Discovery of pyrrolo[2,3-b]pyridine (1,7-dideazapurine) nucleoside analogues as anti-Trypanosoma cruzi agents

[Title page]

Discovery of pyrrolo[2,3-*b*]pyridine (1,7-dideazapurine) nucleoside analogues as anti-*Trypanosoma cruzi* agents

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Abstract (max 150 words)

Trypanosoma cruzi is the causative pathogen of Chagas disease and the main culprit for cardiac-related mortality in Latin-America triggered by an infective agent. Uncapable of synthesizing purines *de novo*, this parasite depends on acquisition and processing of host-derived purines, making purine (nucleoside) analogues a potential source of antitrypanosomal agents. In this respect, hitherto 7-deazaadenosine (tubercidin) analogues attracted most attention. Here, we investigated analogues with an additional nitrogen (N1) removed. Structure-activity relationship investigation showed that C7 modification afforded analogues with potent antitrypanosomal activity. Halogens and small, linear carbon-based substituents were preferred. Compound **11** proved most potent *in vitro*, showed full suppression of parasitemia in a mouse model of acute infection and elicited 100 % animal survival after oral dosing at 25 mg/kg b.i.d. for five and fifteen days. Cyclophosphamide-induced immunosuppression led to recrudescence. Washout experiments demonstrated a lack of complete clearance of infected cell cultures, potentially explaining the *in vivo* results.

Introduction

Chagas disease (CD) is a neglected tropical disease (NTD) caused by the protist parasite *Trypanosoma cruzi* (*T. cruzi*).[1-2] Although originally confined to Latin-American countries, it has become a global health concern in recent years as a result of increased migration and due to non-vector mediated transmission.[3] In endemic countries, the disease is prevalent mostly in rural, impoverished areas and spread via bites of infected vectors, triatomine or "kissing" bugs, which release parasites while defecating during the acquisition of a blood meal. Oral transmission via parasite contaminated food is another important mode of transmission. In non-endemic countries, vertical transmission (mother to child), as well as iatrogenic spread via blood products and donor organs render this infectious disease a significant health concern of increasing importance.[3-4]

Clinically, CD is characterized by two disease stages. A first, acute stage occurs when patients are just infected, leading to the formation of a 'chagoma' or Romaña's sign, inflammatory reactions at the site of infection. Further symptoms are non-specific (fever, malaise), but can also be absent. After the initial parasitemia fades due to activation of the host immune system, a sub-patent parasitism is sustained, leading to a so-called indeterminate state, which can last for years and even decades. Between thirty to forty percent of patients will ultimately develop chronic disease, resulting in progressive cardiac (cardiomyopathy) and/or gastro-intestinal dysfunction (e.g. megacolon and megaesophagus).[4] Overall, CD can be considered a 'silent disease', due to the absence of pronounced symptoms, which leads to unawareness of patients and non-treatment, causing severe organ damage and often, sudden death.

Presently, there is no vaccine available for the prevention of CD, and even though vector control

programs have reduced the intra-domiciliary contraction of CD in endemic regions, it requires continuous efforts, and does not address the issues posed by the other transmission modes, particularly congenital infection.[4] Therefore, effective chemotherapy is set as one of the key goals to eliminate this NTD. Currently, two drugs are in use: nifurtimox and benznidazole,[1, 5] which both suffer from significant limitations, including limited efficacy – especially for the chronic stage of the disease – and significant side-effects. The latter is an eminent cause of premature treatment discontinuation.[4] There is an urgent need to develop safer and more efficacious treatment options. In this regard, several recent reports have described the discovery of new lead compounds, offering new hope to treat this NTD.[1-2, 6-7]

T. cruzi parasites lack the enzymes of the *de novo* purine salvage pathway,[8] in contrast to most mammalian cells. Being purine auxotroph, they rely on the uptake and salvage machinery to 'steal' pre-formed purines (purine nucleosides and/or nucleobases) from their host, rendering the evaluation of (synthetically) modified purine analogues an attractive strategy to find new hits.[8]

Representative deazapurine nucleoside analogues[9] known to display activity against *T. cruzi* are depicted in Figure 1.[8, 10-12] The interest in deazapurine nucleoside analogues as antitrypanosomal agents can, in part, be traced back to the natural nucleoside antibiotic tubercidin (7-deazaadenosine, **2**), which elicits a broad spectrum of *in vitro* biological effects, including antitumor, antiviral and antitrypanosomal activity, but is also notoriously toxic to mammalian cells.[9] Several reports have focused on the introduction of additional modifications to improve selectivity and potency, predominantly as antiviral and/or anticancer agents.[9, 13] Our group recently reported on 7-modified (3'-deoxy-) 7-deazaadenosine derivatives with

pronounced antitrypanosomal activity (**3** & **4**).[14-15] We thus became interested in analogues that feature an additional N-deletion (*i.e.* so-called 1,7-dideazapurine [or pyrrolo[2,3-*b*]pyridine]) nucleosides (Figure 1).

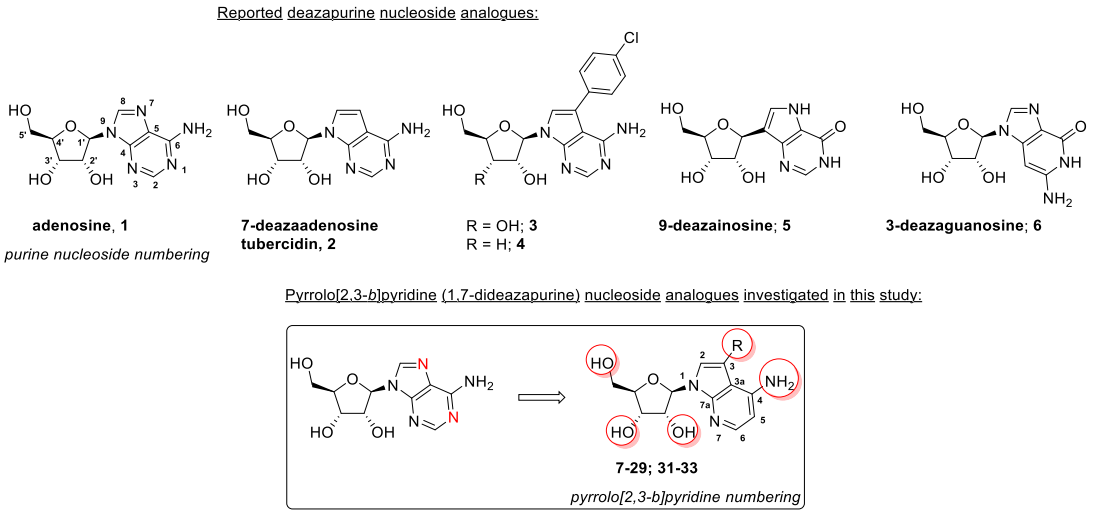


Figure 1: Deazapurine nucleoside analogues with reported antitrypanosomal activity (upper line). Structure of target adenosine analogues in the present work, indicated are the different groups to be modified (lower line).

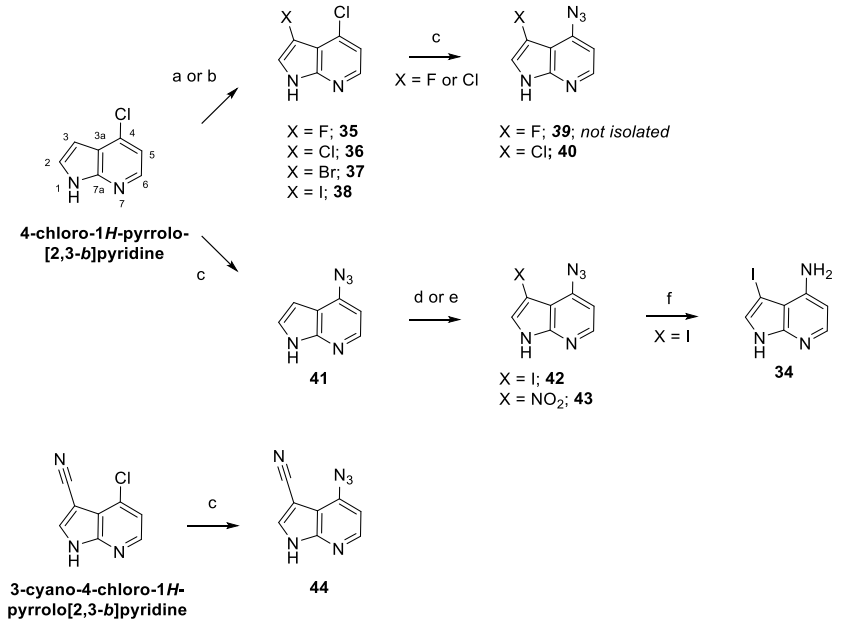
In the present study, we describe the discovery and structure-activity relationships of pyrrolo[2,3-*b*]pyridine nucleoside analogues with significant *in vitro* anti-*T. cruzi* activity, and the evaluation of one analogue in a mouse model of acute Chagas disease.

Results and discussion

Chemistry

For the synthesis of the target pyrrolo[2,3-*b*]pyridine nucleoside analogues we first prepared the required heterocyclic building blocks (**35-44**; Scheme 1). Halogen-containing heterocycles

were obtained by electrophilic halogenation at the C3 position of 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine or C4-azido analogue **41** with the appropriate *N*-halosuccinimide or Selectfluor®. To circumvent issues with the transformation of the 4-chlorine into an amino functionality at the nucleoside stage (also see Scheme 2), due to the inherently higher electron density in the pyrrolo[2,3-*b*]pyridine ring as compared to the pyrrolo[2,3-*d*]pyrimidine system found in 7-deazapurine nucleoside analogues,[16] it was decided to introduce an azido functionality before the glycosylation step (*vide infra*) as an amino group precursor. Azidation was effected under acidic conditions,[16-17] since initial attempts with sodium azide alone or in the presence of 15-crown-5 failed to deliver any appreciable amounts of **41** (data not shown). The azido functionality of compound **42** was reduced under Staudinger reaction conditions giving rise to **34**. Nitration of the C3 position of **41** under previously reported conditions[18] gave **43** in modest yield.

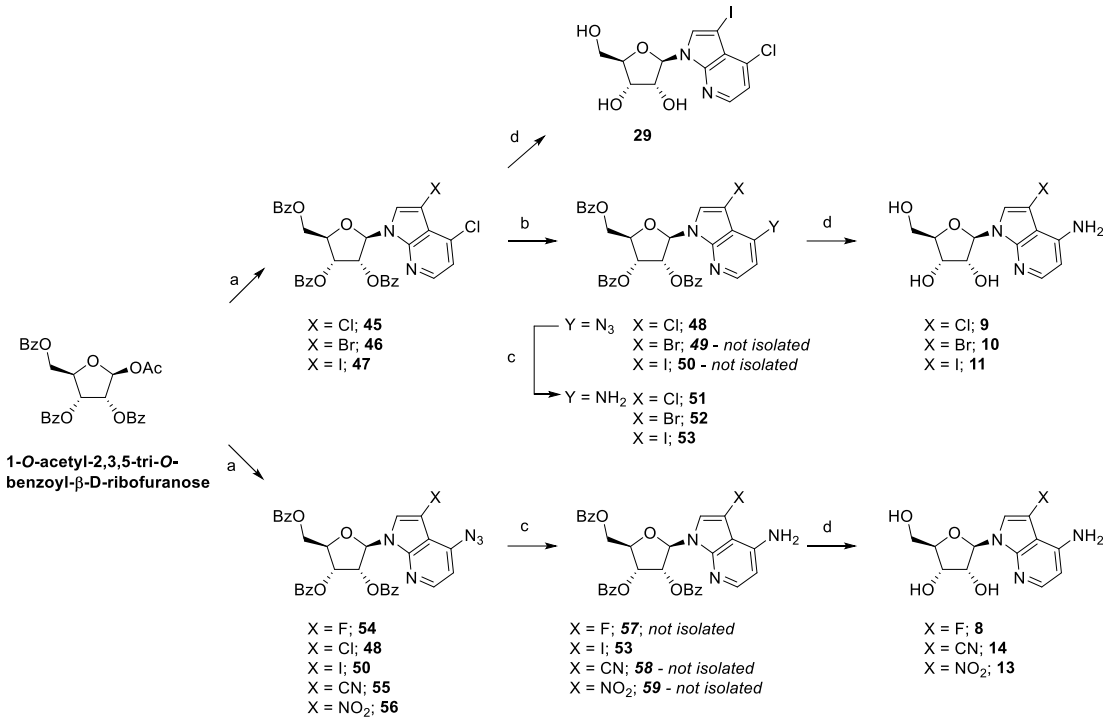


Scheme 1: Reagents and conditions: a) NXS, DMF, 92% (**36**, Cl), 96% (**37**, Br), 92% (**38**, I); b) Selectfluor®, AcOH, MeCN, 70 °C, 29% (**35**, F); c) NaN_3, NH_4Cl, DMF, 110 °C, 71% (**40**),

66% (**41**), 60% (**44**); d) NIS, DMF, 93% (**42**, I); e) H_2SO_4, HNO_3, H_2O (3:1:1 ratio), 0 °C, 35% (**43**, NO_2); f) 1. PMe_3 in THF, THF; 2. aq. HOAc, MeCN, 65 °C, 50%.

Next, Vorbrüggen glycosylation allowed to covert the pyrrolo[2,3-*b*]pyridines (**36-44**) to the corresponding ribonucleosides employing conditions that were recently reported by us (Scheme 2).[16] Notably, no regio-isomeric N7-nucleoside products have been isolated. Interestingly, we also discovered that 3-unmodified 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine could be glycosylated efficiently (data not shown). To the best of our knowledge, this is the first extensive report on a practical glycosylation protocol for substituted pyrrolo[2,3-*b*]pyridines under Vorbrüggen reaction conditions. Previous studies only concerned two substrates,[19] employing anion glycosylation[20] or acid-catalyzed fusion.[21]

We initially envisioned to substitute the 4-chlorine of **45** with sodium azide, but found that this could only be effected at 110 °C in the presence of 15-crown-5. Due to competing side-reactions and incomplete conversion, isolation of sufficiently pure **48** (as well as **49** and **50**), was troublesome, resulting in low yields. Therefore, these crude 4-azido analogues (**49** and **50**) were immediately subjected to Staudinger reduction to give the purifiable amines **51**, **52** and **53**. These issues led us to execute the glycosylation with the 4-azido modified heterocycles (Scheme 2).[16] Subsequent Staudinger reduction and deprotection with 7N NH_3/MeOH gave the desired compounds **9-11**, **13** and **14**. 4-Chloro analogue **29** was prepared via immediate deprotection of **47** with saturated ammonia in methanol.



Scheme 2: Reagents and conditions: a) **36**, **37**, **38**, **39**, **40**, **42**, **43**, **44**, BSA (*N*,*O*-bis(trimethylsilyl)acetamide), TMSOTf, MeCN, 80 °C, 88% (**45**), 77% (**46**), 79% (**47**), 70% (**48**), 76% (**50**), 40% (**54**), 85% (**55**), 43% (**56**); b) NaN_3, 15-crown-5, DMF, 110 °C, 52% (**48**); c) 1. 1M PMe_3 in THF, THF; 2. aq. HOAc, MeCN, 65 °C, 86% (**51**), 28% (**52**, 2 steps from **46**), 21% (**53**, 2 steps from **47**), 76% (**53** from **50**); d) 7N NH_3/MeOH, 51% (**8**, 2 steps), 83% (**9**), 95% (**10**), 78% (**11**), 39% (**13**, 2 steps), 70% (**14**, 2 steps), 63% (**29**).

Correct assignment of the stereo- (β-configuration) and regiochemistry (N1) of the final nucleoside analogues was ascertained by ^{1}H-^{1}H NOESY and ^{1}H-^{13}C HMBC experiments (Supporting Information). The structure of **11** was further confirmed by single crystal X-ray diffraction analysis (Figure 2 & Supporting Information).

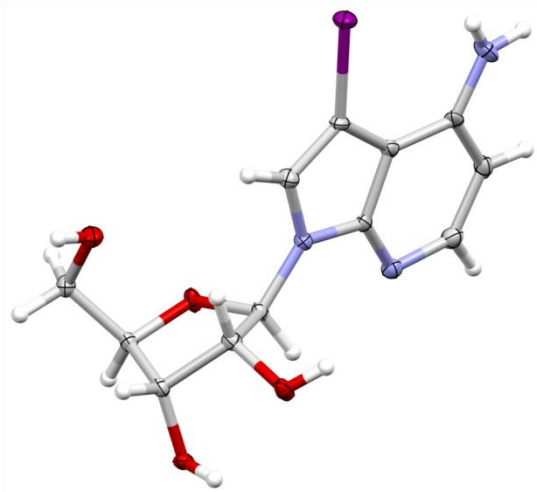
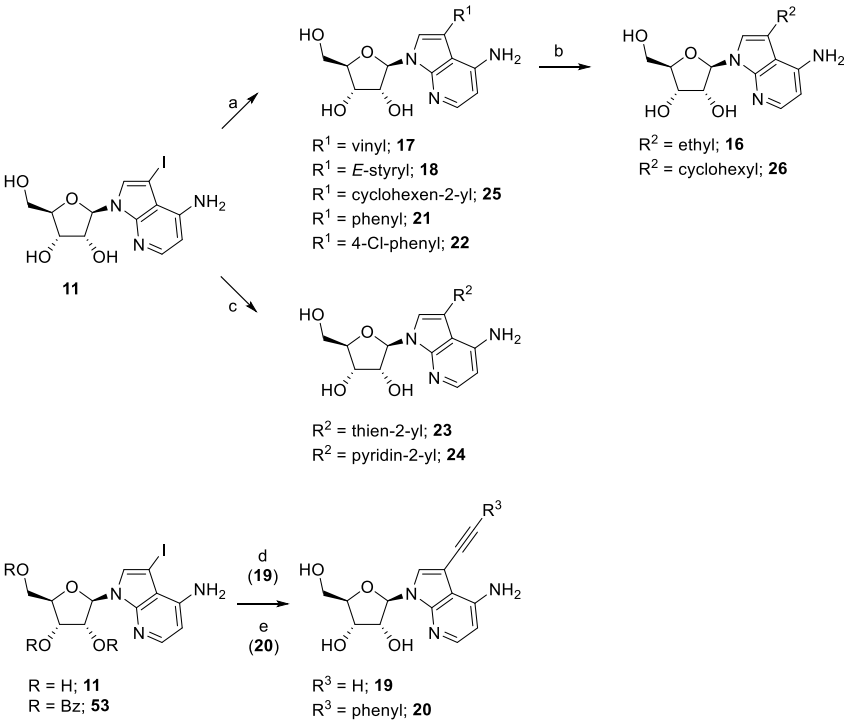


Figure 2: Molecular structure of **11**, showing thermal displacement ellipsoids at the 50% probability level.

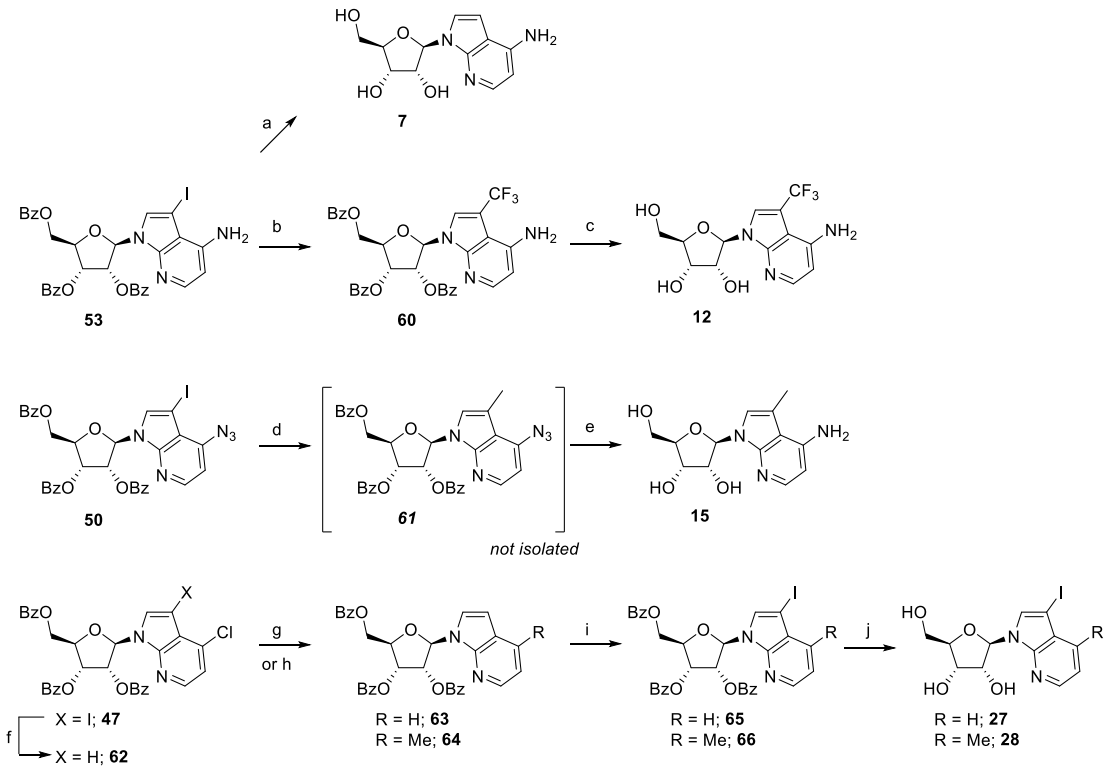
Various palladium-catalyzed cross-coupling reactions were employed to introduce a range of different substituents at the 3-position, using the 3-iodo derivative **11** or its ribose protected precursor **53** as the starting material (Scheme 3). The pyrrolo[2,3-*b*]pyridine system was found to be equally amenable to cross-coupling conditions as its pyrrolo[2,3-*d*]pyrimidine counterpart.[13, 15] Commercially available boronic acids or the corresponding trifluoroborates were employed for the Suzuki coupling. 3-Ethyl (**16**) or cyclohexyl (**26**) analogues were obtained after catalytic hydrogenation of the unsaturated derivatives **17** and **25**.



Scheme 3: Reagents and conditions: appropriate boronic acid (for **18**, **21**, **22**) or potassium trifluoroborate (for **17**, **25**), Na_2CO_3 (or Cs_2CO_3 for the synthesis of **17**), TPPTS, $Pd(OAc)_2$, MeCN/water (1:2 mixture), 100 °C, 35% (**17**), 34% (**18**), 50% (**21**), 60% (**22**), 22% (**25**); b) Pd/C, H_2, MeOH, 40% (**16**); 60% (**26**); c) 2-(tributylstannyl)thiophene or -pyridine, $Pd(PPh_3)_4$, CuI, DMF, 100 °C, 45% (**23**); 22% (**24**); d) 1. trimethylsilylacetylene, CuI, TPPTS, $Pd(PPh_3)_2Cl_2$, Et_3N, THF, 50 °C; 2. 7N NH_3/MeOH, 40% (two steps); e) phenylacetylene, CuI, TPPTS, $Pd(PPh_3)_2Cl_2$, Et_3N, THF, 50 °C, 55%.

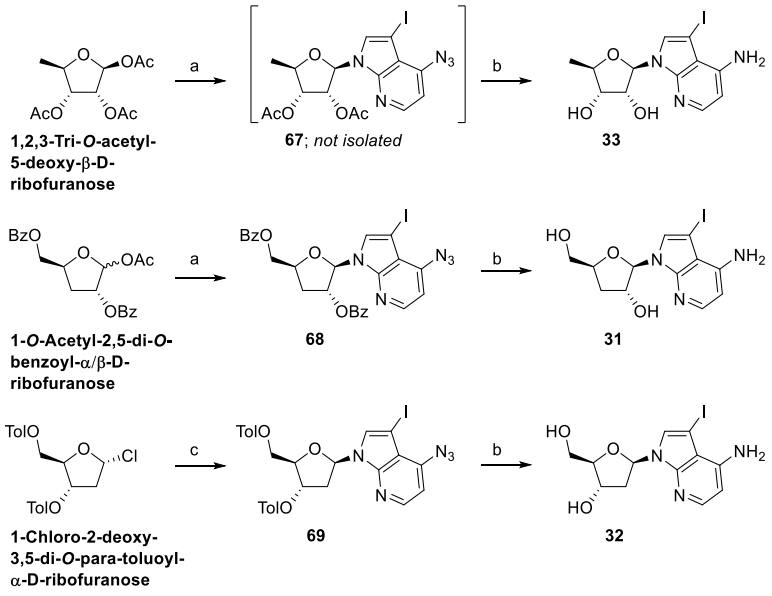
Further elaboration at the 3- and/or the 4-position of the nucleoside analogue is outlined in Scheme 4. Removal of the halogen at the 3-position was effected by catalytic dehydrohalogenation with Pd/C (**7**) or via iodine/Mg exchange using Knochel's i-PrMgCl·LiCl (**62**). Introduction of the 3-trifluoromethyl group (analogue **60**) was achieved by using the *in situ* prepared $CuCF_3$ from the Ruppert-Prakash reagent in NMP/DMF.[22] Methylation at 3-

position was effected via Pd-mediated cross-coupling with $AlMe_3$.[23] Modification of the 4-position was achieved by first removing the 3-iodo halogen (**62**), after which the 4-chloro group was either removed by catalytic dehydrogenation or methylated with $AlMe_3$ to give **63** and **64**, respectively. The halogen was re-introduced at C3 with NIS. Final deprotection was accomplished by ammonolysis with saturated NH_3/MeOH.



Scheme 4: Reagents and conditions: a) 1. Pd/C, H_2, aq. NaOAc, MeOH; 2. NaOMe/MeOH, 47% (two steps); b) KF, CuI, $TMSCF_3$, DMF/NMP (1:1 ratio), 100 °C, 80%; c) 7N NH_3/MeOH, 58%; d) 2M $AlMe_3$ in toluene, $Pd(Ph_3P)_4$, THF, 80 °C, 46%; e) 1. 1M PMe_3 in THF, THF; 2. aq. HOAc, MeCN, 65 °C; 3. 7N NH_3/MeOH, 32% (three steps); f) 1. 1.3M i-PrMgCl·LiCl in THF, THF, -20 °C; 2. 1M aq. HCl, 75%; g) Pd/C, H_2 (balloon), MeOH, 80% (**63**); h) 2M $AlMe_3$ in toluene, $Pd(PPh_3)_4$, THF, 100 °C, 66%, (**64**); i) NIS, DMF, 65% (**65**), 71% (**66**); j) 7N NH_3/MeOH, 68% (**27**), 46% (**28**).

To modify the ribofuranose part of the nucleoside, we started from the appropriate glycosyl donors, *i.e.* commercially available tri-*O*-acetyl-5-deoxyribofuranose, 1-chloro-2-deoxyribofuranose and 3'-deoxyribofuranose[14] (Scheme 5). 3'-Deoxy and 5'-deoxy analogues were obtained by Vorbrüggen glycosylation, while anion glycosylation was used for the synthesis of the 2'-deoxy analogue **69**. It was found that glycosylation with the 2-*O*-acetyl donor resulted in lower yields as compared to the 2-*O*-benzoyl donors. This has also been observed for pyrrolo[2,3-*d*]pyrimidine acceptors.[14, 16] The nucleoside was elaborated as described above.



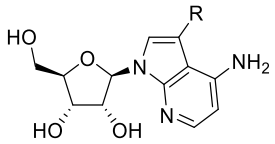
Scheme 5: Reagents and conditions: a) **42**, BSA, TMSOTf, MeCN, 80 °C, 47% (**68**); b) 1. 1M PMe_3 in THF, THF; 2. aq. HOAc, MeCN, 65 °C; 3. 7N NH_3/MeOH, 40% (**31**, two steps), 62% (**32**, two steps), 21% (**33**, three steps); c) **42**, NaH, DMF, 0 °C–rt, 37%.

Biological evaluation

In vitro evaluation

All synthesized compounds were evaluated *in vitro* against intracellular *T. cruzi* amastigotes (Tulahuen strain expressing β-galactosidase) with benznidazole as a reference drug. Cytotoxicity of the compounds was evaluated in MRC-5 fibroblasts. Results are depicted in Tables 1 and 2.

Table 1. Activity of C3 modified pyrrolo[2,3-*b*]pyridine nucleoside analogues against intracellular *T. cruzi* amastigotes. [a]



Cpd.	R	*T. cruzi*[a] EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI	Cpd.	R	*T. cruzi*[a] EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI
7	H	*2.27*	17.9 ± 4.4	7.9	**17**	vinyl	0.81 ± 0.27	>64.0	>79
8	F	8.8 ± 2.4	>64.0	>7.3	**18**	*E*-styryl	>64.0	>64.0	--
9	Cl	0.19 ± 0.05	>64.0	>337	**19**	ethynyl	0.080 ± 0.026	59.0 ± 5.0	730
10	Br	0.20 ± 0.02	>64.0	>320	**20**	phenylethynyl	>64.0	>64.0	--
11	I	0.040 ± 0.013	>64.0	>1600	**21**	phenyl	>64.0	>64.0	--
12	CF_3	1.56 ± 0.85	0.73 ± 0.12	0.47	**22**	4-chlorophenyl	>64.0	>64.0	--
13	NO_2	*47.7*	*>64.0*	>1.3	**23**	2-thienyl	36.9 ± 0.7	>64.0	>1.7
14	CN	4.00 ± 0.00	18.5 ± 15.2	4.6	**24**	2-pyridyl	>64.0	>64.0	--
15	Me	1.14 ± 0.61	51.6 ± 12.4	45	**25**	cyclohexen-1-yl	>64.0	>64.0	--
16	Et	>64.0	>64.0	--	**26**	cyclohexyl	>64.0	>64.0	--
Benznidazole		2.36 ± 0.14	ND	--					

Table 1: [a]Tulahuen strain expressing β-galactosidase, assayed with MRC-5 fibroblasts as the host cell. EC_{50} values are expressed in µM and represent the mean values and SEM from two or three independent experiments. Values in *italics* are the result of a single determination. SI = Selectivity Index, EC_{50}(MRC-5)/EC_{50}(*T. cruzi*). ND; Not Determined.

We found that 1,7-dideazaadenosine **7**[21] displayed only modest antitrypanosomal activity, and that C3 arylated derivatives (**21** and **22**) were devoid of activity, in strong contrast to their previously reported pyrrolo[2,3-*d*]pyrimidine analogues.[14-15] This trend also held for the heteroaryl substituted analogues (**23** & **24**), and for the cyclohexyl and -hexenyl derivatives **25** and **26**.

Halogenated analogues **9**, **10** and **11** on the other hand all displayed submicromolar activity. The 3-iodo substituted **11** emerged as the most potent analogue, displaying an EC_{50} = 0.040 ± 0.013 μM and no discernable *in vitro* cytotoxicity (SI > 1600). Noteworthy, the activity mapped with size of the 3-substituent (I> Br ≈ Cl > F).

We next evaluated three other electron-withdrawing substituents: trifluoromethyl (**12**), nitro (**13**), and cyano (**14**), none of which was able to surpass the micromolar activity cut-off. The C3-methyl substituted derivative **15** showed low micromolar potency, while its ethyl homologue **16** proved inactive. Interestingly, vinyl analogue **17** showed submicromolar activity with a good selectivity index (SI > 79). The activity and selectivity of the alkynyl analogue **19** was even more pronounced (EC_{50} = 0.080 ± 0.026 μM, SI = 730). Further elongation of these analogues (**18** & **20** respectively) proved detrimental to the antitrypanosomal activity.

Interestingly, the cytotoxicity for this new class of nucleosides was generally found to be low, with the trifluoromethyl analogue (**12**) being a notable exception. Having identified the optimal 3-iodo substituent, we further focused the SAR investigation on the C4 position, and the ribofuranose ring of **11** (Table 2).

Replacement of the 6-amino group by either a chlorine (**29**), methyl (**28**) or hydrogen (**27**) led to a significant drop in activity (compare **27**, **28** and **29** with **11**), indicating the absolute

requirement for the NH_2 functionality for potent activity. The matched pyrrolo[2,3-*d*]pyrimidine nucleoside **30** was roughly 20-fold less potent *in vitro*, and much less selective, demonstrating the unique profile of the 1,7-dideazapurine nucleoside analogues.

Table 2. Activity of C4 and ribofuranose modified pyrrolo[2,3-*b*]pyridine nucleoside analogues against intracellular *T. cruzi* amastigotes. [a]

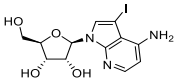
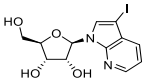
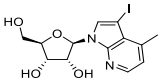
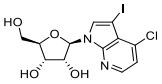
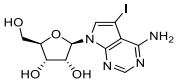
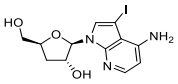
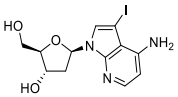
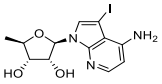
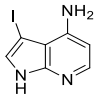
Cpd.	Structure	*T. cruzi* EC_{50} (µM)	MRC-5 EC_{50} (µM)	SI
11		0.040 ± 0.013	>64.0	>1600
27		1.75 ± 0.99	52.9 ± 11.2	>30
28		7.98 ± 2.83	2.29 ± 1.71	0.29
29		*2.14*	*2.18*	1.0
30		0.80 ± 0.02	5.35 ± 0.31	6.7
31		>64.0	>64.0	--
32		>64.0	>64.0	--
33		>64.0	>64.0	--
34		>64.0	>64.0	--
Benznidazole		2.36 ± 0.14	ND	--

Table 2: [a]Tulahuen strain expressing β-galactosidase, assayed with MRC-5 fibroblasts as the host cell. EC_{50} values are expressed in µM and represent the mean values and SEM from two or three independent experiments. Values in *italics* are the result of a single determination. SI = Selectivity Index, EC_{50}(MRC-5)/EC_{50}(*T. cruzi*). ND; Not Determined.

Keeping the 3-iodo-4-amino pyrrolo[2,3-*b*]pyridine base unaltered, we next focused our attention to the sugar ring and investigated the importance of each hydroxy group of the ribofuranose ring by their selective removal (**31**, **32** & **33**). Deletion of either OH resulted in analogues that were devoid of activity at the highest tested concentration. Interestingly, this significantly contrasts with the previously found SAR trends in the related pyrrolo[2,3-*d*]pyrimidine nucleoside series. Neither the addition of aryl groups in the heterocylic part[15] nor the removal of the 3'-OH[24] was found to be beneficial for *in vitro* activity, but surprisingly led to a complete loss of activity, suggesting a distinct uptake and/or activation pathway of the described nucleoside analogue series compared to their pyrrolo[2,3-*d*]pyrimidine counterpart. Lastly, an intact ribofuranose ring was shown to be essential for activity and the aglycon pyrrolo[2,3-*b*]pyridine **34** was also found inactive.

*In vitro metabolic stability of **11***

The metabolic stability of derivative **11** was evaluated by incubating it with mouse, rat, dog and pooled human microsomal fractions. This *in vitro* assay mimics both phase I (NADPH) and phase II (UGT) mediated liver metabolism. The results are depicted in Table 3.

Phase I / II	Time	MOUSE % 11 remaining		RAT % 11 remaining		DOG % 11 remaining		HUMAN % 11 remaining	
		Mean	STDEV	Mean	STDEV	Mean	STDEV	Mean	STDEV
	0	100	-	100	-	100	-	100	-
CYP - NADPH	15	96	1.3	88	2.3	97	1.5	99	4.3
	30	95	0.5	87	1.5	96	0.4	96	0.3
	60	94	8.2	85	4.3	92	1.7	92	2.0
	0	100	-	100	-	100	-	100	-
UGT Enz.	15	102	0.6	97	1.3	94	1.1	99	1.5
	30	103	4.6	94	4.4	94	3.7	99	0.5
	60	101	0.5	94	2.0	84	0.8	100	0.6

Table 3: Assessment of *in vitro* metabolic stability of nucleoside analogue **11** using male mouse, rat, dog and pooled human S9 microsomal fractions. The depicted values are the percentage of remaining parent compound. The various time points of incubation are indicated (0-15-30-60 min). Data originate from two independent experiments of two biological replicates. Reference drugs diclofenac (susceptible to phase I and phase II metabolism) and fluconazole (stable in phase I metabolism) were employed to ensure assay performance (data not shown).

Evaluating the percentage remaining compound at the 30 min time-mark indicated that analogue **11** was metabolically stable. Given that it also displayed interesting *in vitro* activity against *T. cruzi* intracellular amastigotes, and that it was devoid of cytotoxicity against human fibroblasts, analogue **11** was selected for further evaluation in a mouse model of acute *T. cruzi* infection.

*In vivo evaluation of **11***

Compound **11** was evaluated in a stringent model of acute Chagas disease, which employed Y-strain *T. cruzi* in Swiss male mice.[25] The compound was administered at 25 mg/kg b.i.d. by oral

gavage for five consecutive days, with treatment initiated at 5 days dpi (Figure 3). Orally administered benznidazole (BZ, 100 mg/kg, s.i.d.) for five consecutive days was used as a positive control. All non-treated animals developed peak parasitemia at 8dpi and succumbed to the infection by 15 dpi (Panel A & B). Compound- and BZ-treated mice showed 95 % and 100 % parasitemia reduction at 8dpi (Panel A), with blood parasitemia being undetectable at all later timepoints up to 30 dpi upon tail vein blood examination. All treated animals (compound **11** & BZ) survived up to 40 dpi (Panel B), with no relapses, as measured by circulating blood parasitemia and showed no signs of weight loss nor other visual clinical symptoms that would indicate compound toxicity. Next, the surviving animals were immunosuppressed by administration of cyclophosphamide (three cycles of 50 mg/kg s.i.d.), which unfortunately caused re-appearance of blood parasitemia in all animals in both the compound and BZ treatment groups (Panel C). The latter has been observed in other studies as well,[14, 26-28] and this stringent model requires a significantly longer BZ-treatment schedule to establish sterile cure.[26, 28] In order to further investigate the potential of **11** as a potential therapeutic option for CD, we employed an extended (15 day) treatment protocol in the same model of *T. cruzi* infection (Panels D, E, F). Oral administration of **11** again resulted in complete reduction of parasitemia by 9dpi (Panel D), comparable to the reference BZ. All treated animals showed negative parasitemia up to 30 dpi and survived up to 55 dpi (Panels D & E, respectively). No clear signs of compound toxicity (such as weight loss) could be recorded with this extended treatment regime. Then, cyclophosphamide was administered to check whether the mice were completely cured. Regrettably, all mice relapsed, in compound **11** as well as BZ treatment groups (Panel F).

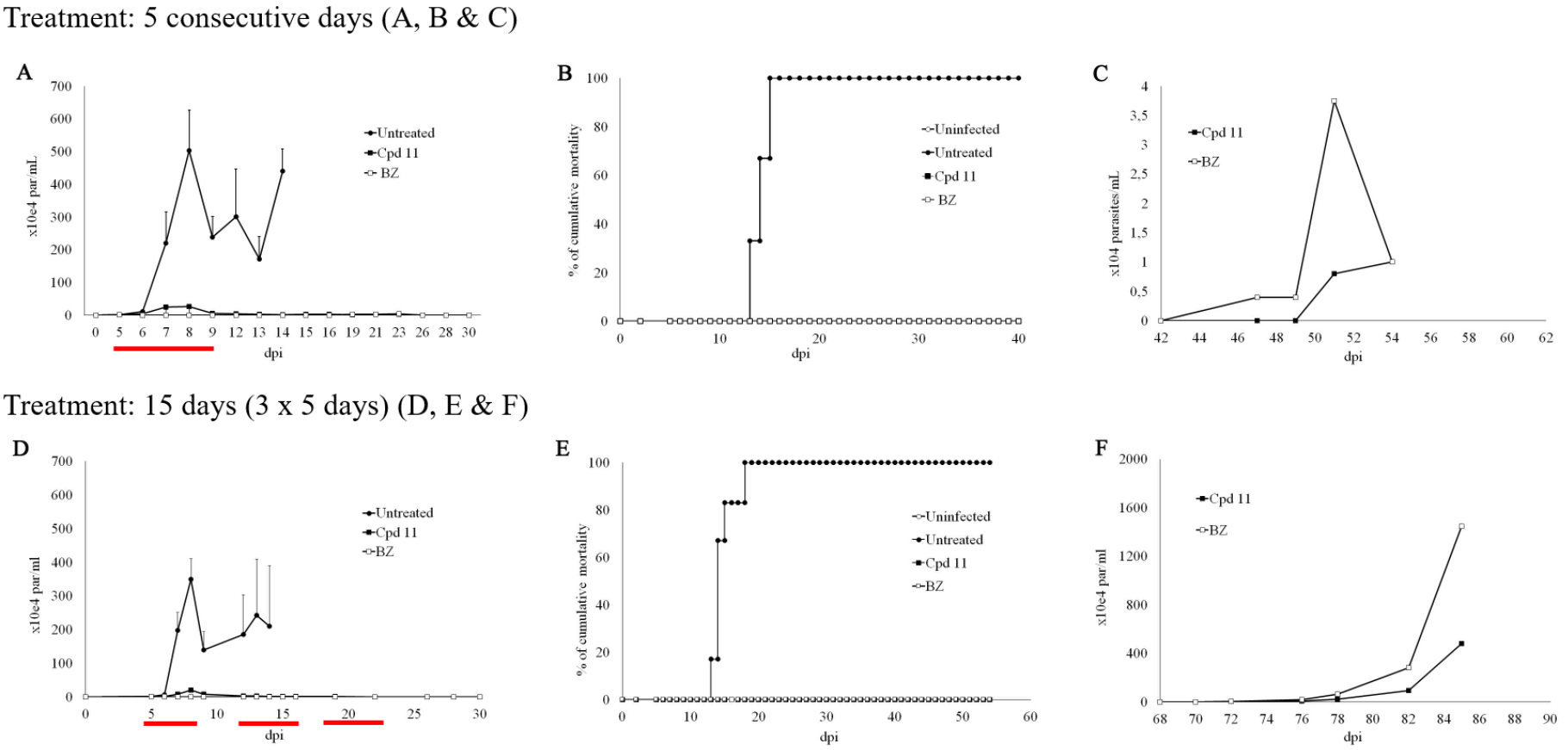


Figure 3: *In vivo* evaluation of analogue **11** in a Y-strain *T. cruzi* mouse model of acute infection. Panels A & D depict blood parasitemia, determined microscopically after tail vein puncture. Panels B & E depict cumulative mortality of animals. Panels C & F depict tail vain blood parasitemia after

cyclophosphamide treatment. Male Swiss mice were infected i.p. with 10^4 bloodstream form trypomastigotes (Y-strain) at day 0. Compound treatment was initiated at 5 dpi and is indicated with a red line. Two different treatment regimens are depicted: compound administration for 5 consecutive days (Panels A, B & C) and 15 days (3 x 5 consecutive days, 2 days apart; Panels D, E, F). Each group consisted of six mice. Benznidazole was included as a reference drug given at 100 mg/kg, s.i.d. with the same dosing regimen as analogue **11**. Cyclophosphamide-induced immunosuppression was done by three cycles of 50 mg/kg s.i.d. administration for 4 consecutive days, followed by 3 drug-free days. When different treatment groups are not depicted separately in the graph, they coincide on the x-axis.

To gain insights into potential reasons for the failure of compound **11** to achieve a sterile cure, we investigated the effects of this derivative (and several other 1,7-dideazapurine nucleosides) on bloodstream form trypomastigotes (Table 3). As the animal model was performed with Y-strain *T. cruzi*, drug sensitivity against intracellular amastigotes of this strain was determined as well. Additionally, cytotoxicity was evaluated against primary mouse cardiac cells and L929 mouse fibroblasts. From these results it became apparent that all 3-substituted pyrrolo[2,3-*b*]pyridine nucleosides assayed, elicited weak to no potency against bloodstream form trypomastigotes, which is reminiscent of what has been found for several CYP51 inhibitors,[29] such as the antifungal posaconozale.[30] Analogue **11** displayed comparable *in vitro* activity against Y-strain intracellular amastigotes as it did against amastigotes of Tulahuen strain (Table 3). Of note is the complete lack of *in vitro* cytotoxicity of all assayed analogues against cardiac as well as against L929 fibroblasts.

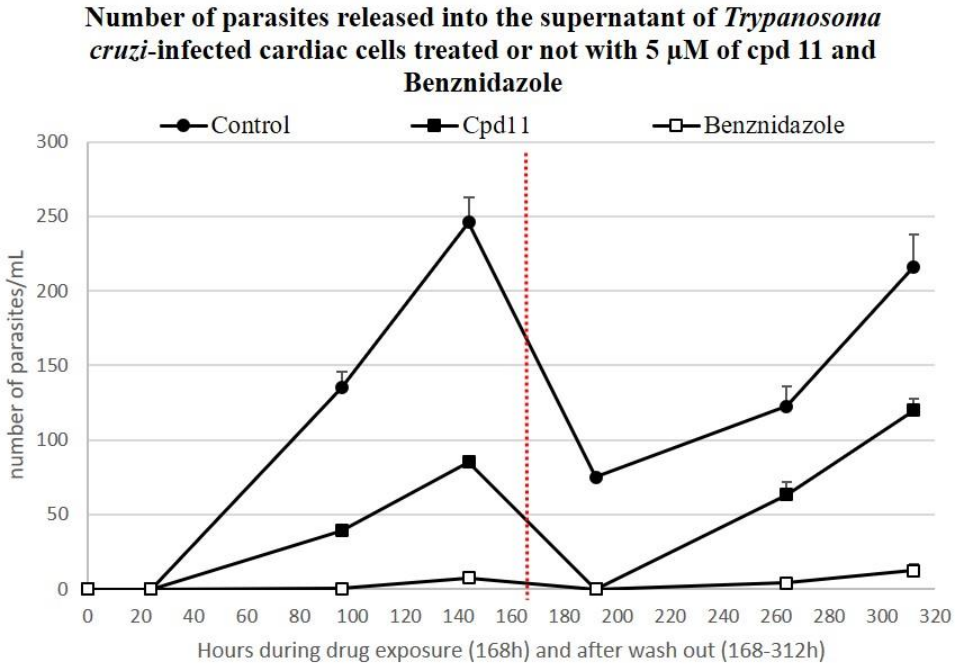
Cpd.	Y-strain trypomastigotes EC_{50} (µM)	Y-strain intracellular amastigotes[a] EC_{50} (µM)	Primary cardiac cells EC_{50} (µM)	L929 cells EC_{50} (µM)
9	>50.0	--	>400	>200
11	>70.0	0.089 ± 0.0	>400	>200
15	>50.0	--	--	>200
17	>50.0	--	--	>200
19	40.5 ± 5.0	--	--	>200
27	36.3 ± 1.7	--	--	>200
Benznidazole	5.7 ± 0.6	0.35 ± 0.2	>400	>200

Table 3: *In vitro* effects of selected analogues against Y-strain *T. cruzi* bloodstream trypomastigotes and intracellular amastigotes (primary cardiac cells as host cell). Cytotoxicity

was simultaneously evaluated against primary mouse cardiac cells and L929 cells. EC_{50} values are reported in μM and represent mean and SE of two independent determinations. [a]The effects of analogues against intracellular amastigotes is measured as the reduction of infection index (percentage of infected host cells x number of parasites per host cell).

Finally, we investigated the potential of **11** to completely clear a *T. cruzi* infection of *in vitro* cultures. Thus, infected cardiac cells (Y-strain *T. cruzi*) were exposed to increasing concentrations of analogue **11**. After 168 h of incubation, cell medium was replaced by fresh, drug-free medium. After incubation for another 168 h, drug sensitivity read-out was performed in a similar way as for the assay without washout. Additionally, at several time points, the cell medium was investigated for the presence of released trypomastigotes (only quantified by light microscopy readout for the 5 μM concentration level). Results of the washout experiment are depicted in Figure 4.

A



B

Cpd.	Y-strain intracellular amastigotes 168 h exposure EC_{50} (µM)	Y-strain intracellular amastigotes 168 h exposure & 168 h washout EC_{50} (µM)
11	0.089 ± 0.0	>5.0
BZ	0.35 ± 0.2	2.7 ± 0.0

Figure 4: Washout experiments of *T. cruzi* (Y-strain) infected cardiac cell cultures. Panel A: Number of culture-released trypomastigotes as a function of incubation time. Data shown are from the 5 µM drug concentration level. Infected cultures are incubated for 168 h with **11** or BZ and then another 168 h with drug-free medium before assay readout. Data represent mean ± SD of two independent experiments. The red line indicates the time point at which compound exposure is halted by changing to drug-free medium. Panel B: Comparison of the drug sensitivity before (168 h drug exposure) and after washout (168 h drug-free medium).

The washout experiment showed that **11** is unable to clear the parasite from infected cardiac

cell culture, since a time-dependent increase in the number of released trypomastigotes was noted, after removal of drug pressure. Further, when the drug sensitivity values for **11** before and after washout were compared (derived from reduction of infection index) a significant increase was observed, possibly related to a trypanostatic rather than trypanocidal behavior of **11**. Thus, the abovementioned results (inability to affect the bloodstream trypomastigote stage and the potential trypanostatic activity) provide an explanation for the lack of sterile cure seen in the *in vivo* experiments.

Conclusion

In the present paper we have described the synthesis of diversely modified pyrrolo[2,3-b]pyridine nucleoside analogues and investigated their activity against *Trypanosoma cruzi*. This 'neglected' purine scaffold was in general devoid of cytotoxicity and halogen or small, linear carbon-based substituents in the 3-position conferred the highest *in vitro* activity against intracellular *T. cruzi* amastigotes. The most potent analogue, **11**, was metabolically stable *in vitro*, and has been evaluated in a Y-strain *T. cruzi* mouse model of acute CD. Oral dosing at 25 mg/kg b.i.d. for five consecutive days resulted in complete suppression of blood parasitemia and enabled survival of all test animals. Immunosuppression resulted in recrudescence, which was also observed in an experiment with a three-fold longer treatment schedule. We observed that this class of analogues generally exhibited low activity against bloodstream trypomastigotes. Washout experiments of infected cultures indicate that **11** is trypanostatic rather than trypanocidal. Follow-up research might entail the combination of this analogue with a known trypanocidal compound,[31-32] particularly given that it was well-tolerated by the mice

and devoid of notable *in vitro* cytotoxicity. Additionally, **11** and several other analogues might serve as tool compounds for the investigation of *T. cruzi* nucleoside transporters.

Experimental section

Chemistry

Reagents and solvents were purchased from standard commercial sources. They were of analytical grade and used as received. Nucleoside analogue **30** was prepared according to a literature procedure.[33] Compounds **36-38**, **40-42** were prepared as described previously.[16]

Analytical TLC was performed on Macherey-Nagel® precoated F254 aluminum plates. TLC plates were first visualized by UV and then developed by staining with basic aq. $KMnO_4$ and heating. Silica gel column chromatography was performed using Macherey-Nagel® 60M silica gel (40-63 μm) or on a Reveleris X2 (Grace/Büchi) automated flash apparatus. The latter employed commercially available prepacked silica columns. Preparative RP-HPLC was performed on a Waters AutoPurification system (equipped with ACQUITY QDa (mass; 100–1000 amu)) and 2998 Photodiode Array (220–400 nm)), employing a Phenomenex® Luna Omega Polar (250x21 mm, 5 μm) column, at a flow rate of 20 mL/min. A gradient of 0.2 % (V/V) formic acid in water/MeCN was used, of which the specific gradient is mentioned for each compound. Exact mass measurements were recorded on a Waters LCT Premier XE time-of-flight (ToF) mass spectrometer equipped with a standard electrospray (ESI) and modular Lockspray® interface. NMR spectra were recorded on a Varian Mercury 300 MHz (^{1}H at 300 MHz, ^{13}C at 75 MHz and ^{19}F at 282 MHz) spectrometer. Chemical shifts values are given in ppm. Spectra were referenced on the residual solvent peak signal. The coupling constants (*J*)

are reported in hertz (Hz). The stereochemistry at C-1' was ascertained by 2D NMR techniques (^{1}H-^{1}H 2D NOESY and ^{1}H-^{13}C gHMBC respectively). In ^{19}F-NMR, signals were referenced to the $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to $\delta = 0$ ppm. Melting points were performed 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 HCOOH in H_2O (0.2 %, v/v)/MeCN at a flow rate of 1.44 mL/min, 95:05 to 00:100 in 6.5 minutes. All target compounds possessed a purity of at least 95 %, as assayed by analytical HPLC (integration of UV signal: total UV chromatogram as well as wavelength selected at 254 nm).

General procedure for glycosylation (based on reference [16] and [33]) – General procedure A

In a flame-dried two-neck round bottom flask under argon was added the appropriate heterocycle (1 eq.). Next, anhydrous MeCN (7.5 mL/mmol starting material (SM)) was added. To the stirring suspension was added BSA (1.1 eq.) in one portion. The resulting mixture was stirred at room temperature for ~10min, after which the glycosyl donor (1.1 eq.) was added in one portion, immediately followed by TMSOTf (1.2 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 ~1 h). Then, the mixture was cooled to ambient temperature. Next, EA (ethyl acetate) was added, and aq. sat. $NaHCO_3$. 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 (generally isocratic, with an eluent consisting of 15 % EA/hexane or petroleum ether (PE)).

General procedure for Staudinger reaction and subsequent iminophosphorane hydrolysis (as described in reference [15]) – General procedure B

The azido-nucleoside (1 eq.) was dissolved in THF (10 mL/mmol). Then, PMe_3 solution (1M in THF; 2.66 eq.) was added and the mixture stirred at ambient temperature. When TLC analysis showed complete disappearance of the starting material (typically 1 h), the solution was evaporated, and re-dissolved in MeCN (10 mL/mmol). To this solution was added a 1M aq. HOAc solution (3.33 eq.), after which the flask was heated in a pre-heated oil bath at 65 °C for 1 h. Then, the mixture was cooled to ambient temperature and poured into sat. aq. $NaHCO_3$ solution. DCM was added, the layers were separated, and the water layer extracted two more times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. Purification by column chromatography (EA/PE gradient) provided the amino nucleoside intermediate.

General procedure for Suzuki Coupling (as described in references [13] and [14]) – General procedure C

11 (1 eq.), boronic acid (1.5 eq.), Na_2CO_3 (9 eq.), $Pd(OAc)_2$ (0.05 eq.) and TPPTS (0.15 eq.) were added to a 10 mL round-bottom flask, equipped with a stir bar. Next, the air was removed and backfilled with argon. This was repeated thrice. Then, a mixture of degassed MeCN/water (1/2 ratio, 6 mL/mmol SM) was added to the solids. The mixture was then stirred at ambient temperature (~5 min), and then heated to 100 °C. The reaction was monitored by analytical LC/MS for consumption of the iodonucleoside **11** (~0.5 to 3 h), after which it was allowed to

cool to ambient temperature. Then, 0.5 M aq. HCl was added to neutralize the mixture (pH ~ 7). Then, the volatiles were removed *in vacuo*, and the residue resuspended in MeOH and evaporated, which was repeated thrice. Then the residue was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~ 5 cm) with 20 % MeOH/DCM. The obtained solution was evaporated until dryness and purified by column chromatography (MeOH/DCM gradient).

4-Amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (7) 53 (0.056 g, 0.080 mmol) was dissolved in MeOH (3 mL) and 0.5 M aq. NaOAc solution (0.20 mL) was added. The flask was purged with N_2 gas, and a catalytic amount of Pd/C was added. The mixture was stirred at ambient temperature under H_2-atmosphere (balloon; bubbling) until LC-MS analysis showed full conversion of the starting material (~5 h). Then, the mixture was purged with N_2, and subsequently filtered over a short pad of Celite®. The filtrate was evaporated until dryness and immediately deprotected with a cat. amount of 5.4 M NaOMe in MeOH (3 mL). After 1 h, the mixture was neutralized with 0.5 M aq. HCl solution and evaporated. The residue was purified by column chromatography 5 → 20 % MeOH/DCM to give **7** (0.010 g, 0.038 mmol) as a white solid in 47 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.51 (dd, *J* = 12.3, 3.6 Hz, 1H, H-5''), 3.62 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 3.90 (q, *J* = 3.0 Hz, 1H, H-4'), 4.07 (q, *J* = 2.4 Hz, 1H, H-3'), 4.55 (t, *J* = 6.0 Hz, 1H, H-2'), 5.13 (br. s, 3H, OH-2', OH-3', OH-5'), 5.93 (d, *J* = 6.3 Hz, 1H, H-1'), 6.20 (d, *J* = 5.4 Hz, 1H, H-5), 6.28 (br. s, 2H, NH_2), 6.55 (d, *J* = 3.6 Hz, 1H, H-3), 7.26 (d, *J* = 3.9 Hz, 1H, H-2), 7.69 (d, *J* = 5.4 Hz, 1H, H-6). Spectral data are in accordance with literature values.[21]

3-Fluoro-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (8) According to General procedure B, **54** (0.55 g, 0.88 mmol) gave rise to the amino-intermediate (0.30 g, 0.50 mmol) as a yellow foam in 57 % yield. Purification: 40 → 60 % EA/Hex. Next the amino-intermediate (0.30 g) was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **8** (0.13 g, 0.45 mmol) as a white solid in 51 % over two steps. Melting point: 200 - 202 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.49 (ddd, *J* = 12.0, 6.4, 3.8 Hz, 1H, H-5''), 3.60 (ddd, *J* = 11.7, 7.6, 4.1 Hz, 1H, H-5'), 3.85 (q, *J* = 3.7 Hz, 1H, H-4'), 4.02 - 4.07 (m, 1H, H-3'), 4.36 (q, *J* = 6.2 Hz, 1H, H-2'), 5.03 (d, *J* = 4.7 Hz, 1H, OH-3'), 5.21 (d, *J* = 6.4 Hz, 1H, OH-2'), 5.36 (dd, *J* = 6.4, 5.0 Hz, 1H, OH-5'), 6.05 (dd, *J* = 6.3, 1.5 Hz, 1H, H-1'), 6.16 (br. s, 2H, NH_2), 6.23 (d, *J* = 5.5 Hz, 1H, H-5), 7.28 (d, *J* = 1.7 Hz, 1H, H-2), 7.74 (d, *J* = 5.5 Hz, 1H, H-6). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -170.64. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5'), 70.6 (C-3') , 73.3 (C-2'), 84.8 (C-4'), 87.2 (C-1'), 97.4 (d, *J* = 13.8 Hz, 1C, C-3a), 100.6 (C-5), 104.6 (d, *J* = 26.5 Hz, 1C, C-2), 143.3 (d, *J* = 241.9 Hz, 1C, C-3), 144.7 (d, *J* = 2.3 Hz, 1C, C-7a), 144.9 (C-6), 147.5 (d, *J* = 2.3 Hz, 1C, C-4). HRMS (ESI): calculated for $C_{12}H_{15}FN_3O_4$ ($[M+H]^+$): 284.1041, found: 284.1040.

3-Chloro-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (9) 51 (0.10 g, 0.16 mmol) was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (6 % MeOH/DCM) to give **9** (0.040 g, 0.14 mmol) as a white solid in 83 % yield. Melting point: 208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.48 – 3.55 (m, 1H, H-5''), 3.58 – 3.65 (m, 1H, H-5'), 3.87 (dd, *J* = 6.6, 3.6 Hz, 1H, H-4'), 4.04 – 4.08 (m, 1H, H-3'), 4.40

(dd, *J* = 11.4, 6.3 Hz, 1H, H-2'), 5.06 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.24 (d, *J* = 6.3 Hz, 1H, OH-2'), 5.40 (dd, *J* = 6.6, 4.8 Hz, 1H, OH-5'), 6.04 (d, *J* = 6.3 Hz, 1H, H-1'), 6.17 (br. s, 2H, NH_2), 6.28 (d, *J* = 5.4 Hz, 1H, H-5), 7.51 (s, 1H, H-2), 7.76 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5'), 70.6 (C-3'), 73.4 (C-2'), 85.0 (C-4'), 87.5 (C-1'), 101.4 (C-5), 101.9 (C-3), 104.2 (C-3a), 119.6 (C-2), 144.7 (C-6), 147.2 (C-7a), 148.5 (C-4). HRMS (ESI): calculated for $C_{12}H_{14}ClN_3O_4$ ($[M+H]^+$): 300.0746, found: 300.0732.

3-Bromo-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (10) 52 (0.18 g, 0.28 mmol) was dissolved in 7N NH_3/MeOH (5 mL). The resulting mixture was stirred at ambient temperature overnight and evaporated until dryness. The residue was purified by column chromatography (6 % MeOH/DCM) to give **10** (0.095 g, 0.28 mmol) as a white solid in 95 % yield. Melting point: 240 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.48 – 3.55 (m, 1H, H-5''), 3.58 – 3.65 (m, 1H, H-5'), 3.87 (dd, *J* = 6.3, 3.6 Hz, 1H, H-4'), 4.04 – 4.08 (m, 1H, H-3'), 4.37 – 4.43 (m, 1H, H-2'), 5.05 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.24 (d, *J* = 6.3 Hz, 1H, OH-2'), 5.40 (dd, *J* = 6.6, 4.5 Hz, 1H, OH-5'), 6.04 (d, *J* = 6.3 Hz, 1H, H-1'), 6.15 (br. s, 2H, NH_2), 6.30 (d, *J* = 5.4 Hz, 1H, H-5), 7.56 (s, 1H, H-2), 7.77 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5'), 70.6 (C-3'), 73.4 (C-2'), 85.0 (C-4'), 85.7 (C-1'), 87.5 (C-3), 101.5 (C-5), 105.0 (C-3a), 122.2 (C-2), 144.5 (C-6), 147.6 (C-7a), 148.6 (C-4). HRMS (ESI): calculated for $C_{12}H_{14}BrN_3O_4$ ($[M+H]^+$): 344.0240, found: 344.0234.

3-Iodo-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (11) 53 (0.15 g, 0.21 mmol) was dissolved in 7N NH_3/MeOH. The mixture was stirred at ambient temperature overnight and evaporated. The residue was purified by column chromatography (5 → 7.5 % MeOH/DCM) to give **11** (0.065 g, 0.17 mmol) as a white solid in 78 % yield. Melting point: 218 - 220 °C. ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 3.51 (dd, *J* = 12.0 Hz, 3.3 Hz, 1H, H-5''), 3.61 (dd, *J* = 12.0, 3.3 Hz, 1H, H-5'), 3.88 (dd, *J* = 6.6, 3.3 Hz, 1H, H-4'), 4.04 – 4.08 (m, 1H, H-3'), 4.41 (dd, *J* = 11.4, 6.0 Hz, 1H, H-2'), 5.05 (d, *J* = 4.5 Hz, 1H, OH-5'), 5.23 (d, *J* = 6.6 Hz, 1H, OH-3'), 5.49 (br. s, 1H, OH-2'), 6.02 (d, *J* = 6.3 Hz, 1H, H-1'), 6.10 (br. s, 2H, NH_2), 6.30 (d, *J* = 5.4 Hz, 1H, H-5), 7.59 (s, 1H, H-2), 7.77 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 49.8 (C-3), 61.7 (C-5'), 70.6 (C-3'), 73.4 (C-2'), 85.1 (C-4'), 87.5 (C-1'), 101.5 (C-5), 106.7 (C-3a), 127.7 (C-2), 143.9 (C-6), 147.9 (C-4), 148.8 (C-7a). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_4$: 392.0102, found: 392.0102.

3-Trifluoromethyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (12) 60 (0.10 g, 0.16 mmol) was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **12** (0.030 g, 0.090 mmol) as a white solid in 58 % yield. Melting point: 224 - 226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.50 - 3.61 (m, 1H, H-5''), 3.61 - 3.71 (m, 1H, H-5'), 3.92 (d, *J* = 3.2 Hz, 1H, H-4'), 4.10 (br. s, 1H, H-3'), 4.46 (br. s, 1H, H-4'), 5.13 (br. s, 1H, OH-3'), 5.36 (br. s, 1H, OH-2'), 5.45 (t, *J* = 5.1 Hz, 1H, OH-5'), 5.85 (s, 2H, NH_2), 6.11 (d, *J* = 5.9 Hz, 1H, H-1'), 6.49 (d, *J* = 5.6 Hz, 1H, H-5), 7.89 (d, *J* = 5.3 Hz, 1H, H-6), 8.13 (s, 1H, H-2). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -52.56. ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.4 (C-5'), 70.4 (C-3'), 73.6 (C-2'), 85.2 (C-4'), 87.9 (C-1'), 102.1 (q, *J* = 36.9 Hz, 1C, C-3), 102.8 (C-3a), 102.9 (C-5), 124.24 (q, *J* = 266.0 Hz, 1C, CF_3), 124.7 (q, *J* = 5.8 Hz, 1C, C-2), 145.0 (C-6), 147.7 (C-4), 149.0 (C-7a). HRMS (ESI): calculated for $C_{13}H_{15}F_3N_3O_4$ ($[M+H]^+$): 334.1009, found: 334.1009.

3-Nitro-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (13) According to General

procedure B, **56** (0.13 g, 0.20 mmol) gave rise to the amino-intermediate as a yellow foam. Purification: 40 → 60 % EA/PET. Next the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **13** (0.024 g, 0.080 mmol) as a white solid in 39 % yield over two steps. Melting point: 219 - 221 °C . ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.60 (ddd, *J* = 12.0, 5.9, 2.9 Hz, 1H, H-5''), 3.73 (ddd, *J* = 12.0, 8.5, 3.5 Hz, 1H, H-5'), 3.93 - 4.01 (m, 1H, H-4'), 4.13 (q, *J* = 4.9 Hz, 1H, H-3'), 4.38 (q, *J* = 5.0 Hz, 1H, H-2'), 5.14 (d, *J* = 5.2 Hz, 1H, OH-3'), 5.42 (t, *J* = 5.3 Hz, 1H, OH-5'), 5.49 (d, *J* = 5.8 Hz, 1H, OH-2'), 6.16 (d, *J* = 4.9 Hz, 1H, H-1'), 6.52 (d, *J* = 5.6 Hz, 1H, H-5), 6.94 (br. s, 2H, NH_2), 7.88 (d, *J* = 5.6 Hz, 1H, H-6), 8.91 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 60.7 (C-5'), 69.7 (C-3'), 74.3 (C-2'), 85.2 (C-4'), 88.4 (C-1'), 99.1 (C-3a), 104.4 (C-5), 128.8 (C-3), 129.1 (C-2), 145.9 (C-6), 147.5 (C-7a), 148.7 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}N_4O_6$ ($[M+H]^+$): 311.0986, found: 311.0966.

3-Cyano-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (14) According to General procedure B, **55** (0.20 g, 0.31 mmol) gave rise to the amino-intermediate as a yellow foam. Purification: 40 → 60 % EA/PET. Next, the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **14** (0.065 g, 0.22 mmol) as a white solid in 70 % yield over two steps. Melting point: 225 - 226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (ddd, *J* = 12.8, 6.7, 3.5 Hz, 1H, H-5''), 3.66 (ddd, *J* = 12.0, 8.2, 3.8 Hz,1H, H-5'), 3.93 (q, *J* = 3.4 Hz, 1H, H-4'), 4.07 – 4.12 (m, 1H, H-3'), 4.40 (q, *J* = 5.7 Hz, 1H, H-2'), 5.15 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.39 (d,

J = 6.2 Hz, 1H, OH-2'), 5.40 (dd, *J* = 6.7, 4.7 Hz, 1H, OH-5'), 6.07 (d, *J* = 5.8 Hz, 1H, H-1'), 6.11 (br. s, 2H, NH_2), 6.47 (d, *J* = 5.6 Hz, 1H, H-5), 7.89 (d, *J* = 5.5 Hz, 1H, H-6), 8.38 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.4 (C-5'), 70.3 (C-3'), 73.8 (C-2'), 81.7 (CN), 85.4 (C-4'), 88.3 (C-1'), 103.0 (C-5), 105.8 (C-3), 116.5 (C-3a), 132.6 (C-2), 145.6 (C-6), 147.6 (C-7a), 148.6 (C-4). HRMS (ESI): calculated for $C_{13}H_{15}N_4O_4$ ($[M+H]^+$): 291.1088, found: 291.1094.

3-Methyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine formic acid salt (15)

According to the General procedure for Staudinger reaction. **61** (0.19 g, 0.31 mmol) gave rise to the amino-intermediate as a yellow foam. Purification: 40 → 60 % EA/PET. Next the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 →10 % MeOH/DCM) to give **15** (35 mg), which was purified by preparative RP-HPLC gradient: 0.2 % formic acid in water:MeCN; 98:2 to 70:30 (8min), then: 66:34 (2min), then: 0:100 (5min), which yielded **15** as a white solid (formic acid salt, 0.032 g, 0.098 mmol) as a white solid in 32 % yield over three steps. Melting point: 80 – 85 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.38 (d, *J* = 1.2 Hz, 3H, CH_3), 3.49 (dd, *J* = 11.7, 3.5 Hz, 1H, H-5''), 3.59 (dd, *J* = 12.0, 3.2 Hz, 1H, H-5'), 3.86 (q, *J* = 3.3 Hz, 1H, H-4'), 4.04 (dd, *J* = 5.1, 2.8 Hz, 1H, H-3'), 4.46 (dd, *J* = 6.4, 5.3 Hz, 1H, H-2'), 5.88 (br. s, 2H, NH_2), 5.91 (d, *J* = 6.4 Hz, 1H, H-1'), 6.20 (d, *J* = 5.6 Hz, 1H, H-5), 6.98 (s, 1H, H-2), 7.65 (d, *J* = 5.3 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 12.3 (CH_3), 62.2 (C-5'), 71.0 (C-3'), 72.9 (C-2'), 84.9 (C-4'), 88.0 (C-1'), 101.0 (C-5),108.0 (C-3), 108.7 (C-3a), 120.5 (C-2), 143.1 (C-6), 148.8 (C-7a), 149.6 (C-4). HRMS (ESI): calculated for $C_{13}H_{18}N_3O_4$ ($[M+H]^+$): 280.1292, found: 280.1292.

3-Ethyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (16) 17 (0.15 g, 0.50 mmol) was dissolved in MeOH (5 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 (2 h). Then, the H_2-balloon was removed, the mixture purged again with N_2 and filtered over Celite®. The filtrate was evaporated until dryness and purified by column chromatography (1 → 10 % MeOH/DCM) to give **16** as a white solid (0.059 g, 0.20 mmol) in 40 % yield. Melting point: 201 - 206 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21 (t, *J* = 7.3 Hz, 3H, CH_3), 2.81 (q, *J* = 7.4 Hz, 2H, CH_2), 3.50 (dd, *J* = 12.0, 2.3 Hz, 1H, H-5''), 3.60 (dd, *J* = 11.7, 3.2 Hz, 1H, H-5'), 3.87 (q, *J* = 3.2 Hz, 1H, H-4'), 4.05 (dd, *J* = 6.7, 3.8 Hz, 1H, H-3'), 4.44 – 4.54 (m, 1H, H-2'), 4.99 (d, *J* = 4.4 Hz, 1H, OH-3'), 5.13 (br. s, 1H, OH-2'), 5.75 (br. s, 1H, OH-5'), 5.84 (br. s, 2H, NH_2), 5.93 (d, *J* = 6.6 Hz, 1H, H-1'), 6.22 (d, *J* = 5.3 Hz, 1H, H-5), 7.00 (s, 1H, H-2), 7.66 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 15.1 (CH_3), 19.6 (CH_2), 62.1 (C-5'), 70.9 (C-3'), 72.8 (C-2'), 85.0 (C-4'), 88.2 (C-1'), 101.2 (C-5), 107.4 (C-3), 115.9 (C-3a), 119.3 (C-2), 143.0 (C-6), 148.9 (C-7a), 149.4 (C-4). HRMS (ESI): calculated for $C_{14}H_{20}N_3O_4$ ($[M+H]^+$): 294.1448, found: 294.1442.

3-Ethenyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (17) 17 was prepared according to General procedure C, except for the use of Cs_2CO_3 instead of Na_2CO_3. **11** (0.16 g, 0.4 mmol) was transformed into **17** (0.040 g, 0.14 mmol) as a white solid in 35 % yield. Chromatography: 0 → 10 % MeOH/DCM. Melting point: 180 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.52 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5''), 3.63 (dd, *J* = 12.0 , 2.9 Hz, 1H, H-5'), 3.89 (q, *J* = 3.5 Hz, 1H, H-4'), 4.06 - 4.10 (m, 1H, H-3'), 4.49 (q, *J* = 5.8 Hz, 1H, H-2'), 5.03 (d, *J* = 4.4

Hz, 1H, OH-3'), 5.06 (dd, *J* = 11.1, 1.8 Hz, 1H, H_2C=), 5.21 (d, *J* = 5.9 Hz, 1H, OH-2'), 5.49 (dd, *J* = 17.1, 1.9 Hz, 1H, H_2C=), 5.65 (br. s, 1H, OH-5'), 5.99 (br. s, 2H, NH_2), 6.01 (d, *J* = 6.4 Hz, 1H, H-1'), 6.29 (d, *J* = 5.6 Hz, 1H, H-5), 7.13 (dd, *J* = 17.4, 10.8 Hz, 1H, =CH), 7.59 (s, 1H, H-2), 7.70 (d, *J* = 5.3 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5'), 70.1 (C-3'), 73.1 (C-2'), 85.1 (C-4'), 88.1 (C-1'), 102.1 (C-5), 105.9 (C-3), 111.8 (C-3a), 113.6 (H_2C=), 120.1 (C-2), 130.0 (=CH), 143.3 (C-6), 148.7 (C-7a), 149.4 (C-4). HRMS (ESI): calculated for $C_{14}H_{18}N_3O_4$ ($[M+H]^+$): 292.1292, found: 292.1296.

3-*E*-Styryl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (18) 18 was prepared according to General procedure C: **11** (0.16 g, 0.40 mmol) was transformed into **18** (0.050 g, 0.14 mmol) as a white solid in 34 % yield. Chromatography: 2 → 10 % MeOH/DCM. Melting point: 195 - 197 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.56 (dd, *J* = 12.0, 3.8 Hz, 1H, H-5''), 3.66 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5'), 3.92 (q, *J* = 3.4 Hz, 1H, H-4'), 4.11 (dd, *J* = 7.6, 4.4 Hz, 1H, H-3'), 4.51 (t, *J* = 5.7 Hz, 1H, H-2'), 5.08 (d, *J* = 4.4 Hz, 1H, OH-3'), 5.27 (br. s, 1 H, OH-2'), 6.05 (d, *J* = 6.2 Hz, 1H, H-1'), 6.30 (br. s, 2 H, NH_2), 6.35 (d, *J* = 5.6 Hz, 1H, H-5), 6.95 (d, *J* = 16.1 Hz, 1H, CH=CH), 7.17 - 7.24 (m, 1H, H_{Phe}), 7.35 (t, *J* = 7.3 Hz, 2H, H_{Phe}), 7.56 (d, *J* = 16.1 Hz, 1H, CH=CH), 7.64 (d, *J* = 7.3 Hz, 2H, H_{Phe}), 7.75 (d, *J* = 5.6 Hz, 1H, H-6), 7.80 (s, 1H, H-2). (OH-5' proton was not observed). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5'), 70.7 (C-3'), 73.3 (C-2'), 85.2 (C-4'), 88.0 (C-1'), 102.4 (C-5), 106.1 (C-3a), 113.7 (C-3), 120.1(C-2), 121.3 (CH=CH), 126.2 (C_{Phe}), 126.6 (C_{Phe}), 126.7 (C_{Phe}), 128.4 (C_{Phe}), 137.7 (CH=CH), 142.6 (C-6), 148.1 (C-7a), 150.0 (C-4). HRMS (ESI): calculated for $C_{20}H_{22}N_3O_4$ ($[M+H]^+$): 368.1605, found: 368.1608.

3-Ethynyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (19) 53 (0.30 g, 0.43

mmol, 1 eq.) was added to a 10 mL round bottom flask, after which CuI (0.016 g, 0.090 mmol, 0.2 eq.), TPPTS (0.048 g, 0.080 mmol, 0.2 eq.) and $Pd(PPh_3)_2Cl_2$ (0.030 g, 0.040 mmol, 0.1 eq.) were added. The flask was evacuated and backfilled with argon three times. Then, under argon was added anhydrous, degassed THF (2 mL, 4 mL/mmol SM) and degassed Et_3N (0.3 mL, 2.13 mmol, 5 eq.), followed by TMS acetylene (0.3 mL, 2.1 mmol, 5 eq.). The resulting solution was stirred at 50 °C overnight and then evaporated until dryness. The residue was purified by column chromatography 0 → 5 % MeOH/DCM to give the intermediate. The intermediate TMS ethynyl nucleoside was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. Next, the mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **19** (0.040 g, 0.14 mmol) as a white solid in 40 % yield for two steps. Melting point: 199 - 202 °C.^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.49 - 3.56 (m, 1H, H-5''), 3.60 - 3.66 (m, 1H, H-5'), 3.90 (d, *J* = 3.2 Hz, 1H, H-4'), 4.08 (dd, *J* = 7.3, 4.4 Hz, 1H, H-3'), 4.20 (s, 1H, HC≡), 4.45 (q, *J* = 5.9 Hz, 1H, H-2'), 5.07 (d, *J* = 4.4 Hz, 1H, OH-3'), 5.27 (d, *J* = 6.2 Hz, 1H, OH-2'), 5.53 (dd, *J* = 6.7, 4.4 Hz, 1H, OH-5'), 6.00 (d, *J* = 6.4 Hz, 1H, H-1'), 6.11 (br. s, 2H, NH_2), 6.31 (d, *J* = 5.6 Hz, 1H, H-5), 7.76 (s, 1H, H-2), 7.78 (d, *J* = 5.6 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5'), 70.7 (C-3'), 73.4 (C-2'), 78.7 (≡C), 82.2 (HC≡), 85.2 (C-4'), 88.1 (C-1'), 92.9 (C-5), 101.5 (C-3), 106.9 (C-3a), 128.3 (C-2), 144.7 (C-6), 147.4 (C-7a), 149.1 (C-4). HRMS (ESI): calculated for $C_{14}H_{16}N_3O_4$ ($[M+H]^+$): 290.1135, found: 290.1121.

3-Phenylethynyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (20) 11 (0.16 g, 0.4 mmol, 1 eq.) was added to a 10 mL round bottom flask, after which CuI (0.015 g, 0.080 mmol, 0.2 eq.), TPPTS (0.11 g, 0.080 mmol, 0.2 eq.) and $Pd(P(Ph)_3)_2Cl_2$ (0.028 g, 0.040

mmol, 0.1 eq.) were added. The flask was evacuated and backfilled with argon three times. Then, under argon was added anhydrous, degassed THF (2 mL, 4 mL/mmol SM) and degassed Et_3N (0.7 mL, 2.0 mmol, 5 eq.), followed by phenylacetylene (0.22 mL, 2.0 mmol, 5 eq.). The resulting solution was stirred at 50 °C overnight and then evaporated until dryness. The residue was purified by column chromatography (0 → 5 % MeOH/DCM) to give **20** (0.080 g, 0.22 mmol) as a white solid in 55 % yield. Melting point: 225 - 227 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.32 - 3.57 (m, 1H, H-5''), 3.62 - 3.67 (m, 1H, H-5'), 3.92 (d, *J* = 3.2 Hz, 1H, H-4'), 4.10 (dd, *J* = 7.6, 4.7 Hz, 1H, H-3'), 4.47 (dd, *J* = 11.1, 5.9 Hz, 1H, H-2'), 5.09 (d, *J* = 4.4 Hz, 1H, OH-3'), 5.29 (d, *J* = 6.4 Hz, 1H, OH-2'), 5.54 (br. s, 1H, OH-5'), 6.05 (d, *J* = 6.2 Hz, 1H, H-1'), 6.15 (br. s, 2 H, NH_2), 6.38 (d, *J* = 5.3 Hz, 1H, H-5), 7.32 - 7.49 (m, 3H, H_{Phe}), 7.54 – 7.58 (m, 2H, H_{Phe}), 7.81 (d, *J* = 5.6 Hz, 1H, H-6), 7.84 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5'), 70.7 (C-3'), 73.6 (C-2'), 84.4 (C-3), 85.3 (C-4'), 88.0 (C-1'), 90.5 (C≡C), 93.7 (C≡C), 101.9 (C-5), 106.8 (C-3a), 122.9 (C_{Phe}), 127.9 (C_{Phe}), 128.2 (C-2), 128.7 ($2C_{Phe}$), 130.9 ($2C_{Phe}$), 144.7 (C-6), 147.7 (C-7a), 149.2 (C-4). HRMS (ESI): calculated for $C_{20}H_{20}N_3O_4$ ($[M+H]^+$): 366.1448, found: 366.1439.

3-Phenyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (21) 21 was prepared according to General procedure C: **11** (0.18 g, 0.46 mmol) was transformed into **21** (0.078 g, 0.23 mmol) as a white solid in 50 % yield. Chromatography: 2 → 10 % MeOH/DCM. Melting point: 117 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.56 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5''), 3.64 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5'), 3.95 (q, *J* = 3.0 Hz, 1H, H-4'), 4.08 – 4.12 (m, 1H, H-3'), 4.51 (t, *J* = 5.7 Hz, 1H, H-2'), 5.08 (d, J = 4.2 Hz, 1H, OH-2'), 5.20 – 5.86 (br. s, 4H, OH-3', OH-5', NH_2), 6.07 (d, *J* = 6.6 Hz, 1H, H-1'), 6.43 (d, *J* = 5.7 Hz, 1H, H-5), 7.33 – 7.51 (m, 5H,

H_{Phe}), 7.47 (s, 1H, H-2), 7.86 (d, *J* = 6.0 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5'), 70.8 (C-3'), 73.5 (C-2'), 85.4 (C-4'), 88.2 (C-1'), 101.8 (C-5), 105.2 (C-3a), 116.3 (C-3), 122.0 (C-2), 126.8 (C_{Phe}), 128.7 (2C, C_{Phe}), 128.8 (2C, C_{Phe}), 135.2 (C_{Phe}), 142.4 (C-6), 149.4 (C-4). 1C atom is missing, corresponding to C7a. HRMS (ESI): calculated for $C_{18}H_{20}N_3O_4$ ($[M+H]^+$): 342.1448, found: 342.1441.

3-(4-Chlorophenyl)-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (22) 22 was prepared according to General procedure for Suzuki coupling; **11** (0.18 g, 0.46 mmol) was transformed into **22** (0.078 g, 0.23 mmol) as a white solid in 60 % yield. Chromatography: 2 → 10 % MeOH/DCM. Melting point: 135 - 137 °C. ^1H NMR (300 MHz, DMSO-d_6) δ: 3.52 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5''), 3.63 (dd, *J* = 12.0, 3.6 Hz, 1H, H-5'), 3.91 (q, *J* = 3.3 Hz, 1H, H-4'), 4.10 (dd, *J* = 5.1, 3.0 Hz, 1H, H-3'), 4.51 – 4.55 (m, 1H, H-2'), 5.51 (br. s, 5H, OH-2', OH-3', OH-5', NH_2), 6.10 (d, *J* = 6.6 Hz, 1H, H-1'), 6.36 (d, *J* = 5.4 Hz, 1H, H-5), 7.44 – 7.52 (m, 4H, H_{Phe}), 7.47 (s, 1H, H-2), 7.80 (d, *J* = 5.6 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5'), 70.8 (C-3'), 73.3 (C-2'), 85.1 (C-4'), 88.0 (C-1'), 102.0 (C-5), 105.2 (C-3a), 114.4 (C-3), 122.3 (C-2), 128.6 (2C, C_{Phe}), 130.3 (2C, C_{Phe}), 131.1 (C_{Phe}), 134.3 (C_{Phe}), 143.7 (C-6), 148.8 (C-4), 148.8 (C-7a). HRMS (ESI): calculated for $C_{18}H_{19}ClN_3O_4$ ($[M+H]^+$): 376.1059, found: 376.1059.

3-(Thien-2-yl)-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (23) 11 (0.16 g, 0.4 mmol, 1 eq.) was added to a 10 mL round bottom flask, after which $Pd(P(Ph)_3)_4$ (0.069 g, 0.06 mmol, 0.15 eq.) and CuI (4 mg, 0.02 mmol, 0.05 eq.) were added. The flask was evacuated and backfilled with argon three times. Then, anhydrous, degassed DMF (2 mL, 4 mL/mmol SM) was added under argon and followed by 2-(tributylstannyl)thiophene (0.19 mL, 0.60 mmol, 1.5

eq.). After stirring at ambient temperature for 5 min, the resulting solution was heated to 100 °C. When the starting material was completely consumed (1 h), the mixture was cooled to ambient temperature. Then, EA (10 mL) and water (10 mL) were added into the solution, the precipitation filtered and the layers were separated. Then the water layer was extracted for two more times, and the organic extracts were washed with brine, dried over Na_2SO_4, filtered and then evaporated until dryness. The residue was purified by column chromatography (0 → 10 % MeOH/DCM) to give **23** (0.060 g, 0.18 mmol) as a white solid in 45 % yield. Melting point: 145 - 149 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5''), 3.63 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5'), 3.91 (q, *J* = 3.2 Hz, 1H, H-4'), 4.06 - 4.12 (m, 1H, H-3'), 4.48 - 4.54 (m, 1H, H-2'), 5.06 (d, *J* = 4.4 Hz, 1H, OH-3'), 5.26 (d, *J* = 6.4 Hz, 1H, OH-2'), 5.59 (br. s, 1 H, OH-5'), 5.69 (s, 2H, NH_2), 6.08 (d, *J* = 6.4 Hz, 1H, H-1'), 6.34 (d, *J* = 5.6 Hz, 1H, H-5), 7.10 (dd, *J* = 3.5, 1.2 Hz, 1H, $H_{thiophene}$), 7.16 (dd, *J* = 5.3, 3.5 Hz, 1H, $H_{thiophene}$), 7.53 (s, 1H, H-2), 7.54 (dd, *J* = 5.3, 1.2 Hz, 1H, $H_{thiophene}$) 7.81 (d, *J* = 5.3 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5'), 70.8 (C-3'), 73.4 (C-2'), 85.2 (C-4'), 88.0 (C-1'), 101.9 (C-5), 105.5 (C-3a), 107.6 ($C_{thiophene}$), 123.0 (C-2), 125.5 ($C_{thiophene}$), 126.3 ($C_{thiophene}$), 128.1 ($C_{thiophene}$), 136.7 (C-3), 143.9 (C-6), 148.4 (C-4), 148.9 (C-7a). HRMS (ESI): calculated for $C_{16}H_{18}N_3O_4S$ ($[M+H]^+$): 348.1013, found: 348.1006.

3-(Pyrid-2-yl)-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (24) 11 (0.16 g, 0.40 mmol, 1 eq.) was added to a 10 mL round bottom flask, after which $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.15 eq.) and CuI (4 mg, 0.02 mmol, 0.05 eq.) were added. The flask was evacuated and backfilled with argon three times. Then, anhydrous, degassed DMF (2 mL, 4 mL/mmol SM) was added under argon and followed by 2-(tributylstannyl)pyridine (0.19 mL,

0.60 mmol, 1.5 eq.). After stirring at ambient temperature for 5 min, the resulting solution was heated to 100 °C. When starting material was consumed (1 h), the mixture was cooled to room temperature. Then, EA (10 mL) and water (10 mL) were poured into the solution, and the precipitation was filtered and the layers were separated. Then water layer extracted for two more times, and the organic extracts was washed by brine, dried via Na_2SO_4, filtered, and then evaporated until dryness. The residue was purified by column chromatography (0 → 10 %) MeOH/DCM to give **24** (0.030 g, 0.090 mmol) as a white solid in 22 % yield. Melting point: 246 - 250 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.56 (ddd, *J* = 11.7, 7.3, 3.2 Hz, 1H, H-5''), 3.68 (ddd, *J* = 12.0, 8.2, 3.8 Hz, 1H, H-5'), 3.94 – 3.94 (m, 1H, H-4'), 4.13 (dd, *J* = 7.6, 4.1 Hz, 1H, H-3'), 4.56 (q, *J* = 6.0 Hz, 1H, H-2'), 5.06 (d, *J* = 4.7 Hz, 1H, OH-3'), 5.30 (d, *J* = 6.2 Hz, 1H, OH-2'), 5.69 (dd, *J* = 6.9, 4.2 Hz, 1H, OH-5'), 6.08 (d, *J* = 6.2 Hz, 1H, H-1'), 6.30 (d, *J* = 5.3 Hz, 1H, H-5), 7.19 (t, *J* = 5.9 Hz, 1H, H_{Pyr}), 7.72 (d, *J* = 5.3 Hz, 1H, H-6), 7.81 (t, *J* = 7.8 Hz, 1H, H_{Pyr}), 7.93 (d, *J* = 8.2 Hz, 1H, H_{Pyr}), 8.18 (s, 1H, H-2), 8.52 (d, *J* = 5.0 Hz, 1H, H_{Pyr}). (NH_2 proton was not observed). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5'), 70.6 (C-3'), 73.1 (C-2'), 85.2 (C-4'), 88.4 (C-1'), 101.9 (C-5), 105.5 (C-3a), 115.9 (C-3), 120.3 (C_{Pyr}), 120.4 (C_{Pyr}), 124.5 (C-2), 137.5 (C_{Pyr}), 143.9 (C-6), 147.8 (C_{Pyr}), 149.6 (C-4), 150.7 (C-7a), 153.9 (C_{Pyr}). HRMS (ESI): calculated for $C_{17}H_{19}N_4O_4$ ($[M+H]^+$): 343.1401, found: 343.1399.

3-(Cyclohex-1-en-1-yl)-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (25) 25 was prepared according to General procedure C: **11** (0.16 g, 0.40 mmol) was transformed into **25** (0.030 g, 0.090 mmol) as a white solid in 22 % yield. Chromatography: 2 → 10 % MeOH/DCM. Melting point: 147 - 151 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.62 - 1.65 (m, 2H, CH_2), 1.72 - 1.76 (m, 2H, CH_2), 2.13 - 2.23 (m, 2H, CH_2), 2.29 - 2.36 (m, 2H, CH_2), 3.51

(dd, *J* = 12.1, 3.4 Hz, 1H, H-5''), 3.61 (dd, *J* = 11.9, 3.4 Hz, 1H, H-5'), 3.88 (q, *J* = 3.2 Hz, 1H, H-4'), 4.04 - 4.08 (m, 1H, H-3'), 4.49 (dd, *J* = 11.9, 6.4 Hz, 1H, H-2'), 5.01 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.17 (d, *J* = 6.6 Hz, 1H, OH-2'), 5.70 (br. s, 4H, OH5', NH_2, H-$2_{cyclohex}$), 5.99 (d, *J* = 6.6 Hz, 1H), 6.28 (d, *J* = 5.4 Hz, 1H, H-5), 7.26 (s, 1H, H-2), 7.72 (d, *J* = 5.4 Hz, 1H, H-6). (OH-5' proton was not observed). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.6 (CH_2), 22.6 (CH_2), 25.0 (CH_2), 30.3 (CH_2), 62.0 (C-5'), 70.8 (C-3'), 73.0 (C-2'), 85.0 (C-4'), 88.1 (C-1'), 101.5 (C-5), 105.4 (C-3), 117.6 (C-3a), 120.6 (C-2), 125.4 (C_{olefin}), 132.4 (C_{olefin}), 143.1 (C-6), 148.2 (C-7a), 148.9 (C-4). HRMS (ESI): calculated for $C_{18}H_{24}N_3O_4$ ($[M+H]^+$): 346.1761, found: 346.1759.

3-Cyclohexyl-4-amino-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (26) 25 (0.10 g, 0.29 mmol) was dissolved in MeOH (5 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 was stirred until TLC showed full conversion of the SM (overnight). Then, the H_2-balloon was removed, the mixture purged with N_2 and filtered over Celite®. The filtrate was evaporated until dryness and purified by column chromatography (1 → 10 % MeOH/DCM) to give **26** (0.060 g, 0.17 mmol) as a white solid in 60 % yield. Melting point: 145 - 149 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.15 - 1.33 (m, 3H, CH_2), 1.43 - 1.58 (m, 2H, CH_2), 1.68 - 1.77 (m, 3H, CH_2), 2.00 - 2.03 (m, 2H, CH_2), 2.87 - 2.96 (m, 1H, CH), 3.47 - 3.63 (m, 1H, H-5''), 3.56 - 3.65 (m, 1H, H-5'), 3.87 (q, *J* = 3.2 Hz, 1H, H-4'), 4.03 - 4.08 (m, 1H, H-3'), 4.48 - 4.54 (m, 1H, H-2'), 4.98 (d, *J* = 4.5 Hz, 1H, OH-3'), 5.12 (d, *J* = 6.7 Hz, 1H, OH-2'), 5.75 (br. s, 1H, OH-5'), 5.76 (br. s, 2H, NH_2), 5.92 (d, *J* = 6.6 Hz, 1H, H-1'), 6.24 (d, *J* = 5.4 Hz, 1H, H-5), 7.01 (s, 1H, H-2), 7.66 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 26.0

($3C_{cyclohex}$), 34.5 ($2C_{cyclohex}$), 34.9 ($C_{cyclohex}$), 62.1 (C-5'), 70.9 (C-3'), 72.7 (C-2'), 85.0 (C-4'), 88.5 (C-1'), 101.5 (C-5), 107.0 (C-3), 118.3 (C-3a), 120.7 (C-2), 142.7 (C-6), 148.6 (C-7a), 149.2 (C-4). HRMS (ESI): calculated for $C_{18}H_{26}N_3O_4$ ($[M+H]^+$): 348.1918, found: 348.1921.

3-Iodo-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (27) 65 (0.17 g, 0.25 mmol) was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue was purified by column chromatography (0 → 10 % MeOH/DCM). Product containing fractions were pooled, evaporated and purified by RP-preparative HPLC: gradient (0.2 % formic acid in water/MeCN): 95:5 (hold for 2min), then, 47.5:52.5 (9 min), then 47.5:52.5 (hold for 3min), then 2/98 (3 min). This gave rise to **27** (0.063 g, 0.17 mmol) as a white solid in 68 % yield. Melting point: 155 - 158 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.49 - 3.58 (m, 1H, H-5''), 3.59 - 3.69 (m, 1H, H-5'), 3.91 (q, *J* = 3.8 Hz, 1H, H-4'), 4.10 (dd, *J* = 7.9, 4.7 Hz, 1H, H-3'), 4.44 (q, *J* = 6.2 Hz, 1H, H-2'), 5.10 – 5.14 (m, 2H, OH-3' and OH-5'), 5.33 (d, *J* = 6.4 Hz, 1H, OH-2'), 6.24 (d, *J* = 6.4 Hz, 1H, H-1'), 7.25 (dd, *J* = 7.9, 4.7 Hz, 1H, H-5), 7.73 (dd, *J* = 7.9, 1.5 Hz, 1H, H-4), 8.02 (s, 1H, H-2), 8.30 (dd, *J* = 4.7, 1.2 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.2 (C-3), 61.6 (C-5'), 70.5 (C-3'), 73.8 (C-2'), 85.0 (C-4'), 86.7 (C-1'), 117.4 (C-5), 123.1 (C-3a), 128.9 (C-4), 130.6 (C-2), 143.7 (C-6), 147.3 (C-7a). HRMS (ESI): calculated for $C_{12}H_{14}IN_2O_4$ ($[M+H]^+$): 376.9993, found: 376.9979.

3-Iodo-4-methyl-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (28) 66 (0.070 g, 0.10 mmol) was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue was purified by column chromatography (0→10 % MeOH/DCM) to give **28** (0.018 g, 0.046 mmol) as a white solid in

46 % yield. Melting point: 206 - 208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.78 (s, 3 H, CH_3), 3.53 (ddd, *J* = 12.0, 6.2, 3.8 Hz, 1H, H-5''), 3.63 (ddd, *J* = 11.7, 4.7, 4.1 Hz, 1H, H-5'), 3.89 (q, *J* = 3.7 Hz, 1H, H-4'), 4.05 - 4.14 (m, 1H, H-3'), 4.39 (dd, *J* = 11.4, 6.2 Hz, 1H, H-2'), 5.09 - 5.15 (m, 2H, OH-3' and OH-5'), 5.29 (d, *J* = 6.4 Hz, 1H, OH-2'), 6.21 (d, *J* = 6.3 Hz, 1H, H-1'), 6.97 (dd, *J* = 4.8, 0.7 Hz, 1H, H-5), 7.96 (s, 1H, H-2), 8.14 (d, *J* = 4.8 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 17.6 (CH_3), 52.3 (C-3), 61.8 (C-5'), 70.5 (C-3'), 73.8 (C-2'), 85.0 (C-4'), 88.8 (C-1'), 118.8 (C-5), 119.4 (C-3a), 131.0 (C-2), 140.4 (C-4), 143.2 (C-6), 146.8 (C-7a). HRMS (ESI): calculated for $C_{13}H_{16}IN_2O_4$ ($[M+H]^+$): 391.0149, found: 391.0150.

3-Iodo-4-chloro-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (29) 47 (0.23 g, 0.32 mmol) was dissolved in 7N NH_3/MeOH (6 mL) and stirred at ambient temperature for 2 days. The mixture was then evaporated, and the residue purified by column chromatography (0.4 → 5 % MeOH/DCM) to give **29** (0.083 g, 0.20 mmol) as a white solid in 63 % yield. Melting point: 198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.51 – 3.58 (m, 1H, H-5''), 3.61 – 3.68 (m, 1H, H-5'), 3.91 (dd, *J* = 7.2, 3.6 Hz, 1H, H-4'), 4.08 – 4.12 (m, 1H, H-3'), 4.38 (dd, *J* = 11.4, 6.3 Hz, 1H, H-2'), 5.08 (t, *J* = 5.4 Hz, 1H, OH-5'), 5.14 (d, *J* = 4.8 Hz, 1H, OH-3'), 5.35 (d, *J* = 6.3 Hz, 1H, OH-2'), 6.24 (d, *J* = 6.3 Hz, 1H, H-1'), 7.29 (d, *J* = 5.4 Hz, 1H, H-5), 8.14 (s, 1H, H-2), 8.24 (d, *J* = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 51.5 (C-3), 61.4 (C-5'), 70.4 (C-3'), 74.0 (C-2'), 85.1 (C-4'), 86.8 (C-1'), 117.2 (C-3a), 117.9 (C-5), 132.9 (C-2), 135.4 (C-4), 143.8 (C-6), 147.7 (C-7a). HRMS (ESI): calculated for $C_{12}H_{13}ClIN_2O_4$ ($[M+H]^+$): 410.9603, found: 410.9644.

3-Iodo-4-amino-N1-(3'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (31) According to General procedure B, **68** (0.20 g, 0.33 mmol) gave rise to the amino-intermediate as a yellow

foam. Purification: 40 → 60 % EA/PET. Next, the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **31** (0.061 g, 0.16 mmol) as a white solid 40 % yield over two steps. Melting point: 236 - 239 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.87 (ddd, *J* = 12.9, 6.4, 3.5 Hz, 1H, H-3''), 2.17 (ddd, *J* = 13.0, 8.1, 6.2 Hz, 1H, H-3''), 3.48 (ddd, *J* = 11.9, 5.9, 4.1 Hz, 1H, H-5''), 3.64 (ddd, *J* = 12.0, 5.1, 3.4 Hz, 1H, H-5'), 4.19 - 4.32 (m, 1H, H-4'), 4.33 - 4.45 (m, 1H, H-2'), 5.15 (t, *J* = 5.6 Hz, 1H, OH-5'), 5.49 (d, *J* = 4.4 Hz, 1H, OH-2'), 6.04 (d, *J* = 2.9 Hz, 1H, H-1'), 6.05 (br. s, 2H, NH_2), 6.29 (d, *J* = 5.6 Hz, 1H, H-5), 7.60 (s, 1H, H-2), 7.79 (d, *J* = 5.6 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.5 (C-3'), 49.4 (C3), 62.8 (C5'), 74.6 (C-2'), 79.6 (C-4'), 90.4 (C-1'), 101.4 (C-5), 106.4 (C-3a), 126.9 (C-2), 144.1 (C-6), 147.7 (C-7a), 148.5 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}N_3O_3$ ($[M+H]^+$): 376.0153, found: 376.0156.

3-Iodo-4-amino-N1-(2'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (32) According to General procedure B, **69** (0.16 g, 0.25 mmol) gave rise to the amino-intermediate as a yellow foam. Purification: 40 → 60 % EA/PET. Next the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **32** (0.059 g, 0.16 mmol) as a white solid in 62 % over two steps. Melting point: 206 - 207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.10 (ddd, *J* = 12.9, 5.9, 2.3 Hz, 1H, H-2''), 2.42 - 2.48 (m, 1H, H-2'), 3.45 - 3.61 (m, 2H, H-5'), 3.79 - 3.82 (m, 1H, H-4'), 4.32 (dd, *J* = 5.6, 3.5 Hz, 1H, H-3'), 5.21 (d, *J* = 4.1 Hz, 1H, OH-3'), 5.22 (br. s, 1H, OH-5'), 6.07 (br. s, 2H, NH_2), 6.28 (d, *J* = 5.4 Hz, 1H, H-5), 6.52 (dd, *J* = 8.4, 5.9 Hz, 1H, H-1'), 7.58 (s, 1H, H-

2), 7.77 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 39.6 (C-2'), 49.8 (C-3), 62.1 (C-5'), 71.1 (C-3'), 83.3 (C-1'), 87.3 (C-4'), 101.5 (C-5), 106.5 (C-3a), 127.0 (C-2), 144.1 (C-6), 147.7 (C-7a), 148.6 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_3$ ($[M+H]^+$): 376.0153, found: 376.0162.

3-Iodo-4-amino-N1-(5'-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (33) According to General procedure B, **67** (0.18 g, 0.50 mmol) gave rise to the amino-intermediate as a yellow foam. Purification: 40 → 60 % EA/PET. Next the amino-intermediate was dissolved in 7N NH_3/MeOH and stirred at ambient temperature overnight. The resulting mixture was evaporated until dryness and the residue purified by column chromatography (0 → 10 % MeOH/DCM) to give **33** (0.11 g, 0.29 mmol) as a white solid in 21 % yield over three steps. Melting point: 163 - 167 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.35 (d, *J* = 6.4 Hz, 3H, CH_3), 3.77 (q, *J* = 5.8 Hz, 1H, H-3'), 4.05 (quin, *J* = 6.2 Hz, 1H, H-4'), 4.16 (dd, *J* = 5.1, 3.4 Hz, 1H, H-2'), 5.06 (d, *J* = 5.9 Hz, 1H, OH-3'), 5.98 (br. s, 1H, OH-2'), 6.25 (d, *J* = 7.0 Hz, 1H, H-5), 6.27 (d, *J* = 3.2 Hz, 1H, H-1'), 6.93 (br. s, 2H, NH_2), 7.15 (s, 1H, H-2), 7.73 (d, *J* = 7.2 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 18.4 (CH_3), 46.1 (C-3), 74.7 (C-3'), 74.8 (C-2'), 79.5 (C-4'), 92.3 (C-1'), 98.0 (C-5), 107.6 (C-3a), 129.5 (C-6), 141.3 (C-2), 146.5 (C-7a), 151.3 (C-4). HRMS (ESI): calculated for $C_{12}H_{15}IN_3O_3$ ($[M+H]^+$): 376.0153, found: 376.0152.

3-Iodo-4-amino-1*H*-pyrrolo[2,3-*b*]pyridine (34) According to General procedure B, **42** (0.20 g, 0.70 mmol) gave rise to **34** (0.091 g, 0.35 mmol) as a yellow solid in 50 % yield. Purification: 2 → 10 % MeOH/DCM. Melting point: 148 - 150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.99 (br. s, 2H, NH_2), 6.22 (d, *J* = 5.6 Hz, 1H, H-5), 7.29 (s, 1H, H-2), 7.76 (d, *J* = 5.6 Hz, 1H, H-6), 11.62 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 48.1 (C-3), 100.5 (C-5), 105.9 (C-

3a), 126.8 (C-2), 144.2 (C-6), 148.3 (C-7a). (C-4 carbon was not observed). HRMS (ESI): calculated for $C_7H_7IN_3$ ($[M+H]^+$): 259.9679, found: 259.9666.

3-Fluoro-4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (35) 4-Chloro-1*H*-pyrrolo[2,3-*b*]pyridine (2.00 g, 13.1 mmol, 1 eq.) and Selectfluor® (6.99 g, 19.7 mmol, 1.5 eq.) were added in a 250 mL round bottom flask, followed by the addition of dry acetonitrile (100 mL) and AcOH (20 mL). The flask was evacuated and backfilled with argon three times. The solution was then heated and stirred at 70 °C overnight. After cooling to ambient temperature, the solvent was removed *in vacuo* and co-evaporated with toluene (50 mL x 2). The crude was dissolved in a mixture of DCM/EA (1:1) and filtered through a pad of silica gel which was thoroughly washed. The combined washings were evaporated. The residue was purified by column chromatography (4:1 DCM/EA) to give **35** (0.65 g, 0.38 mmol) as a white solid in 29 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.23 (d, *J* = 4.7 Hz, 1H, H-5), 7.59 (t, *J* = 2.3 Hz, 1H, H-2), 8.21 (d, *J* = 5.3 Hz, 1H, H-6), 11.88 (br.s., 1H, NH). ^{19}F-NMR (282 MHz, DMSO-d_6) δ: -172.74. HRMS (ESI): calculated for $C_7H_5FClN_2$ ($[M+H]^+$): 171.0120, found: 171.0123.

3-Nitro-4-azido-1*H*-pyrrolo[2,3-*b*]pyridine (43) Into a solution of H_2SO_4 (246 µL, 4.53 mmol, 3.6 eq., 98 wt%) was charged **41** (0.20 g, 1.26 mmol, 1 eq.) in portions over 10 min at 0 °C. HNO_3 (96 µL, 1.51 mmol, 1.2 eq., 70 wt%) was added over 15 min, followed by cold water (27 µL, 1.51 mmol, 1.2 eq.). The reaction mixture was stirred at 0 °C for 30 min. Cold water (0 °C) (20 mL) was then slowly added at 0 °C. The resulting suspension was filtered. The residual cake was washed with water (20 mL), 20 wt % K_2HPO_4 solution (10 mL), and water (10 mL), sequentially. The resulting cake was dried under high vacuum to afford **43** (0.090 g, 0.44 mmol)

as a beige solid in 35% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.30 (d, *J* = 5.3 Hz, 1H, H-5), 8.38 (d, *J* = 5.3 Hz, 1H, H-6), 8.76 (s, 1H, H-2), 13.30 (br. s, 1H, NH). HRMS (ESI): calculated for $C_7H_5N_6O_2$ ($[M+H]^+$): 205.0468, found: 205.0471.

3-Cyano-4-azido-1*H*-pyrrolo[2,3-*b*]pyridine (44) *[Caution: this reaction employs a large amount of NaN_3, as well as mild acid under substantial heating. This is a potential hazard (explosion due to the formation of HN_3). Suitable protective measures (blast shield – closed hood sash) need to be taken.]* 3-Cyano-4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (0.60 g, 3.5 mmol, 1 eq.) and NH_4Cl (0.94 g, 17.5 mmol, 5 eq.) were suspended in DMF (11 mL, 3 mL/mmol SM). Then, NaN_3 (1.14 g, 17.5 mmol, 5 eq.) was added and the resulting mixture heated at 110 °C for 6 h, behind a blast shield. After cooling to ambient temperature, the mixture was diluted with EA, and poured in to half-saturated aq. $NaHCO_3$ solution. The layers were separated, and the water layer extracted twice with EA. The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography 30 % EA/Hex to give **44** (0.37 g, 2.10 mmol) as a grey powder in 60 % yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.21 (d, *J* = 5.3 Hz, 1H, H-5), 8.35 (d, *J* = 5.3 Hz, 1H, H-6), 8.40 (s, 1H, H-2), 12.92 (br. s, 1H, NH). HRMS (ESI): calculated for $C_8H_5N_6$ ($[M+H]^+$): 185.0570, found: 185.0566.

3,4-Dichloro-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (45)

45 was prepared according to General procedure A. **36**[16] (0.19 g, 1.0 mmol) gave rise to **45** (0.56 g, 0.88 mmol) as a white foam in 88 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5''), 4.76 - 4.80 (m, 1H, H-4'), 4.86 (dd, *J* = 12.0, 3.3 Hz, 1H, H-5'), 6.12 (dd, *J* = 6.0, 4.8 Hz, 1H, H-3'), 6.20 (t, *J* = 5.4 Hz, 1H, H-2'), 6.74 (d, *J* = 5.4 Hz, 1H, H-

1'), 7.12 (d, *J* = 5.1 Hz, 1H, H-5), 7.33 - 7.42 (m, 5H OBz (4H), H-2), 7.46 - 7.64 (m, 5H, OBz), 7.92 - 7.95 (m, 2H, OBz), 7.97 - 8.00 (m, 2H, OBz), 8.10 - 8.13 (m, 2H, OBz), 8.18 (d, *J* = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.8 (C-5'), 71.6 (C-3'), 74.0 (C-2'), 80.2 (C-4'), 86.7 (C-1'), 106.2 (C-3), 116.5 (C-3a), 118.9 (C-5), 123.4 (C-2), 128.60, 128.65, 128.7, 128.8, 128.9, 129.6, 129.85, 129.96, 129.99, 133.6, 133.8 (2C), 136.8 (C-4), 144.6 (C-6), 147.3 (C-7a), 165.2 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{33}H_{24}Cl_2N_2O_7$ ($[M+H]^+$): 630.0961, found: 631.1021.

3-Bromo-4-chloro-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (46) 46 was prepared according to General procedure glycosylation. **37**[16] (0.35 g, 1.5 mmol) gave rise to **46** (0.777 g, 1.15 mmol) as a white foam in 77 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5''), 4.76 - 4.80 (m, 1H, H-4'), 4.86 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 6.13 (dd, *J* = 5.7, 4.5 Hz, 1H, H-3'), 6.21 (dd, *J* = 5.7, 5.4 Hz, 1H, H-2'), 6.75 (d, *J* = 5.4 Hz, 1H, H-1'), 7.12 (d, *J* = 5.1 Hz, 1H, H-5), 7.33 - 7.41 (m, 4H, OBz), 7.45 (s, 1H, H-2), 7.46 - 7.64 (m, 5H, OBz), 7.92 - 8.00 (m, 4H, OBz), 8.10 - 8.13 (m, 2H, OBz), 8.17 (d, *J* = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.8 (C-5'), 71.6 (C-3'), 74.1 (C-2'), 80.3 (C-4'), 86.7 (C-1'), 89.7 (C-3), 117.3 (C-3a), 118.9 (C-5), 126.1 (C-2), 128.59, 128.63, 128.7, 128.85, 128.91,129.5, 129.9, 129.99, 133.6, 133.8 (2C), 137.1 (C-4), 144.5 (C-6), 147.6 (C-7a), 165.2 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{33}H_{25}BrClN_2O_7$ ($[M+H]^+$): 675.0528, found: 675.0544.

3-Iodo-4-chloro-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (47) 47 was prepared according to General procedure A. **38**[16] (0.42 g, 1.5 mmol) gave rise to **47** (0.854 g, 1.18 mmol) as a yellow foam in 79 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd,

J = 12.0, 3.6 Hz, H-5''), 4.77 - 4.80 (m, 1H, H-4'), 4.87 (dd, *J* = 12.0, 3.3 Hz, 1H, H-5'), 6.14 (dd, *J* = 5.7, 4.8 Hz, 1H, H-3'), 6.22 (dd, *J* = 5.7, 5.4 Hz, 1H, H-2'), 6.73 (d, *J* = 5.4 Hz, 1H, H-1'), 7.11 (d, *J* = 5.1 Hz, 1H, H-5), 7.33 - 7.42 (m, 4H, OBz), 7.47 - 7.64 (m, 6H, H-2; OBz), 7.93 - 8.00 (m, 4H, OBz), 8.10 - 8.14 (m, 2H, OBz), 8.16 (d, *J* = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 52.4 (C-3), 63.8 (C-5'), 71.6 (C-3'), 74.2 (C-2'), 80.3 (C-4'), 86.8 (C-1'), 118.7 (C-3a), 118.8 (C-5), 128.57, 128.62, 128.7, 128.88, 128.91, 129.5, 129.85, 129.93, 130.0 (2C), 131.8 (C-2), 133.5, 133.8, 137.6 (C-4), 144.2 (C-6), 147.7 (C-7a), 165.2 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{33}H_{25}ClIN_2O_7$ ($[M+H]^+$): 723.0389, found: 723.0400.

3-Chloro-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (48)

Method 1: **45** (0.095 g, 0.15 mmol, 1 eq.) was dissolved in DMF (1.5 mL, 10 mL/mmol SM) and NaN_3 (0.098 g, 1.5 mmol, 10 eq.) was added. Then, 15-crown-5 (0.060 mL, 0.30 mmol, 2 eq.) was added and the mixture heated at 110 °C for 8 h. After cooling to ambient temperature, EA was added and the mixture partitioned between EA and half saturated aq. $NaHCO_3$. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated until dryness. The residue was purified by column chromatography (16 % EA/Hex) to give **48** (0.050 g, 0.078 mmol) as a slightly yellow foam in 52 % yield.

Method 2: **48** was prepared according to General procedure A. **40**[16] (0.48 g, 2.5 mmol) gave rise to **48** (1.10 g, 1.73 mmol) as a yellow foam in 70 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.67 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5''), 4.75 - 4.79 (m, 1H, H-

4'), 4.85 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 6.12 (dd, *J* = 5.7, 4.8 Hz, 1H, H-3'), 5.19 (t, *J* = 5.4 Hz, 1H, H-2'), 6.73 (d, *J* = 5.4 Hz, 1H, H-1'), 6.89 (d, *J* = 5.4 Hz, 1H, H-5), 7.27 (s, 1H, H-2), 7.33 - 7.41 (m, 4H, OBz), 7.46 - 7.63 (m, 5H, OBz), 7.92 - 7.95 (m, 2H, OBz), 7.96 - 7.99 (m, 2H, OBz), 8.10 - 8.13 (m, 2H, OBz), 8.25 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.8 (C-5'), 71.6 (C-3'), 74.0 (C-2'), 80.2 (C-4'), 86.5 (C-1'), 105.6 (C-3), 106.9 (C-5), 110.7 (C-3a), 122.3 (C-2), 128.59, 128.63, 128.76, 128.83, 128.9, 129.6, 129.87, 129.98, 130.01, 133.6, 133.8 (2C), 142.4 (C-4), 145.4 (C-6), 148.0 (C-7a), 165.3 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{33}H_{24}ClN_5O_7$ ($[M+H]^+$): 638.1437, found: 638.1448.

3-Iodo-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (50)

50 was prepared according to General procedure A. **42**[16] (1.4 g, 5.0 mmol) gave rise to **50** (2.65 g, 3.60 mmol) as a yellow foam 76 % yield. Purification: 15 % EA/PET. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.67 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5''), 4.75 - 4.79 (m, 1H, H-4'), 4.85 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 6.13 (dd, *J* = 5.7, 4.8 Hz, 1H, H-3'), 6.20 (t, *J* = 5.7 Hz, 1H, H-2'), 6.72 (d, *J* = 5.4 Hz, 1H, H-1'), 6.89 (d, *J* = 5.4 Hz, 1H, H-5), 7.33 - 7.40 (m, 4H, OBz), 7.41 (s, 1H, H-2), 7.47 - 7.64 (m, 5H, OBz), 7.92 - 7.99 (m, 4H, OBz), 8.10 - 8.14 (m, 2H, OBz), 8.25 (d, *J* = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 51.3 (C-3), 63.8 (C-5'), 71.6 (C-3'), 74.2 (C-2'), 80.2 (C-4'), 86.7 (C-1'), 106.9 (C-5), 113.7 (C-3a), 128.59, 128.62, 128.8, 128.89, 128.94, 129.6, 129.9, 129.96, 130.01, 130.4 (C-2), 133.5, 133.8 (2C), 142.4 (C-4), 145.1 (C-6), 148.8 (C-7a), 165.2 (C=O), 165.5 (C=O), 166.3 (C=O). HRMS (ESI): calculated for $C_{33}H_{25}IN_5O_7$ ($[M+H]^+$): 730.0793, found: 730.0807.

3-Chloro-4-amino-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (51) 51 was prepared according to General procedure B. **48** (0.13 g, 0.21 mmol) gave rise to **51**

as a slight yellow foam (0.11 g, 0.18 mmol) in 86 % yield. Purification: 40 → 75 % EA/Hex. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.66 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5''), 4.72 – 4.76 (m, 1H, H-4'), 4.83 (dd, *J* = 11.7, 3.0 Hz, 1H, H-5'), 4.93 (br. s, 2H, NH_2), 6.07 – 6.16 (m, 2H, H-2', H-3'), 6.25 (d, *J* = 5.4 Hz, 1H, H-5), 6.79 (d, *J* = 4.5 Hz, 1H, H-1'), 7.07 (s, 1H, H-2), 7.32 - 7.46 (m, 4H, OBz), 7.46 - 7.63 (m, 5H, OBz), 7.93 - 7.97 (m, 5H, OBz, H-6), 7.98 - 8.15 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 64.0 (C-5'), 71.6 (C-3'), 73.9 (C-2'), 80.0 (C-4'), 85.8 (C-1'), 102.7 (C-5), 105.3, 105.7, 118.4 (C-2), 128.5, 128.6, 128.8, 129.0, 129.7, 129.9, 130.0, 130.1, 133.5, 133.7 (2C), 145.7 (C-6), 148.1 (C-7a), 165.34 (C=O), 165.59 (C=O), 166.36 (C=O). (C-4 carbon was not observed) HRMS (ESI): calculated for $C_{33}H_{27}ClN_3O_7$ ($[M+H]^+$): 612.1532, found: 612.1545.

3-Bromo-4-amino-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (52) 46 (0.718 g, 1.06 mmol, 1 eq.) was dissolved in DMF (11 mL, 10 mL/mmol SM) and NaN_3 (0.690 g, 10.6 mmol, 10 eq.) was added followed by 15-crown-5 (0.42 mL, 2.1 mmol, 2 eq.). The resulting mixture was heated at 110 °C for approximately 10 h, after which it was allowed to cool to ambient temperature. EA was added, and the mixture poured into half saturated aq. $NaHCO_3$ solution. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated until dryness. The residue was purified by column chromatography (0 → 25 % EA/Hex). Product containing fractions were pooled and evaporated. The crude azido-nucleoside intermediate was directly used in the next step – General procedure for B. Purification 20 → 60 % EA/Hex gave **52** (0.195 g, 0.297 mmol) as a white foam in 28 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.66 (dd, *J* = 11.7, 3.6 Hz, 1H, H-5''), 4.72 - 4.76 (m, 1H, H-4'), 4.83 (dd, *J* = 11.7, 3 Hz, 1H, H-5'),

4.97 (br. s, 2H, NH_2), 6.10 (dd, *J* = 6, 4.5 Hz, 1H, H-3'), 6.15 (dd, *J* = 5.7, 5.4 Hz, 1H, H-2'), 6.25 (d, *J* = 5.7 Hz, 1H, H-5), 6.78 (d, *J* = 5.1 Hz, 1H, H-1'), 7.13 (s, 1H, H-2), 7.33 - 7.39 (m, 4H, OBz), 7.46 - 7.63 (m, 5H, OBz), 7.93 - 7.98 (m, 5H, H-6, OBz), 8.12 - 8.15 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 64.0 (C-5'), 71.7 (C-3'), 73.9 (C-2'), 80.0 (C-4'), 85.9 (C-1'), 88.8 (C-3), 102.8 (C-5), 106.5 (C-3a), 120.9 (C-2), 128.5, 128.6, 128.8, 129.0, 129.7, 129.9, 130.0, 130.1, 133.5, 133.7, 145.7 (C-6), 148.1 (C-7a), 165.3 (C=O), 165.6 (C=O), 166.3 (C=O). (C-4 carbon was not observed) HRMS (ESI): calculated for $C_{33}H_{27}BrN_3O_7$ ($[M+H]^+$): 656.1027, found: 656.1070.

3-Iodo-4-amino-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (53)

***Method 1*: 53** was prepared as has been described for **52**. **47** (0.80 g, 1.1 mmol) gave rise to **53** (0.16 g, 0.23 mmol) as a white foam in 21 % yield.

***Method 2*: 53** was prepared according to General procedure B. **50** (1.66 g, 2.28 mmol) gave rise to **53** (1.22 g, 1.73 mmol) as a white foam in 76 % yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.66 (dd, *J* = 12.0, 3.9 Hz, 1H, H-5''), 4.72 - 4.76 (m, 1H, H-4'), 4.84 (dd, *J* = 12.0, 3.0 Hz, 1H, H-5'), 4.98 (br. s, 2H, NH_2), 6.10 (dd, *J* = 5.7, 4.2 Hz, 1H, H-3'), 6.15 (dd, *J* = 5.7, 5.4 Hz, 1H, H-2'), 6.25 (d, *J* = 5.4 Hz, 1H, H-5), 6.78 (d, *J* = 5.1 Hz, 1H, H-1'), 7.22 (s, 1H, H-2), 7.32 - 7.39 (m, 4H, OBz), 7.47 - 7.63 (m, 5H, OBz), 7.93 - 7.98 (m, 5H, H-6, OBz), 8.12 - 8.15 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 51.0 (C-3), 64.0 (C-5'), 71.7 (C-3'), 74.0 (C-2'), 80.1 (C-4'), 85.9 (C-1'), 102.9 (C-5), 108.0 (C-3a), 126.4 (C-2), 128.54, 128.59, 128.9, 129.0, 129.7, 129.9, 130.0, 130.1, 133.5, 133.7, 145.4 (C-6), 148.1 (C-7a), 165.3 (C=O), 165.6 (C=O), 166.3 (C=O). (C-4 was not observed) HRMS (ESI): calculated for $C_{33}H_{27}IN_3O_7$ ($[M+H]^+$): 704.0888, found: 704.0888.

3-Fluoro-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine

(54) *[Caution: this reaction employs a large amount of NaN_3, as well as mild acid under substantial heating. This is a potential hazard (explosion due to the formation of HN_3). Suitable protective measures (blast shield – closed hood sash) need to be taken.]* **35** (0.60 g, 3.50 mmol, 1 eq.) and NH_4Cl (0.94 g, 17.5 mmol, 5 eq.) were suspended in DMF (11 mL, 3 mL/mmol SM). Then, NaN_3 (1.14 g, 17.5 mmol, 5 eq.) was added and the resulting mixture heated at 110 °C for 6 h, behind a blast shield. After cooling to ambient temperature, the mixture was diluted with EA, and poured in to half-saturated aq. $NaHCO_3$ solution. The layers were separated, and the water layer extracted twice with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography 30 % EA/Hex to give the intermediate azido heterocycle as a grey powder. The intermediate azido heterocycle was used directly in the glycosylation step (General procedure A). This gave rise to **54** (0.87 g, 1.4 mmol) as a yellow foam in 40 % yield for two steps. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.66 (dd, *J* = 12.3, 3.8 Hz, 1H, H-5''), 4.75 (q, *J* = 3.5 Hz, 1H, H-4'), 4.83 (dd, *J* = 11.7, 3.2 Hz, 1H, H-5'), 6.09 (dd, *J* = 5.9, 4.4 Hz, 1H, H-3'), 6.18 (t, *J* = 5.9 Hz, 1H, H-2'), 6.78 (d, *J* = 5.9 Hz, 1H, H-1'), 6.83 (d, *J* = 5.6 Hz, 1H, H-5), 7.31 - 7.67 (m, 10H, OBz and H-2), 7.92 - 8.00 (m, 4H, OBz), 8.11 - 8.14 (m, 2H, OBz), 8.24 (d, *J* = 5.3 Hz, 1H, H-6). ^{19}F-NMR (282 MHz, $CDCl_3$) δ: -165.46. HRMS (ESI): calculated for $C_{33}H_{25}FN_5O_7$ ($[M+H]^+$): 622.1733, found: 622.1743.

3-Cyano-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine

(55) 55 was prepared according to General procedure A. **44** (0.66 g, 1.35 mmol) gave rise to **55** (0.70 g, 1.11 mmol) as a yellow foam in 85 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.70 (dd, *J* = 12.0, 3.8 Hz, 1H, H-5''), 4.82 (dt, *J* = 5.0, 3.5 Hz, 1H, H-4'), 4.89 (dd, *J* = 12.0, 3.2 Hz, 1H,

H-5'), 6.15 (t, *J* = 5.0 Hz, 1H, H-3'), 6.24 (dd, *J* = 5.6, 5.0 Hz, 1H, H-2'), 6.62 (d, *J* = 5.0 Hz, 1H, H-1'), 6.99 (d, *J* = 5.6 Hz, 1H, H-5), 7.33 - 7.67 (m, 9H, OBz), 7.85 (s, 1H, H-2), 7.90 - 7.95 (m, 2H, OBz), 7.97 - 8.03 (m, 2H, OBz), 8.06 - 8.13 (m, 2H, OBz), 8.30 (d, *J* = 5.3 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{34}H_{25}N_6O_7$ ($[M+H]^+$): 629.1779, found: 629.1812.

3-Nitro-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (56) **56** was prepared according to General procedure A. **43** (0.10 g, 0.49 mmol) gave rise to **56** (0.14 g, 0.21 mmol) as a yellow foam in 43 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.72 - 4.93 (m, 3H, H-5' and H-4'), 6.13 (t, *J* = 5.4 Hz, 1H, H-3'), 6.23 (t, *J* = 5.0 Hz, 1H, H-2'), 6.69 (d, *J* = 4.7 Hz, 1H, H-1'), 7.08 (d, *J* = 5.3 Hz, 1H, H-5), 7.30 - 7.65 (m, 9H, OBz), 7.86 - 7.96 (m, 2H, OBz), 7.97 - 8.02 (m, 2H, OBz), 8.06 - 8.14 (m, 2H, OBz), 8.30 (d, *J* = 5.0 Hz, 1 H, H-6), 8.39 (s, 1 H, H-2). HRMS (ESI): calculated for $C_{33}H_{25}N_6O_9$ ($[M+H]^+$): 649.1678, found: 649.1686.

3-Trifluoromethyl-4-amino-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (60) Potassium fluoride (0.050 g, 0.85 mmol, 3 eq.) and cuprous iodide (0.16 g, 0.85 mmol, 3 eq.) were placed in a 25 mL round bottom flask, equipped with a stir bar. Next, the flask was evacuated and refilled with argon three times. Then, a solvent mixture of DMF and NMP (1:1, 2.0 mL/2.0 mL) was added to the solids under argon, followed by the addition of $TMSCF_3$ (125 µL, 0.85 mmol, 3 eq.) over 30 min. The mixture was stirred at ambient temperature until the solids were dissolved. Then **53** (0.20 g, 0.28 mmol, 1 eq.) in DMF/NMP (2.0 mL) was added. After 5 min of stirring at ambient temperature, the mixture was heated to 100 °C in a pre-heated oil bath. When the starting material was fully consumed (1 h), the mixture was cooled to ambient temperature, and water and EA were added. The copper precipitation was filtered through a pad of Celite® which was thoroughly washed with EA. The

layers were separated and the organic layer extracted two more times with water. The organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated until dryness. The crude was purified by column chromatography using 40 → 60 % EA/PET to give **60** (0.15 g, 0.23 mmol) as a yellow foam in 80 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.66 (br. s, 2H, NH_2), 4.69 (dd, *J* = 11.9, 3.4 Hz, 1H, H-5''), 4.75 - 4.82 (m, 1H, H-4'), 4.86 (dd, *J* = 12.0, 2.9 Hz, 1H, H-5'), 6.15 (dd, *J* = 5.6, 4.4 Hz, 1H, H-3'), 6.24 (t, *J* = 5.4 Hz, 1H, H-2'), 6.40 (d, *J* = 5.6 Hz, 1H, H-1'), 6.77 (d, *J* = 5.6 Hz, 1H, H-5), 7.30 - 7.63 (m, 10 H, OBz and H-2), 7.88 - 8.02 (m, 4H, OBz), 8.06 (d, *J* = 5.3 Hz, 1H, H-6), 8.08 - 8.15 (m, 2H, OBz). ^{19}F-NMR (282 MHz, $CDCl_3$) δ: -54.72. HRMS (ESI): calculated for $C_{34}H_{26}F_3N_3O_7$ ($[M+H]^+$): 646.1796, found: 646.1803.

3-Methyl-4-azido-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (61) A 25 mL round button flask was charged with **50** (0.30 g, 0.41 mmol, 1 eq.) and $Pd(PPh_3)_4$ (0.047 g, 0.041 mmol, 0.1 eq.). The flask was evacuated and backfilled with argon three times. Then, anhydrous THF (6 mL, 15 mL/mmol SM) was added. $AlMe_3$ (2 M in toluene, 0.25 mL, 0.49 mmol, 1.2 eq.) was added dropwise. After the mixture was stirred for 15 min, the reaction was transferred to a pre-heated oil bath at 80 °C. Heating was continued until full consumption of the starting material was observed by LC-MS (2 h). Then, the mixture was cooled to ambient temperature. Next, EA and 0.5 M aq. HCl solution were added. The layers were separated and the water layer extracted twice more with EA. Then, the organic layers were combined, neutralized with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The resulting oil was purified by column chromatography using 20 → 33 % EA/PET to give **61** as a yellow foam, which was used immediately in the next reaction. HRMS (ESI): calculated for $C_{34}H_{28}N_5O_7$

([M+H]$^+$): 618.1983, found: 618.1972.

4-Chloro-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (62) A 25 mL round bottom flask was charged with **47** (0.50 g, 0.69 mmol, 1 eq.). The flask was evacuated and backfilled with argon three times. Then, anhydrous THF (4 mL, 5 mL/mmol SM) was added and the solution was stirred at -20 °C for 15 min. Next, iPrMgCl·LiCl (1.3 M in THF, 0.59 mL, 0.76 mmol, 1.1 eq.) was added dropwise over 5 min. When full consumption of the starting material was observed by TLC (1 h), a mixture of ice water and sat. NH_4Cl (5 mL) was added to the mixture followed by EA (10 mL). The layers were separated and the water layer extracted twice more with EA (2 x 10 mL). Then, organic layers were combined, dried over Na_2SO_4, filtered and evaporated. Slightly impure **62** (0.31 g) was obtained as a yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd, *J* = 11.7, 3.8 Hz, 1H, H-5''), 4.80 (dd, *J* = 7.3, 3.8 Hz, 1H, H-4'), 4.85 (dd, *J* = 11.4, 2.9 Hz, 1H, H-5'), 6.14 (dd, *J* = 5.7, 4.2 Hz, 1H, H-3'), 6.24 (t, *J* = 5.9 Hz, 1H, H-2'), 6.64 (d, *J* = 3.8 Hz, 1H, H-3), 6.94 (d, *J* = 5.6 Hz, 1H, H-1'), 7.18 (d, *J* = 5.6 Hz, 1H, H-5), 7.26 - 7.55 (m, 10H, OBz and H-2), 7.96 (dd, *J* = 8.2, 1.2 Hz, 2H, OBz), 8.04 (d, *J* = 7.3 Hz, 2H, OBz), 8.12 (dd, *J* = 8.3, 1.3 Hz, 2H, OBz), 8.22 (d, *J* = 5.3 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{33}H_{26}ClN_2O_7$ ([M+H]$^+$): 597.1423, found: 597.1414.

N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (63) 62 (0.31 g, 0.51 mmol, 1 eq.) was dissolved in MeOH (5 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 was stirred until TLC showed full conversion of the SM (overnight). Then, the H_2-balloon was removed, the mixture purged again with N_2 and filtered over Celite®.

The filtrate was evaporated until dryness and purified by column chromatography 1 → 35 % EA/PET to give **63** as a white solid (0.23 g, 0.41 mmol) in 80 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd, *J* = 11.7, 4.1 Hz, 1H, H-5''), 4.77 (dd, *J* = 8.2, 4.1 Hz, 1H, H-4'), 4.84 (dd, *J* = 11.4, 3.2 Hz, 1H, H-5'), 6.19 (dd, *J* = 6.4, 4.4 Hz, 1H, H-3'), 6.31 (t, *J* = 5.9 Hz, 1H, H-2'), 6.50 (d, *J* = 3.8 Hz, 1H, H-3), 6.84 (d, *J* = 5.9 Hz, 1H, H-1'), 7.10 (dd, *J* = 7.8, 4.8 Hz, 1H, H-5), 7.31 - 7.61 (m, 10H, OBz and H-2), 7.88 (dd, *J* = 7.9, 1.8 Hz, 1H, H-4), 7.92 - 8.02 (m, 4H, OBz), 8.10 - 8.15 (m, 2H, OBz), 8.33 (dd, *J* = 4.7, 1.5 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{33}H_{27}N_2O_7$ ($[M+H]^+$): 563.1813, found: 563.1813.

4-Methyl-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (64) A 25 mL round bottom flask was charged with **62** (0.41 g, 0.69 mmol, 1 eq.) and $Pd(PPh_3)_4$ (0.080 g, 0.069 mmol, 0.1 eq.). The flask was evacuated and backfilled with argon three times. Then, anhydrous THF (7 mL, 10 mL/mmol SM) was added. $AlMe_3$ (2 M in toluene, 0.42 mL, 0.83 mmol, 1.2 eq.) was added dropwise over 5 min added to the mixture while stirring. After the mixture was stirred for 15 min, the reaction was transferred to a pre-heated oil bath at 100 °C. Heating was continued until full consumption of the starting material was observed by TLC (overnight). Then, the mixture was cooled to ambient temperature. Next, EA and aq. 0.5 M HCl solution were 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 resulting oil was purified by column chromatography using 20 → 33 % EA/PET to give **64** (0.26 g, 0.45 mmol, 66 %) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.52 (s, 3H, CH_3), 4.69 (dd, *J* = 11.7, 3.8 Hz, 1H, H-5''), 4.75 - 4.81 (m, 1H, H-4'), 4.85 (dd, *J* = 11.7, 3.2 Hz, 1H, H-5'), 6.16 (dd, *J* = 5.7, 4.2 Hz, 1H, H-3'), 6.28 (t, *J* = 5.9 Hz, 1H, H-2'), 6.53 (d, *J* = 3.8 Hz,

1H, H-3), 6.90 (d, *J* = 6.2 Hz, 1H, H-1'), (dq, *J* = 5.3 Hz, 0.9 Hz, 1H, H-5), 7.31 - 7.61 (m, 10H, OBz and H-2), 7.94 - 8.01 (m, 4H, OBz), 8.10 - 8.16 (m, 2H, OBz), 8.25 (d, *J* = 5.0 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{34}H_{29}N_2O_7$ ($[M+H]^+$): 577.1969, found: 577.1961.

3-Iodo-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (65) 63 (0.23 g, 0. 40 mmol, 1 eq.) and NIS (0.095 g, 0.042 mmol, 1.05 eq.) were added in a round bottom flask, followed by the addition of dry DMF (2 mL, 3 mL/mmol SM). The solution was stirred at ambient temperature for 2 h. Then, EA (10 mL) and saturated aq. $Na_2S_2O_3$ solution (10 mL) were added into the reaction mixture. The layers were separated and the water layer extracted two more times with EA. The organic extracts were washed with brine, dried over Na_2SO_4, filtered, and then evaporated until dryness. The residue was purified by column chromatography 1 → 35 % EA/PET to give **65** (0.18 g, 0.26 mmol) as a yellow foam in 65 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.68 (dd, *J* = 12.0, 3.8 Hz, 1H, H-5''), 4.77 (dd, *J* = 7.9, 3.5 Hz, 1H, H-4'), 4.86 (dd, *J* = 11.7, 3.2 Hz, 1H, H-5'), 6.16 (dd, *J* = 5.7, 4.5 Hz, 1H, H-3'), 6.25 (t, *J* = 5.9 Hz, 1H, H-2'), 6.79 (d, *J* = 5.3 Hz, 1H, H-1'), 7.18 (dd, *J* = 7.9, 4.7 Hz, 1H, H-5), 7.32 - 7.63 (m, 10H, OBz and H-2), 7.70 (dd, *J* = 7.9, 1.5 Hz, 1H, H-4), 7.92 - 8.01 (m, 4H, OBz), 8.10 - 8.15 (m, 2H, OBz), 8.32 (dd, *J* = 4.7, 1.5 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{33}H_{26}IN_2O_7$ ($[M+H]^+$): 689.0779, found: 689.0782.

3-Iodo-4-methyl-N1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (66) 64 (0.080 g, 0.14 mmol) was dissolved in DMF (1.5 mL, 10 mL/mmol SM) and NIS (0.038 g, 0.17 mmol, 1.2 eq.) was added. The resulting mixture was stirred at ambient temperature for 2 h. Then, EA (5 mL) and water (5 mL) were added. The layers were separated and the water

layer extracted twice more with EA (2 x 5 mL). Then, organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The resulting oil was purified by column chromatography using 20 → 33 % EA/PET to give **66** (0.070 g, 0.10 mmol) as a yellow foam in 71 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.88 (s, 3H, CH_3), 4.67 (dd, *J* = 12.0, 3.5 Hz, 1H, H-5''), 4.77 (q, *J* = 3.5 Hz, 1H, H-4'), 4.85 (dd, *J* = 12.0, 3.2 Hz, 1H, H-5'), 6.12 (dd, *J* = 5.6, 4.4 Hz, 1H, H-3'), 6.17 (t, *J* = 5.9 Hz, 1H, H-2'), 6.89 (d, *J* = 5.6 Hz, 1H, H-1'), 6.92 (d, *J* = 5.9 Hz, 1H, H-5), 7.31 - 7.61 (m, 10H, OBz and H-2), 7.98 (dd, *J* = 13.5, 7.0 Hz, 4H, OBz), 8.13 (d, *J* = 7.0 Hz, 2 H, OBz), 8.19 (d, *J* = 5.0 Hz, 1 H, H-6). HRMS (ESI): calculated for $C_{34}H_{28}IN_2O_7$ ($[M+H]^+$): 703.0936, found: 703.0956.

3-Iodo-4-azido-N1-(5'-deoxy-2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (67) 67 was prepared according to General procedure A. **42** (0.37 g, 1.40 mmol) gave rise to **67** as a yellow foam, which was used immediately. HRMS (ESI): calculated for $C_{16}H_{17}IN_5O_5$ ($[M+H]^+$): 486.0269, found: 486.0279.

3-Iodo-4-azido-N1-(3'-deoxy-2',5'-di-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (68) 68 was prepared according to General procedure A. **42** (0.20 g, 0.70 mmol) gave rise to **68** (0.20 g, 0.33 mmol) as a yellow foam in 47 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.40 (ddd, *J* = 14.1, 5.5, 1.8 Hz, 1H, H-3''), 2.73 (ddd, *J* = 14.1, 10.3, 5.9 Hz, 1H, H-3'), 4.59 (dd, *J* = 12.0, 4.7 Hz, 1H, H-5''), 4.71 (dd, *J* = 12.3, 3.2 Hz, 1H, H-5'), 4.85-4.76 (m, 1H, H-4'), 5.89 (dt, *J* = 5.9, 1.4 Hz, 1H, H-2'), 6.55 (d, *J* = 1.8 Hz, 1H, H-1'), 6.88 (d, *J* = 5.3 Hz, 1H, H-5), 7.33 - 7.54 (m, 10H, OBz and H-2), 7.54 - 7.67 (m, 2H, OBz), 7.96 - 8.14 (m, 4H, OBz), 8.23 (d, *J* = 5.3 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{26}H_{21}IN_5O_5$ ($[M+H]^+$): 610.0582, found: 610.0562.

3-iodo-4-azido-N1-(2'-deoxy-3',5'-di-*O*-(4-toluoyl)-β-D-ribofuranosyl)-pyrrolo[2,3-*b*]pyridine (69) Sodium hydride (0.034 g, 0.84 mmol, 1.2 eq., 60% dispersion in mineral oil) was added in an oven-dried flask. The flask was evacuated and backfilled with argon three times. DMF (1 mL) was introduced and suspension cooled to 0 °C. **42** (0.20 g, 0.70 mmol, 1 eq.) was added over 2min as a solution in DMF (1 mL) via syringe ***[Caution: H_2 gas released]*** The resulting clear solution (5 min) was allowed to warm to room temperature and 1-chloro-2-deoxy-3,5-di-(4-methylbenzoyl)- D-ribose (0.27 g, 0.70 mmol, 1 eq.) in DMF (1 mL) was added over 5 min. The reaction mixture was stirred at ambient temperature overnight. Then the mixture solution was poured into water (10 mL) and extracted with EA (3 x 10 mL). The organic extracts was washed with brine (20 mL), dried over Na_2SO_4 and filtered and evaporated. The residue was purified by column chromatography 30 % EA/Hex to give **69** (0.17 g, 0.26 mmol) as a white solid in 37% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.43 (d, *J* = 2.3 Hz, 6H, CH_3), 2.69 - 2.81 (m, 2H, H-2', H-2''), 4.57 (dd, *J* = 6.2, 3.8 Hz, 1H, H-5'), 4.66 - 4.72 (m, 2H, H-4', H-5''), 5.73 (dt, *J* = 5.6, 2.6 Hz, 1 H, H-3'), 6.88 (dd, J = 8.4, 6.0 Hz, 1H, H-1'), 6.89 (d, *J* = 5.4 Hz, 1H, H-5), 7.26 - 7.32 (m, 4H, OTol), 7.43 (s, 1H, H-2), 7.92 - 8.02 (m, 4 H, OTol), 8.26 (d, *J* = 5.6 Hz, 1H, H-6). HRMS (ESI): calculated for $C_{28}H_{25}IN_5O_5$ ($[M+H]^+$): 638.0895, found: 638.0909.

Biology

In vitro evaluation

In vitro evaluation of nucleoside compounds was performed exactly as described previously,[14] which includes the drug sensitivity assays on intracellular amastigotes of Tulahuen (β-

galactosidase expressing) strain *T. cruzi* in MRC-5 fibroblasts and Y-strain *T. cruzi* in primary mouse cardiac cells. Drug sensitivity assays on bloodstream Y-strain trypomastigotes and cytotoxicity on MRC-5 fibroblasts and primary mouse cardiac cells have been performed as described.[14]

In vitro cytotoxicity assay against L929 cells was performed exactly as described previously.[31]

In vitro washout experiment with **11**:

After 24 hours of plating, cardiac cell cultures were infected for 24 h at 37 °C with bloodstream trypomastigotes of *T. cruzi* (Y strain) employing a parasite: host cell ratio of 10:1. Then, the cultures were washed to remove free parasites and treated for 168 h at 37 °C with a serial dilution of the compounds (up to 5 μM) in culture medium. The culture medium was replaced every 48 hours with new medium containing compound. After 168 h of drug exposure, the cells were rinsed using phosphate buffered saline (PBS) and drug-free culture medium was added for another 168 h of incubation. Parasites released in the medium (from untreated and 5 μM treated samples) were quantified using light microscopy (Neubauer chambers). Finally, after 168 h of drug exposure and a 168 h washout period, cultures were fixed and stained with Giemsa as described previously.[34] The mean number of infected host cells and of parasites per infected cells was 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.[34] The compound activity was estimated by calculating the inhibition levels of the inhibition index (II - percentage of infected cells versus mean number of parasites per infected cell).

Microsomal stability assays

Evaluation of the *in vitro* metabolic stability was performed exactly as previously described.[14]

***In vivo* evaluation**

Male Swiss Webster mice (18-20 g; 4 – 5 weeks of age) were obtained from the animal facilities of ICTB (Institute of Science and Biomodels Technology / Fiocruz / RJ / Brazil). Housing of animals was with a maximum of 6 animals per cage, in a specific-pathogen-free (SPF) room at 20 - 24 °C under a 12-h light and 12-h dark cycle. All animals were provided sterilized water and chow *ad libitum*. After procurement of the animals, they were acclimatized for 7 days before the experiments were initiated. At the day of infection (0 dpi), animals were infected by i.p. administration of 10^4 bloodstream trypomastigotes (Y-strain) originating from an infected donor mouse. Non-infected control mice were age-matched and housed under identical conditions.[34]

Each experimental group consisted of six animals each: uninfected (non-infected and non-treated), untreated (infected but treated only with vehicle), and treated (infected and treated with the compounds: **11** or the reference benznidazole). Treatment was initiated at the onset of parasitemia (*i.e.* 5 dpi), only using mice with a detectable parasitemia. Compound **11** was administered by oral gavage for five consecutive days at 25 mg/kg twice daily. Formulation of this derivative was done at 2.0 mg/mL in 10 % (v/v) EtOH, 0.1 M aq. citrate buffer (pH = 3.02) and then dosed according to body weight of the animals. The reference drug benznidazole was dosed at 100 mg/kg once daily by oral gavage. Alternatively, **11** was administered at 25 mg/kg b.i.d. for 15 days (three rounds of 5 consecutive days, with two days no administration) also starting at 5 dpi. In this dosing regime, a matched reference control group of mice receiving benznidazole at 100 mg/kg s.i.d. oral gavage for

the same treatment period was included. All compound formulations were freshly prepared before every administration.

All animals were individually checked for circulating blood parasitemia by counting the number of parasites in 5 μL of blood taken from the tail vein and investigated under a light microscope. Parasitemia was checked until 30 dpi, while mortality was checked daily up to 30 days after the administration of the last dose (*i.e.* 40 dpi or 53 dpi for the 5 and 15 days treatment schedule, respectively). Mortality is given as percentage of cumulative mortality (CM) as described before.[34] Mice consistently presented negative parasitemia up to 30 days post treatment (*i.e.* 40 dpi or 53 dpi) were administered three cycles of cyclophosphamide (50 mg/kg/day). Each cycle consisted of 4 consecutive days of i.p. administration of cyclophosphamide, which was followed by a three-day drug-free period.[26]

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 LW16/14).

Ancillary information

Supporting Information

Copies of ^{1}H, ^{13}C and ^{19}F NMR spectra of compounds **8** – **34**, **45** – **47**, **50** and ^{1}H-^{13}C gHMBC and 2D NOESY spectra of compounds **8**, **13**, **14 31** – **33**, **45** – **47**, **50** can be found in the Supporting Information. Additional data from the XRD experiments of **11** are also presented in the Supporting Information.

A file containing molecular-formula strings is provided as a Supplementary Data file (CSV

format).

Accession codes

CCDC 1920234 contains additional supplementary information on the structure determination by single X-ray diffraction, which will be released by the authors upon acceptance of this manuscript.

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Abbreviations used:

BSA, *N*,*O*-bis(trimethylsilyl)acetamide; CD, Chagas disease; EA: Ethyl acetate; NTD, Neglected tropical disease; SM: Starting material; *T. cruzi*, *Trypanosoma cruzi*; TMSOTf, Trimethylsilyl trifluoromethanesulfonate; TPPTS, Trisodium 3-bis(3-sulfonatophenyl)phosphanylbenzenesulfonate.

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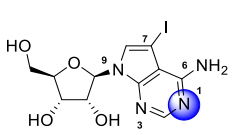
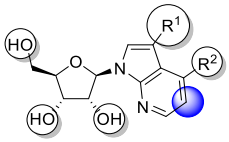
Table of Contents graphic



Iodotubercidin, **30**
pyrrolo[2,3-*d*]pyrimidine
(7-deazapurine) nucleoside

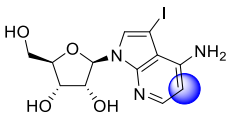
T. cruzi EC_{50}: 0.80 ± 0.025 μM
MRC-5 EC_{50}: 5.35 ± 0.31 μM

Poorly selective



pyrrolo[2,3-*b*]pyridine
(1,7-deazapurine) nucleoside

poorly studied nucleoside modification



11

T. cruzi EC_{50}: 0.040 ± 0.013 μM
MRC-5 EC_{50}: > 64.0 μM

Excellent selectivity
Activity *in vivo*