H-OBz$_{ortho}$), 8.60 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.2 (C-3'), 64.6 (C-5'), 78.6 (C-2'), 78.7 (C-4'), 89.5 (C-5), 90.3 (C-1'), 116.0 (C-4a), 126.8 (C-6), 128.8 (2C, C-OBz$_{meta}$), 128.8 (2C, C-OBz$_{meta}$), 129.2 (C-OBz$_{ipso}$), 129.5 (C-OBz$_{ipso}$), 129.8 (2C, C-OBz$_{ortho}$), 130.0 (2C, C-OBz$_{ortho}$), 133.6 (C-OBz$_{para}$), 133.9 (C-OBz$_{para}$), 150.1 (C7a), 151.6 (C-2), 152.7 (C-4),

165.7 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{25}H_{20}BrClN_3O_5$ ([M+H]$^+$): 556.0269, found: 556.0278.

4-amino-5-bromo-N7-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (33) 32 (1.8 g, 3.3 mmol) was suspended in 7N NH_3/MeOH (100 mL) inside a stainless-steel pressure vessel. The vessel was carefully closed and heated to 130 °C overnight. After cooling to ambient temperature, the mixture was evaporated and purified by column chromatography 0 → 8 % MeOH / DCM, to yield **33** (0.65 g, 2.0 mmol) as a white solid in 60 % yield. Melting point: 223 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.87 (ddd, *J* = 13.2, 6.3, 3.3 Hz, 1H, H-3''), 2.14 – 2.23 (m, 1H, H-3'), 3.50 (ddd, *J* = 12.0, 5.4, 4.2 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 5.4, 3.3 Hz, 1H, H-5'), 4.24 – 4.31 (m, 1H, H-4'), 4.34 – 4.39 (m, 1H, H-2'), 5.03 (t, *J* = 5.7 Hz, 1H, OH-5'), 5.57 (d, *J* = 4.5 Hz, 1H, OH-2'), 6.04 (d, *J* = 2.7 Hz, 1H, H-1'), 6.77 (br. s, 2H, NH_2), 7.65 (s, 1H, H-6), 8.11 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.3 (C-3'), 62.5 (C-5'), 75.0 (C-2'), 80.1 (C-4'), 86.3 (C-4), 90.1 (C-1'), 100.9 (C-4a), 121.4 (C-6), 149.0 (C-7a), 152.4 (C-2), 156.9 (C-4). HRMS (ESI): calculated for $C_{11}H_{14}BrN_4O_3$ ([M+H]$^+$): 329.0244, found: 329.0240.

Biology

In vitro evaluation

Trypanosoma cruzi (intracellular amastigotes) – Tulahuen CL2 β galactosidase strain

Drug activity against *T. cruzi* was tested with the nifurtimox-sensitive Tulahuen CL2 β galactosidase strain.[56] This strain was maintained on MRC-5_{SV2} (human lung fibroblast) cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37°C/5% CO_2. Assays were with 4×10^3 MRC-5 cells/well and 4×10^4 parasites/well. Impact of test compound dilution series (10 concentrations of a 4-fold compound dilution series starting at 64 µM) on parasite growth was analyzed after 7 days incubation by adding the substrate chlorophenolred β-D-galactopyranoside. The change in color was measured

spectrophotometrically at 540 nm after 4 hours incubation at 37°C. The results were expressed as % parasite reduction compared to control wells and used to calculate EC_{50} values.

Activity against *T. cruzi* Y-strain bloodstream trypomastigote form

Bloodstream trypomastigote forms of *T. cruzi* (Y strain) were obtained by cardiac puncture of infected Swiss Webster mice on the peak of parasitemia. For trypanocidal activity assays, 5.0 x 10^6 parasites per ml were incubated for 24 h at 37°C in RPMI-1640 medium (Sigma-Aldrich) supplemented with 5% fetal bovine serum (FBS) in the presence or absence of increasing concentrations of the compounds (0 to 32 µM) as already described.[57] After 24 h of incubation, the parasite death rates were determined by light microscopy using a Neubauer chamber that allows the direct quantification of live parasites. Then the concentration that induced 50% of parasite lysis (EC_{50}) was determined.

Activity against *T. cruzi* Y-strain intracellular amastigote form

Primary cultures of embryonic cardiomyocytes were obtained from Swiss mice, as previously reported.[58] After purification, the cardiac cells were seeded at a density of 10^5 cells/well into 24-well culture plates containing gelatin-coated cover slips. The cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% horse serum, 5% FBS, 2.5 mM $CaCl_2$, 1 mM L-glutamine, and 2% chicken embryo extract. All cultures were maintained at 37°C in an atmosphere of 5% CO_2 and air, and the assays were run twice in duplicate.

After 24 hours of plating, cardiac cell cultures were infected for 24 hours at 37 °C with bloodstream trypomastigotes employing a parasite/host cell ratio of 10/1. After the initial host-parasite contact (24h), the cultures were washed to remove free parasites and treated for 168 hours at 37°C with graded concentrations (up to 2 µM) of the compounds diluted in culture medium. The culture medium was replaced every 48 hours. After drug exposure, the infected cultures were fixed and stained with Giemsa as reported.[59] The mean number of infected host cells and parasites per infected cells were scored in 200 host cells in two independent experiments each run in duplicate. Only characteristic parasite nuclei and kinetoplasts were considered as surviving parasites since irregular structures could represent parasites undergoing cell death.[59] The compound activity was estimated by calculating the inhibition levels of the

infection index (II - percentage of infected cells multiplied by the mean number of parasites per infected cell).

Cytotoxicity on MRC-5 fibroblasts

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

Cytotoxicity on cardiac cells

Primary cultures of mouse embryonic cardiac cells (CC) were obtained from Swiss mice (18 days of gestation) following a previously described method.[58] After purification, the CCs were seeded into 96-well microplates containing gelatin-coated coverslips at densities of 50,000 cells per well.[60] The CC cultures were then sustained in Dulbecco's modified Eagle medium (DMEM; without phenol red; Sigma-Aldrich) supplemented with 2 % chicken embryo extract, 5% FBS, 10 % horse serum, 1 mM L-glutamine and 2.5 mM $CaCl_2$ at 37°C. CCs cultures were incubated at 37°C for 24 h, with increasing concentrations of each compound (up to 200 µM) diluted in DMEM without phenol red. Next, mammalian cell morphology and spontaneous contractibility were evaluated by light microscopy, and cell viability was determined by a colorimetric assay using PrestoBlue-(CC).[45, 57] After 24 h of incubation, absorbance at 570 and 600 nm was measured by a UV spectrophotometer and the results were calculated according to the manufacturer's instructions. EC_{50} values with Standard Error of the Mean (SEM) and 95 % confidence intervals were calculated through non-linear regression analysis in the GraphPad Prism v 6.0 software package.

Microsomal stability assays

Male mouse, male rat, male dog and pooled human liver microsomes were purchased from a commercial source (Corning) and stored at -80 °C. NADPH generating system solutions A and B and UGT reaction mix solutions A and B (Corning) were kept at -20°C. The test compound and the reference compounds diclofenac (MW 296.15) and fluconazole (MW 306.27) were formulated in DMSO at 10 mM. The microsomal stability assay was carried out based on the BD Biosciences Guidelines for Use (TF000017 Rev1.0) with minor adaptations. The metabolic stability of the compounds was studied through the CYP_{450} superfamily (Phase I metabolism) by fortification with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and through uridine glucuronosyl-transferase (UGT) enzymes (Phase II metabolism) by fortification with uridine diphosphate glucuronic acid (UDPGA). For the CYP_{450} and other NADPH dependent enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in potassium phosphate buffer in a reaction started by the addition of 1 mM NADPH and stopped at 0, 15, 30 and 60 minutes. At these time points, 20 µl was withdrawn from the reaction mixture and 80 µl cold acetonitrile (ACN) containing the internal standard tolbutamide, was added to inactivate the enzymes and precipitate the proteins. The mixture was vortexed for 30 s and centrifuged at 4 °C for 5 min at 15,000 rpm. The supernatant was stored at -80°C until analysis. For the UGT enzymes, both compounds were incubated at 5 µM together with 0.5 mg/mL liver microsomes in a reaction started by the addition of 2 mM UDPGA cofactor. The corresponding loss of parent compound was determined using liquid chromatography (UPLC) (Waters Aquity™) coupled with tandem quadrupole mass spectrometry (MS^2) (Waters Xevo™), equipped with an electrospray ionization (ESI) interface and operated in multiple reaction monitoring (MRM) mode.

***In vivo* evaluation**

Male Swiss Webster mice (18-20 g; 4 – 5 weeks of age) obtained from the animal facilities of ICTB (Institute of Science and Biomodels Technology / Fiocruz / RJ / Brazil) were housed at a maximum of

6 per cage, kept in a specific-pathogen-free (SPF) room at 20 to 24°C under a 12-h light and 12-h dark cycle, and provided sterilized water and chow ad libitum. The animals were allowed to acclimate for 7 days before starting the experiments. Infection was performed by intraperitoneal (i.p.) injection of 10^4 bloodstream trypomastigotes (Y-strain). Age-matched non-infected mice were maintained under identical conditions.[57]

The animals were divided into the following groups (n=6): uninfected (non-infected and non-treated), untreated (infected but treated only with vehicle), and treated (infected and treated with the compounds). The *T. cruzi* (Y-strain) infected mice were treated for five consecutive days, starting at the 5th day post-infection (dpi), which in this experimental model corresponds to the time of parasitemia onset, using 25 mg/kg (oral gavage, b.i.d.) of the tested compounds **2** and **5**, and 100 mg/kg/day BZ administered orally (oral gavage, s.i.d.). Nucleoside test compounds **2** and **5** were formulated in 10 % (v/v) EtOH, 0.1 M aq. citrate buffer (pH = 3.02) at 2.0 and 1.8 mg/mL, respectively and dosed according to body weight. Formulations were prepared freshly before each administration. Only mice with positive parasitemia were used in the infected groups. Parasitemia was individually checked by direct microscopic counting of the number of parasites in 5 µl of blood, and mice were checked for mortality daily until 30 days post-treatment (corresponding to 40 dpi). Mortality is expressed as the percent cumulative mortality (CM) as described before.[57]

Mice that presented a consistently negative parasitemia up to 30 days post treatment (= 40 dpi) were submitted to three cycles of immunosuppression with cyclophosphamide (50 mg/kg/day), each cycle including four consecutive days of cyclophosphamide administration (i.p.) and a three-day interval of non-administration.[46]

Ethics statement

All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA LW16/14).

Ancillary information

Supporting information

Copies of ^{1}H, ^{13}C and ^{19}F NMR spectra of compounds **3–32** and ^{1}H-^{13}C gHMBC and 2D NOESY spectra of compound **32** can be found in the Supporting Information.

Single X-ray crystal data for compound **5** can be found in the Supporting Information. CCDC 1833253 contains the additional supplementary crystallographic data for compound **5** and will be released upon acceptance of the manuscript for publication.

Availability of Molecular Formula Strings (CSV) when submitted.

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Abbreviations used:

BSA, N,O-bis(trimethylsilyl)acetamide; TPPTS, trisodium 3-bis(3-sulfonatophenyl)phosphanylbenzenesulfonate.

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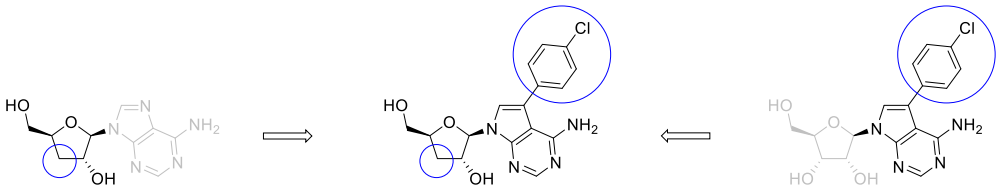
Table of contents graphic



Cordycepin, **1d**
T. cruzi EC_{50}: 2.51 ± 0.22 μM

5
T. cruzi EC_{50}: 0.047 ± 0.006 μM
In vivo activity 25 mg/kg, bid, po, 5d

2
T. cruzi EC_{50}: 0.47 ± 0.25 μM
In vivo activity 25 mg/kg, bid, po, 5d