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Article

Revisiting Pyrazolo[3,4-*d*]pyrimidine Nucleosides as Anti-*Trypanosoma cruzi* and Antileishmanial Agents

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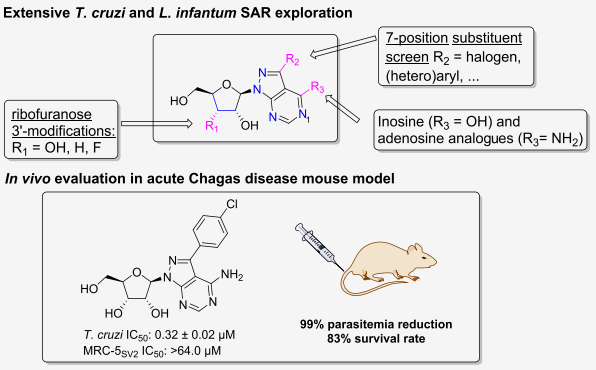
ABSTRACT: Chagas disease and visceral leishmaniasis are two neglected tropical diseases responsible for numerous deaths around the world. For both, current treatments are largely inadequate, resulting in a continued need for new drug discovery. As both kinetoplastid parasites are incapable of *de novo* purine synthesis, they depend on purine salvage pathways that allow them to acquire and process purines from the host to meet their demands. Purine nucleoside analogues therefore constitute a logical source of potential antiparasitic agents. Earlier optimization efforts of the natural product tubercidin (7-deazaadenosine) involving modifications to the nucleobase 7-position and the ribofuranose 3′-position led to analogues with potent anti-*Trypanosoma* brucei and anti-*Trypanosoma cruzi* activities. In this work, we report the design and synthesis of pyrazolo[3,4-*d*]pyrimidine nucleosides with 3′- and 7-modifications and assess their potential as anti-*Trypanosoma cruzi* and antileishmanial agents. One compound was selected for *in vivo* evaluation in an acute Chagas disease mouse model.



INTRODUCTION

Chagas disease and leishmaniasis are two vector-borne communicable diseases responsible for numerous deaths every year. Characterized by the WHO as neglected tropical diseases (NTDs), they occur mainly in populations living in poverty in developing regions around the world and have a severe impact on the lives of affected persons and their families.[1,2] Chagas disease, caused by *Trypanosoma cruzi*, is endemic to Latin America, where it is still one of the most prevalent public health problems.[3] Migration and specific transmission modes have enabled spreading beyond these geographical boundaries so that it is now considered a global issue.[4] Chagas disease starts with an acute symptomatic phase characterized by high-grade parasitemia, which progresses into an asymptomatic chronic state after a few weeks. While most people stay asymptomatic for life, 30−40% will develop severe clinical manifestations after 10−30 years.[5,6] Treatment options are limited to nifurtimox and benznidazole, two old drugs that have only limited efficacy in the chronic disease phase and are associated with severe adverse reactions.[4] At present, no vaccine is available and effective antiparasitic chemotherapy is therefore key in eliminating this NTD. As the current Chagas disease pipeline is almost empty, there is a pressing need to develop novel, safe, and efficacious treatments.[7−9]

Leishmaniasis is endemic in 60 countries with Brazil, India, Ethiophia, Somalia, Kenya, South Sudan, and Sudan reporting more than 90% of all cases.[10] Depending on the causative *Leishmania* species, the disease exists in two main clinical forms: visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). The most severe systemic VL form is usually fatal within two years without treatment[11] and is responsible for up to 30 000 deaths every year. Most antileishmanial drugs have been repurposed from other indications. Efficacy varies by geographical region, and treatment courses are long, require hospitalization, and are associated with significant side effects.[12,13] While several new chemical entities with distinct mechanisms of action have recently entered clinical trials,[14−19] new drug discovery efforts are required to fill the early-stage pipeline.[20] New drug candidates should be suitable for field conditions and allow for global use, oral dosing, and a short treatment course.[7,20]

Unlike their mammalian hosts, both *Trypanosoma cruzi* and *Leishmania* spp. are obligate auxotrophs for purines, meaning that they lack *de novo* purine biosynthesis and rely on the salvage of purines (nucleosides and/or nucleobases) to meet their purine demand.[21−23] Consequently, they have developed a complex set of purine salvage enzymes that allows them to

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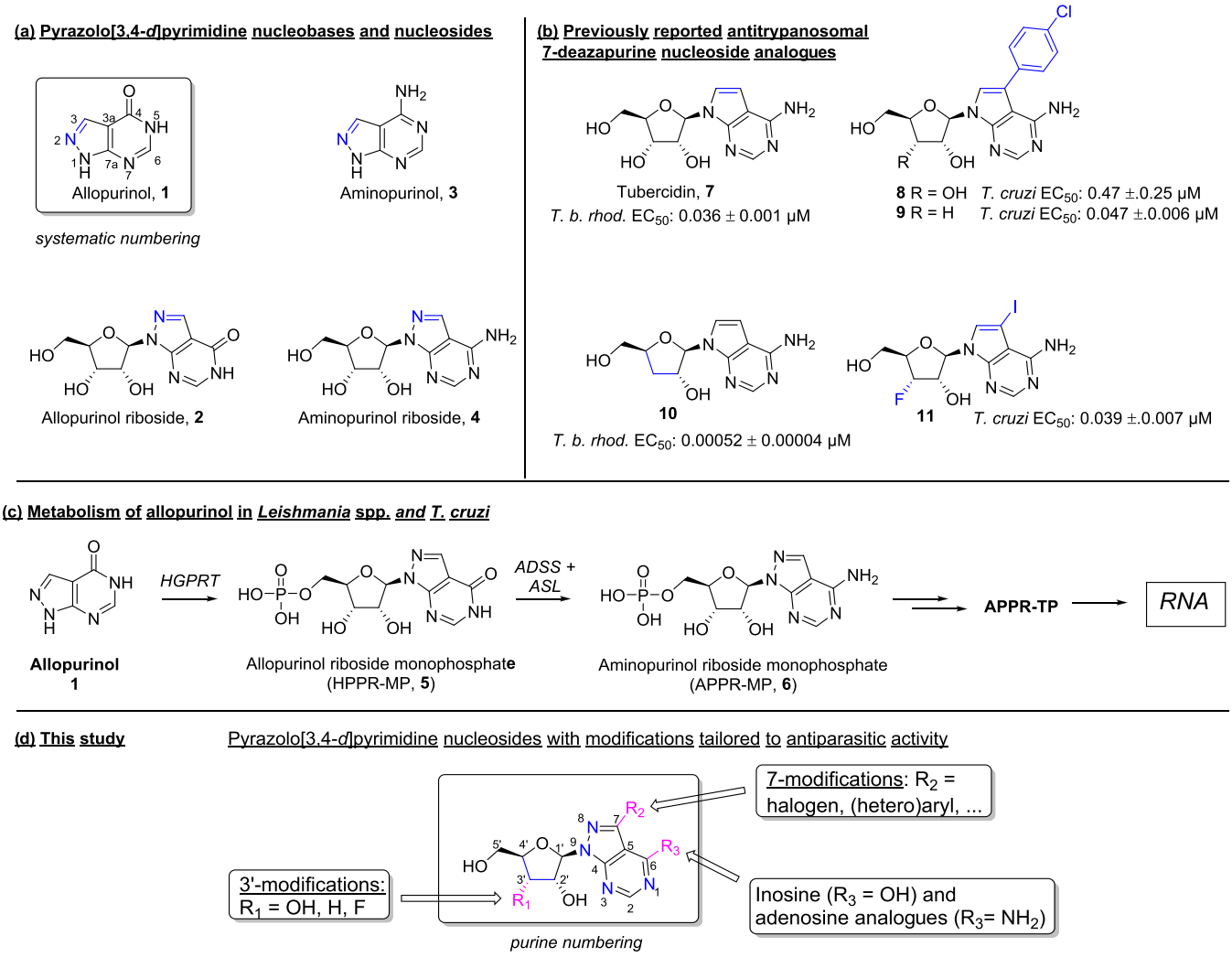

Figure 1. (a) Structures of pyrazolo[3,4-*d*]pyrimidines. (b) Antitrypanosomal 7-deazapurine nucleosides previously reported by our group. (c) Metabolism of allopurinol in *Leishmania* spp. and *T. cruzi*. (c, d) Pyrazolo[3,4-*d*]pyrimidine nucleosides reported in this study. Throughout the text, purine numbering is used as shown in Figure 1d, while in the experimental part, systematic numbering is employed.

acquire and process purines from their hosts. Purine (nucleoside) analogues therefore constitute an interesting pool of potential antiparasitic agents. Indeed, several analogues have been reported that act as inhibitors of enzymes of the purine salvage pathway or as "subversive substrates", which are selectively activated by salvage enzymes of the invading parasite.[24−26]

An example of such a subversive substrate is allopurinol (**1**) (Figure 1a). Next to its use as a treatment for gout, allopurinol inhibits the growth of several *Leishmania* and *Trypanosoma* species[27] in which its mechanism of action has been well-investigated.[28,29] It is selectively metabolized by the parasite to inosine- and adenosine-like nucleotide derivatives **5** and **6** and ultimately to an ATP analogue (i.e., APPR-TP) that is incorporated in RNA (Figure 1c).[28−30] Allopurinol has been evaluated in humans for VL treatment with mixed results. Although it achieved full cures in some patients, it was not satisfactory in monotherapy as first-line treatment due to its static rather than cidal character.[31] Nevertheless, allopurinol is still used in certain combination regimens for the treatment of CL,[11,32,33] and it is the treatment of choice for VL and CL in dogs.[34] Allopurinol has also been investigated for the treatment of Chagas disease with mixed outcomes.[35−37] For example, it proved effective in treating reactivation after heart transplantation.[38,39] Both in leishmaniasis[33,40−42] and in Chagas disease,[43−45] allopurinol has demonstrated synergism with currently used drugs, demonstrating that a nucleobase/nucleoside analogue could be a valuable addition to the therapeutic arsenal. The *in vitro* antileishmanial activity of the ribonucleoside of allopurinol (**2**) is several times higher than allopurinol, but **2** was not further evaluated *in vivo* due to production difficulties and limited benefit over allopurinol.[46−48] The 6-amino congeners aminopurinol **3** and riboside derivative **4** (Figure 1a) were generally more active *in vitro* than allopurinol and **2**,[46,49,50] which may be due to faster conversion to the same active triphosphate. However, **3** and **4** suffered from cytotoxicity and selectivity concerns. Yet, Avila et al. found aminopurinol to be effective in animal models of both VL and Chagas disease at dosages as much as 300-fold lower than used for allopurinol and well below the toxic dose in humans.[46,51] Despite these promising data, neither aminopurinol **3** nor its ribonucleoside **4** was further evaluated for the treatment of leishmaniasis or Chagas disease.

Our group recently reported several 7-deazapurine nucleosides derived from the natural product tubercidin (**7**) that display potent activity against *Typanosoma brucei* and *T. cruzi*

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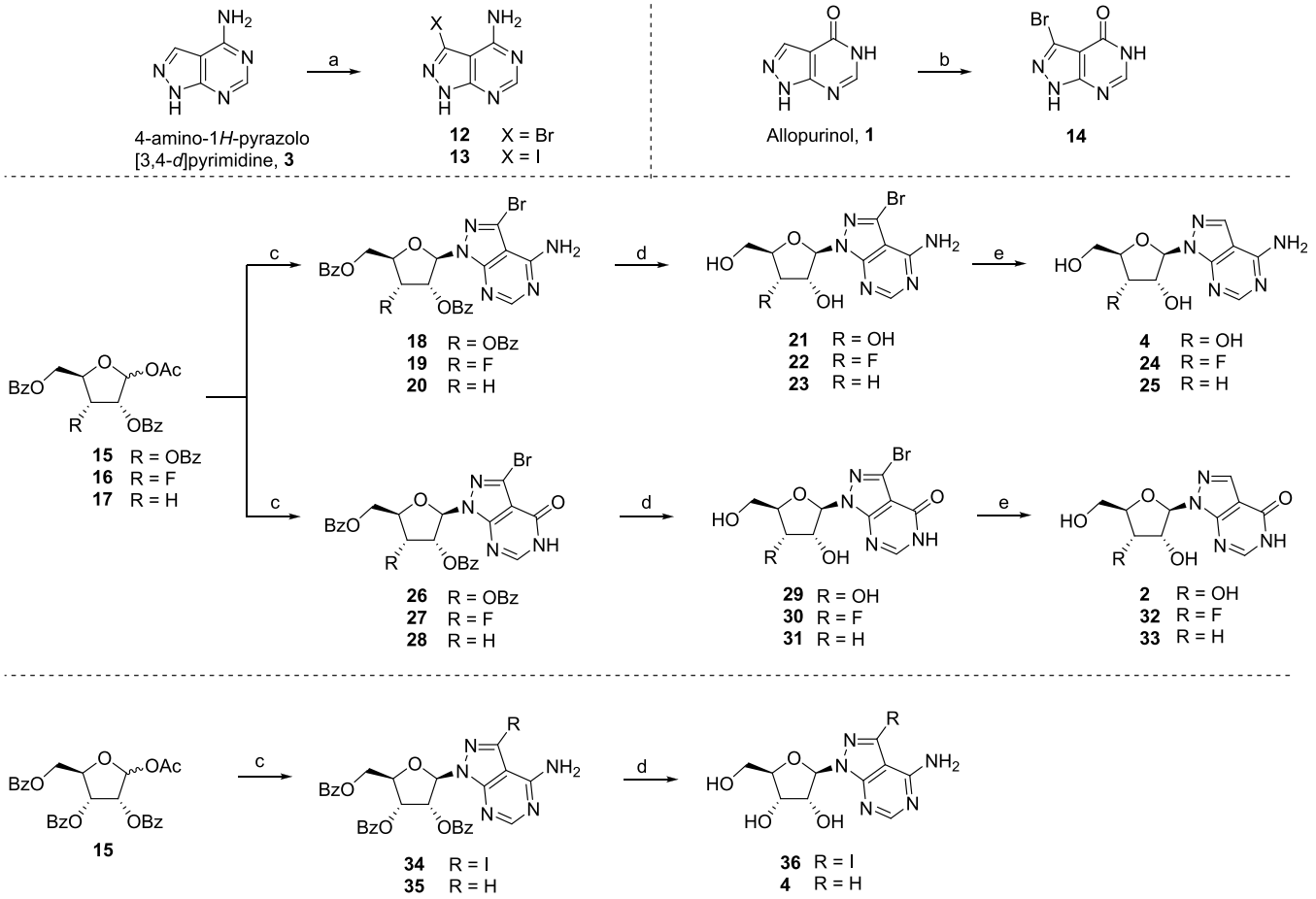
Scheme 1. Synthesis of Ribofuranose-Modified Pyrazolo[3,4-*d*]pyrimidine Nucleosides[a]



[a]Reagents and conditions: (a) NBS, DMF, 60 °C, 91% (for **12**), NIS, DMF, 80 °C, 93% (for **13**); (b) NBS, water, 90 °C, 87%; (c) **12** (for **18**–**20**), **14** (for **26**–**28**), or **13** (for **34**) $BF_3{\cdot}OEt_2$, $MeNO_2$, reflux, 77% (**34**); (d) 0.5 M NaOMe in MeOH, 70% over 2 steps (**21**), 19% over 2 steps (**22**), 20% over 2 steps (**23**), 38% over 2 steps (**29**), 11% over 2 steps (**30**), 6% over 2 steps (**31**), 64% (**36**); (e) Pd/C, H_2, 1 M aq. NaOAc, MeOH, 61% (**4**), 82% (**24**), 66% (**25**), 54% (**2**), 83% (**32**), 80% (**33**).

(Figure 1b).[52−56] The introduction of selected substituents on the 7-position (as in **8, 9**) led to selective anti-*T. cruzi* and anti-*T. brucei* agents.[52] Further deletion of the hydroxyl group on position 3′ afforded compound **10**, which was able to fully cure mice with CNS-stage sleeping sickness,[55] and **9**, which displayed high potency against *T. cruzi*.[53] A fluorine atom in position 3′ also proved favorable, with **11** displaying high anti-*T. cruzi* activity.[57] Compound **9** was evaluated in a Chagas mouse model but failed to deliver sterile cure. Furthermore, none of these 7-deazapurine nucleosides displayed selective activity against the phylogenetically related *Leishmania*, which is remarkable given that several nucleoside analogues display antileishmanial potential,[47,48,58−61] and many nucleosides combine antichagasic and antileishmanial activities.[46,48,61] Nevertheless, known antileishmanial nucleoside analogues (*e.g.*, the pyrazolo[3,4-*d*]pyrimidines (*vide supra*), carbocyclic inosine,[62] 9-deazainosine,[58] Formycin B[58]) contain nucleobase surrogates other than 7-deazapurine, leading us to assume that a 7-deazapurine base is detrimental for antileishmanial activity.

In the search for nucleoside analogues with improved antileismanial activity, we decided to explore nucleosides featuring a pyrazolo[3,4-*d*]pyrimidine (8-aza-7-deazapurine) nucleobase. Next to 7-deazapurines, pyrazolo[3,4-*d*]-pyrimidines are the only purine isosteres that allow derivatization *via* 7-modifications,[63,64] such as the ones found beneficial to increase the potency and selectivity of (3′-deoxy)tubercidin. Compared to tubercidin **1**,[65] aminopurinol riboside **4** is significantly less toxic.[51] Although several 7-modified and 3′-modified pyrazolo[3,4-*d*]pyrimidine nucleosides have been reported,[66−68] they have not been explored for antiparasitic activity. One exception is 7-bromo-allopurinol riboside, which proved more potent than allopurinol.[69]

In this study, we describe the synthesis and initial evaluation of a library of 7-substituted and ribofuranose-modified pyrazolo[3,4-*d*]pyrimidine nucleosides, comprising both inosine-like (allopurinol riboside) and adenosine-like (aminopurinol riboside) analogues. The structure−activity relationships for anti-*T. cruzi* and antileishmanial activity are discussed, and one analogue was evaluated in an acute Chagas disease mouse model.

RESULTS AND DISCUSSION

Chemistry. The required brominated and iodinated nucleobase analogues **12** and **13** were readily obtained from aminopurinol **3** *via* reaction with *N*-bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS) in *N*,*N*-dimethylformamide (DMF) at elevated temperature (Scheme 1). While we were also interested in the chloro and fluoro derivatives,

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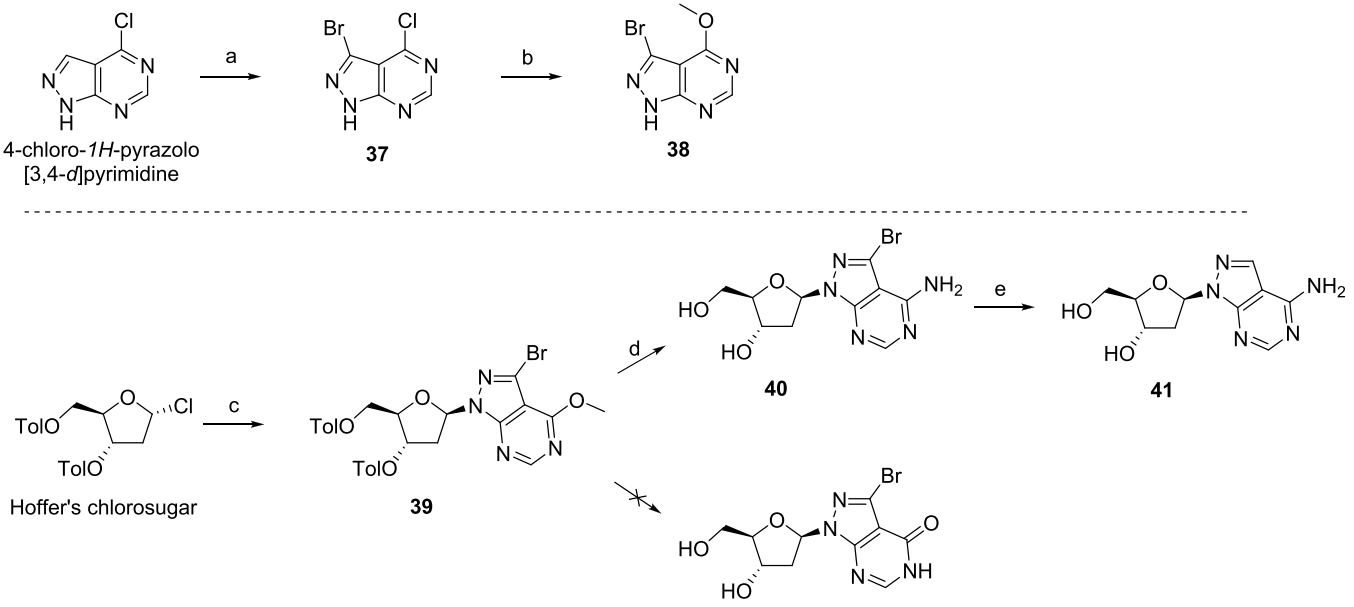
Scheme 2. Reagents and Conditions: (a) NBS, DMF, 50 °C; (b) NaOMe, MeOH, 70 °C, 51% Over 2 steps; (c) 38, KOH, TDA-1, MeCN, 8%; (d) 7 N NH_3 in MeOH, 90 °C, 76%; (e) H_2, $Pd(OH)_2/C$, NaOAc, MeOH, 46%



chlorination of aminopurinol **3** with *N*-chlorosuccinimide (NCS) or fluorination with diethylaminosulfur trifluoride (DAST) failed to deliver the desired halogenated heterocycles. Bromination of allopurinol with bromine in water at 90 °C afforded **14**.

7-Bromoallopurinol **14** and 7-bromoaminopurinol **12** were coupled with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose, its 3′-deoxy counterpart **17**,[53] and 3′-deoxy-3′-fluoro analogue **16**.[57] Coupling of these three donors with 7-bromoaminopurinol **12** was performed with $BF_3{\cdot}OEt_2$ in refluxing nitromethane, as described for **18**.[69] For the 7-bromoaminopurinol nucleosides **18**−**20**, a single product was obtained and no regioisomers were observed. Deprotection with sodium methoxide resulted in **21**−**23**, and reductive dehalogenation with H_2 and Pd/C in buffered methanol afforded **4, 24**, and **25**. Remarkably, the combined glycosylation-deprotection yields were much lower (∼20%) for the 3′-modified analogues compared to that of the ribofuranose **4** (∼70%). The correct regiochemistry of the final compounds was confirmed *via* comparison of the ^{13}C NMR spectra of **24** and **25** with that of the literature compound **4**. The chemical shifts of *C*-7 (∼133 ppm) and *C*-5 (∼154 ppm) (systematic numbering) were identical to reported values, whereas in the *N*-8 regioisomer, *C*-7 would be shifted upfield about 10 ppm and *C*-5 shifted downfield about 5−6 ppm.[70,71] In the ^1H-^{13}C HMBC spectra, *H*-1′−*C*-7 coupling was absent, providing further evidence of the desired *N*-9 regiochemistry.

7-Bromoallopurinol **14** was glycosylated under the same conditions, but for each donor three different products with the same mass were observed on thin-layer chromatography (TLC), corresponding to the *N*-9, *N*-8, and *N*-1 regioisomers. The higher-running, less polar spot was the major product and was presumed to be the correct regioisomer[72] and was isolated. Glycosylation yields were generally lower (∼50%) than for the corresponding aminopurinols. The correct regiochemistries of **32** and **33** were verified *via* comparison of their ^{13}C NMR spectra with that of **2**.[72] Compared to the 6-aminonucleosides, the upfield shift of *C*-7 in the *N*-8 isomer is reported to be less pronounced,[72] but the values of *C*-7 (∼135 ppm) and *C*-5 (∼148 ppm) (systematic numbering) were again very similar. Similarly, in this case, *H*-1′−*C*-7 coupling was again absent in the ^1H-^{13}C HMBC spectra, further confirming *N*-9 attachment of the heterocycle.

Glycosylation of **15** with 7-iodoaminopurinol afforded **34** in high yield, and deprotection in methanolic ammonia furnished **36**. Both **34** and **36** were used for further modifications (*vide infra*). Compound **4** could also be obtained from direct glycosylation of 4-aminopyrazolo[3,4-*d*]pyrimidine, followed by deprotection.

The 2′-deoxy analogues were prepared as described by Seela et al. (Scheme 2).[64,73−75] Compound **38** was obtained efficiently by bromination of 4-chloro-1*H*-pyrazolo[3,4-*d*]-pyrimidine with NBS, followed by nucleophilic aromatic substitution with sodium methoxide. Anion glycosylation of **38** with commercially available Hoffer's chlorosugar afforded **39** in 8% yield. Simultaneous deprotection and introduction of the 6-amino group to afford **40** was achieved by overnight heating in methanolic ammonia. Reductive dehalogenation *via* hydrogenation over Pd/C in buffered methanol afforded **41**. Attempted conversion of **39** to the corresponding 6-oxo congener in dilute NaOH solution, as described by Seela for the 3-unsubstituted analogue,[71] resulted in glycosidic bond breakage.

Further modifications focused on the introduction of substituents on the 7-position of **21**. Different phenyl rings were introduced *via* aqueous Suzuki coupling reactions with the appropriate arylboronic acids to furnish **42**−**63** (Scheme 3). Reaction with 4-chloro-3-cyano-phenylboronic acid gave rise to significant amounts of biphenyl product **75**, which was also isolated. Except for the 2-pyridyl and 2-thiophene substituents in **65** and **67**, which were introduced *via* Stille coupling, other heterocyclic substituents were also introduced under aqueous Suzuki coupling conditions to furnish **64**, **66**, and the substituted 2-thienyl analogues **68**−**70**. The vinyl- and isopropenyl-compounds **71** and **72** were synthesized *via* the Suzuki reaction with the respective potassium trifluoroborate salts, while *trans*-2-vinylphenylboronic acid was used to obtain **73**. The synthesis of **74** required multiple additions of

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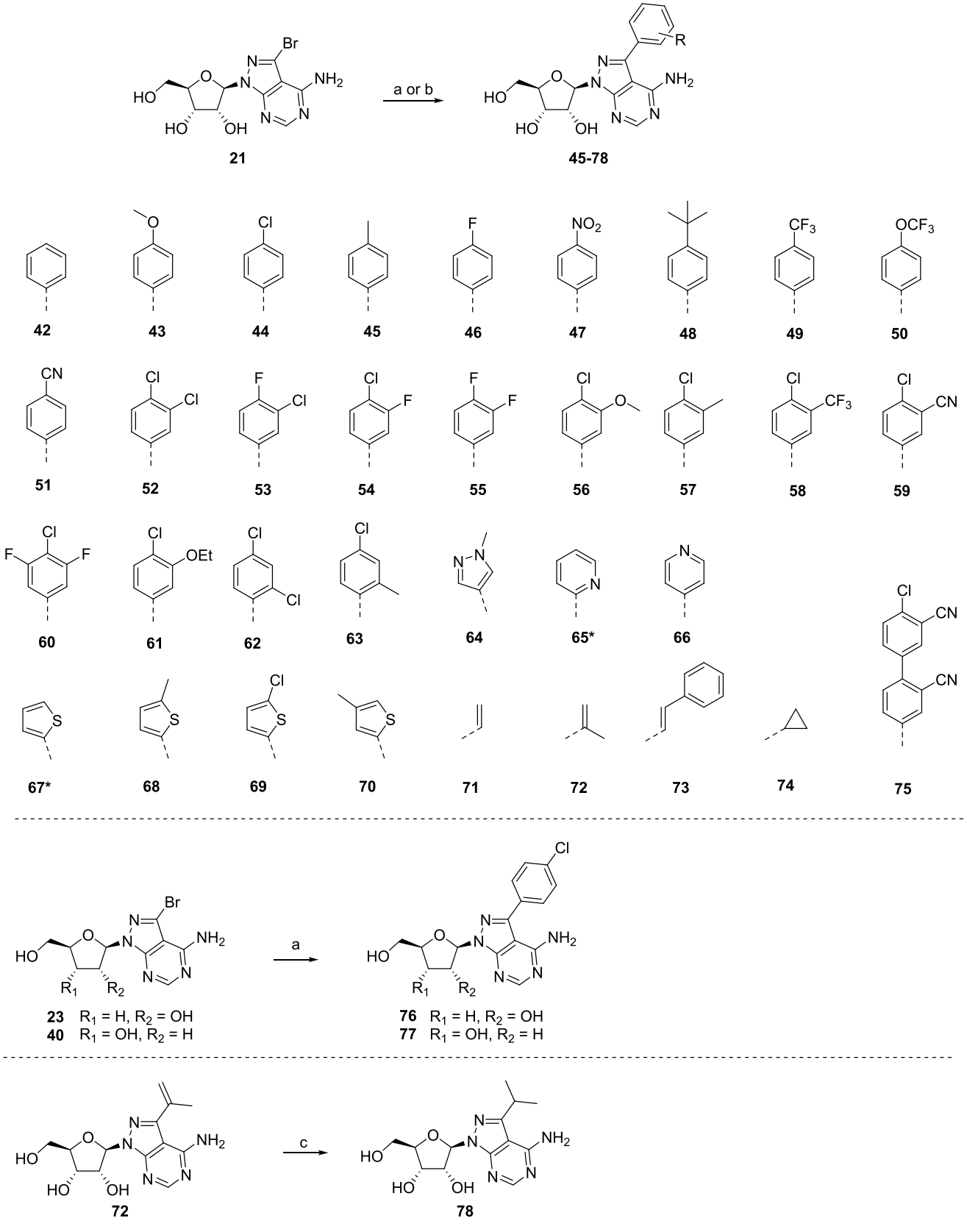
Scheme 3. Reagents and Conditions: (a) Boronic Acid or Trifluoroborate Salt, $Pd(OAc)_2$, TPPTS, Na_2CO_3 (in the Case of a Boronic Acid) or Cs_2CO_3 (When a Trifluoroborate Salt Was Used), $MeCN/H_2O$ 1:2, 16−81% (For All Compounds Except for 67 and 65); (b) Tributylstannylated Heterocycle, $Pd(PPh_3)_4$, CuI, DMF, 24% (67)*, 66% (65)*; (c) H_2, $Pd(OH)_2/C$, MeOH, 81%



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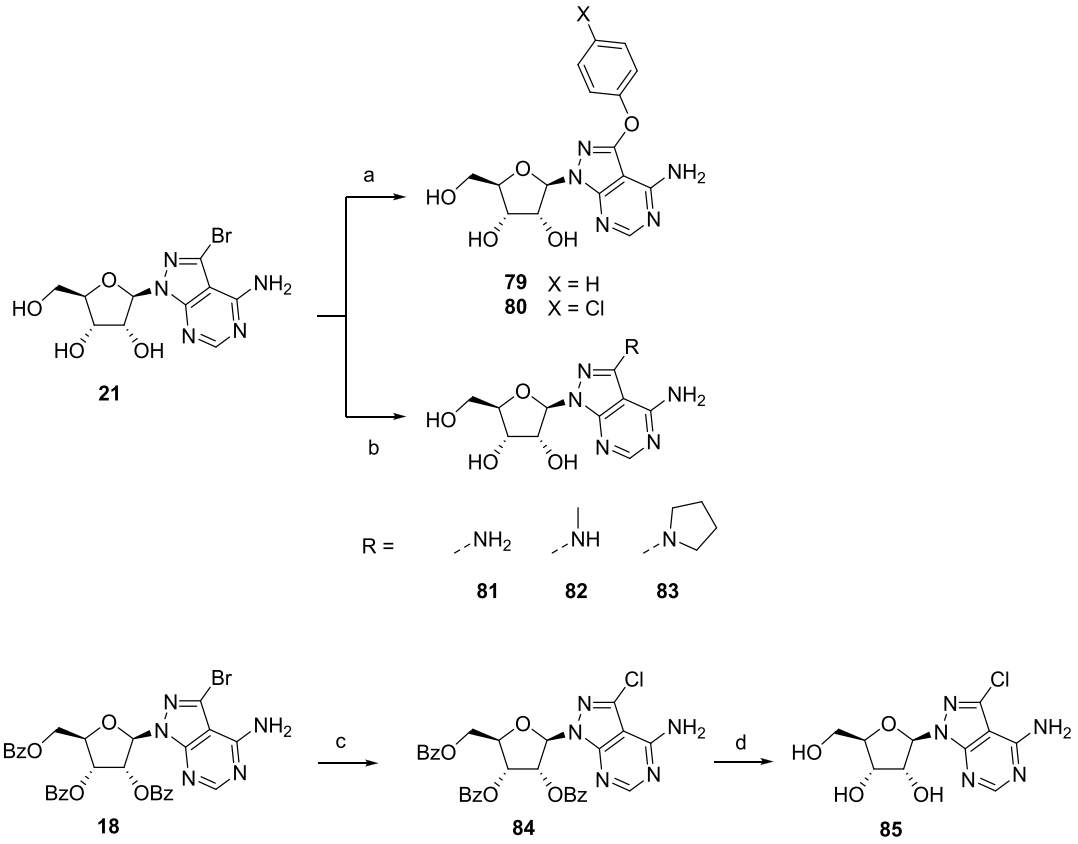
Scheme 4. Reagents and Conditions: (a) Phenol (for 79) or 4-Chlorophenol (for 80), CuI, *N*,*N*-Dimethylglycine, Cs_2CO_3, DMA, 120 °C, 5% (79), 3% (80); (b) CuCl, aq. NH_4OH (for 81) or aq. NHMe (for 82) or Pyrrolidine, 1,4-Dioxane/H_2O 1:2 (for 83), 120 °C, 19% (81), 5% (82), 16% (83); (c) Me_4NCl, Cu_2O, L-Proline, 2-Methoxyethanol, 120 °C, 7 days; (d) 0.5 M NaOMe in MeOH, 34% Over 2 Steps



cyclopropylboronic acid, since reaction with potassium cyclopropyltrifluoroborate proved unsuccessful. Likewise, the 3′-deoxy and 2′-deoxy bromonucleosides **23** and **40** were subjected to the Suzuki reaction with 4-chlorophenylboronic acid to afford **76** and **41**. Finally, the isopropenyl group of previously obtained **72** was reduced *via* catalytic hydrogenation to afford **78**.

Ullman coupling of **21** with phenol or 4-chlorophenol under conditions described for other pyrazolo[3,4-*d*]pyrimidines[76] afforded **79** and **80**, respectively (Scheme 4). The yields were very low (5 and 3%) but provided sufficient amounts of product for preliminary evaluation. The synthesis of **81** *via* Ullman coupling in aqueous ammonia has already been described,[69] and methylamine and pyrrolidine could be coupled under similar conditions to furnish **82** and **83**. To introduce a 7-chloro, **18** was subjected to a copper-catalyzed *retro*-Finkelstein reaction.[77] Although this reaction was sluggish, **85** was obtained in decent yield after deprotection.

The 7-iodo analogue **36** served as a useful precursor for another set of analogues (Scheme 5). The Sonogashira reaction with phenylacetylene furnished **86**, which was further reduced to **87** *via* catalytic hydrogenation. The Sonogashira reaction with ethynyltrimethylsilane yielded **88**, which was either further reduced to **89** or reacted with azidotrimethylsilane in a copper(I)-catalyzed azide-alkyne cycloaddition to **90**. A nitrile substituent was introduced *via* a palladium-catalyzed coupling reaction with $Zn(CN)_2$ to furnish **91**.[66] The nitrile group was further transformed to a tetrazole **92** *via* a 1,3-dipolar cycloaddition reaction or reduced to the aminomethyl analogue **93** *via* hydrogenation over Raney nickel. Alternatively, hydration of the nitrile in basic hydrogen peroxide solution furnished **94**. Attempted hydrolysis of nitrile **91**[66] failed to deliver the carboxylic acid but resulted in cleavage of the *N*-glycosidic bond instead. A trifluoromethyl substituent was introduced *via* a cross-coupling reaction with *in situ*-formed $CuCF_3$[56,78] to afford **95**. Deprotection using sodium methoxide then furnished **96**.

To gain access to the methyl-substituted base **98**, 5-amino-4-cyano-3-methyl-1*H*-pyrazole **97** was synthesized *de novo* from malonitrile and acetyl chloride according to the method of Haneman (Scheme 6).[79] Ring closure of **97** with thioacetamide instead of formamide furnished the bismethylated heterocycle **99**. Glycosylation of **98** afforded **100**, which was deprotected to **101**. Unfortunately, attempted glycosylation of **99** under the same conditions was not successful.

Since a carboxylic acid could not be obtained from the cyano analogue **91**, we looked at other methods to introduce a carbonyl group on the 7-position. Vilsmeier−Haack formylation of **35** failed and only led to *N*-formylation of the 6-amino group. Palladium-catalyzed carbonylation reactions of **34** with different CO equivalents were also unsuccessful.[80−82] Finally, we tried to convert a vinyl substituent into the corresponding aldehyde (Scheme 7). To minimize side reactions, we chose to start from the benzoyl-protected iodide precursor **36**. Suzuki reaction with potassium vinyl trifluoroborate[83] afforded **102** in acceptable yields. Oxidative cleavage of the vinyl group **102** was accomplished *via* the Lemieux−Johnson oxidation to afford aldehyde **103**. The carboxylic acid analogue **108** could

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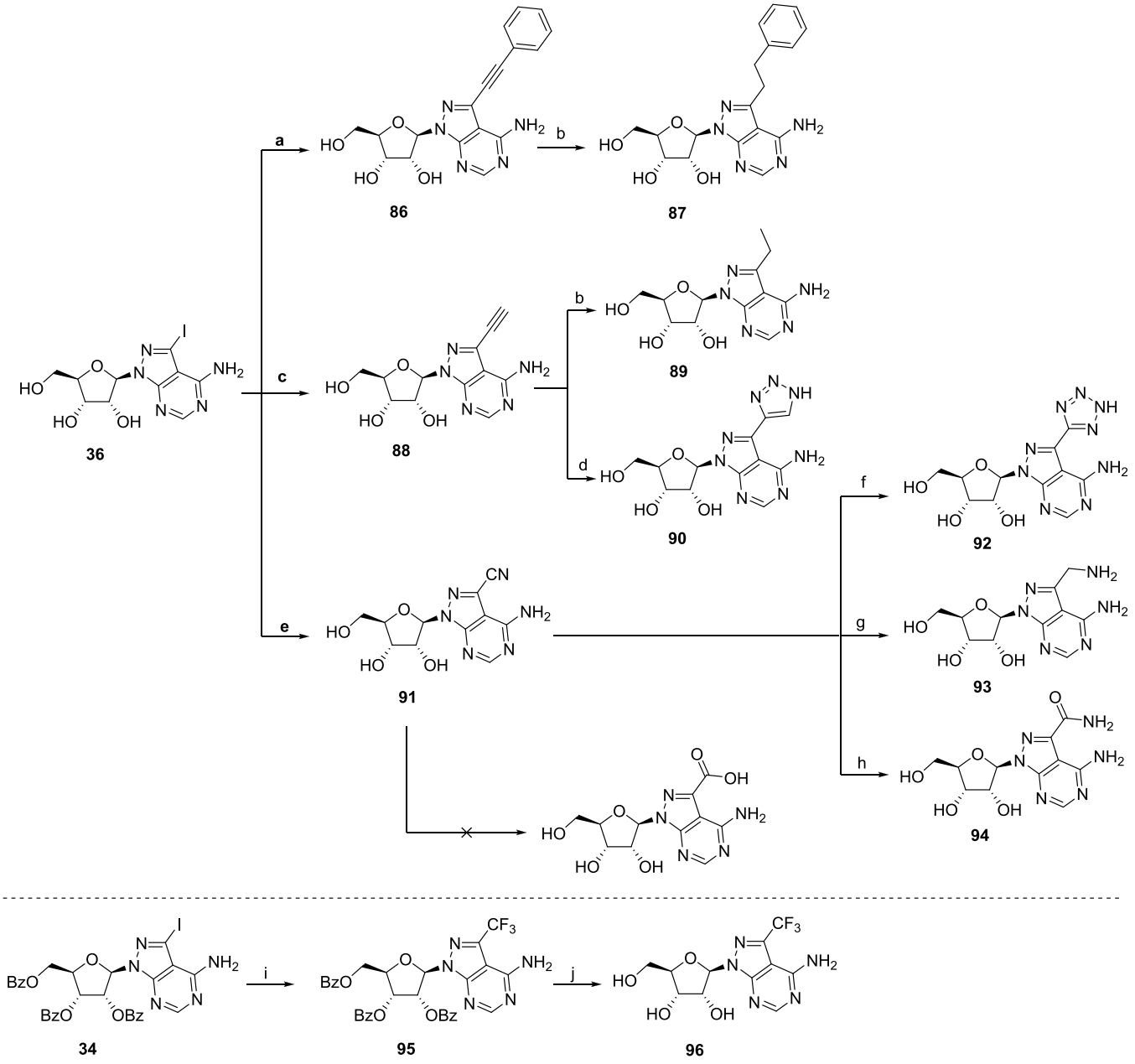
Scheme 5. Reagents and Conditions: (a) Phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, DMF/Et_3N 4:1, 40%; (b) H_2, $Pd(OH)_2/C$, MeOH, 89% (87), 74% (89); (c) (i) Ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, DMF/Et_3N 4:1; (ii) 7 N NH_3 in MeOH, 19%; (d) $TMSN_3$, CuI, DMF/MeOH 9:1, 100 °C, 40%; (e) $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, DMF, 150 °C, 28%; (f) NaN_3, NH_4Cl, LiCl, DMF, 100 °C, 25%; (g) H_2, Raney Nickel, MeOH, 10%; (h) NH_4OH, H_2O_2, 25%; (i) $TMSCF_3$, CuI, KF, DMF/NMP 1:1, Reflux; (j) 0.5 M NaOMe in MeOH, 14% Over 2 Steps



now efficiently be obtained *via* the Pinnick oxidation of **103**. Alternatively, the aldehyde functionality of **103** was further elaborated to a methyl-*N*-morpholino substituent *via* reductive amination (**104**) or to a difluoromethyl substituent *via* reaction with DAST (**106**). Deprotection under basic conditions afforded **105** and **107**. The carboxylic acid functionality was further derivatized to different amides *via* HCTU-mediated coupling to afford analogues **113**−**116** after benzoate deprotection. Attempted cyclopropanation of **102** with *in situ*-generated difluorocarbene (from $BrCF_2CO_2Na$[84]) or dichlorocarbene (generated from $CHCl_3$ and NaOH) failed.

Biological Evaluation. The prepared nucleoside analogues were evaluated *in vitro* for their activity against *T. cruzi* and *Leishmania infantum*. Cytotoxicity was assayed against MRC-5_{SV2} cells (*T. cruzi* host cell) and primary mouse macrophages (PMM, *L. infantum* host cell) (Table 1).

Although the anti-*T. cruzi* and antileishmanial activities of allopurinol (**1**), aminopurinol (**3**), and their ribonucleosides **2** and **4** are already known, they were included in this study as reference compounds. In accordance with various literature reports,[46,51,85] **1** and **3** displayed good activity against *T. cruzi* and *L. infantum*. Yet, the 20-fold higher activity and selectivity of aminopurinol (**3**) compared to allopurinol (**1**) against *L. infantum* is striking. As mentioned before, **3** has never been evaluated in an *in vivo* VL model although it is known to be safe at low doses.[46,51] Introduction of a bromo substituent in

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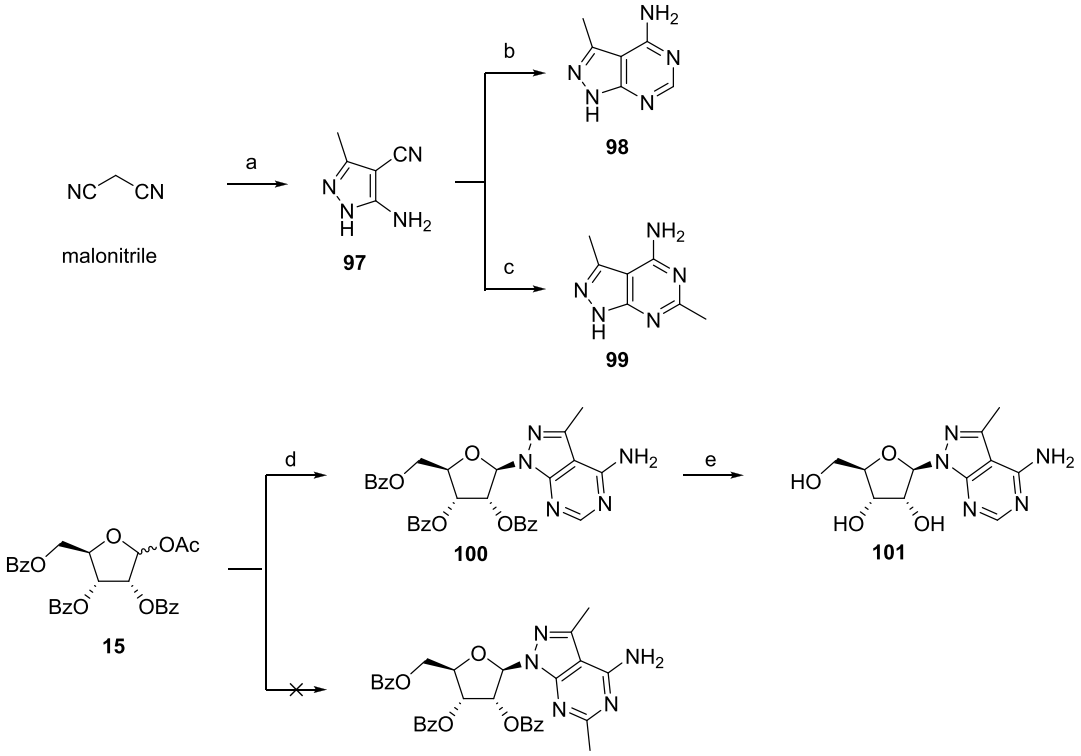
Scheme 6. Reagents and Conditions: (a)(i) NaH, AcCl, Tetrahydrofuran (THF), 0 °C to RT, (ii) Dimethyl Sulfate, Reflux, (iii) $H_2NNH_2{\cdot}H_2O$, Et_3N, 38% Over 3 Steps; (b) Formamide, 180 °C, 23%; (c) Thioacetamide, Reflux, 36%; (d) 98, $BF_3{\cdot}OEt_2$, $MeNO_2$, Reflux; (e) 0.5 M NaOMe in MeOH, 21% Over 2 Steps



position 7 of allopurinol (**1**) or aminopurinol (**3**) led to a drastic drop in activity, possibly due to impeded conversion by PRTases, which are essential for the activation of nucleobase analogues. The 4-APP ribonucleoside **4** displayed potent activity against both *T. cruzi* and *L. infantum* intracellular amastigotes, as could be expected from literature reports on its activity against *T. cruzi* epimastigotes and *Leishmania* promastigotes.[49,50] In both MRC-5_{SV2} cells and PMM cells, **4** failed to show cytotoxicity up to 64 μM, but similar to **3**, it has never been evaluated *in vivo*. Introduction of a halogen atom on the 7-position of **4** (compounds **21** and **36**) led to a severe decrease in activity against *T. cruzi* and *L. infantum*. This is different from earlier reported results in *T. cruzi* epimastigotes and *Leishmania* promastigotes, where the activity was more comparable to the parent compound **4**.[49,50] Activity of allopurinol ribonucleoside **2** was comparable to allopurinol.[85] In our hands, introduction of a bromide on the 7-position of **2** (compound **29**) rendered the compound inactive, which conflicts with a report stating it to be more active than **2** against *L. tropica* intracellular amastigotes.[69]

The combination of aminopurinol (**3**) and allopurinol (**1**) with a 3′-deoxy-3′-fluororibofuranose moiety (**24** and **32**) resulted in inactive compounds. Introduction of a bromide on the 3-position of **24** rendered the compound highly cytotoxic (**22**). It did not display any selective activity, contrasting with the matched 7-deazapurine nucleoside.[57] The same was true for the 3′-deoxynucleosides **23**, **25**, **31**, and **33**. While the inactivity of **33** was already noted by Moorman et al.,[86] the inactivity of **25** was more surprising, as removal of the 3′-hydroxy group resulted in a major increase in activity against *T. brucei* or *T. cruzi* in earlier 7-deazapurine nucleoside series.[53,55] Again, the introduction of a 7-bromo substituent (**23**) afforded a cytotoxic compound without any specific antiparasitic activity. The 2′-deoxynucleosides **41** and **40** were also inactive, as was already reported for **41**.[46]

Based on the structure−activity relationship (SAR) of earlier nucleoside series,[52,53,57,78] which demonstrated that modifications of the 7-position could improve activity, we performed an extensive substituent screen. In a first set of analogues, different substituted phenyl rings were introduced on the 7-position (Table 2). Remarkably, also in this series, a 4-chlorophenyl (**44**) proved to confer the best antitrypanosomal activity (IC_{50} = 0.32 μM) and was about 50-fold more active than the 7-phenyl analogue **42**. The second most potent para-substituted analogue was the 4-methylphenyl substituted compound **45**. Further substitution of the 4-chlorophenyl substituent with a 3-fluoro (**53**), methyl (**57**), or methoxy group (**56**) failed to potentiate its activity. The 2,4-substituted analogues **62** and **63** were less active than the 3,4-disubstituted analogues. Strikingly, the superiority of this 4-chlorophenyl modification has also been observed for other 7-deazapurine nucleosides and suggests that the chloro substituent is involved in a crucial interaction with a parasitic target or transporter, rather than just reducing the electron density of the phenyl ring. Removal of the 3′-OH group as in **76** was expected to result in increased anti-*T. cruzi* activity, based on earlier observations with 7-deazapurine nucleosides,[53] but surprisingly led to a compound that was 3-fold less active than **44**. Nevertheless, **44** is more potent than its 7-deazapurine congener[52] and displays an improved selectivity profile, with no *in vitro* toxicity in MRC-5_{SV2} or PMM cells in concentrations up to 64 μM. The 2′-deoxy analogue **77** displayed reasonably good anti-*T. cruzi* activity but was less potent than both **44** and **76**. All phenyl-substituted analogues displayed in Table 2 were inactive against *L. infantum*.

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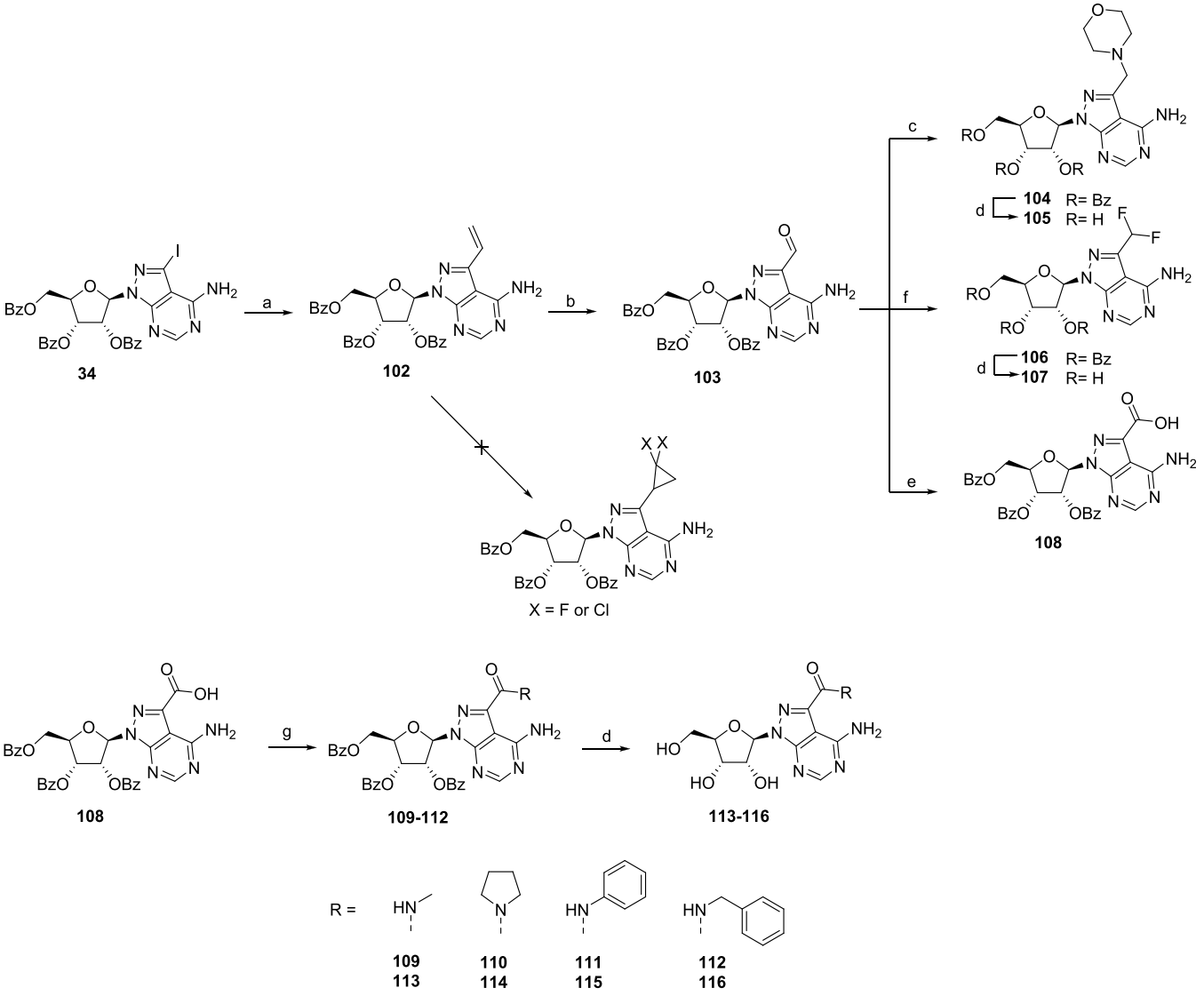
Scheme 7. Reagents and Conditions: (a) Potassium Vinyl Trifluoroborate, $Pd(OAc)_2$, PPh_3, Cs_2CO_3, DMF/H_2O 9:1, 100 °C, 38%; (b) $K_2OsO_4 \cdot 2H_2O$, $NaIO_4$, 2,6-Lutidine, 1,4-Dioxane/H_2O 3:1, 48%; (c) Morpholine, $NaBH_3CN$, AcOH, MeOH/THF 2:1; (d) 0.5 M NaOMe in MeOH, 64% Over 2 Steps (105), 58% Over 2 Steps (107), 29% Over 2 Steps (113), 28% Over 2 Steps (114), 61% Over 2 Steps (115), 44% Over 2 Steps (116); (e) $NaClO_2$, NaH_2PO_4, H_2O_2, THF/H_2O 6:1, 94%; (f) DAST, CH_2Cl_2; (g) Aq. NHMe (109), Pyrrolidine (110), Aniline (111), or Benzylamine (112), HCTU, *N,N*-Diisopropylethylamine (DIPEA), DMF



Bioisosteric replacement of the phenyl ring by a thiophene resulted in a loss of activity (**67**−**70**) (Table 3). The 5-chlorothiophene and 5-methylthiophene analogues **69** and **68** were 5−10-fold less active than their phenyl counterparts and displayed lower selectivity. Introducing an extra atom or linker between the nucleobase and the phenyl ring was also not tolerated, as illustrated by the inactivity of the phenoxy analogues **79** and **80** and the low activity of elongated phenyl analogues **86**, **73**, and **87**. None of these analogues showed any activity against *L. infantum*. A number of nitrogen-containing heterocycles were also introduced on the 7-position (**64**−**66**, **90**, and **92**). A 2- or 4-pyridyl substituent (**65** and **66**), an *N*-methylpyrazole (**64**), and tetrazole (**92**) did not lead to any specific activity against *T. cruzi* or *L. infantum*. While **64** and **92** were devoid of MRC-5_{SV2} cytotoxicity, the triazole analogue **90** was highly cytotoxic and also did not display any specific antiparasitic activity.

Among a series of analogues with small substituents on the 7-position, a nitrile (**91**) led to reasonable activity against both *T. cruzi* and *L. infantum* (Table 4). The chloro analogue **85** was less active against *T. cruzi* but displayed higher selectivity toward MRC-5_{SV2} cells. A trifluoromethyl (**96**) or difluoromethyl (**107**) substituent resulted in cytotoxic compounds that did not display selective antiparasitic activity. Among the carbon-based substituents, the methyl analogue **101** and the ethynyl analogue **88** displayed moderate anti-*T. cruzi* activity. A vinyl (**71**), ethyl (**89**), or cyclopropyl (**74**) substituent led to inactive compounds, while an isopropenyl (**72**) or isopropyl (**78**) group resulted in cytotoxic compounds with no specific antiparasitic activity. An amine (**81**), methylamine (**82**), or pyrrolidine group (**83**) on the 7-position did not lead to any significant antiparasitic activity, as was the case for an aminomethyl (**93**) or morpholinomethyl (**105**) substituent. The activity of the amide-substituted nucleosides (**94**, **113**−

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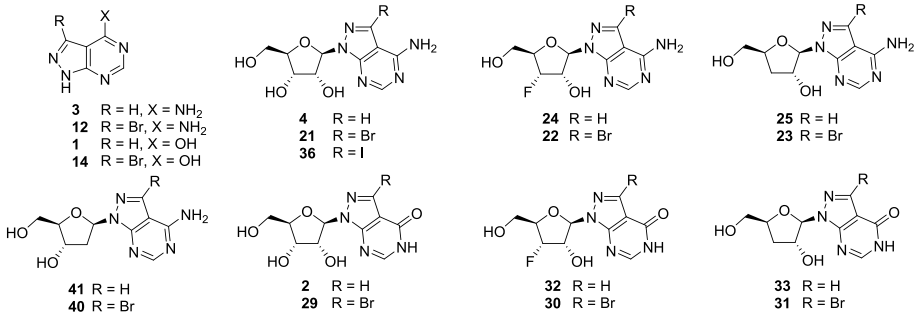
Table 1. Evaluation of Drug Sensitivity of Ribofuranose-Modified Nucleoside Analogues against *T. cruzi* and *L. inf.*[a]



cpd.	structure	*T. cruzi* IC_{50} (μM)	MRC-5 CC_{50} (μM)	SI	*L. infantum.* IC_{50} (μM)	PMM CC_{50} (μM)	SI
Nucleobases							
3	R = H	*0.57*	*2.46*	*5*	*0.18*	*>64.0*	*>355*
12	R = Br	*38.1*	*>64.0*	*>1*	*22.6*	*>64.0*	*>2*
1	R = H	9.75 ± 1.75	>64.0	>7	3.51 ± 1.77	>64.0	>18
14	R = Br	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
Ribonucleosides							
4	X = H	0.29 ± 0.03	>64.0	>219	1.06 ± 0.35	>64.0	>60
21	X = Br	4.37 ± 0.55	>64.0	>15	29.4 ± 21.4	>64.0	>2
36	R = I	*12.62*	*25.4*	*2*	*25.4*	*>64.0*	*>2*
2	R = H	7.18 ± 3.66	>64.0	9	6.66 ± 4.66	>64.0	10
29	R = Br	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
3′-Deoxy-3′-Fluoronucleosides							
24	X = H	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
22	X = Br	*1.06*	*0.23*	0	*0.08*	*0.13*	*1*
32	X = H	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
30	R = Br	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
3′-Deoxynucleosides							
25	R = H	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
23	R = Br	*38.5*	*2.2*	0	*>64.0*	*>64.0*	
33	R = H	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
31	R = Br	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
2′-Deoxynucleosides							
41	R = H	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
40	R = Br	53.2 ± 10.8	24.9 ± 14.0	0	>64.0	>64.0	

[a]Cytotoxicity Was Assayed against Human MRC-5_{SV2} Cells and Primary Mouse Macrophages (PMMs). Values represent mean ± SEM, which originate from 2−3 independent experiments and are expressed in μM. Values in italics represent the result of a single determination because of inactivity or overt cytotoxicity. SI: *in vitro* selectivity index is the ratio of CC_{50} for the host cell (MRC-5_{SV2} for *T. cruzi*, PMM for *L. inf.*) and IC_{50} of the parasite. Benznidazole was included as a reference for *T. cruzi* (IC_{50} = 2.02 ± 0.28 μM) and miltefosine as a reference for *L. infantum* (IC_{50} = 7.47 ± 2.23 μM).

116) varied: a carboxamide group (**94**) again resulted in a cytotoxic compound, while adding a methyl group on the amide nitrogen (**113**) removed all cytotoxic effects and provided a compound with moderate anti-*T. cruzi* activity. Amide analogues with bigger groups (**114**−**116**) were inactive.

Overall, the SAR of the pyrazolo[3,4-*d*]pyrimidine nucleosides for anti-*T. cruzi* and anti-*L. infantum* activities turned out to be completely different from previously reported 7-deazapurine[52,53,57] and 1,7-dideazapurine[78] nucleoside series. As already known from several literature reports, the parent nucleosides aminopurinol riboside **4** and allopurinol riboside **2** displayed good *in vitro* activity against both *T. cruzi* and *L. infantum*. Introduction of a halogen atom on the 7-position had a detrimental effect on the activity against both *T. cruzi* and *L. infantum* and modifications at the 3′-position of the ribose moiety, expected to result in more potent compounds based on SAR studies of earlier nucleoside series, completely abolished activity, or resulted in cytotoxic compounds. A substituent screen of the 7-position of **4** revealed highly varying effects on the antiparasitic activity and cytotoxicity to MRC-5_{SV2} and PMM. A 4-chlorophenyl substituent resulted in a compound with potent anti-*T. cruzi* activity and devoid of cytotoxic effects in MRC-5_{SV2} or PMM at concentrations up to 64 μM. Contrary to expectations, removal of the 3′-hydroxyl group of **44** resulted in a compound that was 2−3-fold less potent (**76**). The insertion of a linker (oxygen, carbon-based, amide) between the oxygen and the phenyl ring was not tolerated. Bioisosteric replacement of the phenyl ring with a thiophene was also not tolerated and resulted in a 10-fold decrease in activity. Other heterocycles in the 7-position resulted in compounds with low antiparasitic activity. The effect of other, smaller substituents varied greatly. Some resulted in moderate anti-*T. cruzi* activity (*e.g.*, chloride, methyl, ethynyl), while other substituents afforded highly cytotoxic nonselective compounds. Overall, none of the 7-modified analogues displayed good activity against *L. infantum*, suggesting that, regardless of the nature of the heterocyclic nucleobase, substituents on this position are not tolerated. **44** was the

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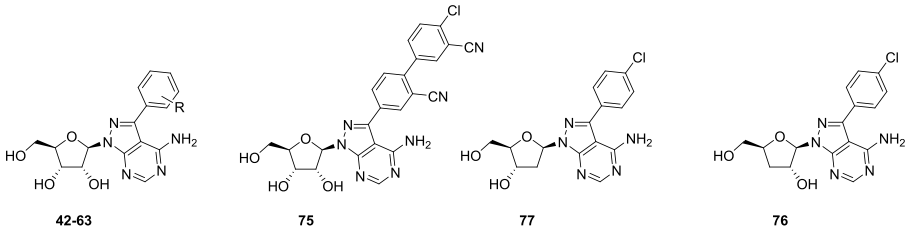
Table 2. Evaluation of Drug Sensitivity of 7-Modified Nucleoside Analogues against *T. cruzi* and *L. infantum*[a]



cpd.	structure (R =)	*T. cruzi* IC_{50} (μM)	MRC-5 CC_{50} (μM)	SI	*L. inf.* IC_{50} (μM)	PMM CC_{50} (μM)	SI
42	H	*13.1*	*>64.0*	*>5*	*>64.0*	*>64.0*	
43	4-OMe	18.2	*>64.0*	>4	*>64.0*	*>64.0*	
44	4-Cl	0.32 ± 0.02	>64.0	>197	>64.0	>64.0	
45	4-Me	1.77 ± 0.92	>64.0	>36	>64.0	>64.0	
46	4-F	3.36 ± 1.88	>64.0	>19	>64.0	>64.0	
47	4-NO_2	10.3 ± 5.8	>64.0	>6	>64.0	>64.0	
48	4-*t*-Bu	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
49	4-CF_3	6.34 ± 1.90	>64.0	>10	57.0 ± 9.3	>64.0	>1
50	4-OCF_3	15.6 ± 6.2	>64.0	>4	>64.0	>64.0	
51	4-CN	*32.0*	*>64.0*	>2	*>64.0*	*>64.0*	
52	3,4-diCl	2.82 ± 1.94	*>64.0*	*>23*	*>64.0*	*>64.0*	
53	3-Cl-4-F	4.66 ± 2.71	>64.0	>14	>64.0	>64.0	
54	4-Cl-3-F	1.19 ± 0.92	>64.0	>54	>64.0	>64.0	
55	3,4-diF	4.24 ± 1.55	>64.0	>15	>64.0	>64.0	
56	4-Cl-3-OMe	1.14 ± 1.02	>64.0	>56	>64.0	>64.0	
57	4-Cl-3-Me	0.81 ± 0.13	>64.0	>79	>64.0	>64.0	
58	4-Cl-3-CF_3	*43.8*	*>64.0*	*>1*	*>64.0*	*>64.0*	
59	4-Cl-3-CN	*>64.0*	*>64.0*		*>64.0*	*>64.0*	
60	4-Cl-3,5-diF	1.33 ± 0.51	>64.0	>17	*>64.0*	*>64.0*	
61	4-Cl-3-OEt	45.3	>64.0	>1	*>64.0*	*>64.0*	
62	2,4-diCl	6.06 ± 2.69	>64.0	>11	52.8 ± 11.2	>64.0	>1
63	4-Cl-2-Me	3.76 ± 0.61	>64.0	>17	*>64.0*	*>64.0*	
75		*>64.0*	*>64.0*		*>64.0*	*>64.0*	
77		2.49 ± 0.42	>64.0	>22	[32.5, >64.0]	[32.0, >64.0]	1
76		1.04 ± 0.32	>64.0	>61	>64.0	>64.0	

[a]Cytotoxicity was assayed against human MRC-5SV2 cells and primary mouse macrophages (PMMs). Values represent mean ± SEM, which originate from 2−3 independent experiments and are expressed in μM. Values in parentheses represent the values of the different determinations, as no correct average can be calculated. Values in italics represent the result of a single determination because of inactivity or overt cytotoxicity. SI: *in vitro* selectivity index is the ratio of CC_{50} for the host cell (MRC-5_{SV2} for *T. cruzi*, PMM for *L. inf.*) and IC_{50} of the parasite. Benznidazole was included as a reference for *T. cruzi* (IC_{50} = 2.02 ± 0.28 μM) and miltefosine as a reference for *L. infantum* (IC_{50} = 7.47 ± 2.23 μM).

most potent analogue for *T. cruzi* and was more active and more selective than its matched 7-deazapurine nucleoside congener.[52] Because of its potent anti-*T. cruzi* activity and favorable selectivity profile, **44** was selected for further evaluation in an acute Chagas disease mouse model.

Metabolic Stability of Compound 44. The *in vitro* metabolic stability of **44** was evaluated using male mice and pooled human liver microsomes (S9 fraction) (Table 5). Compound **44** was not susceptible to Phase-I and Phase-II metabolism in both mouse and human microsomes with 100% of the parent drug remaining after 60 min. These results favored further evaluation of **44** in an *in vivo* laboratory rodent model.

***In Vivo* Evaluation of Compound 44.** To determine its efficacy *in vivo*, **44** was evaluated in an acute Chagas disease model using the Y strain of *T. cruzi* in Swiss male mice.[92,93] **44** was evaluated at 0.25, 2.5, or 25 mg/kg b.i.d or in combination with benznidazole (**44** at 2.5 mg/kg b.i.d + benznidazole at 10 mg/kg q.d.). Compounds were administered orally for 5 consecutive days, starting the administration at parasitemia onset on day 6 postinfection (dpi), which peaked at 8 dpi in untreated animals. **44** at 25 mg/kg gave 99% reduction in the parasitemia peak at 8 dpi, which was similar to the optimal q.d. dose of benznidazole at 100 mg/kg. Lower dosages (0.25 and 2.5 mg/kg) only gave partial reduction of parasitemia (43%). Coadministration of **44** (2.5 mg/kg) and benznidazole (10 mg/kg) reached 71% reduction, which was slightly better than benznidazole alone. However, no mice sustained negative parasitemia, hence failing parasitological cure. In the 2.5 mg/mk and 25 mg/kg treatment groups, 5 out of 6 mice (83%) survived until the end of the experiment (34 dpi), similar to benznidazole at 10 mg/kg. In the benznidazole 100 mg/kg group, all mice survived. In the untreated control group, all mice succumbed to the infection by day 27. In the 0.25 mg/kg, only one mouse survived at 34 dpi (Figure 2).

As the *in vitro T. cruzi* screening was performed with the Tulahuen strain (DTU IV) and *in vivo* assays with the Y strain (DTU II), additional *in vitro* screens were conducted with the latter. The findings confirmed the high potency of **44** against intracellular forms (IC_{50} = 0.26 ± 0.03 μM, SI > 1900) present in cardiomyocytes (Table 6), similar to the values obtained with the Tulahuen strain intracellular amastigotes. In addition,

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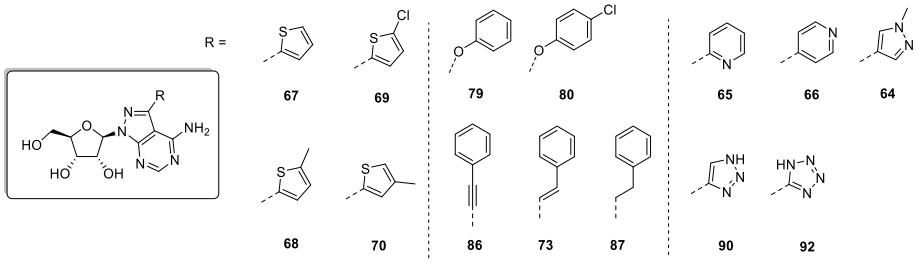
Table 3. Evaluation of Drug Sensitivity of 7-Modified Pyrazolo[3,4-*d*]pyrimidine Nucleoside Analogues against *T. cruzi* and *L. infantum*[a]



cpd.	*T. cruzi* IC_{50} (μM)	MRC-5 CC_{50} (μM)	SI	*L. infantum.* IC_{50} (μM)	PMM CC_{50} (μM)	SI
Thiophenes						
67	*10.4*	*32*	*3*	*>64.0*	*>64.0*	
69	2.56 ± 1.12	47.3 ± 6.12	*14*	>64.0	>64.0	
68	7.10 ± 4.2	>64.0	>9	[32.0,>64.0]	>64.0	>1
70	*32*	*>64.0*	*>2*	*>64.0*	*>64.0*	
Elongated phenyls						
79	*>64.0*	>64.0		*32*	*>64.0*	*>2*
80	*>64.0*	>64.0		*>64.0*	>64.0	
86	29.4 ± 11.1	>64.0	>2	49.4 ± 8.7	>64.0	>1
73	*16.5*	*>64.0*	*>4*	*50.8*	*>64.0*	*>1*
87	*52.3*	*>64.0*	*>1*	*>64.0*	>64.0	
Nitrogen-containing heterocycles						
65	30.1 ± 17.8	36.4 ± 4.1	1	[50.8, >64.0, >64.0]	>64.0	1
66	*21.5*	*>64.0*	*>3*	*25.4*	*>64.0*	*>3*
64	*45.3*	*>64.0*	*>1*	*>64.0*	>64.0	
90	*0.43*	*0.6*	*1*	*43.1*	*>64.0*	*>1*
92	*>64.0*	>64.0		*>64.0*	>64.0	

[a]Cytotoxicity was assayed against human MRC-5_{SV2} cells and primary mouse macrophages (PMMs). Values represent mean ± SEM, which originate from 2−3 independent experiments and are expressed in μM. Values in parentheses represent the values of the different determinations, as no correct average can be calculated. Values in italics represent the result of a single determination because of inactivity or overt cytotoxicity. SI: *in vitro* selectivity index is the ratio of CC_{50} for the host cell (MRC-5_{SV2} for *T. cruzi*, PMM for *L. inf.*) and IC_{50} of the parasite. Benznidazole was included as a reference for *T. cruzi* (IC_{50} = 2.02 ± 0.28 μM) and miltefosine as a reference for *L. infantum* (IC_{50} = 7.47 ± 2.23 μM).

44 did not exert cardiotoxicity in two-dimensional (2D) and three-dimensional (3D) cardiac cell cultures, giving CC_{50} values up to 500 and 200 μM, respectively. After discarding the potential impact of parasite strain on the *in vitro* and *in vivo* outcomes, we next evaluated the activity of **44** (as well as **4**) against the nondividing and highly infective bloodstream trypomastigote form. Both compounds proved to be inactive (IC_{50} > 81 μM), while benznidazole gave an IC_{50} of 5.7 ± 0.6 μM. These results corroborate former studies using pyrrolo-[2,3-*b*]pyridine (1,7-dideazapurine) nucleoside analogues that failed to achieve parasitological cure in treated mice despite parasitemia suppression and high animal survival rates.[78] The inability to kill bloodstream trypomastigotes may explain the parasitemia recrudescence and thus lack of sterile cure, as has also been reported for other nucleoside analogues.[53,78] The lack or low activity against bloodstream trypomastigotes resembles that of the azole ergosterol biosynthesis inhibitors that failed in clinical trials for Chagas disease,[87,88] raising the potential relevance of targeting both the intracellular multiplicative amastigotes and the nonreplicative trypomastigote forms. Recent findings also highlighted the role of metabolic heterogeneity in drug efficacy upon recalcitrant *T. cruzi* infection.[89] The authors reported that limiting exogenous glutamine impairs ergosterol biosynthesis inhibitors (azoles) to act upon intracellular amastigotes. In addition to the occurrence of nonreplicative forms (like dormant amastigotes and trypomastigotes), the impact of metabolic and environmental heterogeneity must be considered in the search for novel anti-*T. cruzi* agents as these factors can modulate drug efficacy.

Effect of the *T. cruzi* Host Cell on Drug Sensitivity to Compound 44. To investigate whether host cell permeability could be a limiting factor for the *in vivo T. cruzi* efficacy or lack of antileishmanial activity of compound **44**, we evaluated the effect of **44** against *T. cruzi* in PMM host cells (Table 7). Surprisingly, **44** was completely inactive against *T. cruzi* in PMM cells, while benznidazole retained its activity in both cell types. To rule out drug efflux as the cause of this effect, the experiment was repeated in the presence of the ABC transporter inhibitors verapamil, cyclosporine A, and probenecid. In all cases, **44** was inactive, indicating that its inactivity in PMM cells is likely due to permeability issues. These findings might offer further explanation as to why **44** was not able to fully clear *T. cruzi* infection *in vivo*. Tissue tropism in Chagas disease has been demonstrated to play an important role in persistence,[90−92] and limited permeability in certain tissues has also been implicated in the ineffectivity of Posaconazole in curing *T. cruzi* infections.[93] These results could also offer an explanation as to why several of the herein-reported nucleoside analogues, as well as others that were previously found to display potent anti-*T. cruzi* activity, are inactive when evaluated against *L. infantum* intracellular amastigotes in PMM host cells.[52,94] The origins of this lack of permeability in PMM cells are currently unclear and require further study. Furthermore, it

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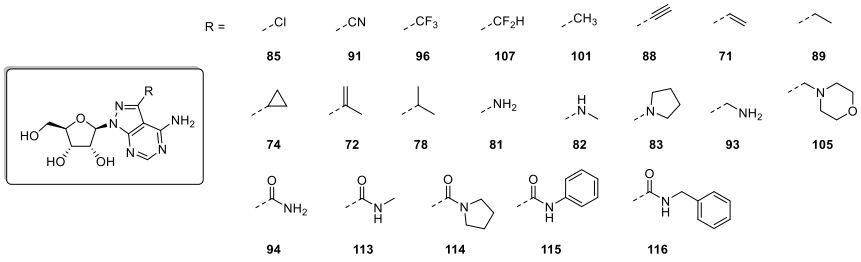
Table 4. Evaluation of Drug Sensitivity of 7-Modified Nucleoside Analogues against *T. cruzi* and *L. infantum*[a]



cpd.	*T. cruzi* IC_{50} (μM)	MRC-5 CC_{50} (μM)	SI	*L. infantum.* IC_{50} (μM)	PMM CC_{50} (μM)	SI
85	2.89 ± 0.23	>64.0	>22	>64.0	>64.0	>1
91	*0.95*	*34.6*	*36*	*8*	*>64.0*	*>8*
96	*27*	*13.3*	*<1*	*>64.0*	>64.0	
107	*8.3*	*2.42*	*<1*	*0.25*	*0.25*	*1*
101	4.68 ± 1.33	57.5 ± 6.5	12	32.7 ± 15.5	40.0 ± 12	1
88	2.78 ± 0.04	[46.5, >64.0]	20	10.4 ± 2.3	[8.00, >64.0]	1
71	*>64.0*	>64.0		*12.7*	*32*	*3*
89	*>64.0*	>64.0		*>64.0*	>64.0	
74	*>64.0*	>64.0		*>64.0*	>64.0	
72	*29.5*	*0.35*	*<1*	*24.1*	*32*	*1*
78	*>64.0*	*10.6*	*<1*	*>64.0*	>64.0	
81	*7.64*	*23*	*3*	*6.82*	*8*	*1*
82	*>64.0*	>64.0		*50.8*	*>64.0*	*>1*
83	*>64.0*	>64.0		*>64.0*	>64.0	
93	*11.1*	*59.3*	*5*	*2*	*8*	*4*
105	>64.0	>64.0		>64.0	>64.0	
94	*0.25*	*0.25*	*1*	*8.11*	*8*	*1*
113	*4*	*>64.0*	*16*	*>64.0*	>64.0	
114	*>64.0*	>64.0		*>64.0*	>64.0	
115	*>64.0*	>64.0		*32.5*	*32*	*1*
116	*>64.0*	>64.0		*>64.0*	>64.0	

[a]Cytotoxicity was assayed against human MRC-5_{SV2} cells and primary mouse macrophages (PMMs). Values represent mean ± SEM, which originate from 2−3 independent experiments and are expressed in μM. Values in parentheses represent the values of the different determinations, as no correct average can be calculated. Values in italics represent the result of a single determination because of inactivity or overt cytotoxicity. SI: *in vitro* selectivity index is the ratio of CC_{50} for the host cell (MRC-5_{SV2} for *T. cruzi*, PMM for *L. inf.*) and IC_{50} of the parasite. Benznidazole was included as a reference for *T. cruzi* (IC_{50} = 2.02 ± 0.28 μM) and miltefosine as a reference for *L. infantum* (IC_{50} = 7.47 ± 2.23 μM).

Table 5. *In Vitro* Metabolic Stability of Compound 44 Using Male Mouse and Pooled Human S9 Microsomal Fractions[a]

		mouse % 44 remaining		human % 44 remaining	
phase-I/II	time	mean	STDEV	mean	STDEV
CYP450-NADPH	0	100		100	
	15	103	11.4	102	10.3
	30	103	12.8	101	7.8
	60	108	16.1	101	8.5
UGT enzymes	0	100		100	
	15	103	3.2	100	2.8
	30	115	0.3	106	9.5
	60	115	3.0	104	7.2

[a]The depicted values are the percentage of remaining parent compound at the various time points of incubation (0−15−30−60 min). Data originate from two independent experiments of two biological replicates. Diclofenac (susceptible to Phase-I and Phase-II metabolism) was included as reference to ensure proper assay performance (data not shown).

would be worthwhile to evaluate if certain nucleoside prodrugs could lead to improved permeability and/or improved *in vivo* efficacy.

CONCLUSIONS

We described the design and synthesis of a library of pyrazolo[3,4-*d*]pyrimidine nucleosides that were evaluated for *in vitro* activity against *T. cruzi* and *L. infantum* intracellular amastigotes. SAR trends were highly different from earlier reported nucleoside series. Modifications of the 3′-position of the parent adenosine- and inosine-like analogues aminopurinol riboside **4** and allopurinol riboside **2** were detrimental to activity and led to inactive compounds. The introduction of a halogen atom on the 7-position led to a significant decrease in activity. An extensive screen of substituents on the 7-position **4** revealed varying effects on antiparasitic activity and selectivity toward MRC-5_{SV2} and PMM cells. A 4-chlorophenyl substituent on the 7-position (**44**) afforded good anti-*T. cruzi* activity and selectivity and was also metabolically stable in human and mouse liver microsomes. **44** was next evaluated in an acute Chagas disease model, resulting in a rapid, almost complete reduction in parasitemia. Treatment with **44** led to the survival of 5 out of 6 mice but failed to induce sterile parasitological cure. None of the new analogues showed good *in vitro* activity against *L. infantum*, which could potentially be attributed to limited permeability in the PMM host cell.

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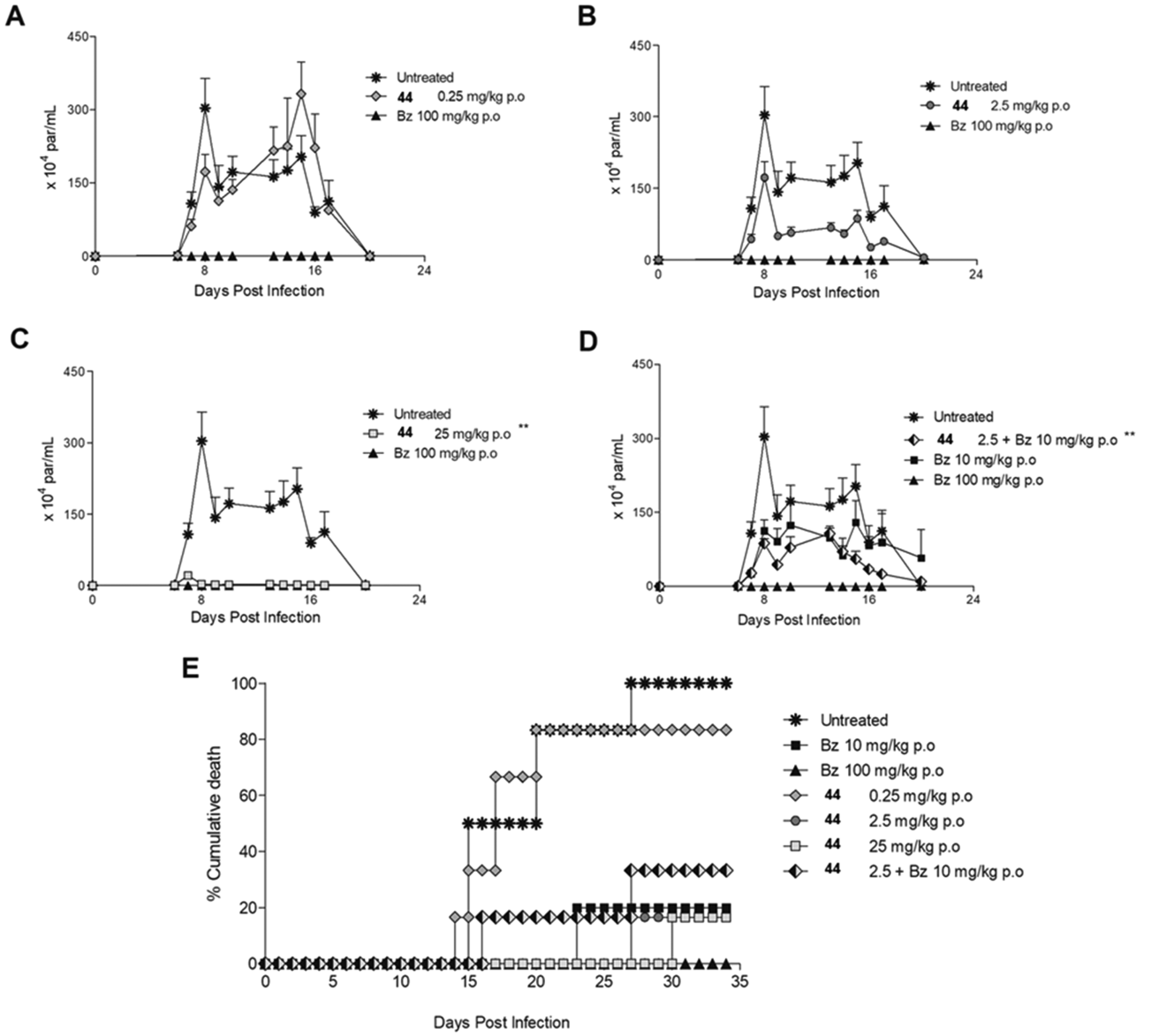

Figure 2. *In vivo* efficacy of **44** administered orally for 5 days in Swiss mice infected with the Y strain of *T. cruzi*. Parasitemia curve for **44** at (A) 0.25 mg/kg/b.i.d, (B) 2.5 mg/kg/b.i.d, (C) 25 mg/kg/b.i.d, and (D) coadministration of **44** at 2.5 mg/kg + benznidazole (Bz) at 10 mg/kg/b.i.d. Mortality rates were up to 35 dpi (E). ** p value ≤ 0.05.

Table 6. *In Vitro* Efficacy of 4 and 44 against *T. cruzi* Y-Strain Bloodstream Trypomastigotes and Intracellular Amastigotes in Cardiac Cells[a]

cpd.	*T. cruzi* Y bloodstream trypomastigotes IC_{50} (μM)	*T. cruzi* Y intracellular amastigotes IC_{50} (μM)	primary cardiac cells LC_{50} (μM)
4	>81	2.46 ± 0.3	>500
44	>81	0.26 ± 0.03	500

[a]IC_{50} values are depicted as means ± SEM of two independent determinations, using duplicates.

EXPERIMENTAL SECTION

Chemistry. *General.* All reagents and solvents were obtained from standard commercial sources and were of analytical grade. Unless otherwise specified, they were used as received. All moisture-sensitive reactions were carried out under an argon atmosphere. Reactions were carried out at ambient temperature, unless otherwise indicated. Reactions were monitored *via* analytical TLC or analytical LCMS. Analytical TLC was performed on Machery−Nagel precoated F254 aluminum plates and visualized by UV followed by staining with basic aq. $KMnO_4$, cerium-molybdate, or sulfuric acid-anisaldehyde spray. Analytical LCMS was performed on a Waters AutoPurification system

Table 7. Evaluation of Drug Sensitivity of 44 and Benznidazole (Bz) against *T. cruzi* in PMM Host Cells, Alone or with Coadministration of Verapamil (8 μM), Probenecid (700 μM), or Cyclosporin A (2 μM)

cpd.	*T. cruzi* (MRC-5) IC_{50} (μM)	*T. cruzi* (PMM) IC_{50} (μM)	*T. cruzi* (PMM) + verapamil IC_{50} (μM)	*T. cruzi* (PMM) + probenecid IC_{50} (μM)	*T. cruzi* (PMM) + cyclosporin A IC_{50} (μM)
44	0.32 ± 0.02	>64.0	>64.0	>64.0	>64.0
Bz	2.02 ± 0.28	1.96 ± 0.25			

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(equipped with ACQUITY QDa (mass; 100−1000 amu)) and 2998 Photodiode Array (220−400 nm) using a Waters Cortecs C18 (2.7 μm, 100 mm × 4.6mm) column and a gradient system of HCOOH in H_2O (0.2%, v/v)/MeCN at a flow rate of 1.44 mL/min (95:05−00:100 in 6.5 min or 50:50−00:100 in 6.5 min), or a Waters Alliance XE separation module using a Phenomenex Kinetix C8 (2.6 μm, 50 mm × 2.10 mm) column and the same gradient system. Preparative HPLC was performed on the same system, using a Phenomenex Luna Omega Polar column (250 mm × 21 mm, 5 μm) and a gradient system of 0.2% formic acid in water/MeCN at a flow rate of 20 mL/min (gradients are specified in the individual procedures). Column chromatography was performed manually using Machery−Nagel 60 M silica gel (40−63 μm) or on a Reveleris X2 (Grace/Büchi) automated flash unit employing prepacked silica columns. Exact mass measurements were performed on a Waters LCT Premier XE time-of-flight (ToF) mass spectrometer equipped with a standard electrospray (ESI) and a modular Lockspray interface. Samples were infused in a MeCN/water (1:1) + 0.1% formic acid mixture at 100 μL/min. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer or a Bruker Avance Neo 400 MHz spectrometer. Chemical shifts (δ) are given in ppm, and spectra are referenced to the residual solvent peak. Coupling constants are given in Hz. Systematic numbering is employed for NMR assignments in the individual procedures. Melting points were determined on a Büchi-545 apparatus and are uncorrected. Purity was assessed by means of liquid chromatography−mass spectrometry (LCMS). All obtained final compounds had purity >95%, as assayed by analytical HPLC (UV), unless otherwise indicated.

General Procedure A: Large-Scale $BF_3{\cdot}OEt_2$-Mediated Glycosylation with Commercially Available 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose. The respective pyrazolo[3,4-*d*]pyrimidine (1.0 equiv) and 1-*O*-acetyl-tri-*O*-benzoyl-β-D-ribofuranose (1.5 equiv) were added to dry nitromethane (2.5 mL/mmol). The mixture was heated to reflux, when $BF_3{\cdot}OEt_2$ (1.5 equiv) was added, upon which the solids started to dissolve. After 90 min of heating at reflux, the solvent was removed *in vacuo*. The resulting oil was dissolved in CH_2Cl_2 (sonicate until fully dissolved) and directly poured on a silica column (preconditioned with CH_2Cl_2). The column was eluted with 100% CH_2Cl_2 until all excess 1-*O*-acetyl-tri-*O*-benzoyl-β-D-ribofuranose had eluted and then with 5% acetone in CH_2Cl_2 to collect the product.

General Procedure B: Small-Scale $BF_3{\cdot}OEt_2$-Mediated Glycosylation with Protected Ribose Derivative. The respective pyrazolo[3,4-*d*]pyrimidine (1.1 equiv) and ribose derivative (1.0 equiv) were added to dry nitromethane (2.5 mL/mmol). The mixture was heated to reflux, when $BF_3{\cdot}OEt_2$ (1.0 equiv) was added, upon which the solids started to dissolve. After 90 min of heating at reflux, the solvent was removed *in vacuo*. The resulting oil was dissolved in CH_2Cl_2, celite (1.5 g/g starting material) was added, and the solvent was removed *in vacuo*. The resulting solid was purified by flash column chromatography to afford the protected nucleoside.

***General Procedure C**: Deprotection with NaOMe.* Protected nucleoside (1.0 equiv) was dissolved in CH_2Cl_2 (0.5 mL/mmol). MeOH (5 mL/mmol) was added, followed by NaOMe in MeOH (5.4 M, 0.2 mL/mmol). The mixture was stirred at room temperature or 60 °C (for **36**) until TLC analysis (20% MeOH in CH_2Cl_2) indicated completion of the reaction. The reaction was neutralized to pH 7 *via* the addition of 4 N HCl, celite (1.5 g/g starting material) was added, and the solvents were removed *in vacuo*. The solid residue was brought onto a silica column and eluted with a mixture of MeOH and CH_2Cl_2 to isolate the final product.

***General Procedure D**: Catalytic Hydrogenation in Buffered MeOH.* The nucleoside analogue was dissolved in a mixture of MeOH (8 mL/mmol) and aq. 1M NaOAc (2 mL/mmol). The flask was placed under a nitrogen atmosphere, and a catalytic amount of Pd/C was added. The atmosphere was exchanged for H_2 and the mixture was stirred until TLC analysis (20% MeOH in CH_2Cl_2) indicated completion of the reaction. The mixture was then filtered over celite, celite was added to the filtrate, and the solvents were removed *in vacuo*. The resulting solid was purified *via* flash column chromatography to afford the final product.

General Procedure E: Suzuki Reaction. Compound **21** (1 equiv) or **23** (in the case of **76**, 1 equiv), boronic acid (1.5 equiv) or trifluoroborate salt (1.5 equiv) Na_2CO_3 (when a boronic acid was used, 3 equiv) or Cs_2CO_3 (when a trifluoroborate salt was used, 3 equiv), $Pd(OAc)_2$ (0.05 equiv), and TPPTS (0.12 equiv) were added to a 10 mL round-bottom flask, equipped with a stir bar. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, MeCN (2 mL/mmol SM) and H_2O (4 mL/mmol SM) were added to the solids under argon. After 5 min of stirring, the mixture was heated to reflux. When the starting material was fully consumed (usually 1−3 h; as monitored by LCMS analysis), the mixture was cooled to ambient temperature and neutralized (pH ∼ 7) with 4 M aq. HCl. Celite (5 g/mmol) was added, and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography.

General Procedure F: Amide Coupling. The nucleoside carboxylic acid (1.0 equiv) was dissolved in DMF (10 mL/mmol). DIPEA (3.0 equiv) was added, followed by HCTU (2.5 equiv). After 5 min, the respective amine (5.0 equiv) was added and the reaction mixture was stirred overnight. When TLC or LCMS analysis indicated full conversion, the reaction mixture was diluted with excess EtOAc and transferred to a separation funnel. The organic phase was washed with 1 N HCl, aq. sat. $NaHCO_3$, and brine sequentially. The organic phase was then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was used directly in the next reaction, without purification.

*3-Bromo-4-amino-1H-pyrazolo[3,4-d]pyrimidine (**12**).* 4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidine **3** (8.26 g, 61.1 mmol, 1.0 equiv) was dissolved in DMF (60 mL). NBS (11.4 g, 64.2 mmol, 1.05 equiv) was added, and the mixture was heated at 60 °C overnight. The mixture was cooled to room temperature and poured into ice-cold water (350 mL). The resulting suspension was stirred for 10 min at 0 °C and filtered overnight. The solids were collected and dried under a high vacuum overnight to afford **12** (11.9 g, 55.7 mmol, 91% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.91 (1H, br. s, NH_2'), 7.72 (1H, br. s, NH_2''), 8.16 (1H, s, C-6), 13.75 (1H, br. s, NH) ppm. HRMS (ESI): calcd for $C_5H_5BrN_5$ ($[M + H]^+$): 213.9728, found: 213.9731.

*3-Iodo-4-amino-1H-pyrazolo[3,4-d]pyrimidine (**13**).* 4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidine **3** (5.68 g, 42.1 mmol, 1.0 equiv) was dissolved in DMF (40 mL). NIS (10.4 g, 46.3 mmol, 1.1 equiv) was added, and the mixture was heated at 80 °C overnight. The mixture was cooled to room temperature and poured into ice-cold water (350 mL). The resulting suspension was stirred for 10 min at 0 °C and filtered overnight. The solids were collected and dried under a high vacuum overnight to afford **13** (10.2 g, 39.1 mmol, 93% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (1H, s, C-6), 13.80 (1H, br. s, NH) ppm. HRMS (ESI): calcd for $C_5H_5IN_5$ ($[M + H]^+$): 261.9590, found: 261.9594.

*3-Bromo-allopurinol (**14**).* Allopurinol (4.55 g, 33.4 mmol, 1.0 equiv) was suspended in water (300 mL). Bromine (4.29 mL, 83.6 mmol, 2.5 equiv) was added carefully, and a reflux cooler with a septum was placed on top of the flask. The reflux cooler was connected *via* vacuum tubing to a large flask containing excess aq. 2 M $Na_2S_2O_3$ solution. The reaction mixture was heated at 90 °C overnight and cooled down to room temperature. A mixture of aq. sat. $NaHCO_3$ (75 mL) and aq. 2 M $Na_2S_2O_3$ (75 mL) was added through the reflux cooler, after which the reaction mixture turned white. The suspension was cooled down further to 0 °C, stirred for 10 min, and filtered. The solids were washed with ice-cold water (3×), collected, and dried over a high vacuum overnight to afford **14** (6.26 g, 29.1 mmol, 87%) as a light-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (1H, d, J = 3.8 Hz), 12.22 (1H, br. s.), 13.98 (1H, br. s) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 104.5 (C-3a), 121.9 (C-3), 149.4 (C-7a), 154.6 (C-6), 157.0 (C-4) ppm. HRMS (ESI): calcd for $C_5H_4BrN_4O$ ($[M + H]^+$): 214.9568, found: 214.9572.

3-Bromo-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine[69] *(**18**).* Compound **12** (10.3 g, 48.1 mmol, 1.0 equiv) and 1-*O*-acetyl-tri-*O*-benzoyl-β-D-ribofuranose

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(36.4 g, 72.2 mmol, 1.5 equiv) were subjected to general procedure A to afford **18** (20.3 g, 30.8 mmol, 64% yield) as a brown oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.51−4.67 (2 H, m, H-5′, H-5″), 4.82−4.89 (1H, m, H-4′), 6.10 (1H, t, J = 5.7 Hz, H-3′), 6.25 (1H, dd, J = 5.4, 3.4 Hz, H-2′), 6.67 (1H, d, J = 3.2 Hz, H-1′), 7.38−7.70 (9H, m, H_{Phe}), 7.84−8.05 (6H, m, H_{Phe}), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 63.6 (C-5′), 71.3 (C-3′), 74.2 (C-2′), 79.5 (C-4′), 86.4 (C-1′), 100.3 (C-3a), 121.2 (C-3) 128.8 (C_{Phe}), 129.0 (C_{Phe}), 129.2 (C_{Phe}), 129.2 (C_{Phe}), 129.7 (C_{Phe}), 129.8 (C_{Phe}), 129.8 (C_{Phe}), 129.8 (C_{Phe}), 133.9 (C_{Phe}), 134.3 (C_{Phe}), 134.4(C_{Phe}), 155.5 (C-7a), 157.7 (C-6), 157.8 (C-4), 165.0 (C═O), 165.1 (C═O), 165.9 (C═O) ppm. HRMS (ESI): calcd for $C_{31}H_{25}BrN_5O_7$ ([M + H]$^+$): 658.0937, found: 658.0925.

3-Bromo-4-amino-1-(2′,5′-di-O-benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**19**). Compound **12** (0.330 g, 1.54 mmol, 1.1 equiv) and compound **16** (0.563 g, 1.40 mmol) were subjected to general procedure B. Purification by flash column chromatography (automated, 0 → 5% MeOH in CH_2Cl_2) afforded semipure **19**, which was used as such in the next reaction. HRMS (ESI): calcd for $C_{24}H_{20}BrFN_5O_5$ ([M + H]$^+$): 556.0632, found: 556.0590.

3-Bromo-4-amino-1-(2′,5′-di-O-benzoyl-3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**20**). Compound **12** (0.395 g, 1.84 mmol, 1.1 equiv) and compound **17** (0.643 g, 1.67 mmol, 1.0 equiv) were subjected to general procedure B. Purification by flash column chromatography (automated, 0 → 5% MeOH in CH_2Cl_2) afforded semipure **20**, which was used as such in the next reaction. HRMS (ESI): calcd for $C_{24}H_{21}BrN_5O_5$ ([M + H]$^+$): 538.726, found: 538.0706.

3-Bromo-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**21**). Compound **18** (12.7 g, 19.4 mmol) was subjected to general procedure C (reaction time: 2 h). Purification was performed *via* flash column chromatography (manual, first 5% MeOH in CH_2Cl_2 to remove higher-running impurities, and then 15% MeOH in CH_2Cl_2) to isolate **21** (4.68 g, 13.5 mmol, 70% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.38−3.47 (1H, m, H-5′), 3.50−3.60 (1H, m, H-5″), 3.89 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.16 (1H, dd, J = 9.4, 4.7 Hz, H-3′), 4.55 (1H, dd, J = 10.8, 5.6 Hz, H-2′), 4.81 (1H, t, J = 5.9 Hz, OH), 5.16 (1H, d, J = 5.3 Hz, OH), 5.40 (1H, d, J = 5.9 Hz, OH), 6.06 (1H, d, J = 5.0 Hz, H-1′), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.6 (C-3′), 72.9 (C-2′), 85.3 (C-4′), 88.1 (C-1′), 99.7 (C-3a), 119.3 (C-3), 155.0 (C-7a), 157.0 (C-6), 157.4 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}BrN_5O_4$ ([M + H]$^+$): 330.0202, found: 330.0198.

3-Bromo-4-amino-1-(3′-deoxy-3′-fluoro-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**22**). Compound **19** (used directly from the previous reaction) was subjected to general procedure C (reaction time: 90 min). Purification *via* flash column chromatography (automated, 0 →10% MeOH in CH_2Cl_2) afforded **22** (97 mg, 0.279 mmol, 20% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47−3.54 (2H, m, H-5′, H-5″), 4.11−4.27 (1H, m, H-4′), 4.84−5.18 (3H, m, H-3′, H-2′, OH), 5.87 (1H, d, J = 6.7 Hz, OH), 6.06 (1H, d, J = 7.3 Hz, H-1′) ppm, 6.99 (1H, br. s, OH), 8.09 (1H, br. s, OH), 8.24 (1H, s, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ 61.4 (d, J = 10.4 Hz, C-5′), 71.6 (d, J = 16.1 Hz, C-2′), 83.8 (d, J = 20.7 Hz, C-4′), 87.5 (C-1′), 92.9 (d, J = 182.0 Hz, C-3′), 100.5 (C-3a), 120.2 (C-3), 155.9 (C-7a), 157.6 (C-6), 157.9 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −198.19 (1F, dt, J = 54.1, 25.2 Hz) ppm. HRMS (ESI): calcd for $C_{10}H_{12}BrFN_5O_3$ ([M + H]$^+$): 348.0108, found: 348.0090.

3-Bromo-4-amino-1-(3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**23**). Compound **20** (used directly from the previous reaction) was subjected to general procedure C (reaction time: 2 h). Flash column chromatography (automated, 2 → 20% MeOH in CH_2Cl_2) afforded **23** (0.106 g, 0.321 mmol, 19% yield over 2 steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.98 (1H, ddd, J = 12.7, 6.3, 2.1 Hz, H-3′), 2.25 (1H, ddd, J = 12.8, 9.0, 5.7 Hz, H-3″), 3.38−3.50 (2H, m, H-5′, H-5″), 4.31 (1H, ddd, J = 11.6, 9.3, 5.8 Hz, H-4′), 4.55 (1H, dt, J = 5.4, 1.8 Hz, H-2′), 6.11 (1H, d, J = 1.8 Hz, H-1′), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.3 (C-3′), 64.4 (C-5′), 74.6, (C-2′) 81.6 (C-4′), 91.0 (C-1′), 99.8 (C-3a), 119.6 (C-3), 155.0 (C-7a), 157.5 (C-6), 157.7 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}BrN_5O_3$ ([M + H]$^+$): 330.0202, found: 330.0195.

4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine[70] (**4**). Compound **21** (120 mg, 0.347 mmol) was subjected to general procedure D (reaction time: 2 h). Flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) afforded **4** (57 mg, 0.213 mmol, 61% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.38−3.64 (2H, m, H-5′, H-5″), 3.87−3.96 (1H, m, H-4′), 4.22 (1H, dd, J = 9.7, 4.4 Hz, H-3′), 4.60 (1H, dd, J = 10.0, 5.0 Hz, H-2′), 4.88 (1H, t, J = 5.9 Hz, OH), 5.14 (1H, d, J = 4.7 Hz, OH), 5.36 (1H, d, J = 6.4 Hz, OH), 6.09 (1H, d, J = 4.7 Hz, H-1′), 8.18 (1H, s, H-3), 8.20 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.5 (C-5′), 71.0 (C-2′), 73.2 (C-3′), 85.1 (C-4′), 88.6 (C-1′), 100.5 (C-3a), 133.5 (C-3), 154.1 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{14}N_5O_4$ ([M + H]$^+$): 268.1046, found: 268.1032. Spectral data are in accordance with literature values.[70]

4-Amino-1-(3′-deoxy-3′-fluoro-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**24**). Compound **22** (0.058 g, 0.167 mmol) was subjected to general procedure D (reaction time: 15 min). Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **24** (37 mg, 0.137 mmol, 82% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47−3.65 (2H, m, H-5′, H-5″), 4.21 (1H, dt, J = 24.9, 4.7 Hz, H-4′), 4.93−5.31 (3H, m, H-3′, H-2′, OH), 5.83 (1H, d, J = 6.4 Hz, OH), 6.10 (1H, d, J = 7.0 Hz, H-1′), 7.50−8.07 (2H, m, NH_2), 8.20 (2H, s, H-6, H-3) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 61.2 (d, J = 10.4 Hz, C-5′), 71.3 (d, J = 17.3 Hz, C-2′), 83.2 (d, J = 21.9 Hz, C-4′), 87.7 (C-1′), 92.7 (d, J = 182.0 Hz, C-3′), 100.8 (C-3a), 133.6 (C-3), 154.3 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −198.52 (1F, dt, J = 54.1, 25.2 Hz) ppm. HRMS (ESI): calcd for $C_{10}H_{13}FN_5O_3$ ([M + H]$^+$): 270.1002, found: 270.0998.

4-Amino-1-(3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (**25**). Compound **23** (0.050 g, 0.151 mmol) was subjected to general procedure D (reaction time: 3 h). Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **25** (26 mg, 0.100 mmol, 66% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.98 (1H, ddd, J = 12.7, 6.2, 2.2 Hz, H-3′), 2.33 (1H, ddd, J = 12.7, 9.1, 5.7 Hz, H-3″), 3.36−3.53 (2H, m, H-5′, H-5″), 4.32 (1H, ddd, J = 11.4, 9.1, 5.9 Hz, H-4′), 4.51−4.61 (1H, m, H-2′), 4.74 (1H, t, J = 5.7 Hz, OH), 5.53 (1H, d, J = 3.8 Hz, OH), 6.14 (1H, d, J = 1.5 Hz, H-1′), 7.50−7.98 (2H, br. m, NH_2), 8.15 (1H, s, H-3), 8.19 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.1 (C-3′), 64.2 (C-5′), 74.5 (C-2′), 81.0 (C-4′), 90.7 (C-1′), 100.1 (C-3a), 133.2 (C-3a), 153.7 (C-7a), 156.1 (C-6), 158.0 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{14}N_5O_3$ ([M + H]$^+$): 252.1079, found: 252.1075.

3-Bromo-4-oxo-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine[69] (**26**). Compound **14** (6.45 g, 30.0 mmol, 1.0 equiv) and 1-*O*-acetyl-tri-*O*-benzoyl-β-D-ribofuranose (22.7 g, 45.0 mmol, 1.5 equiv) were subjected to general procedure A to afford, after recrystallization from MeOH, **26** (6.26 g, 9.49 mmol, 32% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (1H, dd, J = 12.2, 4.6 Hz, H-5′), 4.80 (1H, dd, J = 12.3, 3.8 Hz, H-5″), 4.84−4.89 (1H, m, H-4′), 6.26 (1H, dd, J = 6.1, 5.3 Hz, H-3′), 6.34 (1H, dd, J = 5.3, 3.0 Hz, H-2′), 6.72 (1H, d, J = 2.9 Hz, H-1), 7.34−7.62 (9H, m, H_{Phe}), 7.94−8.15 (7H, m, H_{Phe}, H-6), 11.91 (1H, br. s., NH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.5 (C-5′), 71.6 (C-2′), 74.6 (C-3′), 80.4 (C-4′), 87.2 (C-1′), 106.0 (C-3a), 124.5 (C-3), 128.5 (C_{Phe}), 128.5 (C_{Phe}), 128.5 (C_{Phe}), 128.6 (C_{Phe}), 128.7 (C_{Phe}), 129.5 (C_{Phe}), 129.8 (C_{Phe}), 129.8 (C_{Phe}), 129.9 (C_{Phe}), 133.2 (C_{Phe}), 133.6 (C_{Phe}), 133.7 (C_{Phe}), 148.1 (H-7a), 153.9 (H-6), 158.5 (H-4), 165.1 (C═O), 165.2 (C═O), 166.3 (C═O) ppm. HRMS (ESI): calcd for $C_{31}H_{24}BrN_4O_8$ ([M + H]$^+$): 659.0778, found: 659.0774.

3-Bromo-4-oxo-1-(2′,5′-tri-O-benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**27**). Compounds **14** (0.338 g, 1.57 mmol, 1.1 equiv) and **16** (0.575 g, 1.43 mmol, 1.0 equiv) were subjected to general procedure B. TLC analysis (5% MeOH in CH_2Cl_2) indicated the presence of a major apolar spot and two smaller more polar spots. The major apolar spot was presumed to

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be the desired *N*-1 regioisomer[72] **27** and was isolated *via* flash chromatography (automated, 0 → 5% MeOH in CH_2Cl_2) and used as such in the next reaction. HRMS (ESI): calcd for $C_{24}H_{19}BrFN_4O_6$ ($[M + H]^+$): 557.0472, found: 557.0457.

3-Bromo-4-oxo-1-(2′,5′-tri-O-benzoyl-3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**28**). Compounds **14** (0.348 g, 1.62 mmol, 1.1 equiv) and **17** (0.565 g, 1.47 mmol, 1.0 equiv) were subjected to general procedure B. TLC analysis (5% MeOH in CH_2Cl_2) indicated the presence of a major apolar spot and two smaller more polar spots. The major apolar spot was presumed to be the desired N-1 regioisomer[72] **28** and was isolated *via* flash chromatography (automated, 0 → 5% MeOH in CH_2Cl_2) and used as such in the next reaction. HRMS (ESI): calcd for $C_{24}H_{20}BrN_4O_6$ ($[M + H]^+$): 539.0566, found: 539.0552.

3-Bromo-4-oxo-1-β-D-ribofuranosyl-pyrazolo[3,4-d]pyrimidine[69] (**29**). Compound **26** (0.530 g, 0.804 mmol) was subjected to general procedure C (reaction time: 1 h). Purification *via* flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) afforded **29** (105 mg, 0.302 mmol, 38% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.36−3.47 (1H, m, H-5′), 3.48−3.59 (1H, m, H-5″), 3.88 (1H, dd, J = 10.3, 4.7 Hz, H-4′), 4.10−4.18 (1H, m, H-3′), 4.44−4.53 (1H, m, H-2′), 4.66−4.80 (1H, m, OH), 5.16 (1H, br. s., OH), 5.42 (1H, br. s., OH), 6.00 (1H, d, J = 5.0 Hz, H-1′), 8.15 (1H, s, H-6), 12.41 (1H, br. s, NH) ppm. HRMS (ESI): calcd for $C_{10}H_{12}BrN_4O_5$ ($[M + H]^+$): 346.9991, found: 347.0004. Spectral data are in accordance with literature values.[69]

3-Bromo-4-oxo-1-(3′-deoxy-3′-fluoro-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**30**). Compound **27** (used directly from the previous step) was subjected to general procedure C (reaction time: 3 h). Flash column chromatography (automated, 2 → 12% MeOH in CH_2Cl_2), followed by an additional purification *via* preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 0:100 in 18 min) afforded **30** (56 mg, 0.160 mmol, 11% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.44−3.62 (2H, m, H-5′, H-5″), 4.21 (1H, dt, J = 25.8, 5.3 Hz, H-4′), 4.90 (1H, ddd, J = 24.6, 7.0, 4.4 Hz, H-2′), 5.08 (1H, dd, J = 54.2, 4.1 Hz, H-3′), 6.03 (1H, d, J = 7.0 Hz, H-1′), 8.18 (1H, s, H-6), 12.43 (1H, br. s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 60.9 (d, J = 10.4 Hz, C-5′), 71.5 (d, J = 16.1 Hz, C-2′), 83.6 (d, J = 20.7 Hz, C-4′), 87.0 (C-1′), 92.3 (d, J = 183.1 Hz, C-3′), 105.4 (C-3a), 122.8 (C-3), 150.3 (C-7a), 154.5 (C-6), 156.3 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −198.39 (1F, dt, J = 54.1, 25.2 Hz) ppm. HRMS (ESI): calcd for $C_{10}H_{11}BrFN_4O_4$ ($[M + H]^+$): 348.9948, found: 348.9996.

3-Bromo-4-oxo-1-(3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (**31**). Compound **28** (used directly from the previous step) was subjected to general procedure C (reaction time: 3 h). Flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification *via* preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 30:70 in 18 min) afforded **31** (31 mg, 0.093 mmol, 6% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 2.10 (1H, ddd, J = 13.1, 6.4, 1.9 Hz, H-3′), 2.48 (1H, ddd, J = 13.2, 9.4, 5.6 Hz, H-3″), 3.62 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.70 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 4.46−4.56 (1H, m, H-4′), 4.67 (1H, dt, J = 5.6, 1.6 Hz, H-2′), 6.24 (1H, d, J = 1.5 Hz, H-1′), 8.05 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, CD_3OD) δ 37.0 (C-3′), 66.1 (C-5′), 77.3 (C-2′), 83.6 (C-4′), 93.3 (C-1′), 107.0 (C-3a), 124.4 (C-3), 150.5 (C-7a), 155.5 (C-6), 159.1 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{12}BrN_4O_4$ ($[M + H]^+$): 331.0042, found: 331.0047.

4-Oxo-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (Allopurinol Riboside)[72] (**2**). Compound **29** (65 mg, 0.187 mmol) was submitted to general procedure D (reaction time: 1 h). Flash column chromatography (automated, 10 → 35% MeOH in CH_2Cl_2) afforded **2** (27 mg, 0.101 mmol, 54% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.43 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.57 (1H, dd, J = 11.7, 4.4 Hz, H-5″), 3.91 (1H, dd, J = 10.0, 5.0 Hz, H-4′), 4.21 (1H, t, J = 4.8 Hz, H-3′), 4.54 (1H, m, J = 4.5, 4.5 Hz, H-2′), 4.77 (1H, br. s., OH), 4.96−5.63 (2H, m, OH, OH), 6.07 (1H, d, J = 4.4 Hz, H-1′), 8.13 (1H, s, H-3), 8.17 (1H, s, H-6), 12.37 (1H, br. s, NH) ppm. HRMS (ESI): calcd for $C_{10}H_{13}N_4O_5$ ($[M + H]^+$): 269.0886, found: 269.0891. Spectral data are in accordance with literature values.[72]

4-Oxo-1-(3′-deoxy-3′-fluoro-β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (**32**). Compound **30** (0.031 g, 0.089 mmol) was subjected to general procedure D (reaction time: 30 min). Purification *via* flash column chromatography afforded **32** (20 mg, 0.074 mmol, 83% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47−3.57 (2H, m, H-5′, H-5″), 4.13−4.29 (1H, m, H-4′), 4.89−5.22 (2H, m, H-3′, H-2′), 6.09 (1H, d, J = 7.0 Hz, H-1′), 8.15 (1H, s, H-6), 8.22 (1H, s, H-4), 12.22 (1H, br. s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 61.0 (d, J = 10.4 Hz, C-5′), 71.6 (d, J = 16.1 Hz, C-2′), 83.4 (d, J = 20.7 Hz, C-4′), 87.1 (C-1′), 92.5 (d, J = 182.0 Hz, C-3′), 106.6 (C-3a), 135.8 (C-3), 148.9 (C-7a), 153.5 (C-6), 157.0 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −198.82 (1F, dt, J = 54.1, 24.8 Hz) ppm. HRMS (ESI): calcd for $C_{10}H_{12}FN_4O_4$ ($[M + H]^+$): 271.0843, found: 271.0834.

4-Oxo-1-(3′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**33**). Compound **31** (0.023 g, 0.069 mmol) was subjected to general procedure D (reaction time: 30 min). Purification *via* flash column chromatography afforded **33** (14 mg, 0.056 mmol, 80% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.91−2.05 (1H, m, H-3′), 2.23−2.37 (1H, m, H-3″), 3.33−3.56 (2H, m, H-5′, H-5″), 4.25−4.42 (1H, m, H-4′), 4.49−4.63 (1H, m, H-2′), 4.85 (1H, br. s, OH), 5.59 (1H, br. s, OH), 6.12 (1H, s, H-1′), 8.13 (1H, s, H-3), 8.14 (1H, s, H-6), 12.2 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.0 (C-3′), 64.1 (C-5′), 74.7 (C-2′), 81.4 (C-4′), 90.7 (C-1′), 105.9 (C-3a), 135.4 (C-3), 148.5 (C-7a), 152.4 (C-6), 157.1 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}N_4O_4$ ($[M + H]^+$): 253.0937, found: 253.0921.

3-Iodo-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine[95] (**34**). Compound **13** (10.1 g, 39.1 mmol, 1.0 equiv) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (29.6 g, 58.7 mmol, 1.5 equiv) were subjected to general procedure A to afford **34** (21.2 g, 30.0 mmol, 77% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56−4.90 (3H, m, H-5′, H-5″, H-4′), 6.20 (1H, t, J = 5.6 Hz, H-3′), 6.36 (1H, dd, J = 5.0, 3.2 Hz, H-2′), 6.76 (1H, d, J = 2.9 Hz, H-2′), 7.35−7.62 (9H, m, H_{Phe}), 7.91−8.14 (6H, m, H_{Phe}), 8.34 (1H, s, H-6) ppm. HRMS (ESI): calcd for $C_{31}H_{25}IN_5O_7$ ($[M + H]^+$): 706.0799, found: 706.0824. Spectral data are in accordance with literature values.[95]

4-Amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidine[70] (**35**). 4-Aminopyrazolo[3,4-*d*]pyrimidine **3** (3.16 g, 23.4 mmol, 1.0 equiv) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (17.7 g, 35.1 mmol, 1.5 equiv) were subjected to general procedure A to afford **35** (8.06 g, 13.9 mmol, 59% yield) as a colorless foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 4.54 (1H, dd, J = 12.3, 4.4 Hz, H-5′), 4.68 (1H, dd, J = 12.3, 3.5 Hz, H-5″), 4.89−4.95 (1H, m, H-4′), 6.17−6.33 (2H, m, H-2′, H-3′), 6.75 (1H, d, J = 2.6 Hz, H-1′), 7.40−7.56 (6H, m, H_{Phe}), 7.59−7.72 (3H, m, H_{Phe}), 7.87−8.03 (6H, m, H_{Phe}), 8.47 (1H, br. s, NH_2), 8.40 (1H, s, H-3), 8.43 (1H, s, H-6), 9.13 (1H, br. s., NH_2′) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 63.2 (C-5′), 71.0 (C-2′), 74.2 (C-3′), 79.1 (C-4′), 86.2 (C-1′), 100.3 (-3a), 128.4 (C_{Phe}), 128.5 (C_{Phe}), 128.6 (C_{Phe}), 128.7 (C_{Phe}), 128.8 (C_{Phe}), 129.2 (C_{Phe}), 129.3 (C_{Phe}), 129.4 (C_{Phe}), 133.5 (C_{Phe}), 133.9 (C_{Phe}), 134.0 (C_{Phe}), 135.7 (C-3), 152.0 (C-7a), 152.8 (C-6), 154.5 (C-4), 164.6 (C═O), 164.7 (C═O), 165.4 (C═O) ppm. HRMS (ESI): calcd for $C_{31}H_{26}N_5O_7$ ($[M + H]^+$): 580.1832, found: 580.1793. Spectral data are in accordance with literature values.[70]

3-Iodo-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine[95] (**36**). Compound **34** (7.43 g, 10.5 mmol) was subjected to general procedure C, with the exception that the temperature was raised to 60 °C to aid dissolution, (reaction time: 2 h). Purification *via* flash column chromatography (manual, 5% MeOH in CH_2Cl_2 to remove higher-running impurities and then 15% MeOH in CH_2Cl_2) afforded **36** (2.63 g, 6.69 mmol, 64% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.43 (1H, dd, J = 11.7, 5.6 Hz, H-5′), 3.55 (1H, dd, J = 11.7, 4.4 Hz, H-5″), 3.89 (1H, dd, J = 10.0, 4.4 Hz, H-4′), 4.16 (1H, t, J = 4.7 Hz, H-3′), 4.53−4.60 (1H, m, H-2′), 4.82 (1H, br. s, OH), 5.15 (1H, br. s, OH), 5.38 (1H, br. s, OH), 6.03 (1H, d, J = 5.0 Hz, H-1′), 8.23 (1H, s, H-6) ppm. ^{13}C NMR (75

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MHz, DMSO-d_6) δ 62.7 (C-5′), 71.1 (C-2′), 73.3 (C-3′), 85.7 (C-4′), 88.7 (C-1′), 103.9 (C-3a), 121.0 (C-3), 154.9 (C-7a), 156.8 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}IN_5O_4$ ([M + H]$^+$): 394.0012, found: 394.0021.

3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine (**37**). 4-Chloropyrazolo[3,4-*d*]pyrimidine (5.00 g, 32.4 mmol, 1.0 equiv) was dissolved in DMF (50 mL). NBS (6.33 g, 35.6 mmol, 1.1 equiv) was added, and the mixture was heated at 50 °C for 3 h. H_2O (100 mL) was added, and the mixture was extracted with EtOAc (3 ×150 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was used crude in the next reaction. HRMS (ESI): calcd for $C_5H_2BrClN_4$ ([M + H]$^+$): 232.9230, found: 232.9242.

3-Bromo-4-methoxypyrazolo[3,4-d]pyrimidine[73] (**38**). Compound **37** (crude) was dissolved in MeOH (50 mL). NaOMe (5.4 M in MeOH, 20 mL) was added, and the mixture was heated at 70 °C for 2 h. The reaction was quenched *via* the addition of aq. sat. NH_4Cl (200 mL) and H_2O (50 mL). The mixture was extracted with EtOAc (3 × 250 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (manual, petroleum ether/EtOAc 60:40) to afford **38** (3.76 g, 16.4 mmol, 51% yield over 2 steps) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.10 (3H, s, CH_3), 8.56 (1H, s, H-6), 14.27 (1H, br. s., NH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 54.4 (C-5′), 101.4 (C-3a), 118.1 (C-3), 156.0 (C-7a), 156.4 (C-6), 163.0 (C-4) ppm. HRMS (ESI): calcd for $C_6H_6BrN_4O$ ([M + H]$^+$): 228.9725, found: 228.9738. Spectral data are in accordance with literature values.[73]

3-Bromo-4-methoxy-1-(2′-deoxy-3′,5′-di-O-(p-toluoyl)-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine[73] (**39**). Powdered KOH (1.60 g, 28.6 mmol, 4.0 equiv) and TDA-1 (0.227 mL, 0.71 mmol, 0.1 equiv) were added to a suspension of **38** (1.64 g, 7.14 mmol, 1.0 equiv) in MeCN (250 mL). The mixture was stirred for 20 min before Hoffer's chlorosugar (2.64 g, 6.78 mmol, 0.95 equiv) was added. The mixture was stirred for another 30 min and filtered over celite. The filtrate was concentrated *in vacuo*, and the residue was adsorbed onto celite and purified *via* flash column chromatography (automated, 5 → 35% EtOAc in petroleum ether) to afford **39** (310 mg, 0.55 mmol, 8% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (3H, s, $CH_{3\ toluoyl}$), 2.44 (3H, s, $CH_{3\ toluoyl}$), 2.67 (1H, ddd, J = 14.3, 6.4, 3.0 Hz, H-2′), 3.52 (1H, dt, J = 14.0, 6.8 Hz, H-2″), 4.19 (3H, s, OCH_3), 4.47−4.68 (3H, m, H-5′, H-5″, H-4′), 5.80−5.92 (1H, m, H-3′), 6.91 (1H, t, J = 6.7 Hz, H-1′), 7.18−7.33 (4H, m, $H_{toluoyl}$), 7.88−7.99 (2H, m, $H_{toluoyl}$), 8.00−8.07 (2H, m, $H_{toluoyl}$), 8.51−8.63 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 21.7 (2 × $CH_{3\ toluoyl}$), 35.8 (OCH_3), 54.6 (C-2′), 64.1 (C-5′), 75.4 (C-3′), 82.8 (C-4′), 85.0 (C-1′), 103.8 (C-3a), 120.4 (C-3), 126.6 ($C_{toluoyl}$), 127.0 ($C_{toluoyl}$), 129.1 ($C_{toluoyl}$), 129.2 ($C_{toluoyl}$), 129.8 ($C_{toluoyl}$), 129.9 ($C_{toluoyl}$), 143.7 ($C_{toluoyl}$), 144.3 ($C_{toluoyl}$), 156.4 (-6), 163.9 (C-4), 165.9 (C═O), 166.3 (C═O) ppm. HRMS (ESI): calcd for $C_{27}H_{26}BrN_4O_6$ ([M + H]$^+$): 581.1036, found: 581.1009. Spectral data are in accordance with literature values.[73]

4-Amino-3-bromo-1-(2′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine[64] (**40**). Compound **39** (0.310 g, 0.48 mmol) was stirred in 7 N NH_3 in MeOH (20 mL) in a pressure tube at 90 °C for 24 h. The reaction vessel was cooled down to room temperature before it was opened, and its contents were transferred to a pear-shaped flask. The volatiles were removed *in vacuo*, and the residue was adsorbed onto celite and purified by flash column chromatography (automated, 2 → 10% MeOH in CH_2Cl_2 + 1% NH_4OH) to afford **40** (120 mg, 0.363 mmol, 76% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (1H, m, J = 13.3, 6.8, 4.2 Hz, H-2′), 2.74 (1H, dt, J = 13.0, 6.3 Hz, H-2″), 3.36 (1H, dt, J = 11.6, 5.9 Hz, H-5′), 3.50 (1H, dt, J = 11.4, 5.6 Hz, H-5″), 3.80 (1H, td, J = 5.7, 3.6 Hz, H-4′), 4.34−4.46 (1H, m, H-3′), 4.75 (1H, t, J = 5.8 Hz, OH), 5.27 (1H, d, J = 4.6 Hz, OH), 6.51 (1H, t, J = 6.4 Hz, H-1′), 8.23 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 37.8 (C-2′), 62.3 (C-5′), 70.8 (C-3′), 83.9 (C-4′), 87.7 (C-1′), 99.8 (C-3a), 119.0 (C-3), 154.5 (C-7a), 157.0 (C-6), 157.4 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}BrN_5O_3$ ([M + H]$^+$): 330.0202, found: 330.0231. Spectral data are in accordance with literature values.[64]

4-Amino-1-(2′-deoxy-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine[74] (**41**). Compound **40** (72 mg, 0.218 mmol) was subjected to general procedure D (reaction time: 30 min). Purification *via* flash column chromatography (automated, 5 → 15% MeOH in CH_2Cl_2) afforded **41** (52 mg, 0.100 mmol, 46% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.23 (1H, ddd, J = 13.1, 6.7, 3.9 Hz, H-2′), 2.80 (1H, dt, J = 13.0, 6.3 Hz, H-2″), 3.33−3.41 (1H, m, H-5′, partially under water peak), 3.52 (1H, dd, J = 11.4, 5.3 Hz, H-5″), 3.81 (1H, td, J = 5.6, 3.5 Hz, H-4′), 4.43 (1H, br. s., H-3′), 4.80 (1H, br. s., OH), 5.24 (1H, br. s., OH), 6.54 (1H, t, J = 6.5 Hz, H-1′), 7.45−7.98 (2H, m, NH_2), 8.15 (1H, s, H-3), 8.19 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 38.0 (C-2′), 62.5 (C-5′), 71.1 (C-3′), 84.0 (C-4′), 87.6 (C-1′), 100.5 (C-3a), 133.1 (C-3), 153.7 (C-7a), 156.0 (C-6), 158.0 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{14}N_5O_3$ ([M + H]$^+$): 252.1097, found: 252.1084. Spectral data are in accordance with literature values.[96]

3-Phenyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine[97] (**42**). Compound **21** (0.132 g, 0.38 mmol) was subjected to general procedure E, using phenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 0:100 to 49:51 in 18 min) afforded **42** (44 mg, 0.118 mmol, 34% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.42−3.53 (1H, m, H-5′), 3.55−3.67 (1H, m, H-5″), 3.93 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.22−4.30 (1H, m, H-3′), 4.60−4.70 (1H, m, H-2′), 4.77−4.89 (1H, m, OH), 5.06−5.20 (1H, m, OH), 5.33−5.49 (1H, m, OH), 6.19 (1H, d, J = 4.7 Hz, H-1′), 7.47−7.62 (3H, m, H_{Phe}), 7.65−7.72 (2H, m, H_{Phe}), 8.29 (1H, s, H-6) ppm. HRMS (ESI): calcd for $C_{16}H_{18}N_5O_4$ ([M + H]$^+$): 344.1359, found: 344.1356. Spectral data are in accordance with literature values.[97]

3-(4-Methoxyphenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**43**). Compound **21** (0.087 g, 0.25 mmol) was subjected to general procedure E, using 4-methoxyphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 1 → 12% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 41:59 in 10.5 min) afforded **43** (42 mg, 0.112 mmol, 45% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 (1H, dd, J = 11.7, 5.6 Hz, H-5′), 3.60 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 3.84 (3H, s, CH_3), 3.93 (1H, dd, J = 9.6, 4.7 Hz, H-4′), 4.26 (1H, t, J = 4.8 Hz, H-3′), 4.65 (1H, t, J = 4.8 Hz, H-2′), 6.18 (1H, d, J = 4.7 Hz, H-1′), 7.05−7.19 (2H, m, H_{Phe}), 7.55−7.67 (2H, m, H_{Phe}), 8.27 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.7 (CH_3), 62.8 (C-5′), 71.4 (C-2′), 73.7 (C-3′), 85.6 (C-4′), 88.8 (C-1′), 98.2 (C-3a), 115.1 (C_{Phe}), 125.3 (C_{Phe}), 130.0 (C_{Phe}), 145.2 (C-3), 155.7 (C-7a), 156.4 (C-6), 158.7 (C-4), 160.3 (C_{Phe}) ppm. HRMS (ESI): calcd for $C_{17}H_{20}N_5O_5$ ([M + H]$^+$): 374.1464, found: 374.1455.

3-(4-Chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**44**). Compound **21** (0.087 g, 0.25 mmol) was subjected to general procedure E, using 4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 1 → 12% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 0:100 to 41:59 in 10.5 min) afforded **44** (38 mg, 0.101 mmol, 40% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.44 (1H, dd, J = 11.7, 5.6 Hz, H-5′), 3.58 (1H, dd, J = 12.0, 4.7 Hz, H-5″), 3.91 (1H, dd, J = 10.0, 5.0 Hz, H-4′), 4.25 (1H, t, J = 4.7 Hz, H-3′), 4.63 (1H, t, J = 4.8 Hz, H-2′), 4.86 (1H, br. s, OH), 5.33 (2H, br. s, OH, OH), 6.17 (1H, d, J = 4.4 Hz, H-1′), 6.99 (2H, br. s, NH_2), 7.57−7.70 (4H, m, H_{Phe}), 8.26 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 129.2 (C_{Phe}), 130.0 (C_{Phe}), 131.4 (C_{Phe}), 133.7 (C_{Phe}), 143.8 (C-3), 155.4 (C-7a), 156.1 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{17}ClN_5O_4$ ([M + H]$^+$): 378.0969, found: 378.0981.

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*3-(4-Methylphenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**45**).* Compound **21** (0.094 g, 0.27 mmol) was subjected to general procedure E, using 4-methylphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 41:59 in 10.5 min) afforded **45** (40 mg, 0.112 mmol, 42% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (3H, s, CH_3), 3.40−3.52 (1H, m, H-5′), 3.54−3.67 (1H, m, H-5″), 3.93 (1H, dd, J = 9.1, 5.0 Hz, H-4′), 4.27 (1H, q, J = 4.7 Hz, H-3′), 4.65 (1H, q, J = 5.0 Hz, H-2′), 4.85 (1H, t, J = 5.7 Hz, OH), 5.14 (1H, d, J = 5.3 Hz, OH), 5.41 (1H, d, J = 5.6 Hz, OH), 6.19 (1H, d, J = 4.4 Hz, H-1′), 7.38 (2H, d, J = 7.9 Hz, H_{Phe}), 7.57 (2H, d, J = 7.9 Hz, H_{Phe}), 8.27 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.9 (CH_3), 62.4 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 128.1 (C_{Phe}), 129.8 (C_{Phe}), 138.4 (C_{Phe}), 144.9 (C-3), 155.3 (C-7a), 156.0 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{20}N_5O_4$ ([M + H]$^+$): 358.1515, found: 358.1500.

*3-(4-Fluorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**46**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-fluorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 32:68 in 12 min) afforded **46** (66 mg, 0.183 mmol, 52% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 (1H, dd, J = 11.9, 5.7 Hz, H-5′), 3.60 (1H, dd, J = 11.7, 4.4 Hz, H-5″), 3.93 (1H, dd, J = 10.0, 4.4 Hz, H-4′), 4.26 (1H, t, J = 4.7 Hz, H-3′), 4.65 (1H, t, J = 4.7 Hz, H-2′), 4.85 (1H, br. s, OH), 5.13 (1H, br. s, OH), 5.40 (1H, br. s, OH), 6.18 (1H, d, J = 4.4 Hz, H-1′), 6.97 (2H, br. s, NH_2), 7.32−7.47 (2H, m, H_{Phe}), 7.63−7.76 (2H, m, H_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.8 (C-5′), 71.3 (C-2′), 73.6 (C-3′), 85.6 (C-4′), 88.8 (C-1′), 98.3 (C-3a), 116.6 (d, J = 21.9 Hz, C-3_{Phe}, C-5_{Phe}), 129.5 (d, J = 3.5 Hz, C-1_{Phe}),130.9 (d, J = 9.2 Hz, C-2_{Phe}, C-6_{Phe}), 144.4 (C-3), 155.8 (C-7a), 156.5 (C-6), 158.6 (C-4), 163.0 (d, J = 245.0 Hz, C-4_{Phe}) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −113.07 to −112.95 (1F, m) ppm. HRMS (ESI): calcd for $C_{16}H_{17}FN_5O_4$ ([M + H]$^+$): 362.1265, found: 362.1265.

*3-(4-Nitrophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**47**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-nitrophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 32:68 in 12 min) afforded **47** (70 mg, 0.181 mmol, 52% yield) as a light-brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.61 (1H, dd, J = 11.7, 4.7 Hz, H-5″), 3.95 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.28 (1H, t, J = 4.4 Hz, H-3′), 4.67 (1H, br. s., H-2′), 4.84 (1H, br. s., OH), 5.16 (1H, br. s., OH), 5.43 (1H, br. s., OH), 6.22 (1H, d, J = 4.7 Hz, H-1′), 7.94 (2H, d, J = 8.8 Hz, H_{Phe}), 8.31 (1H, s, H-6), 8.37−8.45 (2H, m, H_{Phe}) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.5 (C-1′), 98.0 (C-3a), 124.3 (C-3_{Phe}, C-5_{Phe}), 129.6 (C-2_{Phe}, C-6_{Phe}), 138.9 (C-1_{Phe}), 143.1 (C-3), 147.4 (C-4_{Phe}), 155.6 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{17}N_6O_6$ ([M + H]$^+$): 389.1210, found: 389.1211.

*3-(4-tert-Butylphenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**48**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-*tert*-butylphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **48** (55 mg, 0.138 mmol, 39% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (9H, s, 3 × CH_3), 3.45 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.61 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 3.95 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.27 (1H, t, J = 4.7 Hz, H-3′), 4.64 (1H, t, J = 5.0 Hz, H-2′), 4.86 (1H, br. s, OH), 5.17 (1H, br. s, OH), 5.44 (1H, br. s, OH), 6.19 (1H, d, J = 4.4 Hz, H-1′), 7.54−7.68 (4H, m, H_{Phe}), 8.28 (1H, s, C-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.0 ($CH(CH_3)_3$), 34.5 ($\underline{C}H(CH_3)_3$), 62.4 (C-5′), 70.9 (C-2′), 73.3 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 126.0 (C-3_{Phe}, C-5_{Phe}), 127.9 (C-2_{Phe},C-6_{Phe}), 129.8 (C-1_{Phe}), 144.8 (C-3), 151.4 (C-4_{Phe}), 155.3 (C-7a), 156.0 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{20}H_{26}N_5O_4$ ([M + H]$^+$): 400.1985, found: 400.1972.

*3-(4-Trifluoromethylphenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**49**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-trifluoromethylphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **49** (67 mg, 0.163 mmol, 47% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 (1H, dd, J = 11.7, 5.0 Hz, H-5′), 3.60 (1H, dd, J = 11.4, 3.5 Hz, H-5″), 3.94 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.27 (1H, t, J = 4.5 Hz, H-3′), 4.66 (1H, t, J = 4.5 Hz, H-2′), 4.84 (1H, br. s., OH), 5.18 (1H, br. s., OH), 5.45 (1H, br. s., OH), 6.21 (1H, d, J = 4.4 Hz, H-1′), 7.86−7.96 (4H, m, H_{Phe}), 8.30 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.8 (C-5′), 71.3 (C-2′), 73.7 (C-3′), 85.7 (C-4′), 88.9 (C-1′), 98.3 (C-3a), 126.5 (q, J = 3.5 Hz, C-3Phe, C-5Phe), 129.4 (q, J = 32.2 Hz, C-4Phe), 129.5 (C-2Phe, C-6Phe), 137.0 (C-4Phe), 144.0 (C-3), 156.0 (C-7a), 156.6 (C-6), 158.6 (C-4) ppm. 1 quaternary carbon ($\underline{C}F_3$) missing. ^{19}F NMR (282 MHz, DMSO-d_6) δ −61.06 (1F, s) ppm. HRMS (ESI): calcd for $C_{17}H_{17}F_3N_5O_4$ ([M + H]$^+$): 412.1233, found: 412.1225.

*3-(4-Trifluoromethoxyphenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**50**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-trifluoromethoxyphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 4 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 0:100 in 18 min) afforded **50** (40 mg, 0.094 mmol, 27% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.37−3.50 (1H, m, H-5′), 3.53−3.66 (1H, m, H-5″), 3.93 (1H, dd, J = 10.0, 5.6 Hz, H-4′), 4.26 (1H, dd, J = 7.0, 5.0 Hz, H-3′), 4.65 (0 H, dd, J = 8.2, 4.1 Hz, H-2′), 4.84 (1H, t, J = 5.3 Hz, OH), 5.18 (1H, br. s., OH), 5.45 (1H, d, J = 4.4 Hz, OH), 6.19 (1H, d, J = 4.7 Hz, H-1′), 7.54 (2H, dd, J = 8.8, 0.9 Hz, H_{Phe}), 7.71−7.89 (2H, m, H_{Phe}), 8.29 (1H, s, H_{Phe}) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.8 (C-5′), 71.3 (C-2′), 73.7 (C-3′), 85.7 (C-4′), 88.9 (C-1), 98.3 (C-3a), 120.60 (q, J = 255.0 Hz, $\underline{C}F_3$), 122.1 (C-3_{Phe}, C-5_{Phe}), 130.7 (C-2_{Phe}, C-6_{Phe}), 132.3 (C-1), 144.1 (C-3), 149.1 (C-4_{Phe}), 155.9 (C-7a), 156.5 (C-6), 158.7 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −56.61 (1F, s) ppm. HRMS (ESI): calcd for $C_{17}H_{17}F_3N_5O_5$ ([M + H]$^+$): 428.1182, found: 428.1204.

*3-(4-Cyanophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**51**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-cyanophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 4 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **51** (56 mg, 0.152 mmol, 43% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.46 (1H, dd, J = 10.9, 5.2 Hz, H-5′), 3.60 (1H, dd, J = 11.6, 3.6 Hz, H-5″), 3.94 (1H, dd, J = 9.9, 4.8 Hz, H-4′), 4.27 (1H, dd, J = 8.6, 3.6 Hz, H-3′), 4.65 (1H, dd, J = 8.6, 4.9 Hz, H-2′), 4.75−4.90 (1H, m, OH), 5.16 (1H, d, J = 4.0 Hz, OH), 5.43 (1H, d, J = 5.4 Hz, OH), 6.21 (1H, d, J = 4.5 Hz, H-1′), 7.07 (2H, br. s, NH_2), 7.85 (2H, d, J = 8.3 Hz, H-3_{Phe}, H-5_{Phe}), 8.02 (2H, d, J = 8.3 Hz, H-2_{Phe}, H-6_{Phe}), 8.30 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.9 (C-2′), 73.3 (C-3′), 85.3 (C-4′), 88.5 (C-1′), 97.9 (C-3a), 111.3 (C-4_{Phe}), 118.8 ($\underline{C}N$), 129.1 (C-2_{Phe}, C-6_{Phe}), 133.1 (C-3_{Phe}, C-5_{Phe}), 137.1 (C-1_{Phe}), 143.4 (C-3), 155.6 (C-7a), 156.2 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{17}N_6O_4$ ([M + H]$^+$): 369.1311, found: 369.1241.

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*3-(3,4-Dichlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidine (**52**).* Compound **21** (0.118 g, 0.34 mmol) was subjected to general procedure E, using 3,4-dichlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 41:59 in 10.5 min) afforded **52** (45 mg, 0.109 mmol, 32% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.40−3.52 (1H, m, H-5′), 3.55−3.65 (1H, m, H-5″), 3.93 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.26 (1H, t, J = 4.5 Hz, H-3′), 4.65 (1H, t, J = 3.8 Hz, H-2′), 4.83 (1H, br. s., OH), 5.17 (1H, br. s., OH), 5.43 (1H, br. s., OH), 6.18 (1H, d, J = 4.7 Hz, H-1′), 6.95−7.37 (2H, m, NH_2), 7.63 (1H, dd, J = 8.2, 2.1 Hz, H-6_{Phe}), 7.81 (1H, d, J = 8.5 Hz, H-5_{Phe}), 7.85 (1H, d, J = 2.1 Hz, H-2_{Phe}), 8.29 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 128.5 (C-6_{Phe}), 130.1 (C-2_{Phe}), 131.3 (C-5_{Phe}), 131.6 (C-3_{Phe}) 131.7 (C-4_{Phe}), 133.1 (C-1_{Phe}), 142.6 (C-3), 155.4 (C-7a), 156.1 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{16}Cl_2N_5O_4$ ([M + H]$^+$): 412.0579, found: 412.0575.

*3-(3-Chloro-4-fluorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**53**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-chloro-4-fluorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 32:68 in 12 min) afforded **53** (75 mg, 0.197 mmol, 56% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.40−3.51 (1H, m, H-5′), 3.54−3.67 (1H, m, H-5″), 3.93 (1H, dd, J = 10.0, 5.3 Hz, H-4′), 4.26 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.64 (1H, dd, J = 10.5, 5.6 Hz, H-2′), 4.82 (1H, t, J = 5.9 Hz, OH), 5.14 (1H, d, J = 5.6 Hz, OH), 5.40 (1H, d, J = 5.9 Hz, OH), 6.18 (1H, d, J = 4.4 Hz, H-1′), 6.79−7.38 (2H, br. s, NH_2), 7.52−7.69 (2H, m, H-5_{Phe}, H-6_{Phe}), 7.80 (1H, dd, J = 7.0, 2.1 Hz, H-2_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 117.5 (d, J = 21.9 Hz, C-5_{Phe}), 120.1 (d, J = 20.7 Hz, C-3_{Phe}), 129.1 (d, J = 8.1 Hz, C-6_{Phe}), 130.3 (d, J = 3.5 Hz, C-1_{Phe}), 130.4 (C-2_{Phe}), 142.8 (C-3), 155.4 (C-7a), 156.1 (C-6), 158.2 (C-4) ppm. 1 quaternary carbon (C-4_{Phe}) missing. ^{19}F NMR (282 MHz, DMSO-d_6) δ −118.16 to −115.34 (142 F, m) ppm. HRMS (ESI): calcd for $C_{16}H_{16}ClFN_5O_4$ ([M + H]$^+$): 396.0875, found: 396.0891.

*3-(3-Fluoro-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**54**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-fluoro-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **54** (82 mg, 0.207 mmol, 59% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.39−3.52 (1H, m, H-5′), 3.59 (1H, dt, J = 12.0, 4.7 Hz, H-5″), 3.93 (1H, q, J = 4.7 Hz, H-4′), 4.26 (1H, q, J = 5.0 Hz, H-3′), 4.65 (1H, q, J = 5.2 Hz, H-2′), 4.82 (1H, t, J = 5.7 Hz, OH), 5.14 (1H, d, J = 5.6 Hz, OH), 5.41 (1H, d, J = 5.9 Hz, OH), 6.18 (1H, d, J = 4.4 Hz, H-1′), 7.20 (2H, br. s, NH_2), 7.51 (1H, ddd, J = 8.2, 2.1, 0.6 Hz, H-5_{Phe}), 7.63 (1H, dd, J = 10.1, 2.1 Hz, H-2_{Phe}), 7.76 (1H, t, J = 8.1 Hz, H-6_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.8 (C-2′), 73.3 (C-3′), 84.8 (C-4′), 88.3 (C-1′) 97.8 (C-3a), 116.8 (d, J = 20.7 Hz, C-2_{Phe}), 120.0 (d, J = 18.4 Hz, C-4_{Phe}), 125.5 (d, J = 3.5 Hz, C-5_{Phe}), 131.3 (C-6_{Phe}), 133.4 (d, J = 8.1 Hz, C-1_{Phe}), 142.9 (C-3), 155.4 (C-7a), 156.1 (C-6), 158.1 (C-4), 157.4 (d, J = 244.5 Hz, C-3_{Phe}) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −115.13 (dd, J = 10.2, 7.8 Hz) ppm. HRMS (ESI): calcd for $C_{16}H_{16}ClFN_5O_4$ ([M + H]$^+$): 396.0875, found: 396.0860.

*3-(3,4-Difluorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidine (**55**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3,4-difluorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **55** (66 mg, 0.174 mmol, 50% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.40−3.51 (1H, m, H-5′), 3.58 (1H, d, J = 3.8 Hz, H-5″), 3.93 (1H, dd, J = 10.0, 5.3 Hz, H-4′), 4.26 (1H, t, J = 4.5 Hz, H-3′), 4.64 (1H, t, J = 4.7 Hz, H-2′), 4.74−4.88 (1H, m, OH), 5.03−5.29 (1H, m, OH), 5.33−5.51 (1H, m, OH), 6.18 (1H, d, J = 4.4 Hz, H-1′), 6.90−7.33 (2H, m, H_{Phe}), 7.40−7.55 (1H, m, H_{Phe}), 7.55−7.73 (1H, m, H_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.4 (C-2′), 73.3 (C-3′) 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 117.3−118.7 (C$_{Phe}$), 124.9−125.9 (C$_{Phe}$), 129.5−130.4 (C$_{Phe}$), 143.0 (d, J = 2.3 Hz, C-3), 147.9−148.8 (C$_{Phe}$), 151.3 (C-7a), 155.0−155.6 (C$_{Phe}$), 156.1 (C-6), 158.1 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −138.90 to −138.66 (1F, m), −137.66 to −137.43 (1F, m) ppm. HRMS (ESI): calcd for $C_{16}H_{16}F_2N_5O_4$ ([M + H]$^+$): 380.1170, found: 380.1185.

*3-(3-Methoxy-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**56**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-methoxy-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **56** (86 mg, 0.211 mmol, 60% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47 (1H, dd, J = 11.0, 5.4 Hz, H-5′), 3.61 (1H, dd, J = 11.0, 4.7 Hz, H-5″), 3.82−4.04 (4H, m, H-4′, C$\underline{H}_3$), 4.27 (1H, t, J = 4.7 Hz, H-3′), 4.66 (1H, t, J = 4.7 Hz, H-2′), 4.85 (1H, br. s., OH), 5.03−5.24 (1H, m, OH), 5.31−5.57 (1H, m, OH), 6.19 (1H, d, J = 4.7 Hz, H-1′), 7.25 (1H, dd, J = 8.1, 1.9 Hz, H-6_{Phe}), 7.36 (1H, d, J = 1.8 Hz, H-2_{Phe}), 7.59 (1H, d, J = 7.9 Hz, H-5_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.0 (CH_3), 62.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.5 (C-1′), 97.8 (C-3a), 112.8 (C-2_{Phe}), 121.0 (C-6_{Phe}), 121.7 (C-4_{Phe}), 130.5 (C-5_{Phe}), 132.6 (C-1_{Phe}), 144.0 (C-3), 154.8 (C-7a), 155.3 (C-3_{Phe}), 156.0 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{19}ClN_5O_5$ ([M + H]$^+$): 408.1075, found: 408.1099.

*3-(3-Methyl-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**57**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-methyl-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **57** (88 mg, 0.224 mmol, 64% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (3H, s, CH_3), 3.54 (1H, dt, J = 11.8, 6.0 Hz, H-5′), 3.68 (1H, dt, J = 12.0, 4.7 Hz, H-5″), 4.01 (1H, dd, J = 9.4, 4.7 Hz, H-4′), 4.34 (1H, q, J = 5.2 Hz, H-3′), 4.73 (1H, q, J = 5.2 Hz, H-2′), 4.92 (1H, t, J = 5.9 Hz, OH), 5.22 (1H, d, J = 5.6 Hz, OH), 5.48 (1H, d, J = 5.9 Hz, OH), 6.26 (1H, d, J = 4.7 Hz, H-1′), 6.57−7.45 (2H, m, NH_2), 7.55−7.77 (3H, m, H_{Phe}), 8.36 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.8 (CH_3), 62.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.8 (C-3a), 127.4 (C$_{Phe}$), 129.6 (C$_{Phe}$), 130.9 (C$_{Phe}$), 131.4 (C$_{Phe}$), 134.0 (C$_{Phe}$), 136.3 (C$_{Phe}$), 143.9 (C-3), 155.4 (C-7a), 156.1 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{19}ClN_5O_4$ ([M + H]$^+$): 392.1126, found: 392.1073.

*3-(3-Trifluoromethyl-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**58**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-trifluoromethyl-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **58** (50 mg, 0.112 mmol, 32% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 (1H, dd, J = 11.6, 5.7 Hz, H-5′), 3.60 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 3.94 (1H, dd, J = 9.7, 5.0 Hz, H-4′), 4.27 (1H, t, J = 4.5 Hz, H-3′), 4.66 (1H, t, J = 4.4 Hz, H-2′), 4.83 (1H, br. s., OH), 5.16 (1H, br.

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s., OH), 5.40 (1H, br. s., OH), 6.20 (1H, d, J = 4.7 Hz, H-1′), 7.23 (2H, br. s., NH_2), 7.77−8.10 (3H, m, H_{Phe}), 8.30 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.5 (C-1′), 97.9 (C-3a), 122.7 (q, J = 275.0 Hz, CF_3), 127.0 (C_{Phe}), 127.5 (d, J = 4.6 Hz, C_{Phe}), 131.1 (C_{Phe}), 132.0 (C_{Phe}), 132.4 (C_{Phe}), 133.6 (C_{Phe}), 142.6 (C-3), 155.5 (C-7a), 156.1 (C-6), 158.2 (C-4) ppm. ^{19}F NMR (377 MHz, DMSO-d_6) δ −61.4 (s) ppm. HRMS (ESI): calcd for $C_{17}H_{16}F_3ClN_5O_4$ ([M + H]$^+$): 446.0843, found: 446.0853.

*3-(3-Cyano-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**59**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-cyano-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 40:60 in 12 min) afforded **59** (25 mg, 0.062 mmol, 18% yield) as a white solid and **75** (7 mg, 0.014 mmol, 4% yield) as a white solid. Analytical data **59**: ^1H NMR (400 MHz, DMSO-d_6) δ 3.37−3.51 (1H, m, H-5′), 3.59 (1H, ddd, J = 11.5, 9.6, 4.3 Hz, H-5″), 3.93 (1H, q, J = 4.8 Hz, H-4′), 4.26 (1H, q, J = 4.8 Hz, H-3′), 4.65 (1H, q, J = 5.0 Hz, H-2′), 4.82 (1H, t, J = 5.8 Hz, OH), 5.15 (1H, d, J = 5.4 Hz, OH), 5.42 (1H, d, J = 5.6 Hz, OH), 6.19 (1H, d, J = 4.5 Hz, H-1′), 6.79−7.65 (2H, m, NH_2), 7.90 (1H, d, J = 8.1 Hz, H_{Phe}), 7.96 (H, dd, J = 8.3, 2.4 Hz, H_{Phe}), 8.17 (1H, d, J = 2.0 Hz, H_{Phe}), 8.29 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 60.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 97.9 (C-3a), 112.9 (C_{Phe}), 116.0 (C_{Phe}), 130.7 (C_{Phe}), 132.3 (C_{Phe}), 134.2 (C_{Phe}), 134.4 (C_{Phe}), 135.6 (C_{Phe}), 142.1 (C-3), 155.5 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{16}ClN_6O_4$ ([M + H]$^+$): 403.0922, found: 403.0929. Analytical data **75**: ^1H NMR (400 MHz, DMSO-d_6) δ 3.41−3.53 (1H, m, H-5′), 3.61 (1H, dt, J = 11.8, 4.8 Hz, H-5″), 3.95 (1H, dd, J = 10.0, 4.9 Hz, H-4′), 4.29 (1H, dd, J = 10.4, 5.1 Hz, H-3′), 4.65 (1H, dd, J = 10.3, 5.0 Hz, H-2′), 4.82 (1H, t, J = 5.4 Hz, OH), 5.16 (1H, d, J = 5.6 Hz, OH), 5.43 (1H, d, J = 5.8 Hz, OH), 6.22 (1H, d, J = 4.4 Hz, H-1′), 7.15 (2H, br. s, NH_2), 7.84−8.23 (5H, m, H_{Phe}), 8.30 (1H, d, J = 2.0 Hz, H_{Phe}), 8.32 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.8 (C-2′), 73.3 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 98.0 (C-3a), 111.3 (C_{Phe}), 112.6 (C_{Phe}), 115.6 (C_{Phe}), 118.0 (C_{Phe}), 130.6 (C_{Phe}), 131.0 (C_{Phe}), 133.1 (C_{Phe}), 133.4 (C_{Phe}), 133.4 (C_{Phe}), 134.7 (C_{Phe}), 135.1 (C_{Phe}), 136.0 (C_{Phe}), 137.3 (C_{Phe}), 141.1 (C_{Phe}), 142.5 (C-3), 155.5 (C-7a), 156.2 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{24}H_{19}ClN_7O_4$ ([M + H]$^+$): 504.1187, found: 504.1199.

*3-(3,5-Difluoro-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**60**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3,5-difluoro-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **60** (70 mg, 0.169 mmol, 48% yield) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 3.40−3.52 (1H, m, H-5′), 3.60 (1H, dt, J = 11.8, 4.8 Hz, H-5″), 3.93 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.27 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.65 (1H, dd, J = 10.5, 5.3 Hz, H-2′), 4.82 (1H, t, J = 5.9 Hz, OH), 5.14 (1H, d, J = 5.6 Hz, OH), 5.41 (1H, d, J = 5.9 Hz, OH), 6.18 (1H, d, J = 4.7 Hz, H-1′), 7.24 (2H, br. s., NH_2), 7.46−7.65 (2H, m, H_{Phe}), 8.28 (H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.5 (C-1′), 97.9 (C-3a), 108.8 (t, J = 20.7 Hz, C_{Phe}), 112.8 (dd, J = 23.0, 2.3 Hz, C_{Phe}), 133.1 (t, J = 10.4 Hz, C_{Phe}), 142.1 (d, J = 2.3 Hz, C-3), 155.7 (C-7a), 156.2 (C-6), 156.6 (d, J = 3.5 Hz, C_{Phe}), 158.0 (C-4), 159.9 (d, J = 4.6 Hz) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −112.93 (2F, d, J = 7.2 Hz) ppm. HRMS (ESI): calcd for $C_{16}H_{15}ClF_2N_5O_4$ ([M + H]$^+$): 414.0781, found: 414.0733.

*3-(3-Ethoxy-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**61**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 3-ethoxy-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **61** (108 mg, 0.256 mmol, 73% yield) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 1.40 (3H, t, J = 6.9 Hz, CH_3), 3.60 (1H, ddd, J = 12.0, 10.0, 4.7 Hz, H-5′), 3.94 (1H, dd, J = 10.0, 4.4 Hz, H-5″), 4.20 (2H, q, J = 6.9 Hz, CH_2), 4.27 (1H, dd, J = 10.0, 5.0 Hz, H-4′), 4.66 (1H, dd, J = 10.3, 5.0 Hz, H-3′), 4.85 (1H, dd, J = 6.4, 5.6 Hz, H-2′), 5.14 (1H, d, J = 5.6 Hz, OH), 5.40 (1H, d, J = 5.9 Hz, OH), 6.19 (1H, d, J = 4.4 Hz, OH), 6.69−7.23 (2H, m, NH_2), 7.24 (1H, dd, J = 8.2, 1.8 Hz, H-6_{Phe}), 7.34 (1H, d, J = 1.8 Hz, H-2_{Phe}), 7.59 (1H, d, J = 8.2 Hz, H-5_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6 (CH_3), 62.3 (C-5′), 64.3 (CH_2), 70.8 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.5 (C-5′), 97.8 (C-3a), 113.6 (C-2 $_{Phe}$), 120.9 (C-6_{Phe}), 121.9 (C-4 $_{Phe}$), 130.5 (C-5 $_{Phe}$), 132.5 (C-1_{Phe}), 144.1 (C-3), 154.1 (C-3), 155.3 (C-7a), 156.0 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{18}H_{21}ClN_5O_5$ ([M + H]$^+$): 422.1231, found: 422.1141.

*3-(2,4-Dichlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**62**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 2,4-dichlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **62** (42 mg, 0.102 mmol, 29% yield) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 3.37−3.48 (1H, m, H-5′), 3.52−3.63 (1H, m, H-5″), 3.92 (1H, dd, J = 10.0, 4.8 Hz, H-4′), 4.22 (1H, dd, J = 9.7, 5.0 Hz, H-3′), 4.61 (1H, dd, J = 9.7, 4.7 Hz, H-2′), 4.82 (1H, t, J = 5.9 Hz, OH), 5.14 (1H, d, J = 5.6 Hz, OH), 5.41 (1H, d, J = 5.9 Hz, OH), 6.16 (1H, d, J = 4.4 Hz, H-1′), 7.52 (1H, d, J = 8.5 Hz, H-3_{Phe}), 7.57 (1H, dd, J = 8.2, 2.1 Hz, H-5_{Phe}), 7.79 (1H, d, J = 2.1 Hz, H-6_{Phe}), 8.25 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.6 (C-1′), 99.4 (C-3a), 127.8 (C-3_{Phe}), 129.7 (C-6_{Phe}), 130.3 (C-1_{Phe}), 133.2 (C-2_{Phe}), 133.9 (C-5_{Phe}), 134.7 (C-4_{Phe}), 141.2 (C-3), 154.7 (C-7a), 156.2 (C-6), 157.8 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{16}Cl_2N_5O_4$ ([M + H]$^+$): 412.0579, found: 412.0586.

*3-(2-Methyl-4-chlorophenyl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**63**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 2-methyl-4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **63** (83 mg, 0.212 mmol, 61% yield) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 2.18−2.37 (3H, m, CH_3), 3.42 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.58 (1H, dd, J = 11.7, 4.4 Hz, H-5″), 3.93 (1H, dd, J = 9.1, 4.7 Hz, H-4′), 4.23 (1H, t, J = 5.0 Hz, H-3′), 4.60 (1H, t, J = 4.5 Hz, H-2′), 6.19 (1H, d, J = 4.1 Hz, H-1′), 7.30−7.45 (2H, m, NH_2, H_{Phe}), 7.49 (1H, s, H_{Phe}), 8.30 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 19.9 (CH_3), 62.8 (C-5′), 71.4 (C-2′), 73.9 (C-3′), 85.6 (C-4′), 88.9 (C-1′), 99.4 (C-3a), 126.7 (C_{Phe}), 131.0 (C_{Phe}), 132.1 (C_{Phe}), 134.1 (C_{Phe}), 139.8 (C_{Phe}), 143.8 (C-3), 155.0 (C-7a), 156.0 (C-6), 157.9 (C-4) ppm. HRMS (ESI): calcd for $C_{17}H_{19}ClN_5O_4$ ([M + H]$^+$): 392.1126, found: 392.1077.

*3-(1-Methylpyrazol-4-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**64**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 1-methylpyrazole-4-boronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **64** (65 mg, 0.187 mmol, 53% yield) as a white solid. ^1H NMR (300 MHz, DMSO-d_6) δ 3.45 (1H, dt, J = 11.2, 5.4 Hz, H-5′), 3.59 (1H, dt, J = 12.0, 4.2 Hz, H-5″), 3.80−4.02 (4H, m, CH_3, H-4′), 4.24 (1H, dd, J = 9.7, 5.0 Hz, H-3′), 4.62 (1H, dd, J = 9.7, 5.1 Hz, H-2′), 4.86 (1H, t, J = 5.2 Hz, OH), 5.12 (1H, d, J = 5.3 Hz, OH), 5.38 (1H, d, J = 5.9 Hz, OH), 6.13 (1H, d, J = 4.7 Hz, H-1′), 6.67−7.46 (2H, m, NH_2), 7.74 (1H, s, H-$5_{pyrazole}$), 8.10 (1H, s, H-$3_{pyrazole}$), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.9 (C-2′), 73.1 (C-3′), 85.2 (C-4′), 88.4 (C-1′), 98.1 (C-3a), 113.3 (C-$4_{pyrazole}$), 130.4 (C-$5_{pyrazole}$), 137.7 (C-$3_{pyrazole}$), 155.0 (C-7a), 156.0 (C-6), 158.3 (C-4) ppm. 1 quaternary carbon (C-3)

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missing. HRMS (ESI): calcd for $C_{14}H_{18}N_7O_4$ ([M + H]$^+$): 348.1420, found: 348.1418.

*3-(Pyridin-2-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine, Formic Acid Salt (**65**).* Compound **21** (0.173 g, 0.50 mmol, 1.0 equiv), $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.15 equiv), and CuI (0.010 g, 0.05 mmol, 0.1 equiv) were dissolved in dry degassed DMF (2 mL) under argon. 2-(Tributylstannyl)pyridine was added, and the mixture was warmed to 100 °C. After 2 h, LCMS analysis indicated completion of the reaction. The mixture was cooled to room temperature, diluted with MeOH (15 mL) and MeCN (15 mL), and washed with hexanes (2 × 15 mL). The MeOH/MeCN phase was concentrated *in vacuo*. The residue was adsorbed onto celite and purified by flash column chromatography (automated, 4 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) to afford **67** (80 mg, 0.232 mmol, 66% yield) as a white solid. NMR analysis showed the presence of an extra proton, indicating that **65** was isolated as its formic acid salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.43−3.58 (1H, m, H-5′), 3.60−3.74 (1H, m, H-5″), 3.97 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.27−4.41 (1H, m, H-3′), 4.60−4.74 (1H, m, H-2′), 4.88 (1H, br. s., OH), 5.17 (1H, br. s., OH), 5.47 (1H, br. s., OH), 6.21 (1H, d, J = 4.4 Hz, H-1′), 7.51 (1H, ddd, J = 7.5, 5.1, 1.2 Hz, H-4$_{pyr}$), 8.04 (1H, td, J = 7.8, 1.8 Hz, H-5$_{pyr}$), 8.11 (1H, d, J = 3.2 Hz), 8.24 (1H, s, H-6), 8.28 (1H, d, J = 7.9 Hz, H-3$_{pyr}$), 8.74 (1H, dd, J = 4.1, 0.9 Hz, H-6$_{pyr}$), 9.93 (1H, d, J = 3.8 Hz, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 71.0 (C-2′), 73.4 (C-3′), 85.4 (C-4′), 88.6 (C-1′), 98.5 (C-3a), 121.0 (C-3$_{pyr}$), 124.1 (C-5$_{pyr}$), 138.3 (C-3), 143.7 (C-4$_{pyr}$), 148.5 (C-6$_{pyr}$), 150.7 (C-2$_{pyr}$), 155.7 (C-7a), 156.6 (C-6), 158.8 (C-4)ppm. HRMS (ESI): calcd for $C_{15}H_{17}N_6O_4$ ([M + H]$^+$): 345.1311, found: 345.1312.

*3-(Pyridin-4-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**66**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using pyridine-4-boronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **66** (37 mg, 0.056 mmol, 16% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.39−3.55 (1H, m, H-5′), 3.55−3.70 (1H, m, H-5″), 3.94 (1H, dd, J = 9.7, 4.8 Hz, H-4′), 4.28 (1H, dd, J = 10.0, 5.2 Hz, H-3′), 4.66 (1H, dd, J = 9.7, 5.0 Hz, H-2′), 4.84 (1H, t, J = 5.9 Hz, OH), 5.16 (1H, d, J = 5.6 Hz, OH), 5.43 (1H, d, J = 5.6 Hz, OH), 6.21 (1H, d, J = 4.4 Hz, H-1′), 7.18 (2H, br. s., NH_2), 7.67 (2H, d, J = 5.9 Hz, H-3$_{pyr}$, H-5$_{pyr}$), 8.31 (1H, s, H-6), 8.74 (2H, d, J = 5.3 Hz, H-2$_{pyr}$, H-6$_{pyr}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.8 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.6 (C-1′), 97.9 (C-3a), 122.8 (C-3$_{pyr}$, C-5$_{pyr}$), 139.8 (C-3), 142.6 (C-4$_{pyr}$), 150.3 (C-2$_{pyr}$, C-6$_{pyr}$), 155.6 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{15}H_{17}N_6O_4$ ([M + H]$^+$): 345.1311, found: 345.1317.

*3-(Thiophen-2-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**67**).* Compound **21** (0.173 g, 0.50 mmol, 1.0 equiv), $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.15 equiv), and CuI (0.010 g, 0.05 mmol, 0.1 equiv) were dissolved in dry degassed DMF (2 mL) under argon. 2-(Tributylstannyl)thiophene (0.238 mL, 0.75 mmol, 1.5 equiv) was added, and the mixture was warmed to 100 °C. After 2 h, LCMS analysis indicated completion of the reaction. The mixture was cooled to room temperature, diluted with MeOH (15 mL) and MeCN (15 mL), and washed with hexanes (2 × 15 mL). The MeOH/MeCN phase was concentrated *in vacuo*. The residue was adsorbed onto celite and purified by flash column chromatography (automated, 4 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) to afford **67** (42 mg, 0.120 mmol, 24% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 (1H, dt, J = 11.4, 5.4 Hz, H-5′), 3.60 (1H, dt, J = 11.7, 3.8 Hz, H-5″), 3.93 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.14−4.31 (1H, m, H-3′), 4.62 (1H, dd, J = 7.3, 3.2 Hz, H-2′), 4.84 (1H, t, J = 6.2 Hz, OH), 5.16 (1H, br. s., OH), 5.42 (1H, br. s., OH), 6.16 (1H, d, J = 4.7 Hz, H-1′), 6.83−7.19 (2H, m, NH_2), 7.25 (2H, dd, J = 5.0, 3.5 Hz, C-4$_{Het}$), 7.50 (1H, dd, J = 3.7, 1.0 Hz, C-3$_{Het}$), 7.72 (1H, dd, J = 5.1, 1.0 Hz, C-5$_{Het}$), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.9 (C-5′), 71.3 (C-2′), 73.6 (C-3′), 85.8 (C-4′), 88.9 (C-1′), 98.1 (C-3a), 128.3 (C-3$_{Het}$), 128.5(C-4$_{Het}$), 128.9 (C-5$_{Het}$), 134.3 (C-2$_{Het}$), 139.4 (C-3), 155.7 (C-7a), 156.6 (C-6), 158.6 (C-4) ppm. HRMS (ESI): calcd for $C_{14}H_{16}N_5O_4S$ ([M + H]$^+$): 350.0923, found: 350.0932.

*3-(5-Methylthiophen-2-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**68**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 5-methylthiophene-2-boronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **64** (65 mg, 0.187 mmol, 53% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.34 (3H, s, CH_3), 3.44 (1H, dt, J = 11.9, 6.1 Hz, H-5′), 3.59 (1H, dt, J = 11.4, 4.7 Hz, H-5″), 3.91 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.23 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.58 (1H, dd, J = 9.7, 5.0 Hz, H-2′), 4.82 (1H, t, J = 5.9 Hz, OH), 5.13 (1H, d, J = 5.6 Hz, OH), 5.40 (1H, d, J = 5.9 Hz, OH), 6.13 (1H, d, J = 4.7 Hz, H-1′), 6.82−6.97 (1H, m, C-4$_{Het}$), 6.99−7.21 (2H, m, NH_2), 7.27 (1H, d, J = 3.5 Hz, C-3$_{Het}$), 8.25 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9 (CH_3), 62.5 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.4 (C-1′), 97.5 (C-3a), 126.8 (C-4$_{Het}$), 128.0 (C-5$_{Het}$), 131.5 (C-3$_{Het}$), 139.1 (C-2$_{Het}$), 141.3 (C-3), 155.2 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{15}H_{18}N_5O_4S$ ([M + H]$^+$): 364.1079, found: 364.1088.

*3-(5-Chlorothiophen-2-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**69**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 5-chlorothiophene-2-boronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 40:60 in 12 min) afforded **69** (55 mg, 0.143 mmol, 41% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.36−3.51 (1H, m, H-5′), 3.59 (1H, dt, J = 11.6, 5.0 Hz, H-5″), 3.92 (1H, dd, J = 10.3, 5.1 Hz, H-4′), 4.23 (1H, dd, J = 10.0, 5.1 Hz, H-3′), 4.60 (1H, dd, J = 10.0, 5.0 Hz, H-2′), 4.82 (1H, t, J = 5.8 Hz, OH), 5.15 (1H, d, J = 5.6 Hz, OH), 5.42 (1H, d, J = 5.9 Hz, OH), 6.15 (1H, d, J = 4.5 Hz, H-1′), 7.25 (2H, d, J = 4.0 Hz, H-4$_{Het}$), 7.35 (1H, d, J = 3.9 Hz, H-3$_{Het}$), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.4 (C-1′), 97.5 (C-3a), 128.0 (C-3$_{Het}$), 128.2 (C-5$_{Het}$), 129.3 (C-4$_{Het}$), 132.8 (C-2$_{Het}$), 138.1 (C-3), 155.3 (C-7a), 156.3 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{14}H_{15}ClN_5O_4S$ ([M + H]$^+$): 384.0533, found: 384.0550.

*3-(4-Methylthiophen-2-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**70**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to general procedure E, using 4-methylthiophene-2-boronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 40:60 in 12 min) afforded **70** (38 mg, 0.105 mmol, 30% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (3H, d, J = 0.5 Hz, CH_3), 3.40−3.50 (1H, m, H-5′), 3.59 (1H, dt, J = 11.9, 4.8 Hz, H-5″), 3.92 (1H, dd, J = 10.1, 4.9 Hz, H-4′), 4.24 (1H, dd, J = 10.3, 5.0 Hz, H-3′), 4.61 (1H, dd, J = 9.9, 5.1 Hz, H-2′), 4.83 (1H, t, J = 5.8 Hz, OH), 5.15 (1H, d, J = 5.6 Hz, OH), 5.41 (1H, d, J = 5.9 Hz, OH), 6.15 (1H, d, J = 4.5 Hz, H-1′), 7.28 (1H, t, J = 1.1 Hz, H-3$_{Het}$), 7.30 (1H, d, J = 1.1 Hz, H-5$_{Het}$), 8.27 (1H, s) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 15.5 (CH_3), 62.4 (C-5′), 70.9 (C-2′), 73.1 (C-3′), 85.3 (C-4′), 88.4 (C-1′), 97.5 (C-3a), 122.8 (C-5$_{Het}$), 130.0 (C-3$_{Het}$), 133.5 (C-4$_{Het}$), 138.5 (C-3), 139.1 (C-2$_{Het}$), 155.2 (C-7a), 156.2 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{15}H_{18}N_5O_4S$ ([M + H]$^+$): 364.1079, found: 364.1081.

*3-Vinyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**71**).* Compound **21** (0.118 g, 0.34 mmol) was subjected to general procedure E, using potassium vinyl trifluoroborate as the coupling partner and Cs_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **71** (60 mg,

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0.205 mmol, 58% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.41−3.50 (1H, m, H-5′), 3.60 (1H, dt, J = 11.6, 4.8 Hz, H-5″), 3.92 (1H, dd, J = 9.4, 4.8 Hz, H-4′), 4.24 (1H, dd, J = 9.9, 5.5 Hz, h-3′), 4.57 (1H, dd, J = 10.1, 5.0 Hz, H-2′), 4.87 (1H, t, J = 5.8 Hz, OH), 5.12 (1H, d, J = 5.4 Hz, OH), 5.36 (1H, d, J = 5.9 Hz, OH), 5.46 (1H, dd, J = 11.8, 1.0 Hz, CH=C$\underline{H}_2$), 6.05 (1H, dd, J = 17.1, 1.0 Hz, CH=C$\underline{H}_2$), 6.12 (1H, d, J = 4.3 Hz, H-1′), 7.28 (1H, dd, J = 17.1, 11.0 Hz, C$\underline{H}$=CH$_2$), 7.54 (2H, br. s, NH$_2$), 8.18 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.3 (C-4′), 88.5 (C-1′), 98.1 (C-3a), 118.5 (CH=$\underline{C}$H$_2$), 127.3 (C$\underline{H}$=CH$_2$), 141.9 (C-3), 155.0 (C-7a), 155.9 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{12}H_{16}N_5O_4$ ([M + H]$^+$): 294.1202, found: 294.1213.

*3-Isopropenyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**72**).* Compound **21** (0.250 g, 0.72 mmol) was subjected to general procedure E, using potassium isopropenyltrifluoroborate as the coupling partner and Cs_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **72** (170 mg, 0.553 mmol, 77% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (3H, s, C$\underline{H}_3$C=CH$_2$), 3.45 (1H, dt, J = 11.6, 5.9 Hz, H-5′), 3.55−3.65 (1H, m, H-5″), 3.92 (1H, dd, J = 9.7, 4.4 Hz, H-4′), 4.23 (1H, dd, J = 9.7, 4.7 Hz, H-3′), 4.59 (1H, dd, J = 9.4, 5.0 Hz, H-2′), 4.84 (1H, t, J = 5.6 Hz, OH), 5.12 (1H, d, J = 5.3 Hz, OH), 5.29−5.45 (2H, m, OH, CH$_3$C=C$\underline{H}_2$), 5.53 (1H, br. s., CH$_3$C=C$\underline{H}_2$), 6.13 (1H, d, J = 4.1 Hz, H-1′), 7.16 (2H, br. s., NH$_2$), 8.23 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.7 ($\underline{C}$H$_3$C=CH$_2$), 62.4 (C-5′), 70.9 (C-2′), 73.2 (C-3′), 85.2 (C-4′), 88.5 (C-1′), 97.3 (C-3a), 118.6 (CH$_3$C=$\underline{C}$H$_2$), 137.2 (CH$_3\underline{C}$=CH$_2$), 146.2 (C-3), 154.9 (C-7a), 155.9 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{13}H_{18}N_5O_4$ ([M + H]$^+$): 308.1359, found: 308.1367.

*3-(E-Styryl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**73**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to a slightly modified general procedure E, using *trans*-2-vinylphenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H$_2$O/MeCN 98:02 to 33:67 in 12 min) afforded **72** (55 mg, 0.149 mmol, 43% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 (1H, dd, J = 11.7, 5.9 Hz, H-5′), 3.62 (1H, dd, J = 11.7, 4.4 Hz, H-5″), 3.92 (1H, dd, J = 10.0, 4.7 Hz, H-4′), 4.26 (1H, t, J = 4.7 Hz, H-3′), 4.65 (1H, t, J = 5.0 Hz, H-2′), 6.12 (1H, d, J = 4.7 Hz, H-1′), 7.26−7.49 (4H, m, H$_{Phe}$), 7.53−7.71 (3H, m, NH$_2$, H$_{Phe}$), 7.74−7.83 (2H, m, 2 × H$_{vinyl}$), 8.18 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.9 (C-2′), 73.1 (C-3′), 85.3 (C-4′), 88.6 (C-1′), 98.5 (C-3a), 118.0 (C$_{vinyl}$), 127.4 (C$_{Phe}$), 128.0 (C$_{Phe}$), 128.3 (C$_{Phe}$), 128.6 (C$_{Phe}$), 132.2 (C$_{vinyl}$), 141.8 (C-3), 155.1 (C-7a), 155.9 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{18}H_{20}N_5O_4$ ([M + H]$^+$): 370.1515, found: 370.1509.

*3-Cyclopropyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**74**).* Compound **21** (0.120 g, 0.35 mmol) was subjected to a slightly modified general procedure E, using cyclopropylboronic acid as the coupling partner and Na_2CO_3 as the base. After 8 h, a second portion of cyclopropylboronic acid (1.5 equiv) was added, and the reaction was stirred for another 16 h, before LCMS analysis indicated full conversion. Purification *via* flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) afforded **74** (72 mg, 0.234 mmol, 67% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77−1.07 (4H, m, 2 × CH$_{2\ cyclopropyl}$), 2.39−2.48 (1H, m, CH$_{cyclopropyl}$), 3.40 (1H, dt, J = 12.0, 6.0 Hz, H-5′), 3.57 (1H, dt, J = 11.7, 4.7 Hz, H-5″), 3.87 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.20 (1H, dd, J = 10.0, 5.2 Hz, H-3′), 4.49 (1H, dd, J = 10.0, 5.0 Hz, H-2′), 4.81 (1H, dd, J = 6.6, 5.1 Hz, OH), 5.06 (1H, d, J = 5.9 Hz, OH), 5.30 (1H, d, J = 5.9 Hz, OH), 6.01 (1H, d, J = 4.4 Hz, H-1′), 7.41 (2H, br. s., NH$_2$), 8.15 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 7.7 (C$_{cyclopropyl}$), 7.9 (C$_{cyclopropyl}$), 8.5 (C$_{cyclopropyl}$), 62.4 (C-5′), 71.0 (C-2′), 73.2 (C-3′), 85.0 (C-4′), 88.4 (C-1′), 99.5 (C-3a), 147.0 (C-3), 154.9 (C-7a), 155.9 (C-6), 158.3 (C-4) ppm. HRMS (ESI): calcd for $C_{13}H_{18}N_5O_4$ ([M + H]$^+$): 308.1359, found: 308.1342.

*3-(4-Chlorophenyl)-4-amino-1-(3′-deoxy-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**76**).* Compound **23** (0.026 g, 0.079 mmol) was subjected to general procedure E, using 4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 0 → 10% MeOH in CH_2Cl_2 + 1% NH_4OH) afforded **76** (23 mg, 0.064 mmol, 81% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.00 (1H, ddd, J = 12.3, 6.2, 1.6 Hz, H-3′), 2.28−2.46 (1H, m, H-3″), 3.39−3.58 (2H, m, H-5′, H-5″), 4.29−4.41 (1H, m, H-2′), 4.62 (1H, br. s., H-4′), 4.75 (1H, t, J = 5.7 Hz, OH), 5.59 (1H, d, J = 3.8 Hz, OH), 6.24 (1H, s, H-1′), 7.61 (2H, d, J = 8.4 Hz, H$_{Phe}$), 7.67 (2H, d, J = 8.4 Hz, H$_{Phe}$), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 36.1 (C-3′), 64.1 (C-5′), 74.5 (C-2′), 81.2 (C-4′), 90.6 (C-1′), 97.5 (C-3a), 129.2 (C$_{Phe}$), 130.0 (C$_{Phe}$), 131.5 (C$_{Phe}$), 133.6 (C$_{Phe}$), 143.7 (C-3), 155.0 (C-7a), 156.1 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{17}ClN_5O_3$ ([M + H]$^+$): 362.1020, found: 362.0999.

*3-(4-Chlorophenyl)-4-amino-1-(2′-deoxy-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**77**).* Compound **23** (0.042 g, 0.127 mmol) was subjected to general procedure E, using 4-chlorophenylboronic acid as the coupling partner and Na_2CO_3 as the base. Purification *via* flash column chromatography (automated, 1 → 8% MeOH in CH_2Cl_2 + 1% NH_4OH) afforded **76** (28 mg, 0.098 mmol, 81% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (1H, ddd, J = 13.2, 6.8, 4.3 Hz, H-2′), 2.85 (1H, dt, J = 12.9, 6.0 Hz, H-2″), 3.40 (1H, dt, J = 11.7, 6.0 Hz, H-5′), 3.55 (1H, dt, J = 11.4, 5.5 Hz, H-5″), 3.84 (1H, td, J = 5.4, 3.6 Hz, H-4′), 4.42−4.51 (1H, m, H-3′), 4.77 (1H, t, J = 5.8 Hz, OH), 5.27 (1H, d, J = 4.6 Hz, OH), 6.64 (1H, t, J = 6.4 Hz, H-1′), 7.58−7.64 (2H, m, H$_{Phe}$), 7.65−7.72 (2H, m, H$_{Phe}$), 8.27 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 38.0 (C-2′), 62.4 (C-5′), 71.1 (C-3′), 83.9 (C-4′), 87.7 (C-1′), 97.8 (C-3a), 129.2 (C$_{Phe}$), 130.0 (C$_{Phe}$), 131.5 (C$_{Phe}$), 133.6 (C$_{Phe}$), 143.6 (C-3), 155.0 (C-7a), 156.0 (C-6), 158.1 (C-4) ppm. HRMS (ESI): calcd for $C_{16}H_{17}ClN_5O_3$ ([M + H]$^+$): 362.1020, found: 362.1004.

*3-Isopropyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**78**).* Compound **72** (0.060 g, 0.195 mmol) was dissolved in MeOH (5 mL). The flask was placed under a nitrogen atmosphere, and a catalytic amount of $Pd(OH)_2$/C was added. The atmosphere was exchanged for H_2, and the mixture was stirred for 1 h until TLC analysis (10% MeOH in CH_2Cl_2) indicated completion of the reaction. The mixture was filtered over celite, celite was added to the filtrate, and the solvents were removed under reduced pressure. The solid residue was purified *via* flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) to afford **78** (49 mg, 0.158 mmol, 81% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (6H, d, J = 6.7 Hz, CH(C$\underline{H}_3$)$_2$), 3.38−3.65 (3H, m, H-5′, H-5″, C$\underline{H}$(CH$_3$)$_2$), 3.90 (1H, dd, J = 9.4, 4.7 Hz, H-4′), 4.25 (1H, dd, J = 10.3, 5.0 Hz, H-3′), 4.56 (1H, dd, J = 10.0, 5.3 Hz, H-2′), 4.85 (1H, dd, J = 6.7, 5.0 Hz, OH), 5.07 (1H, d, J = 5.6 Hz, OH), 5.32 (1H, d, J = 5.9 Hz, OH), 6.06 (1H, d, J = 4.4 Hz, H-1′), 7.30 (2H, br. s, NH$_2$), 8.15 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.3 (CH($\underline{C}$H$_3$)$_2$), 27.4 ($\underline{C}$H(CH$_3$)$_2$), 63.0 (C-5′), 71.5 (C-2′), 73.7 (C-3′), 85.6 (C-4′), 89.1 (C-1′), 98.6 (C-3a), 151.6 (C-3), 155.5 (C-7a), 156.2 (C-6), 158.5 (C-4) ppm. HRMS (ESI): calcd for $C_{13}H_{20}N_5O_4$ ([M + H]$^+$): 310.1515, found: 310.1494.

*3-O-Phenyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**79**).* Compound **21** (0.220 g, 0.64 mmol, 1.0 equiv), phenol (0.090 g, 0.96 mmol, 1.5 equiv), CuI (0.024 g, 0.13 mmol, 0.2 equiv), *N*,*N*-dimethylglycine (0.040 g, 0.38 mmol, 0.6 equiv), and Cs_2CO_3 (0.417 g, 1.28 mmol, 2.0 equiv) were dissolved in dry degassed DMA (4 mL) under argon. The reaction was heated at 120 °C overnight, cooled down to room temperature, and concentrated *in vacuo*. The residue was taken up in MeOH, celite was added, and the mixture was concentrated under reduced pressure. The solid residue was purified by flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2) to afford **79** (12 mg, 0.033 mmol, 5% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.34−3.42 (1H, m, H-5′, underwater peak), 3.48 (1H, dd, J = 12.0, 4.4 Hz, H-5″), 3.84 (1H, dd, J = 10.3, 4.7 Hz, H-4′), 4.07 (1H, t, J = 4.8 Hz, H-3′), 4.43 (1H, t, J = 4.7 Hz, H-2′), 6.02 (1H, d, J = 4.1 Hz, H-1′), 7.14−7.26 (1H, m,

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H_{Phe}), 7.33−7.49 (4H, m, H_{Phe}), 8.22 (1H, br. s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.7 (C-2′), 73.1 (C-3′), 84.9 (C-4′), 88.1 (C-1′), 119.1 (C_{Phe}), 124.5 (C_{Phe}), 129.6 (C_{Phe}), 152.8 (C_{Phe}), 154.7 (C-7a), 155.0 (C-6), 157.4 (C-4), 157.4 (C-3) ppm. Two quaternary carbons missing (C-3, C-3a). HRMS (ESI): calcd for $C_{16}H_{18}N_5O_5$ ([M + H]$^+$): 360.1308, found: 360.1308.

*3-O-(4-Chlorophenyl)-4-amino1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**80**).* Compound **21** (0.220 g, 0.64 mmol, 1.0 equiv), 4-chlorophenol (0.123 g, 0.96 mmol, 1.5 equiv), CuI (0.024 g, 0.13 mmol, 0.2 equiv), *N*,*N*-dimethylglycine (0.040 g, 0.38 mmol, 0.6 equiv), and Cs_2CO_3 (0.417 g, 1.28 mmol, 2.0 equiv) were dissolved in dry degassed DMA (4 mL) under argon. The reaction was heated at 120 °C overnight, cooled down to room temperature, and concentrated *in vacuo*. The residue was taken up in MeOH, celite was added, and the mixture was concentrated under reduced pressure. The solid residue was purified by flash column chromatography (automated, 2 → 15% MeOH in CH_2Cl_2), followed by an additional purification *via* preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) to afford **80** (8 mg, 0.020 mmol, 3% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.34−3.42 (1H, m, H-5′), 3.46−3.54 (1H, m, H-5″.), 3.84 (1H, dd, J = 10.0, 5.3 Hz, H-4′), 4.02−4.16 (1H, m, H-3′), 4.31−4.52 (1H, m, H-2′), 4.64−4.75 (1H, m, OH), 4.96−5.18 (1H, m, OH), 5.26−5.50 (1H, m, OH), 6.02 (1H, d, J = 4.1 Hz, H-1′), 7.48 (4H, s, H_{Phe}), 8.21 (1H, s, H_{Phe}) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.1 (C-5′), 70.6 (C-2′), 73.0 (C-3′), 84.8 (C-4′), 88.0 (C-1′), 89.8 (C-3a), 120.9 (C_{Phe}), 128.2 ($_{CPhe}$), 129.3 (C_{Phe}), 152.3 (C_{Phe}), 153.4 (C-7a), 154.9 (C-6), 157.3 (C-4), 157.4 (C-3) ppm. HRMS (ESI): calcd for $C_{16}H_{17}ClN_5O_5$ ([M + H]$^+$): 394.0918, found: 394.0927.

*3,4-Diamino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine[69] (**81**).* A mixture of **21** (145 mg, 0.42 mmol, 1.0 equiv), CuCl (0.006 g, 0.042 mmol, 0.1 equiv), and aq. NH_4OH (20−30% wt, 30 mL) was heated in a pressure reactor at 130 °C overnight. The vessel was cooled to room temperature, and the contents were diluted with MeOH and transferred to a pear-shaped flask. Celite was added, and the mixture was concentrated *in vacuo* slowly (NH_3 evolution!). The solid residue was purified by flash column chromatography (4 → 20% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 40:60 in 12 min) to afford **81** (23 mg, 0.082 mmol, 19% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.32−3.45 (1H, m, H-5′), 3.54 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 3.81 (1H, dd, J = 9.4, 4.4 Hz, H-4′), 4.11 (1H, t, J = 4.8 Hz, H-3′), 4.44 (1H, t, J = 5.0 Hz, H-2′), 4.82 (1H, br. s, OH), 5.03 (1H, br. s, OH), 5.23 (1H, br. s, OH), 5.84 (2H, br. s, NH_2), 5.93 (1H, d, J = 4.7 Hz, H-1′), 7.29 (2H, br. s., NH_2), 8.02 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.7 (C-5′), 71.0 (C-2′), 72.6 (C-3′), 84.5 (C-4′), 87.4 (C-1′), 90.6 (C-3a), 148.1 (C-3), 155.0 (C-7a), 156.1 (C-6), 157.8 (C-4) ppm. Spectral data are in accordance with literature values.[69] HRMS (ESI): calcd for $C_{10}H_{15}N_6O_4$ ([M + H]$^+$): 283.1155, found: 283.1179.

*3-N-Methylamino-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**82**).* A mixture of **21** (150 mg, 0.43 mmol, 1.0 equiv), CuCl (0.006 g, 0.043 mmol, 0.1 equiv), and aqueous methylamine (40%, 30 mL) was heated in a pressure reactor at 130 °C over the weekend. The vessel was cooled to room temperature, and the contents were diluted with MeOH and transferred to a pear-shaped flask. Celite was added, and the mixture was concentrated *in vacuo* slowly (NHMe evolution!). The solid residue was purified by flash column chromatography (4 → 20% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) to afford **82** (6 mg, 0.020 mmol, 5% yield) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 2.85 (3H, s, CH_3), 3.76 (1H, dd, J = 12.6, 4.1 Hz, H-5′), 3.82−3.92 (1H, m, H-5″), 4.17 (1H, dd, J = 7.0, 3.8 Hz, H-4′), 4.45 (1H, dd, J = 5.0, 3.8 Hz, H-3′), 4.71−4.80 (1H, m, H-2′, partially underwater peak), 6.07 (1H, d, J = 5.0 Hz, H-1′), 8.04 (1H, s, H-6) ppm. A qualitative ^{13}C NMR spectrum could not be obtained from the amount of product available. HRMS (ESI): calcd for $C_{11}H_{17}N_6O_4$ ([M + H]$^+$): 297.1311, found: 297.1318.

*3-(1-Pyrrolidin-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**83**).* A mixture of **21** (150 mg, 0.43 mmol, 1.0 equiv), CuCl (0.006 g, 0.043 mmol, 0.1 equiv), pyrrolidine (0.706 mL, 8.60 mmol, 20.0 equiv) in 1,4-dioxane (1.5 mL) and H_2O (3 mL) was heated in at 120 °C over the weekend. The mixture was cooled to room temperature and diluted with MeOH. Celite was added, and the mixture was concentrated *in vacuo*. The solid residue was purified by flash column chromatography (4 → 20% MeOH in CH_2Cl_2), followed by an additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) to afford **83** (23 mg, 0.068 mmol, 16% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83−1.96 (4H, m, 2 × $CH_{2\ pyrrolidine}$), 3.29−3.40 (4H, m, 2 × $CH_{2\ pyrrolidine}$), 3.45 (1H, dd, J = 11.7, 5.6 Hz, H-5′), 3.59 (1H, dd, J = 11.7, 4.1 Hz, H-5″), 3.87 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.21 (1H, t, J = 4.8 Hz, H-3′), 4.50 (1H, t, J = 4.5 Hz, H-2′), 4.81 (1H, br. s, OH), 5.02 (1H, br. s, OH), 5.28 (1H, br. s, OH), 6.02 (1H, d, J = 4.4 Hz, H-1′), 6.98 (2H, br. s, NH_2), 8.09 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.7 (2 × $C_{pyrrolidine}$), 50.3 (2 × $C_{pyrrolidine}$), 62.6 (C-5′), 71.1 (C-2′), 73.1 (C-3′), 84.9 (C-4′), 87.9 (C-1′), 92.1 (C-3a), 150.7 (C-3), 155.4 (C-7a), 155.8 (C-6), 157.8 (C-4) ppm. HRMS (ESI): calcd for $C_{14}H_{21}N_6O_4$ ([M + H]$^+$): 337.1624, found: 337.1630.

*3-Chloro-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**84**).* Compound **18** (0.223 g, 0.339 mmol, 1.0 equiv), Cu_2O (0.012 g, 0.085 mmol, 0.25 equiv), Me_4NCl (0.111 g, 1.02 mmol, 3.0 equiv), and L-proline (0.020 g, 0.17 mmol, 0.5 equiv) were suspended in dry degassed 2-methoxy-ethanol (2 mL) under argon. The mixture was heated at 120 °C for 7 days until LCMS analysis indicated ∼80% conversion and further progression had ceased. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was used crude in the next reaction. HRMS (ESI): calcd for $C_{31}H_{25}ClN_5O_7$ ([M + H]$^+$): 614.1443, found: 614.2593.

*3-Chloro-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**85**).* The crude **84** from the previous reaction was subjected to general procedure C (reaction time: 1 h). Flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2), followed by an additional purification *via* preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 88:12 in 4 min, then to 77:23 in 5 min, and then to 33:67 in 3 min) afforded **85** (35 mg, 0.116 mmol, 34% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.42 (1H, dt, J = 12.0, 5.9 Hz, H-5′), 3.55 (1H, dt, J = 11.7, 5.0 Hz, H-5″), 3.89 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.16 (1H, dd, J = 9.7, 4.7 Hz, H-3′), 4.54 (1H, dd, J = 10.5, 5.3 Hz, H-2′), 4.80 (1H, t, J = 5.7 Hz, OH), 5.16 (1H, d, J = 5.3 Hz, OH), 5.40 (1H, d, J = 5.9 Hz, OH), 6.05 (1H, d, J = 4.7 Hz, OH), 7.23 (1H, br. s, NH), 8.03 (1H, s, NH), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.6 (C-2′), 72.9 (C-3′), 85.2 (C-4′), 88.0 (C-1′), 97.5 (C-3a), 132.0 (C-3), 155.1 (C-7a), 157.2 (C-6), 157.3 (C-4) ppm. HRMS (ESI): calcd for $C_{10}H_{13}ClN_5O_4$ ([M + H]$^+$): 302.0656, found: 302.0652.

*3-Phenylethynyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**86**).* Compound **36** (0.250 g, 0.636 mmol, 1.0 equiv), $PdCl_2(PPh_3)_2$ (0.022 g, 0.032 mmol, 0.05 equiv), and CuI (0.012 g, 0.1 equiv) were added to a 10 mL round-bottom flask. The flask was evacuated and backfilled with argon three times. Then, anhydrous, degassed DMF (2 mL), Et_3N (0.5 mL), and phenylacetylene (0.105 mL, 0.96 mmol, 1.5 equiv) were added. The resulting solution was stirred at room temperature for 3 h until LCMS analysis indicated completion of the reaction. The mixture was concentrated *in vacuo*, and the residue was taken up in MeOH, adsorbed onto celite, and purified *via* flash column chromatography (automated, 2 → 12% MeOH in CH_2Cl_2) to afford **86** (93 mg, 0.253 mmol, 40% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46 (1H, dt, J = 11.8, 6.0 Hz, H-5′), 3.59 (1H, dt, J = 11.9, 4.9 Hz, H-5″), 3.93 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.21 (1H, dd, J = 9.4, 5.0 Hz, H-3′), 4.63 (1H, dd, J = 10.8, 5.0 Hz, H-2′), 4.86 (1H, t, J = 5.9 Hz, OH), 5.18 (1H, d, J = 5.6 Hz, OH), 5.43 (1H, d, J = 6.2 Hz, OH), 6.13 (1H, d, J = 5.0 Hz, H-1′), 7.27−7.55 (3H, m, H_{Phe}), 7.67−7.84 (2H, m, H_{Phe}), 8.28 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.7 (C-5′), 71.2 (C-2′), 73.5 (C-3′), 81.1 ($C_{ethynyl}$), 85.8 (C-4′), 88.9 (C-1′), 94.1

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($C_{ethynyl}$), 101.2 (C-3a), 121.5 (C-3), 127.3 (C_{Phe}), 129.1 (C_{Phe}), 130.1 (C_{Phe}), 132.4 (C_{Phe}), 154.7 (C-7a), 157.2 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{18}H_{18}N_5O_4$ ($[M + H]^+$): 368.1359, found: 368.1355.

*3-Phenylethyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**87**).* Compound **86** (0.051 g, 0.139 mmol) was dissolved in MeOH (5 mL). The flask was placed under a nitrogen atmosphere, and a catalytic amount of $Pd(OH)_2/C$ was added. The atmosphere was exchanged for H_2 and the mixture was stirred for 1 h until TLC analysis (20% MeOH in CH_2Cl_2) indicated completion of the reaction. The mixture was filtered over celite, celite was added to the filtrate, and the solvents were removed under reduced pressure. The solid residue was purified *via* flash column chromatography (automated, 2 → 20% MeOH in CH_2Cl_2) to afford **87** (46 mg, 0.124 mmol, 89% yield) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 3.02 (2H, dd, J = 8.6, 7.0 Hz, CH_2), 3.28 (2H, dd, J = 9.2, 7.0 Hz, CH_2), 3.42 (1H, dt, J = 12.2, 6.3 Hz, H-5′), 3.58 (1H, dt, J = 11.7, 4.7 Hz, H-5″), 3.89 (1H, dd, J = 10.3, 4.6 Hz, H-4′), 4.22 (1H, dd, J = 10.5, 5.0 Hz, H-3′), 4.54 (1H, dd, J = 10.3, 5.0 Hz, H-2′), 4.83 (1H, dd, J = 6.4, 5.3 Hz, OH), 5.08 (1H, d, J = 5.6 Hz, OH), 5.31 (1H, d, J = 5.9 Hz, OH), 6.06 (1H, d, J = 4.4 Hz, H-1′), 7.15−7.32 (5H, m, H_{Phe}), 8.16 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.4 (CH_2), 33.4 (CH_2), 62.6 (C-5′), 71.0 (C-2′), 73.2 (C-3′), 85.0 (C-4′), 88.3 (C-1′), 98.8 (C-3a), 125.8 (C_{Phe}), 128.1 (C_{Phe}), 128.5 (C_{Phe}), 141.1 (C_{Phe}), 145.2 (C-3), 155.0 (C-7a), 155.8 (C-6), 158.3 (C-4) ppm. HRMS (ESI): calcd for $C_{18}H_{22}N_5O_4$ ($[M + H]^+$): 372.1672, found: 372.1684.

*3-Ethynyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**88**).* Compound **36** (0.250 g, 0.636 mmol, 1.0 equiv), $PdCl_2(PPh)_3$ (0.022 g, 0.032 mmol, 0.05 equiv), and CuI (0.012 g, 0.1 equiv) were added to a 10 mL round-bottom flask. The flask was evacuated and backfilled with argon three times. Then, anhydrous, degassed DMF (2 mL), Et_3N (0.5 mL), and ethynyltrimethylsilane (0.881 mL, 6.36 mmol, 10 equiv) were added. The resulting solution was stirred at room temperature for 3 h until LCMS analysis indicated completion of the reaction. The mixture was concentrated *in vacuo*, and the residue was taken up in MeOH, adsorbed onto celite, and purified *via* flash column chromatography (automated, 2 → 12% MeOH in CH_2Cl_2). The intermediate TMS-ethynyl nucleoside was stirred overnight in 7 N NH_3 in MeOH (10 mL). The mixture was concentrated *in vacuo*, and the residue was purified again by flash column chromatography (automated, 2 → 12% MeOH in CH_2Cl_2) to afford **88** (35 mg, 0.120 mmol, 19% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.43 (1H, dt, J = 11.7, 6.0 Hz, H-5′), 3.57 (1H, dt, J = 11.7, 5.0 Hz, H-5″), 3.91 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.19 (1H, dd, J = 9.7, 4.7 Hz, H-3′), 4.57 (1H, dd, J = 10.7, 5.2 Hz, H-2′), 4.70 (1H, s, $H_{ethynyl}$), 4.83 (1H, t, J = 5.9 Hz, OH), 5.16 (1H, d, J = 5.3 Hz, OH), 5.41 (1H, d, J = 6.2 Hz, OH), 6.09 (1H, d, J = 4.7 Hz, H-1′), 6.79 (2H, br. s, NH_2), 8.25 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.2 (C-5′), 70.7 (C-2′), 73.0 (C-3′), 74.9 ($C_{ethynyl}$), 85.4 (C-4′), 86.7 ($C_{ethynyl}$), 88.4 (C-1′), 101.1 (C-3a), 126.3 (C-3), 154.0 (C-7a), 156.8 (C-6), 157.7 (C-4) ppm. HRMS (ESI): calcd for $C_{12}H_{14}N_5O_4$ ($[M + H]^+$): 292.1046, found: 292.1049.

*3-Ethyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**89**).* Compound **88** (47 mg, 0.161 mmol) was dissolved in MeOH (5 mL). The flask was placed under a nitrogen atmosphere, and a catalytic amount of $Pd(OH)_2/C$ was added. The atmosphere was exchanged for H_2 and the mixture was stirred for 1 h until TLC analysis (20% MeOH in CH_2Cl_2) indicated completion of the reaction. The mixture was filtered over celite, celite was added to the filtrate, and the solvents were removed under reduced pressure. The solid residue was purified *via* flash column chromatography (automated, 2 → 20% MeOH in CH_2Cl_2) to afford **87** (35 mg, 0.119 mmol, 74% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (3H, t, J = 7.5 Hz, $CH_2C\underline{H_3}$), 2.96 (2H, q, J = 7.5 Hz, $C\underline{H_2}CH_3$), 3.43 (1H, dt, J = 12.0, 6.0 Hz, H-5′), 3.59 (1H, dt, J = 12.0, 4.7 Hz, H-5″), 3.89 (1H, dd, J = 9.7, 4.5 Hz, H-4′), 4.21 (1H, dd, J = 10.3, 5.3 Hz, H-3′), 4.57 (1H, dd, J = 10.0, 5.0 Hz, H-2′), 4.86 (1H, t, J = 6.0 Hz, OH), 5.08 (1H, d, J = 5.6 Hz, OH), 5.30 (1H, d, J = 5.9 Hz, OH), 6.04 (1H, d, J = 4.7 Hz, H-1′), 7.32 (2H, br. s., NH_2), 8.15 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.0 ($CH_2\underline{C}H_3$), 21.4 ($\underline{C}H_2CH_3$), 62.5 (C-5′), 70.9 (C-2′), 73.0 (C-3′), 85.0 (C-4′), 88.3 (C-1′), 98.6 (C-3a), 147.2 (C-3), 155.1 (C-7a), 155.9 (C-6), 158.2 (C-4) ppm. HRMS (ESI): calcd for $C_{12}H_{18}N_5O_4$ ($[M + H]^+$): 296.1359, found: 296.1363.

*3-(1H-1,2,3-Triazol-4-yl)-4-amino-1-β-D-ribofuranosylpyrazolo-[3,4-d]pyrimidine (**90**).* Compound **88** (0.125 g, 0.429 mmol, 1.0 equiv) and CuI (0.004 g, 0.021 mmol, 0.05 equiv) were dissolved in MeOH (0.2 mL) and DMF (1.8 mL). $TMSN_3$ (0.085 mL, 0.644 mmol, 1.5 equiv) was added, and the mixture was heated at 100 °C overnight. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was dissolved in MeOH, celite was added, and the solvents were removed under reduced pressure. The solid residue was purified by flash column chromatography (4 → 20% MeOH in CH_2Cl_2) to afford **90** (57 mg, 0.170 mmol, 40% yield) as a light-brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 (1H, dt, J = 12.0, 5.6 Hz, H-5′), 3.62 (1H, dt, J = 12.0, 4.7 Hz, H-5″), 3.93 (1H, dd, J = 9.4, 4.6 Hz, H-4′), 4.26 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.66 (1H, dd, J = 10.3, 5.2 Hz, H-2′), 4.88 (1H, t, J = 5.6 Hz, OH), 5.13 (1H, d, J = 5.6 Hz, OH), 5.43 (1H, d, J = 5.9 Hz, OH), 6.14 (1H, d, J = 5.0 Hz, H-1′), 8.14 (1H, br. s., $CH_{triazole}$), 8.25 (1H, s, H-6), 8.54 (1H, br. s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.9 (C-2′), 73.0 (C-3′), 85.4 (C-4′), 88.8 (C-), 98.0 (C-3a), 131.1 ($C_{triazole}$), 136.3 (C-3), 140.3 ($C_{triazole}$), 155.1 (C-7a), 156.7 (C-6), 158.4 (C-4) ppm. HRMS (ESI): calcd for $C_{12}H_{15}N_8O_4$ ($[M + H]^+$): 335.1216, found: 335.1205.

3-Cyano-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine[66] *(**91**).* Compound **36** (2.73 g, 6.95 mmol, 1.0 equiv), $Pd_2(dba)_3$ (0.382 g, 0.417 mmol, 0.06 equiv), dppf (0.771 g, 1.39 mmol, 0.2 equiv), and $Zn(CN)_2$ (0.490 g, 4.17 mmol, 0.6 equiv) were dissolved in dry degassed DMF (30 mL) in a flame-dried flask under argon. The mixture was stirred at 150 °C for 90 min until LCMS analysis indicated completion of the reaction. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was taken up in MeOH, celite was added, and the solvents were removed under reduced pressure. The solid residue was purified first by manual flash column chromatography (5 → 20% MeOH in CH_2Cl_2) and then by automated flash column chromatography (4 → 20% MeOH in CH_2Cl_2) to afford **91** (570 mg, 1.95 mmol, 28% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.45 (1H, dt, J = 11.7, 5.9 Hz, H-5′), 3.58 (1H, dt, J = 12.0, 5.0 Hz, H-5″), 3.95 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.22 (1H, dd, J = 9.7, 5.0 Hz, H-3′), 4.60 (1H, dd, J = 10.3, 5.2 Hz, H-2′), 4.82 (1H, t, J = 5.9 Hz, OH), 5.23 (1H, d, J = 5.6 Hz, OH), 5.50 (1H, d, J = 5.9 Hz, OH), 6.17 (1H, d, J = 5.0 Hz, H-1′), 8.35 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.0 (C-5′), 70.6 (C-2′), 73.3 (C-3′), 85.8 (C-4′), 89.1 (C-1′), 101.2 (C-3), 113.0 (CN), 116.9 (C-3a), 154.4 (C-7a), 157.1 (C-6), 157.4 (C-4) ppm. HRMS (ESI): calcd for $C_{11}H_{13}N_6O_4$ ($[M + H]^+$): 293.0998, found: 293.1002. Spectral data are in accordance with literature values.[66]

*3-(1H-Tetrazol-5-yl)-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**92**).* Compound **91** (0.074 g, 0.253 mmol), NaN_3 (0.021 g, 0.329 mmol, 1.3 equiv), NH_4Cl (0.018 g, 0.329 mmol, 1.3 equiv), and a catalytic amount of LiCl were suspended in DMF (3 mL). The mixture was heated at 100 °C overnight, cooled down to room temperature, and concentrated *in vacuo*. The residue was taken up in MeOH, adsorbed onto celite, and purified by flash column chromatography (2 → 40% MeOH in CH_2Cl_2 + 0.1% AcOH) to afford **92** (21 mg, 0.063 mmol, 25% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.50 (1H, dd, J = 11.7, 6.2 Hz, H-5′), 3.65 (1H, dd, J = 11.9, 4.5 Hz, H-5″), 3.97 (1H, dd, J = 10.3, 5.0 Hz, H-4′), 4.34 (1H, t, J = 4.8 Hz, H-3′), 4.70 (1H, t, J = 4.8 Hz, H-2′), 6.20 (1H, d, J = 4.4 Hz, H-1′), 8.34 (1H, s, H-6), 8.45 (1H, br. s., $H_{tetrazole}$), 9.12 (1H, br. s., $NH_{tetrazole}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.4 (C-5′), 70.7 (C-2′), 73.1 (C-3′), 85.7 (C-4′), 89.1 (C-1′), 98.6 (C-3a), 131.3 (C-3), 150.4 ($C_{tetrazole}$), 155.0 (C-7a), 156.4 (C-6), 157.5 (C-4) ppm. HRMS (ESI): calcd for $C_{11}H_{14}N_9O_4$ ($[M + H]^+$): 336.1169, found: 336.1176.

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*3-Aminomethyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**93**).* **88** (47 mg, 0.161 mmol) was dissolved in MeOH (5 mL). The flask was placed under a nitrogen atmosphere, and Raney nickel (slurry in H_2O, 1 mL) was added. The atmosphere was exchanged for H_2 and the mixture was stirred for 1 h until TLC analysis (20% MeOH in CH_2Cl_2) indicated completion of the reaction. The mixture was filtered over celite, celite was added to the filtrate, and the solvents were removed under reduced pressure. The solid residue was purified *via* flash column chromatography (automated, NH_4OH/MeOH/CH_2Cl_2 1/0/99 → 1/25/74), followed by an additional purification *via* preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 95:05 to 68:32 in 6 min) to afford **93** (5 mg, 0.017 mmol, 10% yield) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 2.66 (2H, s, C$\underline{H}_2$NH$_2$), 3.69 (1H, dd, J = 12.3, 4.4 Hz, H-5′), 3.81 (1H, dd, J = 12.3, 3.2 Hz, H-5″), 4.10 (1H, dd, J = 7.9, 3.5 Hz, H-4′), 4.38−4.51 (1H, m, H-3′), 4.73−4.80 (1H, m, H-2′), 6.23 (1H, d, J = 4.7 Hz, H-1′), 8.20 (1H, s, H-6), 8.50 (4H, br. s., 2 × NH_2) ppm. A qualitative ^{13}C NMR spectrum could not be obtained from the amount of product available. HRMS (ESI): calcd for $C_{11}H_{17}N_6O_4$ ([M + H]$^+$): 297.1311, found: 297.1314.

*3-Carboxamido-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine[66] (**94**).* Compound **91** (55 mg, 0.188 mmol) was dissolved in aq. NH_4OH (28−30% wt 4 mL). Aq. H_2O_2 (30% w/w, 1 mL) was added, and the mixture was stirred for 2 h until LCMS analysis indicated full conversion. The residue was diluted with MeOH, celite was added, and the solvents were removed under reduced pressure. The solid residue was purified by flash column chromatography (automated, 2 → 40% MeOH in CH_2Cl_2 + 0.1% AcOH) to afford **92** (21 mg, 0.063 mmol, 25% yield) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.47 (1H, dt, J = 12.0, 6.2 Hz, H-5′), 3.62 (1H, dt, J = 12.0, 5.3 Hz, H-5″), 3.93 (1H, dd, J = 10.3, 4.7 Hz, H-4′), 4.30 (1H, dd, J = 10.3, 4.7 Hz, H-3′), 4.68 (1H, dd, J = 10.5, 5.3 Hz, H-2′), 4.84 (1H, t, J = 5.9 Hz, OH), 5.08 (1H, d, J = 5.6 Hz, OH), 5.44 (1H, d, J = 5.9 Hz, OH), 6.14 (1H, d, J = 4.7 Hz, H-1′), 7.99 (1H, s, NH_2), 8.07 (1H, br. s., NH_2), 8.19 (1H, br. s., NH_2), 8.24 (1H, s, H-6), 8.86 (1H, br. s., NH_2) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.3 (C-5′), 70.8 (C-2′), 73.1 (C-3′), 85.7 (C-4′), 88.9 (C-1′), 99.5 (C-3a), 139.0 (C-3), 155.4 (C-7a), 156.9 (C-6), 158.2 (C-4), 163.8 (C═O) ppm. HRMS (ESI): calcd for $C_{11}H_{15}N_6O_5$ ([M + H]$^+$): 311.1104, found: 311.1110. Spectral data are in accordance with literature values.[66]

*3-Trifluoromethyl-4-amino-1-(2′,3′,5′-tri-O-benzoyl)-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (**95**).* $TMSCF_3$ (0.315 mL, 2.13 mmol, 3.0 equiv) was added dropwise over the course of 1 h to a suspension of CuI (0.406 g, 2.13 mmol, 3.0 equiv) and KF (0.124 g, 2.13 mmol, 3.0 equiv) in a mixture of dry degassed DMF/NMP 1:1 (3 mL). When all solids had dissolved, **34** (0.500 g, 0.709 mmol, 1.0 equiv) in dry degassed DMF/NMP 1:1 (3 mL) was added, and the mixture was heated to reflux. After 3 h, LC/MS analysis showed full conversion of the starting material, and the reaction was cooled to room temperature. The mixture was diluted with EtOAc (15 mL) and water (5 mL), and the solids were filtered off over Celite. The filter cake was washed extensively with additional EtOAc (3 × 25 mL), and the combined filtrates were transferred to a separation funnel. Additional water (40 mL) was added, the phases were separated, and the organic phase was washed twice more with water (25 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was used as such in the next reaction. HRMS (ESI): calcd for $C_{32}H_{25}F_3N_5O_7$ ([M + H]$^+$): 648.1706, found: 648.1723.

*3-Trifluoromethyl-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**96**).* Crude **95** was subjected to general procedure C (reaction time: 1 h). Purification by flash column chromatography (4 → 20% MeOH in CH_2Cl_2), followed by additional purification by preparative RP-HPLC (0.2% formic acid in H_2O/MeCN 98:02 to 33:67 in 12 min) afforded **96** (33 mg, 0.098 mmol, 14% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.44 (1H, dt, J = 12.0, 5.6 Hz, H-5′), 3.58 (1H, dt, J = 12.0, 4.7 Hz, H-5″), 3.94 (1H, dd, J = 9.7, 5.0 Hz, H-4′), 4.22 (1H, dd, J = 8.5, 4.1 Hz, H-3′), 4.61 (1H, dd, J = 9.4, 4.7 Hz, H-2′), 4.83 (1H, t, J = 5.6 Hz, OH), 5.23 (1H, d, J = 5.0 Hz, OH), 5.48 (1H, d, J = 5.6 Hz, OH), 6.18 (1H, d, J = 4.7 Hz, H-1′), 8.36 (1H, s, H-6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 62.1 (C-5′), 70.7 (C-2′), 73.1 (C-3′), 85.7 (C-4′), 88.8 (C-1′), 96.6 (C-3a), 120.7 (q, J = 269.0 Hz, CF_3), 132.8 (q, J = 38.5 Hz, C-3), 155.6 (C-7a), 156.7 (C-6), 157.2 (C-4). ^{19}F NMR (282 MHz, DMSO-d_6) δ −59.61 (1F, s) ppm. HRMS (ESI): calcd for $C_{11}H_{13}F_3N_5O_4$ ([M + H]$^+$): 336.0920, found: 336.0917.

*5-Amino-3-methyl-1H-pyrazole-4-carbonitrile[98] (**97**).* A solution of malonitrile (1.11 mL, 20.0 mmol, 1.0 equiv) in THF (20 mL) was cooled to 0 °C. NaH (60% wt in mineral oil, 1.60 g, 40.0 mmol, 2.0 equiv) was added slowly, and the mixture was stirred for 10 min. Acetyl chloride (1.43 mL, 20.0 mmol, 1.0 equiv) was added, and the mixture was gradually warmed to room temperature. After 1 h, dimethyl sulfate (2.28 mL, 24.0 mmol, 1.2 equiv) was added and the mixture was heated to reflux. After 3 h, the mixture was cooled down to room temperature, and Et_3N (6.97 mL, 50.0 mmol, 2.0 equiv) was added, followed by hydrazine hydrate (1.0 mL, 20.0 mmol, 1.0 equiv). The mixture was again heated at reflux temperature for 1 h and cooled down to room temperature. The mixture was concentrated *in vacuo*, diluted with H_2O and EtOAc, and transferred to a separation funnel. The phases were separated, and the aqueous phase was extracted twice more with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in MeCN, celite was added, and the solvent was removed under reduced pressure. The solid residue was purified by flash column chromatography (manual, petroleum ether/EtOAc 1:1 and 3:7) to afford **97** (0.930 g, 7.61 mmol, 38% yield) as a yellow sticky foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.12 (3H, br. s.), 5.72 (2H, br. s), 11.51 (1H, br. s) ppm. HRMS (ESI): calcd for $C_5H_7N_4$ ([M + H]$^+$): 123.0671, found: 123.0662. Spectral data are in accordance with literature values.[98]

*3-Methyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine[99] (**98**).* Compound **97** (0.441 g, 3.61 mmol, 1.0 equiv) was dissolved in formamide (2 mL). The mixture was heated at 180 °C for 24 h and cooled down to room temperature by pouring into ice-cold water (25 mL). The resulting solids were filtered off and dried under a high vacuum overnight to afford **98** (126 mg, 0.84 mmol, 23% yield) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.52 (3H, s, CH_3), 7.15 (2H, br. s., NH_2), 8.09 (1H, s, H-6), 12.91 (1H, br. s., NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.9 (CH_3), 98.8 (C-3a), 141.3 (C-3), 156.1 (C-7a), 156.3 (C-6), 158.8 (C-4) ppm. HRMS (ESI): calcd for $C_6H_8N_5$ ([M + H]$^+$): 150.0780, found: 150.0768. Spectral data are in accordance with literature values.[99]

*3,6-Dimethyl-4-amino-1H-pyrazolo[3,4-d]pyrimidine (**99**).* Compound **97** (0.206 g, 1.69 mmol, 1.0 equiv) was dissolved in 2-methoxy-ethanol (3 mL). Thioacetamide (0.253 g, 3.37 mmol, 2.0 equiv) was added, and the mixture was heated at reflux overnight. The mixture was cooled down to room temperature, and the solvent was removed *in vacuo*. The residue was taken up in MeOH, adsorbed onto celite, and purified by flash column chromatography (manual, 10% MeOH in CH_2Cl_2) to afford **99** (100 mg, 0.61 mmol, 36% yield) as a light-purple solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.06 (3H, s, CH_3), 2.36 (3H, s, CH_3), 7.14 (2H, br. s, NH_2), 12.80 (1H, br. s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6 (CH_3) 21.8 (CH_3), 97.0 (C-3a), 141.1 (C-3), 157.3 (C-7a), 158.5 (C-4), 164.9 (C-6) ppm. HRMS (ESI): calcd for $C_7H_{10}N_5$ ([M + H]$^+$): 164.0936, found: 164.0914.

*3-Methyl-4-amino-1-(2′,3′,5′-tri-O-benzoyl-1-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**100**).* Compound **98** (0.100 g, 0.67 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.406 g, 0.8 mmol, 1.2 equiv) were subjected to general procedure B. Purification by flash column chromatography (automated, 0 → 5% MeOH in CH_2Cl_2) afforded **100**, which was used as such in the next reaction. HRMS (ESI): calcd for $C_{32}H_{28}N_5O_7$ ([M + H]$^+$): 594.1989, found: 594.2010.

*3-Methyl-4-amino-1-β-D-ribofuranosyl-pyrazolo[3,4-d]-pyrimidine (**101**).* Compound **98** (used directly from the previous reaction) was subjected to general procedure C (reaction time: 1 h). Purification by flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) afforded **101** (40 mg, 0.142 mmol, 21% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.53 (3H,

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s, CH_3), 3.42 (1H, dt, J = 11.9, 6.1 Hz, H-5′), 3.56 (1H, dt, J = 11.4, 4.7 Hz, H-5″), 3.87 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.17 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.56 (1H, dd, J = 10.5, 5.3 Hz, H-2′), 4.86 (1H, t, J = 5.9 Hz, OH), 5.10 (1H, d, J = 5.3 Hz, OH), 5.30 (1H, d, J = 5.9 Hz, OH), 6.02 (1H, d, J = 4.7 Hz, H-1′), 7.35 (2H, br. s., NH_2), 8.14 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5 (CH_3), 62.4 (C-5′), 70.8 (C-2′), 72.8 (C-3′), 84.9 (C-4′), 88.0 (C-1′), 99.3 (C-3a), 141.9 (C-3), 155.1 (C-7a), 156.0 (C-6), 158.4 (C-4) ppm. HRMS (ESI): calcd for $C_{11}H_{16}N_5O_4$ ([M + H]$^+$): 282.1202, found: 282.1216.

*3-Vinyl-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-d]pyrimidine (**102**).* Compound **34** (5.66 g, 8.03 mmol, 1.0 equiv), $Pd(OAc)_2$ (0.090 g, 0.40 mmol, 0.05 equiv), PPh_3 (0.316 g, 1.20 mmol, 0.15 equiv), potassium vinyl trifluoroborate (2.92 g, 10.0 mmol, 1.25 equiv), and Cs_2CO_3 (7.85 g, 24.1 mmol, 3.0 equiv) were dissolved in degassed DMF/H_2O 9:1 (20 mL) under argon. The mixture was stirred at 100 °C for 72 h, cooled down to room temperature, and transferred to a separation funnel. Water (50 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (automated, 40 → 100% EtOAc in petroleum ether) to afford **102** (1.85 g, 3.05 mmol, 38% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.64 (1H, dd, J = 11.7, 4.7 Hz, H-5′), 4.73−4.89 (2H, m, H-5″, H-4′), 5.66 (1H, dd, J = 11.1, 1.2 Hz, HC=C$\underline{H}_2$), 5.98 (1H, dd, J = 17.7, 1.2 Hz, HC=C$\underline{H}_2$), 6.10 (2H, br. s, NH_2), 6.34 (1H, t, J = 5.7 Hz, H-3′), 6.44 (1H, dd, J = 5.3, 3.2 Hz, H-2′), 6.87 (1H, d, J = 3.2 Hz, H-1′), 6.87 (1H, dd, J = 17.9, 11.4 Hz, $\underline{H}$C=CH$_2$), 7.30−7.45 (6H, m, H_{Bz}), 7.48−7.61 (3H, m, H_{Bz}), 7.92−8.03 (4H, m, H_{Bz}), 8.05−8.14 (2H, m, H_{Bz}), 8.35 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.0 (C-5′), 72.0 (C-2′), 74.5 (C-3′), 80.0 (C-4′), 86.7 (C-1′), 98.9 (C-3a), 121.8 (HC=$\underline{C}$H$_2$), 128.2 (H$\underline{C}$=CH$_2$), 128.3 (C_{Bz}), 128.4 (C_{Bz}), 128.4 (C_{Bz}), 128.8 (C_{Bz}), 128.9 (C_{Bz}), 129.6 (C_{Bz}), 129.8 (C_{Bz}), 129.8 (C_{Bz}), 133.0 (C_{Bz}), 133.5 (C_{Bz}), 133.6 (C_{Bz}), 144.3 (C-3), 155.4 (C-7a), 155.4 (C-6), 157.5 (C-4), 165.1 (C=O), 165.3 (C=O), 166.2 (C=O) ppm. HRMS (ESI): calcd for $C_{33}H_{28}N_5O_7$ ([M + H]$^+$): 606.1989, found: 606.1978.

*4-Amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidine-3-carbaldehyde (**103**).* Compound **102** (1.53 g, 2.52 mmol, 1.0 equiv) and $K_2OsO_4{\cdot}2H_2O$ (0.019 g, 0.05 mmol, 0.02 equiv) were dissolved in a mixture of dioxane (18.9 mL, 7.5 mL/mmol) and water (6.3 mL, 2.5 mL/mmol). 2.6-Lutidine (0.584 mL, 5.04 mmol, 2.0 equiv) and $NaIO_4$ (2.16 g, 10.1 mmol, 4.0 equiv) were added, and the mixture was stirred for 3 h until TLC analysis (petroleum ether/EtOAc 50:50) indicated full conversion. Aq. sat. Na_2SO_3 (25 mL) was added, and the mixture was stirred for 1 more hour before it was transferred to a separation funnel. The mixture was extracted with CH_2Cl_2 (3 × 60 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (automated, 15 → 70% EtOAc in petroleum ether) to afford 103 (0.731 g, 1.20 mmol, 48% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.62 (1H, dd, J = 13.2, 5.3 Hz, H-5′), 4.85−4.94 (2H, m, H-5″, H-4′), 6.32 (1H, t, J = 5.6 Hz, H-3′), 6.49 (1H, dd, J = 5.4, 3.7 Hz, H-2′), 6.58 (1H, br. s, NH_2), 6.91 (1H, d, J = 3.5 Hz, H-1′), 7.34−7.47 (6H, m, H_{Bz}), 7.52−7.63 (3H, m, H_{Bz}), 7.90 (1H, br. s., NH_2), 7.94−8.05 (4H, m, H_{Bz}), 8.05−8.16 (2H, m, H_{Bz}), 8.40 (1H, s, H-6), 9.70 (1H, s, C$\underline{H}$O) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.3 (C-5′), 71.8 (C-2′), 74.3 (C-3′), 80.8 (C-4′), 87.3 (C-1′), 99.4 (C-3a), 128.4 (C_{Phe}), 128.5 (C_{Phe}) (C_{Phe}), 129.6 (C_{Phe}), 129.8 (C_{Phe}), 129.8 (C_{Phe}), 133.3 (C_{Phe}), 133.7 (C_{Phe}), 133.8 (C_{Phe}), 144.9 (C-3), 156.1 (C-7a), 156.7 (C-6), 157.5 (C-4), 165.1 (C=O), 165.3 (C=O), 166.0 (C=O), 187.9 (C$\underline{H}$O) ppm. HRMS (ESI): calcd for $C_{32}H_{26}N_5O_8$ ([M + H]$^+$): 608.1781, found: 608.1792.

*3-(Morpholinomethyl)-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**104**).* Compound **103** (0.125 g, 0.206 mmol, 1.0 equiv) was dissolved in a mixture of MeOH (2 mL) and THF (1 mL). Morpholine (0.089 mL, 1.03 mmol, 5.0 equiv) and AcOH (0.236 mL, 4.12 mmol, 20.0 equiv) were added, followed by $NaBH_3CN$ (0.039 g, 0.62 mmol, 3.0 equiv). After 2 h, LCMS analysis indicated completion of the reaction. The reaction mixture was diluted with water (15 mL) and extracted with EtOAc (3 × 25 mL). The combined organic phases were dried over Na_2SO_4, concentrated *in vacuo*, and used crude in the next reaction. HRMS (ESI): calcd for $C_{36}H_{35}N_6O_8$ ([M + H]$^+$): 679.2516, found: 679.2631.

*3-(Morpholinomethyl)-4-amino-1-(β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidine (**105**).* Compound **104** (crude) was subjected to general procedure C (reaction time: 90 min). Purification by flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) afforded **105** (48 mg, 0.131 mmol, 64% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38−2.58 (4H, m, 2 × $CH_{2\ morpholine}$, under DMSO peak), 3.42 (1H, dt, J = 10.5, 5.0 Hz, H-5′), 3.49−3.69 (5H, m, H-5″, 2 × $CH_{2\ morpholine}$), 3.75 (1H, d, J = 14.4 Hz, CH_2N), 3.80 (1H, d, J = 14.4 Hz, CH_2N), 3.88 (1H, dd, J = 9.4, 4.7 Hz, H-4′), 4.18 (1H, dd, J = 8.2, 4.4 Hz, H-3′), 4.58 (1H, dd, J = 9.7, 3.8 Hz, H-2′), 4.87 (1H, t, J = 6.2 Hz, OH), 5.10 (1H, d, J = 3.5 Hz, OH), 5.31 (1H, d, J = 4.1 Hz, OH), 6.02 (1H, d, J = 5.0 Hz, H-1′), 7.79 (1H, br. s., NH_2), 8.17 (1H, s, H-6), 8.55 (1H, br. s., NH_2) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 52.9 (2 × $CH_{2\ morpholine}$), 56.1 (CH_2N), 62.4 (C-5′), 66.0 (2 × $CH_{2\ morpholine}$), 70.8 (C-2′), 72.8 (C-3′), 85.1 (C-4′), 88.4 (C-1′), 99.4 (C-3a), 143.5 (C-3), 155.2 (C-7a), 156.2 (C-6), 158.6 (C-4) ppm. HRMS (ESI): calcd for $C_{15}H_{23}N_6O_5$ ([M + H]$^+$): 367.1730, found: 367.1727.

*3-Difluoromethyl-4-amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**106**).* Compound **103** (0.143 g, 0.235 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5 mL). DAST (0.155 mL, 1.18 mmol, 5.0 equiv) was added, and the mixture was stirred overnight at room temperature. The reaction was quenched *via* the slow addition of aq. sat. $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in CH_2Cl_2, adsorbed onto celite, and purified *via* flash column chromatography (5 → 65% EtOAc in petroleum ether). The obtained product was used directly in the next reaction. HRMS (ESI): calcd for $C_{32}H_{26}F_2N_5O_7$ ([M + H]$^+$): 630.1800, found: 630.1808.

*3-Difluoromethyl-4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (**107**).* Compound **106** (used directly from the previous reaction) was subjected to general procedure C (reaction time: 30 min). Purification *via* flash column chromatography (4 → 20% MeOH in CH_2Cl_2) afforded **107** (43 mg, 0.136 mmol, 58% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.44 (1H, dt, J = 11.8, 6.0 Hz, H-5′), 3.57 (1H, dt, J = 11.7, 5.0 Hz, H-5″), 3.93 (1H, dd, J = 10.0, 4.7 Hz, H-2′), 4.20 (1H, dd, J = 10.0, 5.0 Hz, H-3′), 4.61 (1H, dd, J = 10.5, 5.3 Hz, H-2′), 4.84 (1H, t, J = 5.7 Hz, OH), 5.19 (1H, d, J = 5.3 Hz, OH), 5.43 (1H, d, J = 5.9 Hz, OH), 6.14 (1H, d, J = 4.7 Hz, H-1′), 6.72 (1H, br. s), 7.42 (1H, t, J = 53.3 Hz), 7.94 (1H, br. s), 8.32 (1H, s) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.6 (C-5′), 71.2 (C-2′), 73.4 (C-3′), 85.9 (C-4′), 89.0 (C-1′) 97.4 (C-3a), 111.5 (t, J = 232.6 Hz, $\underline{C}$F$_2$H), 138.5 (t, J = 28.8 Hz, C-3), 155.8 (C-7a), 157.2 (C-6), 157.6 (C-4) ppm. ^{19}F NMR (282 MHz, DMSO-d_6) δ −110.91 (2F, d, J = 52.9 Hz) ppm. HRMS (ESI): calcd for $C_{11}H_{14}F_2N_5O_4$ ([M + H]$^+$): 318.1014, found: 318.1019.

*4-Amino-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidine-3-carboxylic Acid (**108**).* Compound **103** (0.588 g, 0.968 mmol, 1.0 eq) and NaH_2PO_4 (0.035 g, 0.291 mmol, 0.3 equiv) were dissolved in a mixture of THF (9 mL) and H_2O (1.5 mL). Aq. H_2O_2 (30% w/w, 0.110 mL, 0.968 mmol, 1.0 equiv) was added, followed by dropwise addition of a solution of $NaClO_2$ (0.123 g, 1.36 mmol, 1.4 equiv) in H_2O (1.5 mL). The reaction mixture was stirred overnight, diluted with 0.5 M HCl (20 mL), and extracted with EtOAc (3 × 50 mL). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 and adsorbed onto celite. The solid residue was purified by flash column chromatography (0 → 10% MeOH in CH_2Cl_2 + 0.1% HOAc) to afford **108** (0.570 g, 0.914 mmol, 94%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.72 (1H, dd, J = 13.2, 6.4 Hz, H-5′), 4.82−4.94 (2H, m, H-5″, H-4′), 6.32 (1H, t, J = 5.6 Hz, H-3′), 6.58 (1H, dd, J = 5.3, 4.1 Hz, H-2′), 6.83 (1H, d, J = 3.8 Hz, H-1′), 7.31−

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7.70 (9H, m, H_{Bz}), 7.89−8.13 (6H, m, H_{Bz}), 8.18 (1H, s, H-6), 11.50 (2H, br. s, NH_2), 12.60 (1H, br. s, COOH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.8 (C-5′), 71.8 (C-2′), 74.1 (C-3′), 80.7 (C-4′), 89.1 (C-1′), 101.0 (C-3a), 128.4 (C_{Bz}), 128.5 (C_{Bz}), 128.6 (C_{Bz}), 128.8 (C_{Bz}), 129.5 (C_{Bz}), 129.8 (C_{Bz}), 129.9 (C_{Bz}), 132.0 (C_{Bz}), 132.2 (C_{Bz}), 133.2 (C_{Bz}), 133.6 (C_{Bz}), 133.7 (C_{Bz}), 147.0 (C-3), 152.5 (C-7a), 154.1 (C-6), 155.7 (C-4), 165.0 (COOH), 165.1 (C═O), 166.1 (C═O) ppm. HRMS (ESI): calcd for $C_{32}H_{26}N_5O_9$ ([M + H]$^+$): 624.1731, found: 624.1745.

*3-Methylamido-4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-d]-pyrimidine (**113**).* Compound **108** (0.125 g, 0.20 mmol) was subjected to general procedure F, with methylamine (40% wt in H_2O) as the coupling partner. The obtained residue was subjected to general procedure C (reaction time: 1 h). Purification *via* flash column chromatography (automated, 4 → 20% MeOH in CH_2Cl_2) afforded **113** (0.019 g, 0.059 mmol, 29% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (3H, s, CH_3) 3.35−3.50 (1H, m, H-5′, partially underwater peak), 3.63 (1H, dd, J = 12.0, 4.7 Hz, H-5′), 3.93 (2H, dd, J = 10.3, 4.7 Hz, H-4′), 4.30 (1H, t, J = 4.7 Hz, H-3′), 4.67 (2H, t, J = 4.7 Hz, H-2′), 6.13 (1H, d, J = 4.7 Hz, H-1′), 8.24 (1H, s, H-6) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.0 (CH_3), 62.4 (C-5′), 70.8 (C-2′), 73.1 (C-3′), 85.7 (C-4′), 89.0 (C-1′), 99.3 (C-3a), 139.0 (C-3), 155.4 (C-7a), 157.0 (C-6), 158.1 (C-4), 162.1 (C═O) ppm. HRMS (ESI): calcd for $C_{12}H_{17}N_6O_5$ ([M + H]$^+$): 325.1260, found: 325.1265.

*3-Pyrrolidinamido-4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine (**114**).* Compound **108** (0.080 g, 0.128 mmol) was subjected to general procedure F, with pyrrolidine as the coupling partner. The obtained residue was subjected to general procedure C (reaction time: 1 h). Purification *via* flash column chromatography (automated, 1 → 15% MeOH in CH_2Cl_2) afforded **114** (0.006 g, 0.016 mmol, 28% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.90−2.12 (4H, m, 2 × $CH_{2\ pyrrolidine}$), 3.60−3.74 (3H, m, H-5′, $CH_{2\ pyrrolidine}$), 3.78 (1H, dd, J = 12.3, 3.2 Hz, H-5″), 4.05−4.21 (3H, m, H-4′, $CH_{2\ pyrrolidine}$), 4.50 (1H, t, J = 5.1 Hz, H-3′), 4.75 (1H, t, J = 4.7 Hz, H-2′) 6.34 (1H, d, J = 3.8 Hz, H-1′), 8.20 (1H, s, H-6) ppm. A qualitative ^{13}C NMR spectrum could not be obtained from the amount of product available. HRMS (ESI): calcd for $C_{15}H_{21}N_6O_5$ ([M + H]$^+$): 365.1573, found: 365.1567.

*3-Anilinamido-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**115**).* Compound **108** (0.125 g, 0.20 mmol) was subjected to general procedure F, with aniline as the coupling partner. The obtained residue was subjected to general procedure C (reaction time: 1 h). Purification *via* flash column chromatography (automated, 1 → 15% MeOH in CH_2Cl_2) afforded **115** (0.047 g, 0.122 mmol, 61% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.52 (1H, dt, J = 11.6, 6.0 Hz, H-5′), 3.68 (1H, dt, J = 11.7, 5.0 Hz, H-5″), 3.97 (1H, dd, J = 8.8, 4.4 Hz, H-4′), 4.33 (1H, dd, J = 9.1, 4.7 Hz, H-3′), 4.75−5.00 (2H, m, H-2′, OH), 5.15 (1H, d, J = 5.6 Hz, OH), 5.46 (1H, d, J = 5.9 Hz, OH), 6.19 (1H, d, J = 5.0 Hz, H-1′), 7.20 (1H, t, J = 7.3 Hz, H-4$_{aniline}$), 7.41 (2H, t, J = 7.8 Hz, H-2$_{aniline}$, H-6$_{aniline}$), 7.78 (2H, d, J = 7.9 Hz, H-3$_{aniline}$, H-5$_{aniline}$), 8.20 (1H, br. s., NH_2′), 8.29 (1H, s, H-6), 8.54 (1H, br. s., NH_2), 10.45 (1H, s, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.1 (C-5′), 70.7 (C-2′), 72.8 (C-3′), 85.9 (C-4′), 89.4 (C-1′), 99.7 (C-3a), 121.7 (C-2$_{aniline}$, C-6$_{aniline}$), 124.8 (C-4$_{aniline}$), 128.7 (C-3$_{aniline}$, C-5$_{aniline}$), 137.4 (C-1aniline), 138.7 (C-3), 155.5 (C-7a), 157.0 (C-6), 158.1 (C-4), 160.4 (C═O) ppm. HRMS (ESI): calcd for $C_{17}H_{19}N_6O_5$ ([M + H]$^+$): 387.1417, found: 387.1422.

*3-Benzylamido-4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine (**116**).* Compound **108** (0.125 g, 0.20 mmol) was subjected to general procedure F, with benzylamine as the coupling partner. The obtained residue was subjected to general procedure C (reaction time: 1 h). Purification *via* flash column chromatography (automated, 1 → 15% MeOH in CH_2Cl_2) afforded **116** (0.035 g, 0.087 mmol, 44% yield over 2 steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.48 (1H, dt, J = 12.3, 6.2 Hz, H-5′), 3.63 (1H, dt, J = 12.0, 5.3 Hz, H-5″), 3.94 (1H, dd, J = 9.7, 4.7 Hz, H-4′), 4.32 (1H, dd, J = 10.3, 5.0 Hz, H-3′), 4.46−4.63 (2H, m, $CH_{2\ benzyl}$), 4.72 (1H, dd, J = 10.3, 5.0 Hz, H-2′), 4.85 (1H, t, J = 6.2 Hz, OH), 5.10 (1H, d, J = 5.9 Hz, OH), 5.47 (1H, d, J = 5.9 Hz, OH), 6.16 (1H, d, J = 5.0 Hz, H-1′), 7.19−7.38 (5H, m, H_{Phe}), 8.11 (1H, d, J = 3.2 Hz, NH_2), 8.26 (1H, s, H-6), 8.80 (1H, d, J = 3.2 Hz, NH_2′), 9.38 (1H, t, J = 6.2 Hz, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 42.3 ($CH_{2\ benzyl}$), 62.3 (C-5′), 70.7 (C-2′), 73.0 (C-3′), 85.7 (C-4′), 89.0 (C-1′), 99.4 (C-3a), 126.9 (C-4$_{Phe}$), 127.3 (C-2$_{Phe}$, C-6$_{Phe}$), 128.4 (C-3$_{Phe}$, C-5$_{Phe}$), 138.7 (C-1$_{Phe}$), 139.1 (C-3), 155.4 (C-7a), 156.9 (C-6), 158.1 (C-4), 161.7 (C═O) ppm. HRMS (ESI): calcd for $C_{17}H_{19}N_6O_5$ ([M + H]$^+$): 401.1573, found: 401.1571.

Biological Evaluation. All animal experiments were conducted in compliance with institutional guidelines.

Drug Sensitivity Assays. Compound stock solutions were prepared at 20 mM in 100% dimethyl sulfoxide (DMSO). The compounds were serially prediluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%. Compounds were assayed in 10 concentrations of a 4-fold compound dilution series starting at 64 μM.

L. infantum. *L. infantum* [MHOM/MA(BE)/67] was used. This strain was maintained in golden hamsters (*Mesocricetus auratus*) obtained from Janvier (Le Genest Saint Isle, France) following approval by the Ethical Committee of the University of Antwerp (ECD2019−10). Amastigotes were collected from the spleens of infected donor hamsters as described elsewhere,[100] and spleen parasite burdens were assessed using the Stauber technique. Primary peritoneal mouse macrophages (PMMs) were used as host cells and obtained from Swiss mice (Janvier; ethical approval ECD2019−10) after a 2-day peritoneal stimulation with a 2% potato starch suspension. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2. Assays were performed in 96-well microtiter plates, each well containing 10 μL of the compound dilutions together with 190 μL of macrophage/parasite inoculum (3×10^4 cells + 4.5×10^5 parasites/well). The inoculum was prepared in RPMI-1640 medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% inactivated fetal calf serum. The macrophages were infected after 48 h. The compounds were added after 2 h of infection. Parasite multiplication was compared to untreated-infected controls (100% growth) and uninfected controls (0% growth). After 5 days incubation, parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed after staining with a 10% Giemsa solution. The results were expressed as % reduction in parasite burden compared to untreated control wells, and an IC_{50} value was calculated.

PMM Cytotoxicity. PMM toxicity was assessed during the *in vitro* *Leishmania* susceptibility assays *via* microscopic evaluation of cell detachment, lysis, and granulation. Evaluation was done by semi-quantitative scoring (no exact counting was performed) of at least 500 cells distributed over adjacent microscopic fields. The results were expressed as % reduction in normal cells compared to untreated control wells, and a CC_{50} value was determined.

T. cruzi. The β-galactosidase expressing *T. cruzi* Tulahuen CL2 strain (nifurtimox-sensitive) was used. This strain was maintained in MRC-5_{SV2} (human lung fibroblast) cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2.

Assays were performed in sterile 96-well microtiter plates, each well containing 10 μL of the watery compound dilutions together with 190 μL of MRC-5_{SV2} cell/parasite inoculum (4×10^3 cells/well + 4×10^4 parasites/well). For some assays, PMMs were used as *T. cruzi* host cells. For this purpose, 3×10^4 cells were plated per well and infected two days later with 1.5×10^4 parasites. To explore the involvement of ABC transporters, compound exposure was also combined with established inhibitors verapamil (8 μM), probenecid (700 μM), or cyclosporine A (2 μM). *T. cruzi* growth was compared to untreated-infected controls (100% growth) and noninfected controls (0% growth) after 7 days incubation at 37 °C and 5% CO_2. Parasite burdens were assessed after adding the substrate CPRG (chlorophenolred ß-D-galactopyranoside): 50 μL/well of a stock solution containing 15.2 mg of CPRG + 250 μL of Nonidet in 100 mL of PBS.

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The change in color was measured spectrophotometrically at 540 nm after 4 h incubation at 37 °C. The results were expressed as % reduction in parasite burdens compared to control wells, and an IC_{50} value was calculated.

MRC-5$_{SV2}$ Cytotoxicity. MRC-5$_{SV2}$ cells were cultured in MEM + Earl's salts-medium, supplemented with L-glutamine, $NaHCO_3$, and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO_2. Assays were performed in sterile 96-well microtiter plates, each well containing 10 μL of the watery compound dilutions together with 190 μL of MRC-5$_{SV2}$ inoculum (1.5×10^5 cells/mL). Cell growth was compared to untreated control wells (100% cell growth) and medium-control wells (0% cell growth). After 3 days incubation, cell viability was assessed fluorimetrically after addition of 50 μL of resazurin per well. After 4 h at 37 °C, fluorescence was measured (λ_{ex} 550 nm, λ_{em} 590 nm). The results were expressed as % reduction in cell growth/viability compared to control wells, and an IC_{50} value was determined.

Metabolic Stability. Male mouse and pooled human liver microsomes were purchased from a commercial source (Corning) and stored at −80 °C. NADPH generating system solutions A and B and UGT reaction mix solutions A and B (Corning) were kept at −20 °C. The test compound and the reference compound diclofenac were formulated in DMSO at 10 mM. The microsomal stability assay was carried out based on the BD Biosciences Guidelines for Use (TF000017 Rev1.0) with minor adaptations. The metabolic stability of the compounds was studied through the CYP450 superfamily (Phase-I metabolism) by fortification with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and through uridine glucuronosyl-transferase (UGT) enzymes (Phase-II metabolism) by fortification with uridine diphosphate glucuronic acid (UDPGA). For the CYP450 and other NADPH-dependent enzymes, both compounds were incubated at 5 μM together with 0.5 mg/mL liver microsomes in potassium phosphate buffer in a reaction started by the addition of 1 mM NADPH and stopped at the above-listed sampling times. At these time points, 20 μL was withdrawn from the reaction mixture and 80 μL of cold acetonitrile (ACN), containing the internal standard tolbutamide, was added to inactivate the enzymes and precipitate the protein. The mixture was vortexed for 30 s and centrifuged at 4 °C for 5 min at 15 000 rpm. The supernatant was stored at −80 °C until analysis. For the UGT enzymes, both compounds were incubated at 5 μM together with 0.5 mg/mL liver microsomes in a reaction started by the addition of 2 mM UDPGA cofactor. The corresponding loss of the parent compound was determined using liquid chromatography (UPLC) (Waters Aquity) coupled with tandem quadrupole mass spectrometry (MS^2) (Waters Xevo), equipped with an electrospray ionization (ESI) interface and operated in the multiple reaction monitoring (MRM) mode. The optimal MS parameters and control of the chromatographic separation conditions were tuned in a preceding experiment.

T. cruzi Y-Strain Bloodstream Trypomastigote Activity. Bloodstream trypomastigotes of the Y strain were obtained by cardiac puncture of infected Swiss Webster mice on the parasitaemia peak, and drug sensitivity assays were performed as described.[53]

T. cruzi Y-Strain Intracellular Amastigote Activity. After 24 h of plating, 2D cardiac cell cultures were infected for 24 h at 37 °C with bloodstream trypomastigotes of *T. cruzi* (Y strain) employing a parasite/host cell ratio of 10:1. Then, the cultures were washed to remove free parasites and treated for 48 h at 37 °C with a serial dilution of the compound in culture medium. After drug exposure, the cultures were rinsed using phosphate-buffered saline, fixed, and stained with Giemsa as described previously.[78,101] The mean numbers of infected host cells and of parasites per infected cell were scored in 200 host cells in two independent experiments each run in duplicate. Only characteristic parasite nuclei and kinetoplasts were considered as surviving parasites since irregular structures could represent parasites undergoing cell death. The compound activity was estimated by calculating the inhibition levels of the inhibition index (II, percentage of infected cells vs the mean number of parasites per infected cell).

Cytotoxicity on Cardiac Cells. Cardiac 2D and 3D cell cultures were obtained from the heart of Swiss Webster mice embryos, as reported.[102] To prepare the three-dimensional cultures, isolated cardiac cells were seeded in 96-well U plates (25×10^3 cell/well), previously coated with 1% agarose. Both cardiac cultures were sustained in Dulbecco's modified Eagle's medium (DMEM; without phenol red; Sigma-Aldrich) supplemented with 5% fetal bovine serum, 2.5 mM $CaCl_2$, 1 mM L-glutamine, streptomycin, and 2% chicken embryo extract, at 37 °C. Noninfected cultures were incubated for 48 h at 37 °C with crescent concentrations of **44** diluted in supplemented DMEM medium. Morphology was evaluated by light microscopy, and cellular viability was determined using PrestoBlue.[53] The results are expressed as the difference in reduction between treated and nontreated cultures adopting the manufacturer's instructions. The CC_{50} (minimum concentration that reduces 50% of the cellular viability) was then determined.[102]

***In Vivo* Evaluation.** *Compounds.* Bz (2-nitroimidazole; Laboratório Farmacêutico do Estado de Pernambuco [LAFEPE], Brazil) was used as a reference drug and was formulated using 3% Tween 80 in distilled water. The nucleoside analogue **44** was diluted using 10% Tween 80 in sterile water.

Mouse Infection and Treatment. Male Swiss Webster mice (18−20 g; 4−5 weeks of age) were obtained from the animal facilities of ICTB (Institute of Science and Biomodels Technology/Fiocruz/RJ/Brazil). Housing of animals was with a maximum of 6 animals per cage, in a specific-pathogen-free (SPF) room at 20−24 °C under a 12 h light and 12 h dark cycle. All animals were provided sterilized water and chow ad libitum. The animals were acclimatized for 7 days before the experiments. On the day of infection (0 dpi), animals were infected by i.p. administration of 10^4 bloodstream trypomastigotes (Y strain) originating from an infected donor mouse. Noninfected control mice were age-matched and housed under identical conditions.[53] Each experimental group consisted of six animals: untreated (infected vehicle-treated control) and treated (infected and treated with 44 or with benznidazole). Treatment was initiated at the onset of parasitemia (6 dpi) only using mice with detectable parasitemia. **44** was administered by oral gavage for five consecutive days at 25, 2.5, and 0.25 mg/kg twice daily. Benznidazole treatments at 10 mg/kg and at the optimal dose (100 mg/kg p.o.) were run in parallel. The efficacy of **44** in coadministration with benznidazole was also evaluated (**44** at 2.5 mg/kg b.i.d + benznidazole at 10 mg/kg/day). All treatments followed a 5-day (6^{th} to 10^{th} dpi) dosing regimen, and all compound formulations were freshly prepared before administration. Parasitemia levels in *T. cruzi* assays were individually checked by light microscopic counting of parasites in 5 μL of blood, and mortality rates were checked daily until 34 dpi and expressed as a percentage of cumulative mortality, as described previously.[95]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00135.

HPLC traces of selected final compounds and copies of ^1H and ^{13}C NMR spectra of synthesized compounds (PDF)

Molecular formula strings (CSV)

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Notes

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■ ABBREVIATIONS

APP(R), aminopyrazolo[3,4-*d*]pyrimidine (riboside); Bz, benznidazole; CL, cutaneous leishmaniasis; DTU, discrete typing unit; PMM, primary mouse macrophage; PRTase, phosphoribosyltransferase; *L. infantum*, *Leishmania infantum*; SI, selectivity index; T. cruzi, *Trypanosoma cruzi*; TDA-1, tris[2-(2-methoxyethoxy)ethyl]amine; TPPTS, triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt; VL, visceral leishmaniasis

■ REFERENCES

(1) Molyneux, D. Neglected Tropical Diseases. *Community Eye Health* **2013**, *26*, 21−24.

(2) Houweling, T. A. J.; Karim-Kos, H. E.; Kulik, M. C.; Stolk, W. A.; Haagsma, J. A.; Lenk, E. J.; Richardus, J. H.; Vlas, S. J. de. Socioeconomic Inequalities in Neglected Tropical Diseases: A Systematic Review. *PLoS Neglected Trop. Dis.* **2016**, *10*, No. e0004546.

(3) Lee, B. Y.; Bacon, K. M.; Bottazzi, M. E.; Hotez, P. J. Global Economic Burden of Chagas Disease: A Computational Simulation Model. *Lancet Infect. Dis.* **2013**, *13*, 342−348.

(4) Lidani, K. C. F.; Andrade, F. A.; Bavia, L.; Damasceno, F. S.; Beltrame, M. H.; Messias-Reason, I. J.; Sandri, T. L. Chagas Disease: From Discovery to a Worldwide Health Problem. *Front. Public Health* **2019**, *7*, 166.

(5) Chatelain, E.; Konar, N. Translational Challenges of Animal Models in Chagas Disease Drug Development: A Review. *Drug Des. Dev. Ther.* **2015**, *9*, 4807−4823.

(6) Pérez-Molina, J. A.; Molina, I. Chagas Disease. *Lancet* **2018**, *391*, 82−94.

(7) Rao, S. P. S.; Barrett, M. P.; Dranoff, G.; Faraday, C. J.; Gimpelewicz, C. R.; Hailu, A.; Jones, C. L.; Kelly, J. M.; Lazdins-Helds, J. K.; Mäser, P.; Mengel, J.; Mottram, J. C.; Mowbray, C. E.; Sacks, D. L.; Scott, P.; Späth, G. F.; Tarleton, R. L.; Spector, J. M.; Diagana, T. T. Drug Discovery for Kinetoplastid Diseases: Future Directions. *ACS Infect. Dis.* **2019**, *5*, 152−157.

(8) Field, M. C.; Horn, D.; Fairlamb, A. H.; Ferguson, M. A. J.; Gray, D. W.; Read, K. D.; De Rycker, M.; Torrie, L. S.; Wyatt, P. G.; Wyllie, S.; Gilbert, I. H. Anti-Trypanosomatid Drug Discovery: An Ongoing Challenge and a Continuing Need. *Nat. Rev. Microbiol.* **2017**, *15*, 217−231.

(9) De Rycker, M.; Baragaña, B.; Duce, S. L.; Gilbert, I. H. Challenges and Recent Progress in Drug Discovery for Tropical Diseases. *Nature* **2018**, *559*, 498−506.

(10) WHO. *Regional Strategic Framework for Elimination of Kala-Azar from the Sout-East Asia Region (2005-2015)*; New Delhi, 2005.

(11) Burza, S.; Croft, S. L.; Boelaert, M. Leishmaniasis. *Lancet* **2018**, *392*, 951−970.

(12) Sundar, S.; Singh, A. Chemotherapeutics of Visceral Leishmaniasis: Present and Future Developments. *Parasitology* **2018**, *145*, 481−489.

(13) Chakravarty, J.; Sundar, S. Current and Emerging Medications for the Treatment of Leishmaniasis. *Expert Opin. Pharmacother.* **2019**, *20*, 1251−1265.

(14) DNDi. 2019 R&D portfolio in review: Leishmaniasis − DNDi. https://www.dndi.org/2020/media-centre/news-views-stories/news/leishmaniasis-rnd-portfolio-update/ (accessed Apr 20, 2020).

(15) Nagle, A.; Biggart, A.; Be, C.; Srinivas, H.; Hein, A.; Caridha, D.; Sciotti, R. J.; Pybus, B.; Kreishman-Deitrick, M.; Bursulaya, B.; Lai, Y. H.; Gao, M.-Y.; Liang, F.; Mathison, C. J. N.; Liu, X.; Yeh, V.; Smith, J.; Lerario, I.; Xie, Y.; Chianelli, D.; Gibney, M.; Berman, A.; Chen, Y.-L.; Jiricek, J.; Davis, L. C.; Liu, X.; Ballard, J.; Khare, S.; Eggimann, F. K.; Luneau, A.; Groessl, T.; Shapiro, M.; Richmond, W.; Johnson, K.; Rudewicz, P. J.; Rao, S. P. S.; Thompson, C.; Tuntland, T.; Spraggon, G.; Glynne, R. J.; Supek, F.; Wiesmann, C.; Molteni, V. Discovery and Characterization of Clinical Candidate LXE408 as a Kinetoplastid-Selective Proteasome Inhibitor for the Treatment of Leishmaniases. *J. Med. Chem.* **2020**, *63*, 10773−10781.

(16) Thomas, M. G.; De Rycker, M.; Ajakane, M.; Albrecht, S.; Álvarez-Pedraglio, A. I.; Boesche, M.; Brand, S.; Campbell, L.; Cantizani-Perez, J.; Cleghorn, L. A. T.; Copley, R. C. B.; Crouch, S. D.; Daugan, A.; Drewes, G.; Ferrer, S.; Ghidelli-Disse, S.; Gonzalez, S.; Gresham, S. L.; Hill, A. P.; Hindley, S. J.; Lowe, R. M.; MacKenzie, C. J.; MacLean, L.; Manthri, S.; Martin, F.; Miguel-Siles, J.; Nguyen, V. L.; Norval, S.; Osuna-Cabello, M.; Woodland, A.; Patterson, S.; Pena, I.; Quesada-Campos, M. T.; Reid, I. H.; Revill, C.; Riley, J.; Ruiz-Gomez, J. R.; Shishikura, Y.; Simeons, F. R. C.; Smith, A.; Smith, V. C.; Spinks, D.; Stojanovski, L.; Thomas, J.; Thompson, S.; Underwood, T.; Gray, D. W.; Fiandor, J. M.; Gilbert, I. H.; Wyatt, P. G.; Read, K. D.; Miles, T. J. Identification of GSK3186899/DDD853651 as a Preclinical Development Candidate for the Treatment of Visceral Leishmaniasis. *J. Med. Chem.* **2019**, *62*, 1180−1202.

(17) Wyllie, S.; Thomas, M.; Patterson, S.; Crouch, S.; De Rycker, M.; Lowe, R.; Gresham, S.; Urbaniak, M. D.; Otto, T. D.; Stojanovski, L.; Simeons, F. R. C.; Manthri, S.; MacLean, L. M.; Zuccotto, F.; Homeyer, N.; Pflaumer, H.; Boesche, M.; Sastry, L.; Connolly, P.; Albrecht, S.; Berriman, M.; Drewes, G.; Gray, D. W.; Ghidelli-Disse, S.; Dixon, S.; Fiandor, J. M.; Wyatt, P. G.; Ferguson, M. A. J.; Fairlamb, A. H.; Miles, T. J.; Read, K. D.; Gilbert, I. H. Cyclin-Dependent Kinase 12 Is a Drug Target for Visceral Leishmaniasis. *Nature* **2018**, *560*, 192−197.

(18) Khare, S.; Nagle, A. S.; Biggart, A.; Lai, Y. H.; Liang, F.; Davis, L. C.; Barnes, S. W.; Mathison, C. J. N.; Myburgh, E.; Gao, M.-Y.; Gillespie, J. R.; Liu, X.; Tan, J. L.; Stinson, M.; Rivera, I. C.; Ballard, J.; Yeh, V.; Groessl, T.; Federe, G.; Koh, H. X. Y.; Venable, J. D.; Bursulaya, B.; Shapiro, M.; Mishra, P. K.; Spraggon, G.; Brock, A.; Mottram, J. C.; Buckner, F. S.; Rao, S. P. S.; Wen, B. G.; Walker, J. R.; Tuntland, T.; Molteni, V.; Glynne, R. J.; Supek, F. Proteasome Inhibition for Treatment of Leishmaniasis, Chagas Disease and Sleeping Sickness. *Nature* **2016**, *537*, 229−233.

(19) Van den Kerkhof, M.; Mabille, D.; Chatelain, E.; Mowbray, C. E.; Braillard, S.; Hendrickx, S.; Maes, L.; Caljon, G. In Vitro and in Vivo Pharmacodynamics of Three Novel Antileishmanial Lead Series. *Int. J. Parasitol. Drugs Drug Resist.* **2018**, *8*, 81−86.
(20) Katsuno, K.; Burrows, J. N.; Duncan, K.; Van Huijsduijnen, R. H.; Kaneko, T.; Kita, K.; Mowbray, C. E.; Schmatz, D.; Warner, P.; Slingsby, B. T. Hit and Lead Criteria in Drug Discovery for Infectious Diseases of the Developing World. *Nat. Rev. Drug Discovery* **2015**, *14*, 751−758.
(21) Berens, R. L.; Marr, J. J.; LaFon, S. W.; Nelson, D. J. Purine Metabolism in Trypanosoma Cruzi. *Mol. Biochem. Parasitol.* **1981**, *3*, 187−196.
(22) Carter, N. S.; Yates, P.; Arendt, C. S.; Boitz, J. M.; Ullman, B. Purine and Pyrimidine Metabolism in Leishmania. In *Drug Targets in Kinetoplastid Parasites*; Majumder, H. K., Ed.; Springer: New York, NY, 2008; pp 141−154.
(23) Boitz, J. M.; Ullman, B.; Jardim, A.; Carter, N. S. Purine Salvage in Leishmania: Complex or Simple by Design? *Trends Parasitol.* **2012**, *28*, 345−352.
(24) Berg, M.; Van der Veken, P.; Goeminne, A.; Haemers, A.; Augustyns, K. Inhibitors of the Purine Salvage Pathway: A Valuable Approach for Antiprotozoal Chemotherapy? *Curr. Med. Chem.* **2010**, *17*, 2456−2481.
(25) El Kouni, M. H. Potential Chemotherapeutic Targets in the Purine Metabolism of Parasites. *Pharmacol. Ther.* **2003**, *99*, 283−309.
(26) Datta, A. K.; Datta, R.; Sen, B. Antiparasitic Chemotherapy. In *Drug Targets in Kinetoplastid Parasites*; Majumder, H. K., Ed.; Advances In Experimental Medicine and Biology; Springer: New York, NY, 2008; pp 116−132.
(27) Pfaller, M. A.; Marr, J. J. Antileishmanial Effect of Allopurinol. *Antimicrob. Agents Chemother.* **1974**, *5*, 469−472.
(28) Looker, D. L.; Marr, J. J.; Berens, R. L. Mechanisms of Action of Pyrazolopyrimidines in Leishmania Donovani. *J. Biol. Chem.* **1986**, *261*, 9412−9415.
(29) Berens, R. L.; Marr, J. J.; Steele da Cruz, F. S.; Nelson, D. J. Effect of Allopurinol on Trypanosoma Cruzi: Metabolism and Biological Activity in Intracellular and Bloodstream Forms. *Antimicrob. Agents Chemother.* **1982**, *22*, 657−661.
(30) Nelson, D. J.; Bugge, C. J.; Elion, G. B.; Berens, R. L.; Marr, J. J. Metabolism of Pyrazolo(3,4-d)Pyrimidines in Leishmania Braziliensis and Leishmania Donovani. Allopurinol, Oxipurinol, and 4-Aminopyrazolo(3,4-d)Pyrimidine. *J. Biol. Chem.* **1979**, *254*, 3959−3964.
(31) Kager, P. A.; Rees, P. H.; Wellde, B. T.; Hockmeyer, W. T.; Lyerly, W. H. Allopurinol in the Treatment of Visceral Leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* **1981**, *75*, 556−559.
(32) Martinez, S.; Marr, J. J. Allopurinol in the Treatment of American Cutaneous Leishmaniasis. *N. Engl. J. Med.* **1992**, *326*, 741−744.
(33) Yaich, S.; Charfeddine, K.; Masmoudi, A.; Masmoudi, M.; Zaghdhane, S.; Turki, H.; Hachicha, J. Atypical Presentation of Cutaneous Leishmaniasis in a Renal Transplant Recipient Successfully Treated with Allopurinol and Fluconazole. *Ann. Saudi Med.* **2013**, *33*, 187−191.
(34) Travi, B. L.; Cordeiro-da-Silva, A.; Dantas-Torres, F.; Miró, G. Canine Visceral Leishmaniasis: Diagnosis and Management of the Reservoir Living among Us. *PLoS Neglected Trop. Dis.* **2018**, *12*, No. e0006082.
(35) Gallerano, R. H.; Marr, J. J.; Sosa, R. R. Therapeutic Efficacy of Allopurinol in Patients with Chronic Chagas' Disease. *Am. J. Trop. Med. Hyg.* **1990**, *43*, 159−166.
(36) Rassi, A.; Luquetti, A. O.; Rassi, A.; Rassi, G. G.; Rassi, S. G.; DA Silva, I. G.; Rassi, A. G. Specific Treatment for Trypanosoma Cruzi: Lack of Efficacy of Allopurinol in the Human Chronic Phase of Chagas Disease. *Am. J. Trop. Med. Hyg.* **2007**, *76*, 58−61.
(37) Apt, W.; Arribada, A.; Zulantay, I.; Solari, A.; Sánchez, G.; Mundaca, K.; Coronado, X.; Rodríguez, J.; Gil, L. C.; Osuna, A. Itraconazole or Allopurinol in the Treatment of Chronic American Trypanosomiasis: The Results of Clinical and Parasitological Examinations 11 Years Post-Treatment. *Ann. Trop. Med. Parasitol.* **2005**, *99*, 733−741.
(38) Almeida, D. R.; Carvalho, A. C.; Branco, J. N.; Pereira, A. P.; Correa, L.; Vianna, P. V.; Buffolo, E.; Martinez, E. E. Chagas' Disease Reactivation after Heart Transplantation: Efficacy of Allopurinol Treatment. *J. Heart Lung Transplant. Off. Publ. Int. Soc. Heart Transplant.* **1996**, *15*, 988−992.
(39) Bestetti, R. B.; Theodoropoulos, T. A. D. A Systematic Review of Studies on Heart Transplantation for Patients with End-Stage Chagas' Heart Disease. *J. Card. Failure* **2009**, *15*, 249−255.
(40) Martinez, S.; Looker, D. L.; Berens, R. L.; Marr, J. J. The Synergistic Action of Pyrazolopyrimidines and Pentavalent Antimony against Leishmania Donovani and L. braziliensis. *Am. J. Trop. Med. Hyg.* **1988**, *39*, 250−255.
(41) Chunge, C. N.; Gacmra, G.; Muigai, R.; Wasunna, K.; Rashid, J. R.; Chulay, J. D.; Anabwani, G.; Oster, C. N.; Bryceson, A. D. M. Visceral Leishmaniasis Unresponsive to Antimonial Drugs III. Successful Treatment Using a Combination of Sodium Stibogluconate plus Allopurinol. *Trans. R. Soc. Trop. Med. Hyg.* **1985**, *79*, 715−718.
(42) Torrus, D.; Boix, V.; Massa, B.; Portilla, J.; Pérez-Mateo, M. Fluconazole plus Allopurinol in Treatment of Visceral Leishmanlasis. *J. Antimicrob. Chemother.* **1996**, *37*, 1042−1043.
(43) Grosso, N. L.; Alarcon, M. L.; Bua, J.; Laucella, S. A.; Riarte, A.; Fichera, L. E. Combined Treatment with Benznidazole and Allopurinol in Mice Infected with a Virulent Trypanosoma Cruzi Isolate from Nicaragua. *Parasitology* **2013**, *140*, 1225−1233.
(44) Perez-Mazliah, D. E.; Alvarez, M. G.; Cooley, G.; Lococo, B. E.; Bertocchi, G.; Petti, M.; Albareda, M. C.; Armenti, A. H.; Tarleton, R. L.; Laucella, S. A.; Viotti, R. Sequential Combined Treatment with Allopurinol and Benznidazole in the Chronic Phase of Trypanosoma Cruzi Infection: A Pilot Study. *J. Antimicrob. Chemother.* **2013**, *68*, 424−437.
(45) Rial, M. S.; Scalise, M. L.; López Alarcón, M.; Esteva, M. I.; Búa, J.; Benatar, A. F.; Prado, N. G.; Riarte, A. R.; Fichera, L. E. Experimental Combination Therapy Using Low Doses of Benznidazole and Allopurinol in Mouse Models of Trypanosoma Cruzi Chronic Infection. *Parasitology* **2019**, *146*, 305−313.
(46) Avila, J. L.; Casanova, M. A. Comparative Effects of 4-Aminopyrazolopyrimidine, Its 2′-Deoxyriboside Derivative, and Allopurinol on in Vitro Growth of American Leishmania Species. *Antimicrob. Agents Chemother.* **1982**, *22*, 380−385.
(47) Berman, J. D.; Lee, L. S.; Robins, R. K.; Revankar, G. R. Activity of Purine Analogs against Leishmania Tropica within Human Macrophages in Vitro. *Antimicrob. Agents Chemother.* **1983**, *24*, 233−236.
(48) Marr, J. Purine Analogs as Chemotherapeutic Agents in Leishmaniasis and American Trypanosomiasis. *J. Lab. Clin. Med.* **1991**, *118*, 111−119.
(49) Avila, J.; Polegre, M. A.; Robins, R. K. Biological Action of Pyrazolopyrimidine Derivatives against Trypanosoma Cruzi. Studies in Vitro and in Vivo. *Comp. Biochem. Physiol., Part C: Comp. Pharmacol.* **1987**, *86*, 49−54.
(50) Avila, J.; Polegre, M. A.; Avila, A.; Robins, R. K. Action of Pyrazolopyrimidine Derivatives on American Leishmania Spp. Promastigotes. *Comp. Biochem. Physiol., Part C: Comp. Pharmacol.* **1986**, *83*, 285−289.
(51) Avila, J.; Avila, A.; Muñoz, E.; Monzón, H. Trypanosoma Cruzi: 4-Aminopyrazolopyrimidine in the Treatment of Experimental Chagas' Disease. *Exp. Parasitol.* **1983**, *56*, 236−240.
(52) Hulpia, F.; Campagnaro, G. D.; Scortichini, M.; Van Hecke, K.; Maes, L.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. Revisiting Tubercidin against Kinetoplastid Parasites: Aromatic Substitutions at Position 7 Improve Activity and Reduce Toxicity. *Eur. J. Med. Chem.* **2019**, *164*, 689−705.
(53) Hulpia, F.; Van Hecke, K.; França da Silva, C.; da Gama Jaen Batista, D.; Maes, L.; Caljon, G.; de Nazaré C. Soeiro, M.; Van Calenbergh, S. Discovery of Novel 7-Aryl 7-Deazapurine 3′-Deoxy-Ribofuranosyl Nucleosides with Potent Activity against Trypanosoma Cruzi. *J. Med. Chem.* **2018**, *61*, 9287−9300.

https://doi.org/10.1021/acs.jmedchem.1c00135
J. Med. Chem. 2021, 64, 4206−4238

(54) Hulpia, F.; Campagnaro, G. D.; Alzahrani, K. J.; Alfayez, I. A.; Ungogo, M. A.; Mabille, D.; Maes, L.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. Structure−Activity Relationship Exploration of 3′-Deoxy-7-Deazapurine Nucleoside Analogues as Anti-Trypanosoma Brucei Agents. *ACS Infect. Dis.* **2020**, *6*, 2045−2056.
(55) Hulpia, F.; Mabille, D.; Campagnaro, G. D.; Schumann, G.; Maes, L.; Roditi, I.; Hofer, A.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. Combining Tubercidin and Cordycepin Scaffolds Results in Highly Active Candidates to Treat Late-Stage Sleeping Sickness. *Nat. Commun.* **2019**, *10*, No. 5564.
(56) Hulpia, F.; Bouton, J.; Campagnaro, G. D.; Alfayez, I. A.; Mabille, D.; Maes, L.; de Koning, H. P.; Caljon, G.; Van Calenbergh, S. C6−O-Alkylated 7-Deazainosine Nucleoside Analogues: Discovery of Potent and Selective Anti-Sleeping Sickness Agents. *Eur. J. Med. Chem.* **2020**, *188*, No. 112018.
(57) Bouton, J.; Furquim d'Almeida, A.; Maes, L.; Caljon, G.; Van Calenbergh, S.; Hulpia, F. Synthesis and Evaluation of 3′-Fluorinated 7-Deazapurine Nucleosides as Antikinetoplastid Agents. *Eur. J. Med. Chem.* **2021**, *216*, No. 113290.
(58) Berman, J. D.; Hanson, W. L.; Lovelace, J. K.; Waits, V. B.; Jackson, J. E.; Chapman, W. L.; Klein, R. S. Activity of Purine Analogs against Leishmania Donovani in Vivo. *Antimicrob. Agents Chemother.* **1987**, *31*, 111−113.
(59) Berman, J. D.; Keenan, C. M.; Lamb, S. R.; Hanson, W. L.; Waits, V. B. Leishmania Donovani: Oral Efficacy and Toxicity of Formycin B in the Infected Hamster. *Exp. Parasitol.* **1983**, *56*, 215−221.
(60) Rainey, P.; Nolan, P. A.; Townsend, L. B.; Robins, R. K.; Fox, J. J.; Secrist, J. A., III; Santi, D. V. Inosine Analogs as Anti-Leishmanial Agents. *Pharm. Res.* **1985**, *02*, 217−220.
(61) Marr, J. J.; Berens, R. L.; Cohn, N. K.; Nelson, D. J.; Klein, R. S. Biological Action of Inosine Analogs in *Leishmania* and *Trypanosoma Spp. Antimicrob. Agents Chemother.* **1984**, *25*, 292−295.
(62) Morishige, K.; Aji, T.; Ishii, A.; Yasuda, T.; Wataya, Y. Leishmania Donovani: Pilot Study for Evaluation of Therapeutic Effects of Inosine Analogs against Amastigotes in Vitro and in Vivo. *Exp. Parasitol.* **1995**, *80*, 665−671.
(63) Seela, F.; Becher, G. Synthesis of 7-Halogenated 8-Aza-7-Deaza-2′-Deoxyguanosines and Related Pyrazolo[3,4-d]Pyrimidine 2′-Deoxyribonucleosides. *Synthesis* **1998**, *1998*, 207−214.
(64) Seela, F.; Zulauf, M. Synthesis of 7-Alkynylated 8-Aza-7-Deaza-2′-Deoxyadenosines via the Pd-Catalysed Cross-Coupling Reaction. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3233−3240.
(65) Mihich, E.; Simpson, C. L.; Mulhern, A. I. Comparative Study of the Toxicologic Effects of 7-Deazaadenosine (Tubercidin) and 7-Deazainosine. *Cancer Res.* **1969**, *29*, 116−123.
(66) Earl, R. A.; Townsend, L. B. Pyrazolopyrimidine Nucleosides. Part VII. The Synthesis of Certain Pyrazolo [3,4-d] Pyrimidine Nucleosides Related to the Nucleoside Antibiotics Toyocamycin and Sangivamycin. *J. Heterocycl. Chem.* **1974**, *11*, 1033−1039.
(67) Hecht, S. M.; Frye, R. B.; Werner, D.; Fukui, T.; Hawrelak, S. D. Synthesis and Biological Activity of Pyrazolo[3,4-d]Pyrimidine Nucleosides and Nucleotides Related to Tubercidin, Toyocamycin, and Sangivamycin. *Biochemistry* **1976**, *15*, 1005−1015.
(68) Bhat, G. A.; Montero, J. L. G.; Panzica, R. P.; Wotring, L. L.; Townsend, L. B. Pyrazolopyrimidine Nucleosides. 12. Synthesis and Biological Activity of Certain Pyrazolo[3,4-d]Pyrimidine Nucleosides Related to Adenosine. *J. Med. Chem.* **1981**, *24*, 1165−1172.
(69) Cottam, H. B.; Petrie, C. R.; McKernan, P. A.; Goebel, R. J.; Kent Dailey, N.; Davidson, R. B.; Robins, R. K.; Revanka, G. R. Synthesis and Biological Activity of Certain 3,4-Disubstituted Pyrazolo[3,4-d]Pyrimidine Nucleosides1. *J. Med. Chem.* **1984**, *27*, 1119−1127.
(70) Lin, W.; Li, H.; Ming, X.; Seela, F. 1,N6-Etheno-7-Deaza-2,8-Diazaadenosine: Syntheses, Properties and Conversion to 7-Deaza-2,8-Diazaadenosine. *Org. Biomol. Chem.* **2005**, *3*, 1714−1718.
(71) Seela, F.; Steker, H. Facile Synthesis of 2′-Deoxyribofuranosides of Allopurinol and 4-Amino-1H-Pyrazolo[3,4-d]Pyrimidine via Phase-Transfer Glycosylation. *Helv. Chim. Acta* **1985**, *68*, 563−570.
(72) Lichtenthaler, F. W.; Cuny, E. Nucleosides, 381) The Ribonucleosides of Allopurinol. *Chem. Ber.* **1981**, *114*, 1610−1623.
(73) Seela, F.; Zulauf, M.; Becher, G. Unexpected Dehalogenation of 3-Bromopyrazolo[3,4-d]Pyrimidine Nucleosides during Nucleobase-Anion Glycosylation. *Nucleosides Nucleotides* **1997**, *16*, 305−314.
(74) Seela, F.; Kaiser, K. 8-Aza-7-deazaadenine N8- and N8-(B-D-2′-Deoxyribofuranosides): Building Blocks for Automated DNA Synthesis and Properties of Oligodeoxyribonucleotides. *Helv. Chim. Acta* **1988**, *71*, 1813−1823.
(75) Seela, F.; Winter, H.; Möller, M. Pyrazolo[3,4-d]Pyrimidine 2′ -Deoxy- and 2′,3′ -Dideoxyribonucleosides: Studies on the Glycosylation of 4-Methoxypyrazolo[3,4-d]Pyrimidine. *Helv. Chim. Acta* **1993**, *76*, 1450−1458.
(76) Rutaganira, F. U.; Barks, J.; Dhason, M. S.; Wang, Q.; Lopez, M. S.; Long, S.; Radke, J. B.; Jones, N. G.; Maddirala, A. R.; Janetka, J. W.; El Bakkouri, M.; Hui, R.; Shokat, K. M.; Sibley, L. D. Inhibition of Calcium Dependent Protein Kinase 1 (CDPK1) by Pyrazolopyrimidine Analogs Decreases Establishment and Reoccurrence of Central Nervous System Disease by Toxoplasma Gondii. *J. Med. Chem.* **2017**, *60*, 9976−9989.
(77) Feng, X.; Qu, Y.; Han, Y.; Yu, X.; Bao, M.; Yamamoto, Y. Copper-Catalyzed Conversion of Aryl and Heteroaryl Bromides into the Corresponding Chlorides. *Chem. Commun.* **2012**, *48*, 9468−9470.
(78) Lin, C.; Hulpia, F.; da Silva, C. F.; Batista, D. d. G. J.; Van Hecke, K.; Maes, L.; Caljon, G.; Soeiro, M. d. N. C.; Van Calenbergh, S. Discovery of Pyrrolo[2,3-b]Pyridine (1,7-Dideazapurine) Nucleoside Analogues as Anti-Trypanosoma Cruzi Agents. *J. Med. Chem.* **2019**, *62*, 8847−8865.
(79) Hanefeld, U.; Rees, C. W.; White, A. J. P.; Williams, D. J. One-Pot Synthesis of Tetrasubstituted Pyrazoles—Proof of Regiochemistry. *J. Chem. Soc., Perkin Trans. 1* **1996**, *28*, 1545−1552.
(80) Wu, F. P.; Peng, J. B.; Qi, X.; Wu, X. F. Palladium-Catalyzed Carbonylative Transformation of Organic Halides with Formic Acid as the Coupling Partner and CO Source: Synthesis of Carboxylic Acids. *J. Org. Chem.* **2017**, *82*, 9710−9714.
(81) Ueda, T.; Konishi, H.; Manabe, K. Trichlorophenyl Formate: Highly Reactive and Easily Accessible Crystalline CO Surrogate for Palladium-Catalyzed Carbonylation of Aryl/Alkenyl Halides and Triflates. *Org. Lett.* **2012**, *14*, 5370−5373.
(82) Ueda, T.; Konishi, H.; Manabe, K. Palladium-Catalyzed Fluorocarbonylation Using N-Formylsaccharin as CO Source: General Access to Carboxylic Acid Derivatives. *Org. Lett.* **2013**, *15*, 5370−5373.
(83) Molander, G. A.; Brown, A. R. Suzuki−Miyaura Cross-Coupling Reactions of Potassium Vinyltrifluoroborate with Aryl and Heteroaryl Electrophiles. *J. Org. Chem.* **2006**, *71*, 9681−9686.
(84) Oshiro, K.; Morimoto, Y.; Amii, H. Sodium Bromodifluoroacetate: A Difluorocarbene Source for the Synthesis of Gem -Difluorocyclopropanes. *Synthesis* **2010**, *99*, 2080−2084.
(85) Berens, R. L.; Marr, J. J.; Nelson, D. J.; Lafon, S. W. Antileishmanial Effect of Allopurinol and Allopurinol Ribonucleoside on Intracellular Forms of Leishmania Donovani. *Biochem. Pharmacol.* **1980**, *29*, 2397−2398.
(86) Moorman, A. R.; LaFon, S. W.; Nelson, D. J.; Carter, H. H.; Marr, J. J.; Berens, R. L. Antiprotozoal Activity of 3′-Deoxyinosine. *Biochem. Pharmacol.* **1991**, *42*, 207−212.
(87) Soeiro, M. d. N. C.; de Souza, E. M.; da Silva, C. F.; Batista, D. d. G. J.; Batista, M. M.; Pavão, B. P.; Araújo, J. S.; Aiub, C. A. F.; da Silva, P. B.; Lionel, J.; Britto, C.; Kim, K.; Sulikowski, G.; Hargrove, T. Y.; Waterman, M. R.; Lepesheva, G. I. In Vitro and In Vivo Studies of the Antiparasitic Activity of Sterol 14α-Demethylase (CYP51) Inhibitor VNI against Drug-Resistant Strains of Trypanosoma Cruzi. *Antimicrob. Agents Chemother.* **2013**, *57*, 4151−4163.
(88) Chatelain, E.; Ioset, J.-R. Phenotypic Screening Approaches for Chagas Disease Drug Discovery. *Expert Opin. Drug Discovery* **2018**, *13*, 141−153.
(89) Dumoulin, P. C.; Vollrath, J.; Tomko, S. S.; Wang, J. X.; Burleigh, B. Glutamine Metabolism Modulates Azole Susceptibility in Trypanosoma Cruzi Amastigotes. *eLife* **2020**, *9*, No. e60226.

https://doi.org/10.1021/acs.jmedchem.1c00135
J. Med. Chem. 2021, 64, 4206−4238

(90) Nagajyothi, F.; Machado, F. S.; Burleigh, B. A.; Jelicks, L. A.; Scherer, P. E.; Mukherjee, S.; Lisanti, M. P.; Weiss, L. M.; Garg, N. J.; Tanowitz, H. B. Mechanisms of Trypanosoma Cruzi Persistence in Chagas Disease. *Cell. Microbiol.* **2012**, *14*, 634−643.
(91) Fernandes, M. C.; Andrews, N. W. Host Cell Invasion by Trypanosoma Cruzi: A Unique Strategy That Promotes Persistence. *FEMS Microbiol. Rev.* **2012**, *36*, 734−747.
(92) Matos Ferreira, A. V.; Segatto, M.; Menezes, Z.; Macedo, A. M.; Gelape, C.; de Oliveira Andrade, L.; Nagajyothi, F.; Scherer, P. E.; Teixeira, M. M.; Tanowitz, H. B. Evidence for Trypanosoma Cruzi in Adipose Tissue in Human Chronic Chagas Disease. *Microbes Infect.* **2011**, *13*, 1002−1005.
(93) Francisco, A. F.; Lewis, M. D.; Jayawardhana, S.; Taylor, M. C.; Chatelain, E.; Kelly, J. M. Limited Ability of Posaconazole To Cure Both Acute and Chronic Trypanosoma Cruzi Infections Revealed by Highly Sensitive In Vivo Imaging. *Antimicrob. Agents Chemother.* **2015**, *59*, 4653−4661.
(94) Bouton, J.; Maes, L.; Karalic, I.; Caljon, G.; Van Calenbergh, S. Synthesis and Evaluation of a Collection of Purine-like C-Nucleosides as Antikinetoplastid Agents. *Eur. J. Med. Chem.* **2021**, *212*, No. 113101.
(95) Cottam, H. B.; Wasson, D. B.; Shih, H. C.; Raychaudhuri, A.; Di Pasquale, G.; Carson, D. A. New Adenosine Kinase Inhibitors with Oral Antiinflammatory Activity: Synthesis and Biological Evaluation. *J. Med. Chem.* **1993**, *36*, 3424−3430.
(96) Zhao, H.; Leonard, P.; Guo, X.; Yang, H.; Seela, F. Silver-Mediated Base Pairs in DNA Incorporating Purines, 7-Deazapurines, and 8-Aza-7-Deazapurines: Impact of Reduced Nucleobase Binding Sites and an Altered Glycosylation Position. *Chem. − Eur. J.* **2017**, *23*, 5529−5540.
(97) Kraybill, B. C.; Elkin, L. L.; Blethrow, J. D.; Morgan, D. O.; Shokat, K. M. Inhibitor Scaffolds as New Allele Specific Kinase Substrates. *J. Am. Chem. Soc.* **2002**, *124*, 12118−12128.
(98) Gao, Y.; Lam, Y. Synthesis of Pyrazolo[5,1-d][1,2,3,5]-Tetrazine-4(3H)-Ones. *J. Comb. Chem.* **2010**, *12*, 69−74.
(99) Kulkarni, A.; Quang, P.; Curry, V.; Keyes, R.; Zhou, W.; Cho, H.; Baffoe, J.; Török, B.; Stieglitz, K. 1,3-Disubstituted-4-Amino-pyrazolo [3, 4-d] Pyrimidines, a New Class of Potent Inhibitors for Phospholipase D. *Chem. Biol. Drug Des.* **2014**, *84*, 270−281.
(100) Hendrickx, S.; Caljon, G.; Maes, L. In Vitro Growth Inhibition Assays of Leishmania Spp. In *Trypanosomatids: Methods and Protocols*; Michels, P. A. M.; Ginger, M. L.; Zilberstein, D., Eds.; Springer: New York, NY, 2020; pp 791−800.
(101) Batista, D. G. J.; Pacheco, M. G. O.; Kumar, A.; Branowska, D.; Ismail, M. A.; Hu, L.; Boykin, D. W.; Soeiro, M. N. C. Biological, Ultrastructural Effect and Subcellular Localization of Aromatic Diamidines in Trypanosoma Cruzi. *Parasitology* **2010**, *137*, 251−259.
(102) Timm, B. L.; da Silva, P. B.; Batista, M. M.; da Silva, F. H. G.; da Silva, C. F.; Tidwell, R. R.; Patrick, D. A.; Jones, S. K.; Bakunov, S. A.; Bakunova, S. M.; Maria de Nazaré, C. S. In Vitro and In Vivo Biological Effects of Novel Arylimidamide Derivatives against Trypanosoma Cruzi. *Antimicrob. Agents Chemother.* **2014**, *58*, 3720−3726.

https://doi.org/10.1021/acs.jmedchem.1c00135
J. Med. Chem. 2021, 64, 4206−4238