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J. Med. Chem., Just Accepted Manuscript • DOI: 10.1021/acs.jmedchem.9b01275 • Publication Date (Web): 08 Sep 2019

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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 antityrpanosomal 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 antityrpanosomal 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. Therefore, effective chemotherapy is set as one of the key goals to eliminate this NTD. Currently, two drugs are in use: nifurtimox and benznidazole, 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. 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.

*T. cruzi* parasites lack the enzymes of the *de novo* purine salvage pathway, 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.

Representative deazapurine nucleoside analogues known to display activity against *T. cruzi* are depicted in Figure 1. 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. Several reports have focused on the introduction of additional modifications to improve selectivity and potency, predominantly as antiviral and/or anticancer agents. 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).

![Reported deazapurine nucleoside analogues:]

**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-1H-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,\textsuperscript{16} it was decided to introduce an azido functionality before the glycosylation step (\textit{vide infra}) as an amino group precursor. Azidation was effected under acidic conditions,\textsuperscript{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\textsuperscript{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\textsubscript{3}, NH\textsubscript{4}Cl, DMF, 110 °C, 71\% (40),
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).\(^1\) 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,\(^1\)\(^9\) employing anion glycosylation\(^2\)\(^0\) or acid-catalyzed fusion.\(^2\)\(^1\)

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).\(^1\)\(^6\) 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) NaN₃, 15-crown-5, DMF, 110 °C, 52% (48); c) 1. 1M PMe₃ 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 NH₃/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 ¹H-¹H NOESY and ¹H-¹³C HMBC experiments (Supporting Information). The structure of 11 was further confirmed by single crystal X-ray diffraction analysis (Figure 2 & Supporting Information).
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.\textsuperscript{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.

**Figure 2:** Molecular structure of 11, showing thermal displacement ellipsoids at the 50% probability level.
Scheme 3: Reagents and conditions: appropriate boronic acid (for 18, 21, 22) or potassium trifluoroborate (for 17, 25), Na₂CO₃ (or Cs₂CO₃ 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, H₂, MeOH, 40% (16); 60% (26); c) 2-(tributylstannyl)thiophene or -pyridine, Pd(PPh₃)₄, CuI, DMF, 100 °C, 45% (23); 22% (24); d) 1. trimethylsilylacetylene, CuI, TPPTS, Pd(PPh₃)₂Cl₂, Et₃N, THF, 50 °C; 2. 7N NH₃/MeOH, 40% (two steps); e) phenylacetylene, CuI, TPPTS, Pd(PPh₃)₂Cl₂, Et₃N, 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 CuCF₃ from the Ruppert-Prakash reagent in NMP/DMF.²² Methylation at 3-
position was effected via Pd-mediated cross-coupling with AlMe$_3$. 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$_3$P)$_4$, 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 (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. 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. The nucleoside was elaborated as described above.

Scheme 5: Reagents and conditions: a) 42, BSA, TMSOTf, MeCN, 80 °C, 47% (68); b) 1. 1M PMe₃ in THF, THF; 2. aq. HOAc, MeCN, 65 °C; 3. 7N NH₃/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.\(^a\)

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>R</th>
<th>(T. cruzi^{a}) EC(_{50}) (μM)</th>
<th>MRC-5 EC(_{50}) (μM)</th>
<th>SI</th>
<th>Cpd.</th>
<th>R</th>
<th>(T. cruzi^{a}) EC(_{50}) (μM)</th>
<th>MRC-5 EC(_{50}) (μM)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>H</td>
<td>2.27</td>
<td>17.9 ± 4.4</td>
<td>7.9</td>
<td>17</td>
<td>vinyl</td>
<td>0.81 ± 0.27</td>
<td>&gt;64.0</td>
<td>&gt;79</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>8.8 ± 2.4</td>
<td>&gt;64.0</td>
<td>&gt;373</td>
<td>18</td>
<td>E-styryl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>Cl</td>
<td>0.19 ± 0.05</td>
<td>&gt;64.0</td>
<td>&gt;79</td>
<td>19</td>
<td>ethynyl</td>
<td>0.080 ± 0.026</td>
<td>59.0 ± 5.0</td>
<td>730</td>
</tr>
<tr>
<td>10</td>
<td>Br</td>
<td>0.20 ± 0.02</td>
<td>&gt;64.0</td>
<td>&gt;79</td>
<td>20</td>
<td>phenylethynyl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>0.040 ± 0.013</td>
<td>&gt;64.0</td>
<td>&gt;79</td>
<td>21</td>
<td>phenyl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>CF(_3)</td>
<td>1.56 ± 0.85</td>
<td>0.73 ± 0.12</td>
<td>0.47</td>
<td>22</td>
<td>4-chlorophenyl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>13</td>
<td>NO(_2)</td>
<td>47.7</td>
<td>&gt;64.0</td>
<td>&gt;79</td>
<td>23</td>
<td>2-thienyl</td>
<td>36.9 ± 0.7</td>
<td>&gt;64.0</td>
<td>&gt;1.7</td>
</tr>
<tr>
<td>14</td>
<td>CN</td>
<td>4.00 ± 0.00</td>
<td>18.5 ± 15.2</td>
<td>4.6</td>
<td>24</td>
<td>2-pyridyl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>15</td>
<td>Me</td>
<td>1.14 ± 0.61</td>
<td>51.6 ± 12.4</td>
<td>45</td>
<td>25</td>
<td>cyclohexen-1-yl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>16</td>
<td>Et</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
<td>26</td>
<td>cyclohexyl</td>
<td>&gt;64.0</td>
<td>&gt;64.0</td>
<td>--</td>
</tr>
<tr>
<td>Benznidazole</td>
<td></td>
<td>2.36 ± 0.14</td>
<td>ND</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: *Tulahuen* strain expressing \(\beta\)-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 \( EC_{50} = 0.040 \pm 0.013 \) µM and no discernable \textit{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 (\( EC_{50} = 0.080 \pm 0.026 \) 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 NH₂ 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.\(^a\)

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

\(^a\)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, \(EC_{50}(\text{MRC-5})/EC_{50}(\text{T. cruzi})\). 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 heterocyclic part\textsuperscript{15} nor the removal of the 3'-OH\textsuperscript{24} was found to be beneficial for \textit{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.

\textit{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 \textit{in vitro} assay mimics both phase I (NADPH) and phase II (UGT) mediated liver metabolism. The results are depicted in Table 3.
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. 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
caued 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 vein 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,\textsuperscript{29} such as the antifungal posaconazole.\textsuperscript{30} Analogue 11 displayed comparable \textit{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 \textit{in vitro} cytotoxicity of all assayed analogues against cardiac as well as against L929 fibroblasts.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Cpd. & Y-strain trypomastigotes \textit{EC}_{50} (µM) & Y-strain intracellular amastigotes \textit{EC}_{50} (µM) & Primary cardiac cells \textit{EC}_{50} (µM) & L929 cells \textit{EC}_{50} (µM) \\
\hline
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 \\
\textbf{Benznidazole} & 5.7 ± 0.6 & 0.35 ± 0.2 & >400 & >200 \\
\hline
\end{tabular}
\caption{\textit{In vitro} effects of selected analogues against Y-strain \textit{T. cruzi} bloodstream trypomastigotes and intracellular amastigotes (primary cardiac cells as host cell). Cytotoxicity}
\end{table}
was simultaneously evaluated against primary mouse cardiac cells and L929 cells. EC\textsubscript{50} values are reported in µM and represent mean and SE of two independent determinations. 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 \textit{T. cruzi} infection of \textit{in vitro} cultures. Thus, infected cardiac cells (Y-strain \textit{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.
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 \textit{in vitro} cytotoxicity. Additionally, 11 and several other analogues might serve as tool compounds for the investigation of \textit{T. cruzi} nucleoside transporters.

\textbf{Experimental section}

\textbf{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.\textsuperscript{33} Compounds 36-38, 40-42 were prepared as described previously.\textsuperscript{16}

Analytical TLC was performed on Macherey-Nagel\textsuperscript{®} precoated F254 aluminum plates. TLC plates were first visualized by UV and then developed by staining with basic aq. KMnO\textsubscript{4} and heating. Silica gel column chromatography was performed using Macherey-Nagel\textsuperscript{®} 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\textsuperscript{®} 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\textsuperscript{®} interface. NMR spectra were recorded on a Varian Mercury 300 MHz (\textsuperscript{1}H at 300 MHz, \textsuperscript{13}C at 75 MHz and \textsuperscript{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 (\textit{J})
are reported in hertz (Hz). The stereochemistry at C-1’ was ascertained by 2D NMR techniques
(1H-1H 2D NOESY and 1H-13C gHMBC respectively). In 19F-NMR, signals were referenced to
the CDCl₃ or DMSO-d₆ lock resonance frequency according to IUPAC referencing with CFCl₃
set to δ = 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 H₂O (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. NaHCO₃. The layers were separated, and the water layer extracted twice
more with EA. Organic layers were combined, dried over Na₂SO₄, 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/mmoll). 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/mmoll). 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$_2$SO$_4$, 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$_2$CO$_3$ (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/mmoll 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 N₂ gas, and a catalytic amount of Pd/C was added. The mixture was stirred at ambient temperature under H₂-atmosphere (balloon; bubbling) until LC-MS analysis showed full conversion of the starting material (~5 h). Then, the mixture was purged with N₂, 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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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 NH₃/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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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). ¹⁹F-NMR (282 MHz, DMSO-d₆) δ: -170.64. ¹³C NMR (75 MHz, DMSO-d₆) 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 C₁₂H₁₅FN₃O₄ ([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 NH₃/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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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).

13C NMR (75 MHz, DMSO-d₆) 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 C₁₂H₁₄ClN₃O₄ ([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 NH₃/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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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). ¹³C NMR (75 MHz, DMSO-d₆) δ: 61.7 (C-5‘), 70.6 (C-3‘), 73.4 (C-2‘), 85.0 (C-4‘), 85.7 (C-1‘), 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 C₁₂H₁₄BrN₃O₄ ([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 NH₃/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. ¹H
NMR (300 MHz, DMSO-d$_6$) δ: 3.51 (dd, $J = 12.0$ Hz, 3.3 Hz, 1H, H-5”), 3.61 (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, $J = 5.4$ Hz, 1H, H-5), 7.59 (s, 1H, H-2), 7.77 (d, $J = 5.4$ Hz, 1H, H-6).  

13C NMR (75 MHz, DMSO-d$_6$) δ: 49.8 (C-3), 61.7 (C-5’), 70.6 (C-3’), 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$_{15}$N$_3$O$_4$: 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. 1H 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). 19F-NMR (282 MHz, DMSO-d$_6$) δ: -52.56. 13C 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$_{13}$F$_3$N$_3$O$_4$ ([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 NH₃/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 . ¹H NMR (300 MHz, DMSO-d₆) 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-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, NH₂), 7.88 (d, J = 5.6 Hz, 1H, H-6), 8.91 (s, 1H, H-2). ¹³C NMR (75 MHz, DMSO-d₆) 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 C₁₂H₁₅N₄O₆ ([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 NH₃/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 . ¹H NMR (300 MHz, DMSO-d₆) 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).

$^1$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$_4$O$_4$ (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$_4$O$_4$ ([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 N₂, after which a cat. amount of Pd/C was added. Then, the N₂-atmosphere was exchanged for H₂ (balloon; bubbling), and the mixture stirred until TLC showed full conversion of the SM (2 h). Then, the H₂-balloon was removed, the mixture purged again with N₂ 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. ¹H NMR (300 MHz, DMSO-d₆) δ: 1.21 (t, J = 7.3 Hz, 3H, CH₃), 2.81 (q, J = 7.4 Hz, 2H, CH₂), 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, NH₂), 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). ¹³C NMR (75 MHz, DMSO-d₆) δ: 15.1 (CH₃), 19.6 (CH₂), 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 C₁₄H₂₀N₃O₄ ([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 Cs₂CO₃ instead of Na₂CO₃. 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. ¹H NMR (300 MHz, DMSO-d₆) 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, H-4’).
Hz, 1H, OH-3’), 5.06 (dd, J = 11.1, 1.8 Hz, 1H, H-3’), 5.21 (d, J = 5.9 Hz, 1H, OH-2’), 5.49 (dd, J = 17.1, 1.9 Hz, 1H, H-1’), 5.65 (br. s, 1H, OH-5’), 5.99 (br. s, 2H, NH2), 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).13C NMR (75 MHz, DMSO-d6) 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 C14H18N3O4 ([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. 1H NMR (300 MHz, DMSO-d6) 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, NH2), 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, H2Phe), 7.35 (t, J = 7.3 Hz, 2H, H2Phe), 7.56 (d, J = 16.1 Hz, 1H, CH=CH), 7.64 (d, J = 7.3 Hz, 2H, H2Phe), 7.75 (d, J = 5.6 Hz, 1H, H-6), 7.80 (s, 1H, H-2). (OH-5’ proton was not observed).13C NMR (75 MHz, DMSO-d6) 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 (Cphe), 126.6 (Cphe), 126.7 (Cphe), 128.4 (Cphe), 137.7 (CH=CH), 142.6 (C-6), 148.1 (C-7a), 150.0 (C-4). HRMS (ESI): calculated for C20H22N3O4 ([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(PPh₃)₂Cl₂ (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 Et₃N (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 NH₃/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.¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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). ¹³C NMR (75 MHz, DMSO-d₆) 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 C₁₄H₁₆N₃O₄ ([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(PPh₃)₂Cl₂ (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 Et₃N (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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 6.38 (d, J = 5.3 Hz, 1H, H-5), 7.32 - 7.49 (m, 3H, H₃Phe), 7.54 – 7.58 (m, 2H, H₃Phe), 7.81 (d, J = 5.6 Hz, 1H, H-6), 7.84 (s, 1H, H-2). ¹³C NMR (75 MHz, DMSO-d₆) 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 (C₃Phe), 127.9 (C₃Phe), 128.2 (C-2), 128.7 (2C₃Phe), 130.9 (2C₃Phe), 144.7 (C-6), 147.7 (C-7a), 149.2 (C-4). HRMS (ESI): calculated for C₂₀H₂₀N₃O₄ ([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. ¹H NMR (300 MHz, DMSO-d₆) 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’, NH₂), 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$_{\text{Phe}}$), 7.47 (s, 1H, H-2), 7.86 (d, $J$ = 6.0 Hz, 1H, H-6). $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$: 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$_{\text{Phe}}$), 128.7 (2C, C$_{\text{Phe}}$), 128.8 (2C, C$_{\text{Phe}}$), 135.2 (C$_{\text{Phe}}$), 142.4 (C-6), 149.4 (C-4). 1C atom is missing, corresponding to C7a. HRMS (ESI): calculated for C$_{18}$H$_{20}$N$_3$O$_4$ ([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 $\rightarrow$ 10 % MeOH/DCM. Melting point: 135 - 137 °C. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$: 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$_{\text{Phe}}$), 7.47 (s, 1H, H-2), 7.80 (d, $J$ = 5.6 Hz, 1H, H-6). $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$: 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$_{\text{Phe}}$), 130.3 (2C, C$_{\text{Phe}}$), 131.1 (C$_{\text{Phe}}$), 134.3 (C$_{\text{Phe}}$), 143.7 (C-6), 148.8 (C-4), 148.8 (C-7a). HRMS (ESI): calculated for C$_{18}$H$_{19}$ClN$_3$O$_4$ ([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-(tributylstanny1)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$_2$SO$_4$, 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$_6$) $\delta$: 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$_6$) $\delta$: 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$_3$O$_4$S ([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 Na₂SO₄, 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. ¹H NMR (300 MHz, DMSO-d₆) 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, Hₚyr), 7.72 (d, J = 5.3 Hz, 1H, H-6), 7.81 (t, J = 7.8 Hz, 1H, Hₚyr), 7.93 (d, J = 8.2 Hz, 1H, Hₚyr), 8.18 (s, 1H, H-2’), 8.52 (d, J = 5.0 Hz, 1H, Hₚyr). (NH₂ proton was not observed). ¹³C NMR (75 MHz, DMSO-d₆) 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 (Cₚyr), 120.4 (Cₚyr), 124.5 (C-2), 137.5 (Cₚyr), 143.9 (C-6), 147.8 (Cₚyr), 149.6 (C-4), 150.7 (C-7a), 153.9 (Cₚyr). HRMS (ESI): calculated for C₁₇H₁₉N₄O₄ ([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. ¹H NMR (300 MHz, DMSO-d₆) δ: 1.62 - 1.65 (m, 2H, CH₂), 1.72 - 1.76 (m, 2H, CH₂), 2.13 - 2.23 (m, 2H, CH₂), 2.29 - 2.36 (m, 2H, CH₂), 3.51
(dd, \( J = 12.1, 3.4 \text{ Hz}, 1\text{H}, \text{H-5''} \)), 3.61 (dd, \( J = 11.9, 6.4 \text{ Hz}, 1\text{H}, \text{H-2'} \)), 5.01 (d, \( J = 4.5 \text{ Hz}, 1\text{H}, \text{OH-3'} \)), 5.17 (d, \( J = 6.6 \text{ Hz}, 1\text{H}, \text{OH-2'} \)), 5.70 (br. s, 4H, \text{OH5', NH2, H-2-cyclohex})), 5.99 (d, \( J = 6.6 \text{ Hz}, 1\text{H} \)), 6.28 (d, \( J = 5.4 \text{ Hz}, 1\text{H}, \text{H-5} \)), 7.26 (s, 1H, \text{H-2} \), 7.72 (d, \( J = 5.4 \text{ Hz}, 1\text{H}, \text{H-6} \)). (OH-5’ proton was not observed). \(^{13}C\) NMR (75 MHz, DMSO-d\(_6\)) \( \delta \): 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\(_3\)O\(_4\) ([M+H])\(^{+}\): 346.1761, found: 346.1759.

\textbf{3-Cyclohexyl-4-amino-N1-(\(\beta\)-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (26)} \( \text{(0.10 g, 0.29 mmol)} \) 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 \( \text{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\(_6\)) \( \delta \): 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, \( J = 3.2 \text{ Hz}, 1\text{H}, \text{H-4'} \)), 4.03 - 4.08 (m, 1H, H-3’), 4.48 - 4.54 (m, 1H, H-2’), 4.98 (d, \( J = 4.5 \text{ Hz}, 1\text{H}, \text{OH-3}’ \)), 5.12 (d, \( J = 6.7 \text{ Hz}, 1\text{H}, \text{OH-2'} \)), 5.75 (br. s, 1H, OH-5’), 5.76 (br. s, 2H, NH\(_2\)), 5.92 (d, \( J = 6.6 \text{ Hz}, 1\text{H}, \text{H-1'} \)), 6.24 (d, \( J = 5.4 \text{ Hz}, 1\text{H}, \text{H-5} \)), 7.01 (s, 1H, H-2), 7.66 (d, \( J = 5.4 \text{ Hz}, 1\text{H}, \text{H-6} \)). \(^{13}C\) NMR (75 MHz, DMSO-d\(_6\)) \( \delta \): 26.0
(3Cyclohex), 34.5 (2Cyclohex), 34.9 (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),

3-Iodo-N1-(β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (27) 65 (0.17 g, 0.25 mmol) was
dissolved in 7N NH₃/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 2 min),
then, 47.5:52.5 (9 min), then 47.5:52.5 (hold for 3 min), 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. ¹H NMR
(300 MHz, DMSO-d₆) 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). ¹³C NMR (75 MHz, DMSO-d₆) 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 C₁₂H₁₄N₂O₄ ([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 NH₃/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$_2$O$_4$ ([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 (ddd, $J = 7.2$, 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$_2$O$_4$ ([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
Purification: 40 → 60 % EA/PET. Next, the amino-intermediate was dissolved in 7N NH₃/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. ¹H NMR (300 MHz, DMSO-d₆) 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, NH₂), 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).

¹³C NMR (75 MHz, DMSO-d₆) 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 C₁₂H₁₅N₃O₃ ([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 NH₃/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. ¹H NMR (300 MHz, DMSO-d₆) 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.
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 NH3/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. 1H NMR (300 MHz, DMSO-d6) δ: 1.35 (d, J = 6.4 Hz, 3H, CH3), 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, NH2), 7.15 (s, 1H, H-2), 7.73 (d, J = 7.2 Hz, 1H, H-6). 13C NMR (75 MHz, DMSO-d6) δ: 18.4 (CH3), 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 C12H15IN3O3 ([M+H]+): 376.0153, found: 376.0162.

3-Iodo-4-amino-1H-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. 1H NMR (300 MHz, DMSO-d6) δ: 5.99 (br. s, 2H, NH2), 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$_7$H$_7$IN$_3$ ([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$_7$H$_5$FClIN$_3$ ([M+H]$^+$): 171.0120, found: 171.0123.

3-Nitro-4-azido-1$H$-pyrrolo[2,3-b]pyridine (43) Into a solution of H$_2$SO$_4$ (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$_2$HPO$_4$ 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-\(d_6\)) \(\delta\): 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\(_7\)H\(_5\)N\(_6\)O\(_2\) ([M+H]^\+: 205.0468, found: 205.0471.

**3-Cyano-4-azido-1H-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-1H-pyrrolo[2,3-b]pyridine (0.60 g, 3.5 mmol, 1 eq.) and NH\(_4\)Cl (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\(_2\)SO\(_4\), 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-\(d_6\)) \(\delta\): 7.21 (d, \(J = 5.3\) Hz, 1H, H-5), 8.40 (s, 1H, H-2), 12.92 (br. s, 1H, NH). HRMS (ESI): calculated for C\(_8\)H\(_5\)N\(_6\) ([M+H]^\+: 185.0570, found: 185.0566.

**3,4-Dichloro-N1-(2',3',5'-tri-O-benzoyl-\(\beta\)-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\(_3\)) \(\delta\): 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), 128.6, 128.65, 128.7, 128.8, 128.9, 129.6, 129.9, 132.4 (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$_2$N$_2$O$_7$ ([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$^{15}$ (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), 128.69, 128.7, 128.85, 128.91, 129.9, 133.6, 133.8 (2C), 137.1 (C-4), 144.5 (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$_{23}$BrClN$_2$O$_7$ ([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. 1H NMR (300 MHz, CDCl3) 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). 13C NMR (75 MHz, CDCl3) 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 C33H25ClIN2O7 ([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 NaN3 (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. NaHCO3. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na2SO4, 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. 4016 (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$_3$) $\delta$: 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$_3$) $\delta$: 63.8 (C-5’), 71.6 (C-3’), 74.0 (C-2’), 80.2 (C-4’), 86.5 (C-1’), 105.6 (C-1), 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$_5$O$_7$([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$_3$) $\delta$: 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, 2H, OBz), 8.10 - 8.14 (m, 2H, OBz), 8.25 (d, $J = 5.4$ Hz, 1H, H-6). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 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$_5$O$_7$([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

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(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 \rightarrow 75 \% EA/Hex.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 4.66 (dd, \(J = 12.0, 3.0\) Hz, 1H, H-5\(^{\prime\prime}\)), 4.72 – 4.76 (m, 1H, H-4\(^{\prime}\)), 4.83 (dd, \(J = 11.7, 3.0\) Hz, 1H, H-5\(^{\prime}\)), 4.93 (br. s, 2H, NH\(_2\)), 6.07 – 6.16 (m, 2H, H-2\(^{\prime}\), H-3\(^{\prime}\)), 6.25 (d, \(J = 5.4\) Hz, 1H, H-5), 6.79 (d, \(J = 4.5\) Hz, 1H, H-1\(^{\prime}\)), 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\(_3\)) \(\delta\): 64.0 (C-5\(^{\prime\prime}\)), 71.6 (C-3\(^{\prime}\)), 73.9 (C-2\(^{\prime}\)), 80.0 (C-4\(^{\prime}\)), 85.8 (C-1\(^{\prime}\)), 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\(_3\)O\(_7\) ([M+H]\(^+\)): 612.1532, found: 612.1545.

3-Bromo-4-amino-N1-(2\(^{\prime}\),3\(^{\prime}\),5\(^{\prime}\)-tri-O-benzoyl-\(\beta\)-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\(_2\)SO\(_4\), filtered and evaporated until dryness. The residue was purified by column chromatography (0 \rightarrow 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 \rightarrow 60 \% EA/Hex gave 52 (0.195 g, 0.297 mmol) as a white foam in 28 % yield. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 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, NH₂), 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). ¹³C NMR (75 MHz, CDCl₃) 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, 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 carbon was not observed) HRMS (ESI): calculated for C₃₃H₂₇BrN₃O₇ ([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.

¹H NMR (300 MHz, CDCl₃) 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, NH₂), 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). ¹³C NMR (75 MHz, CDCl₃) 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, 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$_3$O$_7$ ([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$_4$Cl (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$_2$SO$_4$, 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$_3$) $\delta$: 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$_3$) $\delta$: -165.46. HRMS (ESI): calculated for C$_{33}$H$_{25}$FN$_5$O$_7$ ([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$_3$) $\delta$: 4.70 (dd,
$J = 12.0, 3.8 \text{ Hz}, 1\text{H}, \text{H-5''}$), 4.82 (dt, $J = 5.0, 3.5 \text{ Hz}, 1\text{H}, \text{H-4'}$), 4.89 (dd, $J = 12.0, 3.2 \text{ Hz}, 1\text{H}, \text{H-5'}$), 6.15 (t, $J = 5.0 \text{ Hz}, 1\text{H}, \text{H-3'}$), 6.24 (dd, $J = 5.6, 5.0 \text{ Hz}, 1\text{H}, \text{H-2'}$), 6.62 (d, $J = 5.0 \text{ Hz}, 1\text{H}, \text{H-1'}$), 6.99 (d, $J = 5.6 \text{ Hz}, 1\text{H}, \text{H-5}$), 7.33 - 7.67 (m, 9H, OBz), 7.85 (s, 1H, H-2), 7.90 - 7.95 (m, 2H, OBz), 8.06 - 8.13 (m, 2H, OBz), 8.30 (d, $J = 5.3 \text{ Hz}, 1\text{H}, \text{H-6}$). HRMS (ESI): calculated for C$_{34}$H$_{25}$N$_6$O$_7$ ([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, $J = 5.4 \text{ Hz}, 1\text{H}, \text{H-3'}$), 6.23 (t, $J = 5.0 \text{ Hz}, 1\text{H}, \text{H-2'}$), 6.69 (d, $J = 4.7 \text{ Hz}, 1\text{H}, \text{H-1'}$), 7.08 (d, $J = 5.3 \text{ Hz}, 1\text{H}, \text{H-5}$), 7.30 - 7.65 (m, 9H, OBz), 7.86 - 7.96 (m, 2H, OBz), 8.06 - 8.14 (m, 2H, OBz), 8.39 (s, 1H, H-2). HRMS (ESI): calculated for C$_{33}$H$_{25}$N$_6$O$_9$ ([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 Na₂SO₄, 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. ¹H NMR (300 MHz, CDCl₃) δ: 4.66 (br. s, 2H, NH₂), 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). ¹⁹F-NMR (282 MHz, CDCl₃) δ: -54.72. HRMS (ESI): calculated for C₃₄H₂₆F₃N₃O₇ ([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(PPh₃)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. AlMe₃ (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. NaHCO₃, dried over Na₂SO₄, 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$_5$O$_7$ ([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$_4$Cl (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$_2$SO$_4$, 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$_2$O$_7$ ([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 H₂-balloon was removed, the mixture purged again with N₂ 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. ¹H NMR (300 MHz, CDCl₃): 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 C₃₃H₂₇N₂O₇ ([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(PPh₃)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. AlMe₃ (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 Na₂SO₄, 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. ¹H NMR (300 MHz, CDCl₃): δ: 2.52 (s, 3H, CH₃),
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), 6.28 (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_{2}O_{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_{2}S_{2}O_{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_{2}SO_{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\(_3\)) \(\delta:\) 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 (d, J = 7.9, 15 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_{2}O_{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 Na₂SO₄, 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, CDCl₃) δ: 2.88 (s, 3H, CH₃), 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 C₁₃H₂₃N₂O₇ ([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 C₁₆H₁₇IN₅O₅ ([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, CDCl₃) δ: 2.40 (dd, 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\(_5\)O\(_5\) ([M+H]\(^+\): 610.0582, found: 610.0562.

3-iodo-4-azido-N1-(2'-deoxy-3',5'-di-O-(4-toluoyl)-\( \beta \)-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 2 min as a solution in DMF (1 mL) via syringe \([\text{Caution: H}_2\text{ gas released}]\)

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\(_2\)SO\(_4\) 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\(_3\)) \( \delta \): 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\(_5\)O\(_5\) ([M+H]\(^+\): 638.0895, found: 638.0909.

Biology

In vitro evaluation
In vitro evaluation of nucleoside compounds was performed exactly as described previously, which includes the drug sensitivity assays on intracellular amastigotes of Tulahuen (β-galactosidase expressing) strain \textit{T. cruzi} in MRC-5 fibroblasts and Y-strain \textit{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.

\textit{In vitro} cytotoxicity assay against L929 cells was performed exactly as described previously.

\textit{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 \textit{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. 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. 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 \textit{in vitro} metabolic stability was performed exactly as previously described.\textsuperscript{14}

\textbf{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 \textit{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 \textit{10}^4 bloodstream trypomastigotes (Y-strain) originating from an infected donor mouse. Non-infected control mice were age-matched and housed under identical conditions.\textsuperscript{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: \textit{11} or the reference benznidazole). Treatment was initiated at the onset of parasitemia (\textit{i.e.} 5 dpi), only using mice with a detectable parasitemia. Compound \textit{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, \textit{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. 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.

**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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**Acknowledgement:**

C.L. thanks the CSC for a PhD-scholarship. F.H. thanks the FWO-Flanders for a PhD-scholarship. KVH expresses his gratitude to the Hercules Foundation (project AUGE/11/029 “3D-SPACE: 3D Structural Platform Aiming for Chemical Excellence”) and the Special Research Fund (BOF) – UGent (project 01N03217) for funding. G.C. is supported by a research
fund of the University of Antwerp (TT-ZAPBOF 33049). The present work has been funded by the FWO-Flanders (GC, LM, SVC; project number G013118N). Izet Karalic, An Matheeussen and Natascha Van Pelt are acknowledged for their excellent technical assistance.

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.
References:


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

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

Excellent selectivity
Activity in vivo

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

Poorly selective

poorly studied nucleoside modification

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