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Research paper

Synthesis of a 3′-*C*-ethynyl-β-D-ribofuranose purine nucleoside library: Discovery of C7-deazapurine analogs as potent antiproliferative nucleosides

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ABSTRACT

A focused nucleoside library was constructed around a 3′-*C*-ethynyl-D-ribofuranose sugar scaffold, which was coupled to variously modified purine nucleobases. The resulting nucleosides were probed for their ability to inhibit tumor cell proliferation, as well as for their activity against a panel of relevant human viruses. While C6-aryl substituted purine nucleosides were found to be weakly active, several C7-substituted 7-deazapurine nucleosides elicited potent antiproliferative activity. Their activity spectrum was evaluated in the NCI-60 tumor cell line panel indicating activity against several solid tumor derived cell lines. Analog **32**, equipped with a 7-deaza 7-chloro-6-amino-purin-9-yl base was evaluated in a metastatic breast tumor (MDA-MB-231-LM2) xenograft model. It inhibited both tumor growth and reduced the formation of lung metastases as revealed by BLI analysis. The dideazanucleoside analog **66** showed interesting activity against hCMV. These results highlight the potential advantages of recombining known sugar and nucleobase motifs as a library design strategy to discover novel antiviral or antitumor agents.

1. Introduction

Nucleoside analogs are the cornerstones for antiviral therapy with notable successes in the treatment of HIV and Hepatitis C virus infections [1]. Furthermore, nucleoside analogs have also found widespread use in oncology, with most approved derivatives active against various forms of lymphomas [1–3].

A focused screening library, comprised of nucleoside analogs surrounding a single modified D-ribofuranose moiety could be a viable strategy to discover attractive hits for the aforementioned disease areas, as evidenced by a number of recent publications [4–14]. In this paper, a rather underrepresented 3′-*C*-ethynylribofuranose motif [15–18] was used as the sugar scaffold to construct a small library of nucleoside analogs. Previous reports mainly focused on pyrimidine (-like) nucleobase moieties [17,19], structurally resembling the cytidine analog, ECyd (**1**, Fig. 1), which emerged as the most promising derivative from the initial discovery of 3′-*C*-ethynyl nucleosides in 1996 [15]. ECyd has been evaluated in clinical trials as a new antitumoral agent [3] and recently attracted renewed interest as a combination therapy, e.g. with carboplatin [3], or as part of a 'duplex drug', in which it is linked to 2′-deoxy-5-fluorouridine [20].

The purine counterparts on the other hand, exemplified by the 3′-*C*-ethynyladenosine analog (EAdo, **5**), have received little attention, which motivated us to combine this peculiar 3′-*C*-ethynyl sugar element with different purine nucleobases to build a focused library. Previously we reported a series of C2- and C6-substituted purine analogs (**4**, Fig. 1) of 3′-*C*-ethynyladenosine (EAdo, **5**) [21]. Considering the interesting biological properties reported for nucleosides comprising a C6 arylpurine [22,23] (e.g. **2**, Fig. 1) or a C7-substituted-7-deazapurine base, which was recently coined a 'privileged scaffold' [24] and is part of the natural nucleoside antibiotic tubercidin (**3**) [24–26], in this contribution we investigate the effect(s) of combining these base moieties with the 3′-*C*-ethynylribofuranose moiety.

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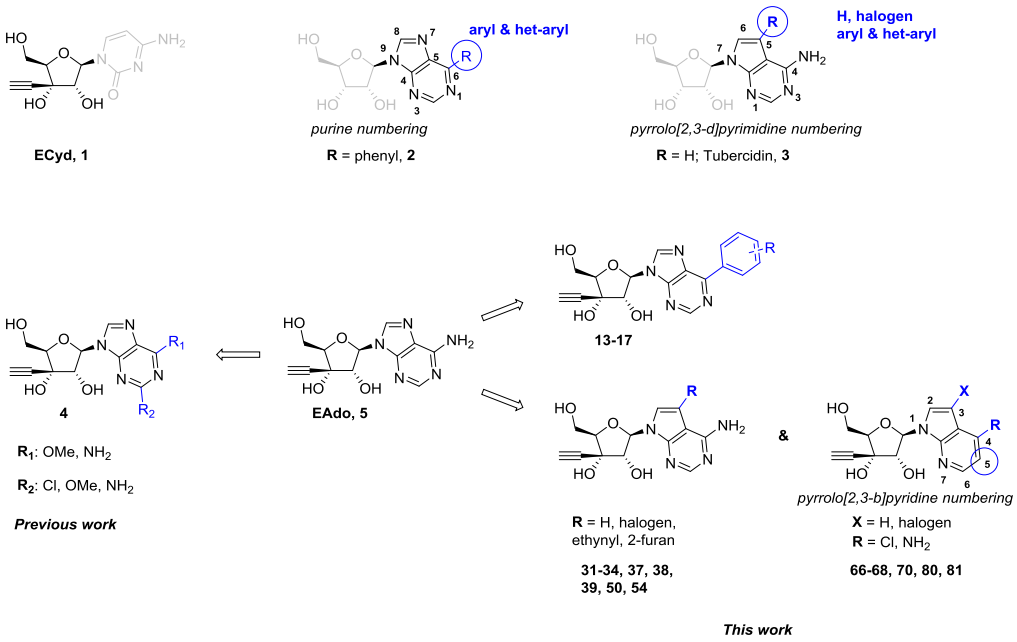
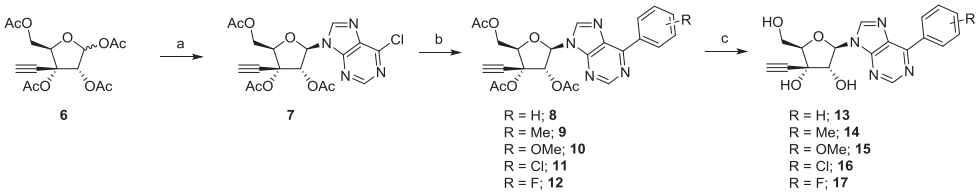


Fig. 1. Overview of previously reported nucleoside analogs featuring a 3′-*C*-ethynylribofuranose (ECyd and EAdo); known purine-modified nucleoside analogs with interesting biological activity (**2** and **3**), and their combination to construct a small library.



Scheme 1. Reagents and conditions: a) (i) 6-chloropurine, HMDS, cat. $(NH_4)_2SO_4$, reflux; (ii) TMSOTf, 1,2-dichloroethane, reflux, 81%; b) (substituted) phenylboronic acid, K_2CO_3, $Pd(Ph_3P)_4$, toluene, 100 °C, 30–52%; c) 7 N NH_3/MeOH, 43–75%.

2. Results and discussion

2.1. Chemistry

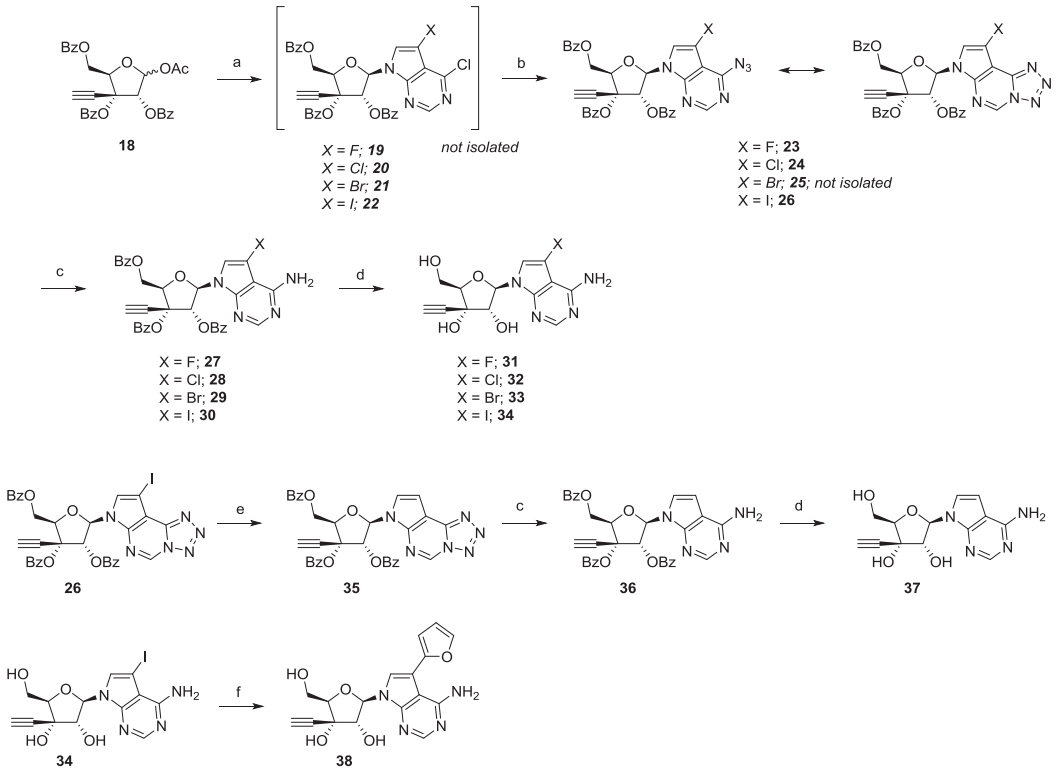
The target nucleoside analogs were prepared by Vorbrüggen glycosylation using either acetate (**6**, Scheme 1) [21] or benzoate (**18**, Scheme 2) [15] protected sugar precursors. Reaction conditions depended on the type of nucleobase (purine [21] vs. 7-deazapurine analogs [27]). Synthesis of the 6-substituted purine analogs **13**–**17** was accomplished via Suzuki reaction of the 6-chloropurine nucleoside **7** with appropriate phenylboronic acids [22]. Final compounds were obtained after deprotection with NH_3/MeOH (Scheme 1).

For the synthesis of the C7-modified 7-deazanucleosides[1] (Scheme 2), the use of the aforementioned sugar precursor **6** only provided the desired glycosylation product in low yield after cumbersome purification procedures. Switching to the benzoate protected sugar derivative **18** [15] improved the coupling yields [27], but the isolated glycosylation products were contaminated with residues of glycosyl donor degradation products (tentative assignment based on ^{1}H NMR data; not shown). Treatment of the crude intermediates **19**–**22** with NH_3/MeOH [27] (or NH_4OH [28]) at elevated (>100 °C) temperatures caused decomposition. Therefore, an alternative protocol to introduce the 6-amino group was employed [29,30]. Nucleophilic displacement of the 6-chloride with sodium azide efficiently delivered the corresponding azide derivatives (**23**–**26**), which generally could be obtained in pure form due to the marked difference in polarity induced by the predominating tetrazolo tautomer. Staudinger reduction furnished the corresponding 6-amino derivatives. Deprotection afforded the final compounds (**31**, **32**, **33**, **34**). To dehalogenate the 7-iodo intermediate **26**, it was subjected to I/Mg exchange using Knochel's iPrMgCl.LiCl [26,31], and the magnesiated intermediate was quenched with aqueous acid to give **35** in good yield. Further conversion to **37** was realized as described above. Introduction of a furan-2-yl moiety was achieved via an aqueous Suzuki reaction on **34** [25]. Remarkably, Suzuki reaction on the 7-iodo-7-deazapurine substrate gave significantly lower yields than reaction with the 6-chloropurine starting material.

A similar glycosylation strategy was followed to synthetize the C7 (*C5*)[2] trifluoromethyl analog **49** from **39** (upper line in Scheme 3) [35,36] by treating commercial 4-chloro-7*H*-pyrrolo[2,3-*d*]

[1] In the body of the text, purine numbering will be used for nucleoside analogs; however in the Experimental section, IUPAC nomenclature and pyrrolo[2,3-*d*]pyrimidine numbering will be applied.

[2] In order to allow for a more facile comparison with the assigned NMR data provided in the Experimental section; pyrrolo[2,3-*d*]pyrimidine numbering is indicated for selected derivates in *italic* and between brackets.



Scheme 2. Reagents and conditions: a) 4-chloro-5-halo-7*H*-pyrrolo[2,3-*d*]pyrimidine (F [32,33], Cl [34], Br [34], I [34]), TMSOTf, MeCN, 80 °C; b) NaN_3, DMF, 65 °C; c) (i) 1.0M PMe_3 in THF, THF; (ii) aq. HOAc, MeCN, 65 °C; d) 7N NH_3/MeOH, 30–95%; e) (i) iPrMgCl.LiCl (1.3M in THF), toluene, −65 °C; (ii) sat. aq. NH_4Cl, 76%; f) furan-2-yl-boronic acid, Na_2CO_3, $Pd(OAc)_2$, TPPTS, MeCN/water (1/2), 100 °C, 29%.

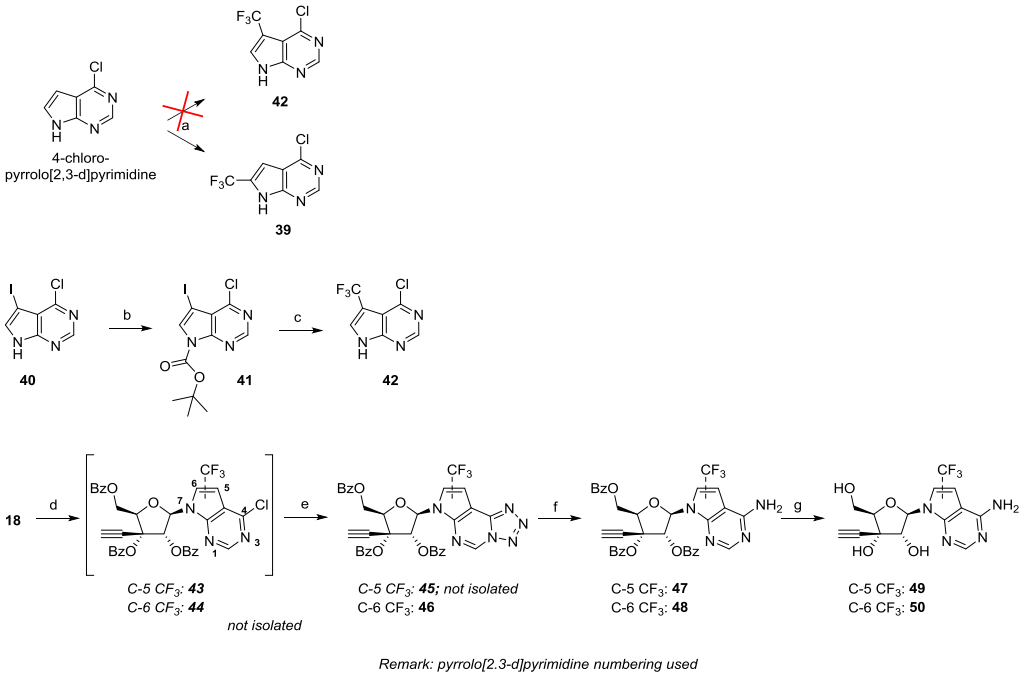
pyrimidine with the Langlois reagent (sodium trifluoromethanesulfinate) [35]. The presence of a $^3J_{H\text{-}1'\text{-}C\text{-}8}$ cross peak in the ^1H-^{13}C gHMBC spectrum of compound **48** (see Supporting Information) allowed to ascertain glycosylation at N9 (*N7*). However, a large coupling constant ($J = 37.8$ Hz) observed for C8 (*C6*) was inconsistent with the CF_3 group being attached to C7 (*C5*). This led us to assign the structure of the trifluoromethylated compound prepared using Langlois' reagent as the C8 (*C6*) regio-isomer. The C7 (*C5*) substituted heterocycle **42** could be obtained from the known C7 (*C5*) iodide **40** [34] by subsequent N-Boc protection and trifluoromethylation using the Ruppert reagent [37] (the N-Boc protecting group was lost during the reaction). Comparison of the ^1H NMR spectra of both regioisomers **39** and **42** (see Supporting Information) led to the confirmation that **39** and thus also **48**, are the C8 (*C6*) substituted isomers. Of note, a recent patent application [38] described both regio-isomers **39** and **42**, in which the regiochemical assignment is opposite to our conclusions. The C7 substituted heterocycle **42** was used to synthetize the desired product **49**, following the same reaction sequence as above.

To obtain the C7 ethynyl substituted analog **54** (Scheme 4) a Sonogashira reaction was envisioned. To avoid selectivity issues with the 3′-*C*-ethynyl group of **18**, this group was protected with a TMS group (**51**) and glycosylated (**52**). After introduction of the C7 ethynyl chain [25], **53** was transformed into **54** employing the same reaction sequence as described above. The synthesis of 3′-*C*-ethyl analog **59** started with catalytic hydrogenation of **18**, giving rise to **55**, which was subjected to glycosylation conditions. The glycosylation product **56** was directly used and elaborated as described above.

For the synthesis of 1,7-dideazapurine (7-azaindole or pyrrolo[2,3-*b*]pyridine)[3] nucleoside analogs (Schemes 5 and 6), commercially available 1*H*-4-chloro-pyrrolo[2,3-*b*]pyridine was halogenated with the appropriate halosuccinimide [34]. Glycosylation products were obtained using the same conditions as for their C7-deazapurine counterparts. Lewis acid-mediated glycosylation with this type of heterocycle has only been reported once [39]. Generally, nucleobase-anion glycosylation [40] or acid-catalyzed fusion [41] are employed to ensure this transformation. Both regio- and stereochemistry were ascertained by ^1H-^{13}C gHMBC and 2D NOESY experiments (see Supporting Information). Deprotection with NH_3/MeOH gave final products **66**, **67** and **68**. De-iodination by I/Mg exchange of **65** gave **69** in good yield, after which deprotection furnished **70**. As expected, introduction of the C6 (*C4*) azido group on e.g. **63** was problematic due to the higher electron density of the pyrrolo[2,3-*b*]pyridine system with respect to the pyrrolo[2,3-*d*]pyrimidine system. No desired product could be detected after reaction with NaN_3 at 65 °C, while gradual increase of the temperature to 100 °C (and higher) only led to degradation.

These issues led us to introduce the azido group [42] before the glycosylation step (Scheme 6). Glycosylation, employing the same conditions as for the chloride-substituted heterocycles, was first attempted with **72**, which afforded two products, **74** and **75**. The identity of each isomer was assigned after Staudinger reduction to **76** and **77** (Scheme 6) to facilitate purification. The ^1H-^{13}C gHMBC spectrum of **76** and **77** showed a markedly different cross-peak pattern between H-1′ and the heterocyclic moiety (see Supporting Information). The synthesis of iodo-substituted **79** was

[3] As for the 7-deazapurine analogs, in the body of the text, purine numbering will be used. In the Experimental section the corresponding pyrrolo[2,3-*b*]pyridine nomenclature is employed. In order to allow for a more facile comparison with the assigned NMR data (Experimental section), pyrrolo[2,3-*b*]pyridine numbering is indicated for selected compounds in *italic* and between brackets.



Scheme 3. Reagents and conditions: a) Langlois reagent, t-BuOOH, DCM/water; b) Boc_2O, DBU, DMAP, 1,4-dioxane, 96%; c) Ruppert reagent ($TMSCF_3$), $B(OMe)_3$, KF, CuI, 1,10-phenanthroline, DMSO, 60 °C, 23%; d) **39** or **42**, BSA, TMSOTf, MeCN, 80 °C; e) NaN_3, DMF, 65 °C, 43% (2 steps, **46**); f) (i) 1.0M PMe_3 in THF, THF; (ii) aq. HOAc, MeCN, 65 °C, 21% (3 steps, **47**), 55 % (**48**); g) 7N NH_3/MeOH, 80% (**49**), 87% (**50**).

accomplished using the same conditions (the tentative N3 (*N7*) isomer could be observed from TLC analysis, but was not isolated nor formally characterized). Both intermediates (**76** and **79**) afforded, after deprotection with saturated NH_3/MeOH, the desired nucleosides **80** and **81**.

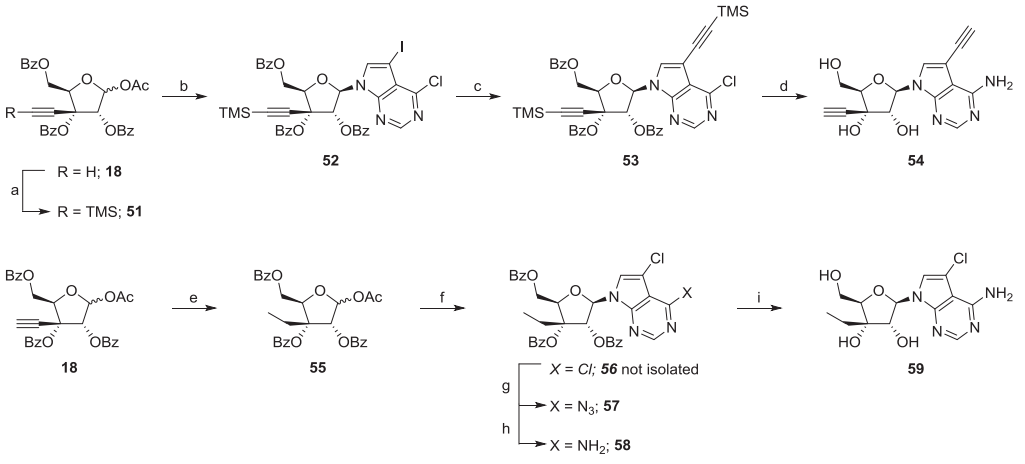
2.2. Biological evaluation

All final nucleoside analogs were assayed for their ability to inhibit cell proliferation of three different tumor cell lines (L1210, CEM and HeLa; Table 1) and for their antiviral activity against a representative panel of human viruses, including herpex simplex virus (HSV) 1 and 2, cytomegalovirus (CMV), varicella zoster virus (VZV), vaccinia virus (VV), adenovirus-2, influenza-A virus (H1N1, H3N2), influenza B virus, feline corona virus, feline herpes virus, para-influenza virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, vesicular stomatitis virus, respiratory syncytial virus (RSV) (Tables 4 and 5).

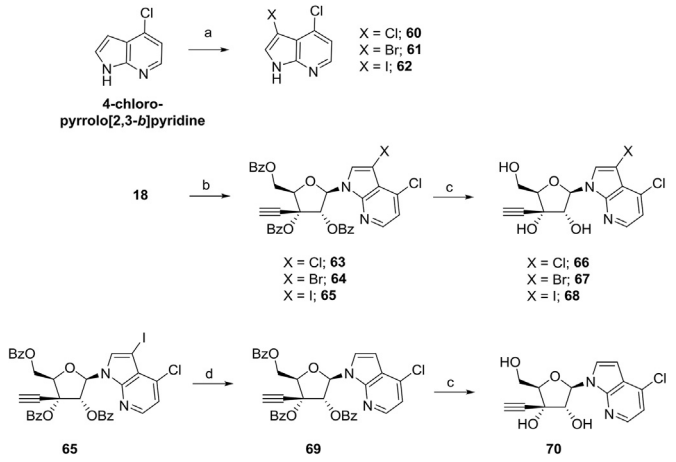
2.2.1. Antiproliferative activity

The results of the inhibition of cell line proliferation are depicted in Table 1.

Introduction of (substituted) phenyl rings in the C6 position of the purine nucleobase, known to confer cytostatic activity in



Scheme 4. Reagents and conditions: a) 1. iPrMgCl.LiCl (1.3M in THF), toluene, −65 °C; 2. TMSCl, 60%; b) 4-chloro-5-iodo-pyrrolo[2,3-*d*]pyrimidine (**40**) [34], BSA, TMSOTf, MeCN, 80 °C, 30%; c) ethynyltrimethylsilane, CuI, $Pd(Ph_3P)_2Cl_2$, Et_3N, DMF, 39%; d) (i) NaN_3, DMF, 65 °C; (ii) 1.0M PMe_3 in THF, THF; (iii) aq. HOAc, MeCN, 65 °C; (iv) 7N NH_3/MeOH, 46%; e) Pd/C, H_2 (balloon), ethyl acetate, 92%; f) 4,5-dichloro-7*H*-pyrrolo[2,3-*d*]pyrmidine [34], BSA, TMSOTf, MeCN, 80 °C; g) NaN_3, DMF, 65 °C, 55% (2 steps); h) (i) 1.0M PMe_3 in THF, THF; (ii) aq. HOAc, MeCN, 65 °C, 88%; i) 7N NH_3/MeOH, 87%.



Scheme 5. Reagents and conditions: a) appropriate *N*-halosuccinimide, DMF, 93% (X = Cl), 96% (X = Br), 92% (X = I); b) **60**–**62**, BSA, TMSOTf, MeCN, 80 °C, 25–39%; c) 7 N NH_3/MeOH, 70–80%; d) (i) iPrMgCl.LiCl (1.3M in THF), toluene, −65 °C; (ii) sat. aq. NH_4Cl, 75%.

ribofuranosylpurine nucleosides [22,23], failed to display any inhibitory activity on the cell line proliferation. Remarkably, 3′-*C*-ethynyl-7-deaza-adenosine (3′-*C*-ethynyltubercidin) **37**, as well as its 7-halogenated analogs **31**–**34** inhibited the proliferation of the different tumor cell lines with nanomolar IC_{50} values. The chloro (**32**) and bromo (**33**) analogs showed the highest antiproliferative activity irrespective of the cell line studied, while unsubstituted derivative **37** showed similar activity as EAdo (**5**). Interestingly, the observed structure-antiproliferative activity relationship from this small subset significantly differs from that observed for the corresponding ribofuranose derivatives [25]. Furan-2-yl substituted analog **38** was found to only weakly inhibit tumor cell proliferation, which contrasts to the potent activity observed for the corresponding 7-(furan-2-yl)-7-deazaadenosine [25]. Similarly, the 7-ethynyl analog **54** only showed weak antiproliferative activity, which also contrasts with the activity observed for the corresponding ribofuranose analog [25]. Saturation of the ethynyl substituent as in **59**, resulted in two orders of magnitude lower IC_{50}'s than those for **32**.

The 7-trifluoromethyl analog **49** gave submicromolar activity, while the corresponding C8 isomer **50** was completely devoid of activity, possibly due to a preferred *anti*-orientation of the purine ring for activity.

To investigate the importance of N1, we synthetized the 1,7-dideazapurine or pyrrolo[2,3-*b*]pyridine analogs **66**–**68**, **70** and **80** and **81**. Interestingly, all the analogs elicited antiproliferative effects, most notably for the CEM cell line (C6 chloride analogs), except for **70**. In the C6 chloride series, the antiproliferative activity correlated with halogen size (I > Br > Cl > H). While analog **80** was significantly less active than the related **32** (approximately 100-fold); this was not the case for the iodo-substituted analog **81**, which displayed potent antiproliferative activity, especially on the L1210 cell line.

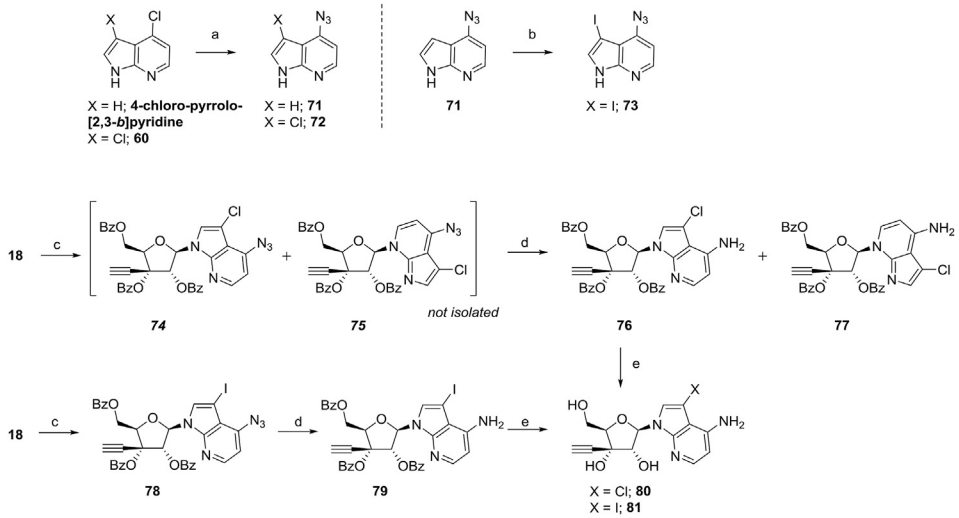
To further explore the potential of these new nucleoside analogs, the most potent analogs (**31**–**34** and **37**) were selected for testing in the NCI-60 cell line panel [43,44]. Tables 2 and 3 summarize the GI_{50} values of representative cell lines. Full assay data as well as mean GI_{50} graphs are provided in the Supporting Information.

Potent growth inhibitory activity was observed for **32**, **33** and **34**, while **31** and **37** were less active. The activity spectrum of the former analogs was found to be broad, and especially pronounced for leukemia cell lines as observed for e.g. clofarabine. GI_{50} values for several solid tumor cell lines (e.g. MCF-7, HCT-116, U251, NCI-H460) are below 100 nM.

Additionally, these analogs were evaluated for their potential to inhibit the cell proliferation of three different endothelial cell types. Agents capable of modifying the tumor vasculature, either by antiangiogenic or vascular-disrupting action, are of interest for antitumor therapies both as single agents and in combination with other chemotherapeutic drugs. Disruption of vascular networks in solid tumors may induce their collapse by deprivation of oxygen and other nutrients [45]. Nucleoside analogs **31**–**34**, **37** were found to potently inhibit the proliferation of the three endothelial cell types studied, with the halogenated derivatives **32** (Cl) and **33** (Br) being most potent. Unfortunately, these analogs also significantly inhibited the proliferation of Hel-fibroblasts.

2.2.2. *In vivo evaluation of compound* **32**

Encouraged by the strong *in vitro* anti-proliferative activity of analogs **31**–**34**, **37**, **49** and **81** against various tumor cell lines



Scheme 6. Reagents and conditions: a) NaN_3, NH_4Cl, DMF, 110 °C, 66% (**71**), 71% (**72**); b) NIS, DMF, 93%; c) **72** or **73**, BSA, TMSOTf, MeCN, 80 °C, 25% (**78**); d) (i) 1.0M PMe_3 in THF, THF; (ii) aq. HOAc, MeCN, 65 °C, 22% (2 steps, **76**), 13% (2 steps, **77**), 74% (**79**); e) 7N NH_3/MeOH, 73% (**80**), 74% (**81**).

Table 1
Effects of different 3′-*C*-ethynyl purine derivatives on the proliferation of three tumor cell lines. IC_{50} values represent the concentration of compound able to inhibit proliferation by 50% (Coulter Counter cell count endpoint).

Cpd.	L1210 IC_{50} (μM)	CEM IC_{50} (μM)	HeLa IC_{50} (μM)	Cpd.	L1210 IC_{50} (μM)	CEM IC_{50} (μM)	HeLa IC_{50} (μM)
5[a]	0.73 ± 0.14	0.61 ± 0.08	0.29 ± 0.11	**38**	30 ± 1	17 ± 5	14 ± 1
13	>250	>250	>250	**49**	0.11 ± 0.03	0.36 ± 0.26	0.75 ± 0.19
14	205 ± 45	154 ± 8	>250	**50**	>250	>250	>250
15	>250	>250	>250	**54**	159 ± 49	63 ± 0	114 ± 20
16	225 ± 23	124 ± 49	170 ± 53	**59**	1.2 ± 0.1	2.6 ± 1.2	4.4 ± 1.5
17	>250	223 ± 21	220 ± 30	**66**	75 ± 19	1.3 ± 0.1	6.3 ± 3.8
31	0.035 ± 0.008	0.16 ± 0.02	0.15 ± 0.01	**67**	30 ± 6	0.56 ± 0.07	0.89 ± 0.11
32	0.014 ± 0.009	0.012 ± 0.001	0.051 ± 0.006	**68**	20 ± 5	0.26 ± 0.03	1.1 ± 0.1
33	0.028 ± 0.013	0.030 ± 0.007	0.093 ± 0.009	**70**	197 ± 62	86 ± 4	135 ± 1
34	0.056 ± 0.012	0.12 ± 0.02	0.18 ± 0.04	**80**	0.98 ± 0.03	5.4 ± 0.8	1.7 ± 0.4
37	0.38 ± 0.04	0.71 ± 0.13	0.88 ± 0.09	**81**	0.044 ± 0.14	0.31 ± 0.27	0.18 ± 0.03

[a] Results are taken from Ref. [21].

(Tables 1–3), analog **32** was selected for *in vivo* evaluation of its antitumor activity in a metastatic breast cancer xenograft mouse model employing MDA-MB-231 (LM2) cells expressing firefly luciferase [46]. Antitumor activity was assessed by measurement of both the BLI signal (Fig. 2, Panel A) and the tumor volume (Panel B). LM2 cells were orthotopically engrafted in SCID mice and treatment commenced once the tumor was palpable (day 10). Compound **32** was injected intratumorally (0.3 mg/kg) 3 times a week for two consecutive weeks. The tumor growth was significantly retarded starting from day 18, i.e. after four i.t. injections, as measured by bioluminescent radiance (Panel A). The reduced tumor growth was also obvious when the tumor size was measured using a digital caliper (Panel B). At day 35, mice were sacrificed and the tumors removed, after which they were macroscopically examined (Panel B). The average control tumor weight was 551 ± 74 mg versus 241 ± 61 mg for the treatment group.

To analyze the effect of **32** on lung metastasis, mice were covered with a black paper during imaging to shield the primary tumor. Interestingly, the total metastasis burden in the lungs was significantly lower in the treated group at day 34 *versus* the vehicle control group (Panel C). This indicates that the nucleoside analog not only exhibits antitumor activity but also reduces breast cancer metastasis to secondary organs.

2.2.3. Antiviral evaluation

Most analogs either showed no antiviral activity up to the highest concentration tested (100 μM) when assayed against a panel of relevant viruses (see Experimental section), or the activity was accompanied by significant toxicity for the host cell, making the derivatives non-specific (data not shown). Only a few analogs combined antiviral activities with acceptable selectivity indices. The results are summarized in Table 5 & Table 6. EAdo (**5**) showed activity against vaccinia virus (Table 5), although it also potently inhibited Hel cell line proliferation (Table 6). Activity against vaccinia virus and HSV-2 was also found for **59**, which, however generally inhibited cell proliferation (see also Table 1).

The most promising antiviral activity was found for compound **66**, which exhibited potent activity against human cytomegalovirus (hCMV), with a reasonable selectivity index (10-fold). Other halogen-substituted derivatives **67** (Br) and **68** (I) displayed elevated cytotoxicity and are therefore non-selective. Apparently, the observed activity is specific for halogen bearing compounds, as

Table 2
Summary of the growth inhibitory potential (expressed as GI_{50}) of selected nucleoside analogs against the NCI-60 tumor cell line panel with Sulforhodamine B (SRB) read-out at 48 h [43,44]. Full details (GI_{50} values for all cell lines) can be found in the Supporting Information. Values represent mean ± SEM of two independent evaluations.

Cpd	Leukemia			Lung		Colon
	CCRF-CEM GI_{50} (μM)	HL-60 GI_{50} (μM)	SR GI_{50} (μM)	A549 GI_{50} (μM)	NCI-H460 GI_{50} (μM)	HCT116 GI_{50} (μM)
31[b]	0.338	0.436	0.185	0.377	0.231	0.153
32	0.248 ± 0.009	0.059 ± 0.025	0.074 ± 0.013	0.188 ± 0.050	[<0.01–0.046][a]	0.020 ± 0.002
33	0.213 ± 0.073	0.047 ± 0.025	0.064 ± 0.02	0.019 ± 0.097	0.034 ± 0.016	0.028 ± 0.002
34	0.272 ± 0.023	0.050 ± 0.018	0.28 ± 0.098	0.443 ± 0.146	0.098 ± 0.039	0.045 ± 0.01
37[b]	2.3	1.57	0.817	1.31	0.473	0.595

[a] Values in brackets represent the obtained GI_{50} values from both experiments.
[b] Compounds **31** and **37** were only tested once.

Table 3
Summary of the growth inhibitory potential (expressed as GI_{50}) of selected nucleoside analogs against the NCI-60 tumor cell line panel with Sulforhodamine B (SRB) read-out at 48 h [43,44]. Full details (GI_{50} values for all cell lines) can be found in the Supporting Information. Values represent mean ± SEM of two independent evaluations.

Cpd	CNS	Melanoma	Prostate		Breast	
	U251 GI_{50} (μM)	Lox IMVI GI_{50} (μM)	PC-3 GI_{50} (μM)	DU145 GI_{50} (μM)	MCF7 GI_{50} (μM)	MDA-MB-231 GI_{50} (μM)
31[b]	0.631	0.279	0.293	0.354	0.103	0.379
32	[<0.01–0.02][a]	0.059 ± 0.023	0.192 ± 0.019	0.134 ± 0.025	0.027 ± 0.005	0.170 ± 0.02
33	0.022 ± 0.009	0.067 ± 0.038	0.107 ± 0.060	0.107 ± 0.044	0.023 ± 0007	0.136 ± 0.08
34	0.074 ± 0.006	0.060 ± 0.021	0.076 ± 0.010	0.131 ± 0.033	[<0.01–0.014][a]	0.186 ± 0.02
37[b]	2.44	0.667	0.808	0.563	0.239	1.53

[a] Values in brackets represent the obtained GI_{50} values from both experiments.
[b] Compounds **31** and **37** were only tested once.

Table 4
Proliferation inhibition on different endothelial cell types: HMEC-1 (Human Dermal Microvascular Endothelial Cells), HMVEC (Human Microvascular Endothelial Cells) and HUVEC (Human Umbilical Vein Endothelial Cells), as well as Hel (Human embryonic lung fibroblasts). IC_{50} values represent the concentration of compound able to inhibit proliferation by 50% (Coulter Counter cell count endpoint).

Cpd.	HMEC-1 IC_{50} (μM)	HMVEC IC_{50} (μM)	HUVEC IC_{50} (μM)	Hel IC_{50} (μM)
31	0.061 ± 0.006	<0.00128	0.067 ± 0.03	0.076 ± 0.019
32	0.0035 ± 0.0009	<0.00128	0.0049 ± 0.0022	0.0092 ± 0.0066
33	0.0045 ± 0.0003	0.0019	0.018 ± 0.006	0.017 ± 0.006
34	0.018 ± 0.015	<0.00128	0.0099 ± 0.0074	0.032 ± 0.023
37	0.061 ± 0.006	N.D	N.D	N.D

Table 5
Antiviral activity against herpes virus-1 (HSV-1), herpes virus-2 (HSV-2), acyclovir-resistant HSV (Thymidine kinase knock-out) and vaccinia virus cultured in Hel (human embryonic lung) cells.

Cpd	HSV-1 (KOS) EC_{50}[a] (μM)	HSV-2 (G) EC_{50}[a] (μM)	HSV-1 (TK^-) KOS ACV^r EC_{50}[a] (μM)	vaccinia virus EC_{50}[a] (μM)	MCC[b] (μM)	CC_{50}[c] (μM)
5	>4	>4	>4	0.35 ± 0.05	20	0.73 ± 0.07
59	2.10 ± 0.97	0.63 ± 0.09	1.27 ± 0.52	0.73 ± 0.33	≥20	N.D.
66	16.3 ± 3.7	>100	10.57 ± 4.84	>100	≥100	5.25 ± 3.80
67	6.67 ± 2.67	>100	5.83 ± 3.17	>100	>100	0.35 ± 0.02
68	>100	>100	>100	>100	>100	0.16 ± 0.0
70	>100	>100	>100	>100	>100	N.D.
Acyclovir	0.2	0.2	10	>250	>250	N.D.
Cidofovir	1.5	1.2	2.0	22	>250	N.D.
Ganciclovir	0.03	0.03	0.5	>100	>100	N.D.

[a] Antiviral activity is expressed as EC_{50} values (μM) and represent the concentration of test compound necessary to reduce viral-induced cytopathogenicity by 50%.
[b] MCC or minimal cytotoxic concentration represents the concentration of test compound that is able to cause a microscopically detectable alteration of normal cell morphology.
[c] CC_{50} represents the concentration (μM) of test compound that reduces cell (Hel) proliferation by 50% as determined by Coulter Counter.

the parent compound without halogen (**70**) was inactive. Furthermore, changing the 6-chloride for an amino group, mimicking a natural adenine, was detrimental for the activity (compare **66** and **80**).

3. Conclusion

We have developed a nucleoside library around a 3′-*C*-ethynylribofuranose moiety, which was combined with several purine nucleobases that bear substituents, which were previously found to confer biological activity when combined with other sugar motifs. While 6-aryl purine nucleoside analogs **13**–**17** were devoid of antiproliferative or antiviral activity, the 7-halogenated 7-deazapurine analogs (**31**–**34**), as well as trifluoromethylated derivative **49** and the C7 unsubstituted analog **37** were found to significantly inhibit the growth of three tumor cell lines. Their spectrum of activity was thoroughly investigated (except for **49**) by assaying against the NCI-60 panel, which also revealed nanomolar activity against several solid tumor derived cell lines. Removal of the N7 nitrogen and introduction of substitutions at C7 (particularly halogens, derivatives **31**–**34**) was shown to lead to significantly more potent antiproliferative nucleosides than the C2/C6 modified derivatives we have previously reported. Additionally, analogs **31**–**34** potently inhibited proliferation of endothelial cell types, which could be particularly interesting for the treatment of solid tumors. As a proof-of-concept, analog **32** was investigated in a metastatic breast cancer xenograft mouse model. This derivative inhibited both tumor growth as well as metastasis as assessed by means of BLI. However, **32** also potently inhibited *in vitro* proliferation of Hel-fibroblasts, requiring further optimization to improve on selectivity. Several 1,7-dideaza-3-*C*-ethynyl analogs were prepared and some of them found to be potent inhibitors of human cytomegalovirus (hCMV) *in vitro*. Particularly, analog **66** requires further evaluation.

In conclusion, the results presented in this paper showcase the utility of screening a focused nucleoside library for both antiviral as well as antiproliferative activity as a means of finding novel hits for further elaboration.

4. Experimental

4.1. General experimental

All reagents and solvents were obtained from standard commercial sources and were of analytical grade. Unless otherwise specified, they were used as received. **6** [21], **18** [15], were prepared according to literature procedures. Halogenated heterocycles 4-chloro-5-halo-pyrrolo[2,3-*d*]pyrimidine: 5-fluoro [32,33], 5-chloro [34], 5-bromo [34], 5-iodo (**40**) [34], were prepared from commercially available 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine employing literature conditions.

All moisture sensitive reactions were carried out under argon atmosphere. Reactions were carried out at ambient temperature unless otherwise indicated. Analytical TLC was performed on Machery-Nagel® pre-coated F254 aluminum plates and were visualized by UV followed by staining with basic aq. $KMnO_4$ or sulfuric acid-anisaldehyde spray. Column chromatography was performed using Davisil® (40–63 μm) or on a Reveleris X2 (Grace/Büchi) automated Flash unit employing pre-packed silica columns. Exact mass measurements were performed on a Waters LCT Premier XE™ Time of Flight (ToF) mass spectrometer equipped with a standard electrospray (ESI) and modular Lockspray™ interface. Samples were infused in a MeCN/water (1:1) + 0.1% formic acid mixture at 100 μL/min. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts (δ) are given in ppm and spectra are referenced to the residual solvent peak. Coupling constants are given in Hz. In ^{19}F NMR, signals were referenced to $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm. After glycosylation, both the correct stereochemistry at C1' (β) and the correct regiochemistry (N9; *purine numbering*) of the glycosylation products was ascertained by means of 2D NMR techniques (2D NOESY,

^{1}H-^{13}C gHMBC, respectively), either on the protected or on the deprotected derivative (depending on peak resolution). Melting points were determined on a Büchi-545 apparatus and are uncorrected. Purity was assessed by means of analytical LC-MS employing either:

(1) Waters Alliance 2695 XE separation Module using a Phenomenex Luna® reversed-phase C18 (2) column (3 μm, 100 × 2.00 mm) and a gradient system of HCOOH in H_2O (0.1%, v/v)/HCOOH in MeCN (0.1%, v/v) at a flow rate of 0.4 mL/min, 10:90 to 0:100 in 9 min. High-resolution MS spectra were recorded on a Waters LCT Premier XE Mass spectrometer.
(2) Waters AutoPurification system (equipped with ACQUITY QDa (mass; 100–1000 amu)) and 2998 Photodiode Array (220–400 nm)) using a Waters Cortecs® C18 (2.7 μm 100 × 4.6 mm) column and a gradient system of HCOOH in H_2O (0.2%, v/v)/MeCN at a flow rate of 1.44 mL/min, 95:05 to 00:100 in 6.5 min.

All obtained final compounds had purity >95%, as assayed by analytical HPLC (UV) unless otherwise indicated.

4.2. Chemistry

4.2.1. General procedure A (Suzuki coupling (6-Cl-purine derivatives))

In a flame-dried 25 mL round-bottom flask, equipped with a stir bar was added under argon, 6-chloro purine nucleoside derivative **6** (0.5 mmol, 1 eq.), the corresponding boronic acid (1.5 eq.), anhydrous K_2CO_3 (1.5 eq.) and $Pd(Ph_3P)_4$ (0.05 eq.). The flask was evacuated and refilled with argon three times. Then, anhydrous degassed toluene (5 mL, 10 mL/mmol SM) was added and the mixture stirred for approximately 5 min before being heated to 100 °C. After TLC monitoring showed full conversion of the starting material (~2–4h), the mixture was allowed to cool to room temperature, filtered and evaporated till dryness. The residue was purified by column chromatography (0 → 5% acetone/DCM).

4.2.2. General procedure B (nucleoside deprotection (ester hydrolysis))

The ester protected nucleoside (1 eq.) was dissolved in 7N NH_3 in MeOH and stirred at ambient temperature until TLC showed full conversion (generally overnight to 36h). Then, the mixture was evaporated to dryness and the residue purified by column chromatography (typically 2 → 10% MeOH/DCM).

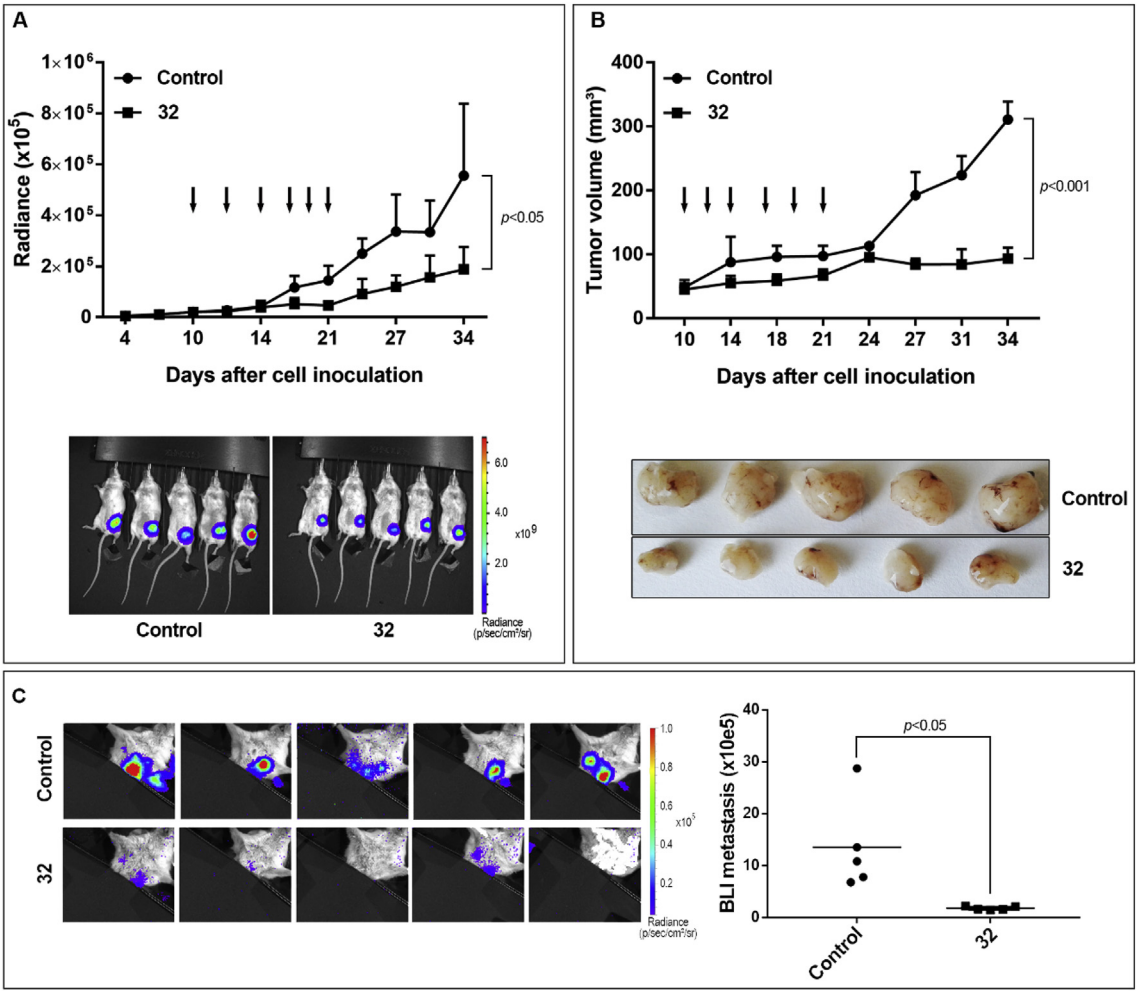


Fig. 2. *In vivo* evaluation of **32**: MDA-MB-231-LM2 cells were orthotopically engrafted in the mammary fat pad of SCID mice. Compound **32** or vehicle were injected i.t. starting 10 days after inoculation and dosed 3 times a week, for 2 consecutive weeks. Arrows indicate compound administration. Panel A: BLI signal at regular time intervals. Data are mean ± STDEV, n = 5. Representative bioluminescence images of vehicle control and **32**-treated mice at day 34 are shown. Panel B: Tumor volumes calculated from caliper measurements. Data are mean ± STDEV, n = 5. At day 35 mice were sacrificed and the corresponding pictures of dissected tumors are shown. Panel C: Lung metastasis at day 34 was quantified after shielding the primary tumor. Data are mean ± STDEV, n = 5. Statistical significance is indicated (multiple *t*-test).

Table 6
Antiviral activity against varicella-zoster virus and human cytomegalovirus.

Cpd.	VZV TK$^+$ (OKA) EC_{50}^{a} (μM)	VZV TK$^-$ (07_01) EC_{50}^{a} (μM)	MCC[b] (μM)	CC_{50}^{c} (μM)	CMV (AD-169) EC_{50}^{d} (μM)	CMV (Davis) EC_{50}^{d} (μM)	MCC[e] (μM)	CC50[f] (μM)
5	1.82 ± 0.24	1.64 ± 0.44	20	0.7 ± 0.1	≥4	≥4	20	0.73 ± 0.07
59	1.71 ± 0.63	3.74 ± 2.69	≥20	3.41 ± 2.25	1.79	>4	4	N.D.
66	16.55	3.06	20	N.D.	0.51 ± 0.0	0.53 ± 0.09	≥20	5.25 ± 3.80
67	2.76 ± 0.09	2.27 ± 1.74	≥100	0.35 ± 0.02	0.36 ± 0.0	0.35 ± 0.02	>100	0.35 ± 0.02
68	9.82 ± 2.21	8.26 ± 2.74	20	0.16 ± 0.0	2.31 ± 1.70	0.66 ± 0.15	20	0.16 ± 0.0
70	>100	>100	>100	N.D.	>100	>100	20	N.D.
80	>100	17.49	>100	N.D.	>100	20	100	N.D.
Acyclovir	1.26 ± 0.73	36.74 ± 2.95	>440	>440	N.D.	N.D.	N.D.	N.D.
Cidofovir	N.D.	N.D.	N.D.	N.D.	1.59 ± 0.35	1.45 ± 0.18	>300	N.D.
Ganciclovir	N.D.	N.D.	N.D.	N.D.	11.75 ± 0.32	6.52 ± 0.53	>350	N.D.

[a]Activity against varicella-zoster virus (VZV) in Hel (human embryonic lung) culture; TK$^-$: thymidylate kinase knock-out strain; EC_{50} values (μM) represent the concentration of compound that reduces virus-induced cytopathicity by 50%.[b,e] MCC or minimal cytotoxic concentration represents the concentration of test compound that causes a microscopically detectable alteration of normal cell morphology.[c,f] CC_{50} represents the concentration (μM) of test compound that reduces cell (Hel) proliferation by 50% as determined by Coulter Counter.[d] Antiviral activity against cytomegalovirus (CMV) in Hel culture; EC_{50} values (μM) represent the concentration of compound that reduce virus-induced cytophathicity by 50%.

4.2.3. General procedure C (Vorbrüggen glycosylation of pyrrolo [2,3-d]pyrimidine and pyrrolo [2,3-b]pyridine derivatives)

In a flame-dried 2-neck round bottom flask, equipped with a stir bar was added the appropriate heterocycle (1.1 eq.) under argon. Then, anhydrous MeCN (7.5 mL/mmol SM) was added, followed by BSA (1.2 eq.). The resulting suspension was stirred at ambient temperature for approximately 10 min; after which a clear solution was obtained. Then, glycosyl donor, **18** [15] (1 eq.) was added in one portion, immediately followed by TMSOTf (1.25 eq.). The resulting solution was stirred at ambient temperature for another 15 min, and then transferred to a pre-heated oil bath at 80 °C. Heating was continued until TLC analysis showed full consumption of the starting material (~2–3h), after which the mixture was cooled to ambient temperature. EA was added, and the mixture poured into a sat. aq. $NaHCO_3$ solution. The layers were separated and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography 15% EA/Hexanes.

4.2.4. General procedure D (nucleophilic displacement with sodium azide)

The appropriate chloro-nucleoside (1 eq.) was dissolved in DMF (10 mL/mmol). Then, NaN_3 (2 eq.) was added and the mixture stirred at 65 °C for 30 min. Then, the mixture was cooled to ambient temperature after which it was poured into half-saturated aq. $NaHCO_3$ solution and EA. The layers were separated and the water layer extracted two more times with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography (EA/Hexanes) to yield the protected azidonucleoside.

4.2.5. General procedure E (Staudinger reduction & iminophosphorane hydrolysis)

The appropriate azidonucleoside (1 eq.) was dissolved in THF (10 mL/mmol). Then, PMe_3 solution (1 M in THF; 2 eq.) was added and the mixture stirred at ambient temperature until TLC analysis showed full conversion of starting material (generally 30 min to 1 h). Next, the solution was evaporated till dryness, and subsequently re-dissolved in MeCN (10 mL/mmol). To this solution was added a 1 M aq. HOAc solution (3.33 eq.), and the mixture heated in a pre-heated oil bath at 65 °C for 1h. Next, the mixture was cooled to ambient temperature and poured into sat. aq. $NaHCO_3$ solution. DCM was added, layers were separated, and the water layer extracted two more times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography (EA/Hexanes) gave rise to the protected nucleoside aminopurine.

4.2.6. N9-β-d-ribofuranosyl-6-phenylpurine (2)[22]

2 was prepared according to a literature procedure [22]. Spectral data are in accordance with literature values [22]. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.56–3.64 (m, 1H, H-5″), 3.69–3.76 (m, 1H, H-5′), 4.00 (dd, J = 7.8, 3.8 Hz, 1H, H-4′), 4.20–4.24 (m, 1H, H-3′), 4.66 (dd, J = 10.8, 5.7 Hz, 1H, H-2′), 5.13 (t, J = 5.7 Hz, 1H, OH-5′), 5.25 (d, J = 5.1 Hz, 1H, OH-3′), 5.56 (d, J = 6.0 Hz, 1H, OH-2′), 6.10 (d, J = 5.4 Hz, 1H, H-1'), 7.58–7.65 (m, 3H, H_{Phe}), 8.82–8.85 (m, 2H, H_{Phe}), 8.93 (s, 1H, H-8), 9.02 (s, 1H, H-2).

4.2.7. 6-chloro-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-d-ribofuranosyl)-purine **(7)**

6-chloropurine (0.46 g, 2.94 mmol, 1.5 eq.) was suspended in HMDS (16 mL, 8 mL/mmol SM) and a catalytic amount of $(NH_4)_2SO_4$ was added. The mixture was then refluxed overnight. After cooling to ambient temperature, the resulting solution was carefully evaporated till dryness, and the resulting oil further dried at high vacuum (~1 h). Then, anhydrous 1,2-dichloroethane (16 mL, 8 mL/mmol SM) was added to dissolve the silylated heterocycle, after which **6** [21] (0.67 g, 1.96 mmol, 1 eq.) was added via syringe, immediately followed by TMSOTf (0.71 mL, 3.91 mmol, 2 eq.). The resulting solution was subsequently refluxed for approximately 30 min and cooled to ambient temperature. Then, the mixture was poured into sat. aq. $NaHCO_3$, and DCM was added. The layers were separated, and the water layer extracted twice more with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography 5 → 7.5% acetone/DCM to give **7** (0.693 g, 1.59 mmol) as a white foam in 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (s, 3H, acetyl-CH_3), 2.14 (s, 3H, acetyl-CH_3), 2.20 (s, 3H, acetyl-CH_3), 2.86 (s, 1H, ethynyl-H), 4.54 (dd, J = 14.7, 5.7 Hz, 1H, H-5″), 4.61–4.68 (m, 2H, H-5′, H-4′), 6.05 (d, J = 4.5 Hz, 1H, H-2′), 6.30 (d, J = 4.8 Hz, 1H, H-1′), 8.52 (s, 1H, H-8), 8.77 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.5 (acetyl-CH_3), 20.9 (acetyl-CH_3), 21.0 (acetyl-CH_3), 63.3 (C-5′), 75.3, 76.0, 77.0, 79.9 (C-2′), 81.6 (C-4′), 86.4 (C-1′), 131.9 (C-5), 143.1 (C-8), 151.7, 152.6 (2C), 168.4 (C=O), 168.7 (C=O), 170.4 (C=O). HRMS (ESI): calculated for $C_{18}H_{19}Cl_1N_5O_7$ ($[M+H]^+$): 452.0968, found: 452.0970. [Remark: correct stereo- and regiochemistry was ascertained by transforming a small amount into 3′-C-ethynyladenosine (**5**) and comparing NMR data to literature reference; [15] which confirmed the correct structure].

*4.2.8. 6-Phenyl-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-D-ribofuranosyl)-purine **(8)***

8 was prepared according to General procedure A. **7** (0.22 g, 0.5 mmol) gave rise to **8** (0.101 g, 0.211 mmol) as a white foam in 42% yield. Purification: 0 → 5% acetone/DCM; second column 0 → 35% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.12 (s, 3H, acetyl-CH_3), 2.16 (s, 3H, acetyl-CH_3), 2.22 (s, 3H, acetyl-CH_3), 2.86 (s, 1H, ethynyl-H), 4.52–4.58 (m, 1H, H-5″), 4.62–4.69 (m, 2H, H-4′, H-5′), 6.12 (d, J = 4.8 Hz, 1H, H-2′), 6.40 (d, J = 4.8 Hz, 1H, H-1′), 7.54–7.61 (m, 3H, H_{Phe}), 8.55 (s, 1H, H-8), 8.75–8.79 (m, 2H, H_{Phe}), 9.04 (s, 1H, H-2). HRMS (ESI): calculated for $C_{24}H_{23}N_4O_7$ ($[M+H]^+$): 479.1561, found: 479.1562.

*4.2.9. 6-(4-Methylphenyl)-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-D-ribofuranosyl)-purine **(9)***

9 was prepared according to General procedure A. **7** (0.22 g, 0.5 mmol) gave rise to **9** (0.107 g, 0.217 mmol) as a white foam in 43% yield. Purification: 0 → 5% acetone/DCM; second column 0 → 35 EA/Hexanes. ^{1}H NMR (300 MHz, CDCl3) δ: 2.12 (s, 3H, acetyl-CH_3), 2.16 (s, 3H, acetyl-CH_3), 2.22 (s, 3H, acetyl-CH_3), 2.46 (s, 3H, CH_3), 2.86 (s, 1H, ethynyl-H), 4.51–4.58 (m, 1H, H-5″), 4.61–4.70 (m, 2H, H-4′, H-5′), 6.12 (d, J = 4.8 Hz, 1H, H-2′), 6.39 (d, J = 5.1 Hz, 1H, H-1′), 7.36–7.39 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.53 (s, 1H, H-8), 8.67–8.70 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 9.01 (s, 1H, H-2). HRMS (ESI): calculated for $C_{25}H_{25}N_4O_7$ ($[M+H]^+$): 493.1718, found: 493.1732.

*4.2.10. 6-(4-Methoxyphenyl)-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-D-ribofuranosyl)-purine **(10)***

10 was prepared according to General procedure A. **7** (0.22 g, 0.5 mmol) gave rise to **10** (0.131 g, 0.258 mmol) as a white foam in 52% yield. Purification: 0 → 5% acetone/DCM; second column 0 → 50 EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (s, 3H, acetyl-CH_3), 2.15 (s, 3H, acetyl-CH_3), 2.22 (s, 3H, acetyl-CH_3), 2.86 (s, 1H, ethynyl-H), 3.91 (s, 3H, OCH_3), 4.54 (dd, J = 14.4, 5.4 Hz, 1H, H-5″), 4.62–4.66 (m, 2H, H-4′, H-5′), 6.11 (d, J = 4.8 Hz, 1H, H-2′), 6.39 (d, J = 5.1 Hz, 1H, H-1′), 7.06–7.09 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.51 (s, 1H, H-8), 8.80–8.83 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 8.97 (s, 1H, H-2). HRMS (ESI): calculated for $C_{25}H_{25}N_4O_8$ ($[M+H]^+$): 509.1667, found: 509.1654.

*4.2.11. 6-(4-Chlorophenyl)-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-D-ribofuranosyl)-purine **(11)***

11 was prepared according to General procedure A. **7** (0.22 g, 0.5 mmol) gave rise to **11** (0.09 g, 0.175 mmol) as a slightly yellow foam in 35% yield. Purification: 0 → 37% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.12 (s, 3H, acetyl-CH_3), 2.16 (s, 3H, acetyl-CH_3), 2.22 (s, 3H, acetyl-CH_3), 2.87 (s, 1H, ethynyl-H), 4.55 (dd, J = 14.7 Hz, 5.7 Hz, 1H, H-5″), 4.62–4.69 (m, 2H, H-4′, H-5′), 6.10 (d, J = 4.8 Hz, 1H, H-2′), 6.39 (d, J = 4.8 Hz, 1H, H-1′), 7.52–7.56 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.55 (s, 1H, H-8), 8.76–8.81 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 9.02 (s, 1H, H-2). HRMS (ESI): calculated for $C_{24}H_{22}ClN_4O_7$ ($[M+H]^+$): 513.1172, found: 513.1170.

*4.2.12. 6-(4-Fluorophenyl)-N9-(2′,3′,5′-tri-O-acetyl-3′-C-ethynyl-β-D-ribofuranosyl)-purine **(12)***

12 was prepared according to General procedure A. **7** (0.22 g, 0.5 mmol) gave rise to **12** (0.075 g, 0.152 mmol) as a slightly yellow foam in 30% yield. Purification: 0 → 37% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (s, 3H, acetyl-CH_3), 2.16 (s, 3H, acetyl-CH_3), 2.22 (s, 3H, acetyl-CH_3), 2.86 (s, 1H, ethynyl-H), 4.55 (dd, J = 15, 5.7 Hz, 1H, H-5″), 4.62–4.69 (m, 2H, H-4′, H-5′), 6.10 (d, J = 4.8 Hz, 1H, H-2′), 6.38 (d, J = 4.8 Hz, 1H, H-1′), 7.21–7.27 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.54 (s, 1H, H-8), 8.82–8.87 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 9.01 (s, 1H, H-2). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −108.6 to −108.5 (m, 1 F). HRMS (ESI): calculated for $C_{24}H_{22}FN_4O_7$ ($[M+H]^+$): 497.1467, found: 497.1469.

*4.2.13. 6-Phenyl-N9-(3′-C-ethynyl-β-D-ribofuranosyl)-purine **(13)***

13 was prepared according to General Procedure B. **8** (0.1 g, 0.209 mmol) gave rise to **13** (0.032 g, 0.091 mmol) as a white solid in 43% yield. Purification: 3% MeOH/DCM. Melting point: 221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.61 (s, 1H, ethynyl-H), 3.73–3.84 (m, 2H, H-5′, H-5″), 4.06 (dd, J = 4.5, 3.0 Hz, 1H, H-4′), 4.91 (t, J = 7.2 Hz, 1H, H-2′), 5.18 (t, J = 5.1 Hz, 1H, OH-5′), 6.00 (d, J = 6.9 Hz, 1H, OH-2′), 6.09 (d, J = 7.5 Hz, 1H, H-1′), 6.14 (s, 1H, OH-3′), 7.59–7.65 (m, 3H, H-3$_{Phe}$, H-4$_{Phe}$, H-5$_{Phe}$), 8.81–8.87 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 8.95 (s, 1H, H-8), 9.03 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 77.3, 78.0 (C-2′), 82.6, 86.2 (C-1′), 87.8 (C-4′), 128.7 (2C, C-3$_{Phe}$, C-5$_{Phe}$), 129.4 (2C, C-2$_{Phe}$, C-6$_{Phe}$), 130.8 (C-5), 131.2 (C-4$_{Phe}$), 135.2 (C-1$_{Phe}$), 145.2 (C-8), 152.0 (C-2), 152.5 (C-4), 153.1 (C-6). HRMS (ESI): calculated for $C_{18}H_{17}N_4O_4$ ($[M+H]^+$): 353.1244, found: 353.1259. [**Remark**: The final product contained ~1.5 eq. of acetamide; which was added to the MW of the product.]

*4.2.14. 6-(4-Methylphenyl)-N9-(3′-C-ethynyl-β-D-ribofuranosyl)-purine **(14)***

14 was prepared according to General Procedure B. **9** (0.1 g, 0.203 mmol) gave rise to **14** (0.040 g, 0.109 mmol) as a white solid in 54% yield. Purification: 3% MeOH/DCM. Melting point: 221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.42 (s, 3H, CH3), 3.61 (s, 1H, ethynyl-H), 3.71–3.87 (m, 2H, H-5′, H-5″), 4.06 (dd, J = 4.5, 3.0 Hz, 1H, H-4′), 4.90 (t, J = 7.2 Hz, 1H, H-2′), 5.19 (dd, J = 5.7, 4.8 Hz, 1H, OH-5′), 5.99 (d, J = 6.9 Hz, 1H, OH-2′), 6.08 (d, J = 7.5 Hz, 1H, H-1′), 6.13 (s, 1H, OH-3′), 7.41–7.44 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.74–8.77 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 8.92 (s, 1H, H-8), 8.99 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.1 (CH3), 61.9 (C-5′), 72.8, 77.3, 78.0 (C-2′), 82.6, 86.2 (C-1′), 87.8 (C-4′), 129.4 (4C, C_{Phe}), 130.6 (C-5), 132.5 (C-1$_{Phe}$), 141.3 (C-4$_{Phe}$), 144.9 (C-8), 151.9 (C-2), 152.4 (C-4), 153.2 (C-6). HRMS (ESI): calculated for $C_{19}H_{19}N_4O_4$ ($[M+H]^+$): 367.1401, found: 367.1387. [Remark: The final product contained ~2.5 eq. of acetamide; which was added to the MW of the product.]

*4.2.15. 6-(4-Methoxyphenyl)-N9-(3′-C-ethynyl-β-D-ribofuranosyl)-purine **(15)***

15 was prepared according to General Procedure B. **10** (0.131 g, 0.258 mmol) gave rise to **15** (0.060 g, 0.157 mmol) as a white solid in 61% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 219 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.60 (s, 1H, ethynyl-H), 3.71–3.85 (m, 2H, H-5′, H-5″), 3.87 (s, 3H, OCH_3), 4.05 (dd, J = 4.5, 3.3 Hz, 1H, H-4′), 4.90 (dd, J = 7.5, 6.9 Hz, 1H, H-2′), 5.20 (dd, J = 5.7, 4.8 Hz, 1H, OH-5′), 5.96 (d, J = 6.9 Hz, 1H, OH-2′), 6.07 (d, J = 7.5 Hz, 1H, H-1′), 6.11 (s, 1H, OH-3′), 7.15–7.18 (m, 2H, H-3$_{Phe}$, H-5$_{Phe}$), 8.84–8.87 (m, 2H, H-2$_{Phe}$, H-6$_{Phe}$), 8.89 (s, 1H, H-8), 8.95 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 55.3 (OCH_3), 61.9 (C-5′), 72.8, 77.3, 77.9 (C-2′), 82.6, 86.2 (C-1′), 87.8 (C-4′), 114.2 (2C, C-3$_{Phe}$, C-5$_{Phe}$), 127.6 (C-1$_{Phe}$), 130.2 (C-5), 131.2 (2C, C-2$_{Phe}$, C-6$_{Phe}$), 144.7 (C-8), 151.9, 152.2, 152.9 (C-4), 161.8 (C-4$_{Phe}$). HRMS (ESI): calculated for $C_{19}H_{19}N_4O_5$ ($[M+H]^+$): 383.1350, found: 383.1359. [Remark: The final product contained ~1.5 eq. of acetamide; which was added to the MW of the product.]

*4.2.16. 6-(4-Chlorophenyl)-N9-(3′-C-ethynyl-β-D-ribofuranosyl)-purine **(16)***

16 was prepared according to General Procedure B. **11** (0.057 g, 0.111 mmol) gave rise to **16** (0.026 g, 0.068 mmol) as a white solid in 61% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 230 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.61 (s, 1H, ethynyl-H), 3.71–3.87 (m, 2H, H-5′, H-5″), 4.06 (dd, J = 4.5, 3.3 Hz, 1H, H-4′), 4.91 (t, J = 7.2 Hz, H-2′), 5.17 (dd, J = 5.7, 4.8 Hz, 1H, OH-5′), 5.99 (d, J = 7.2 Hz, 1H, OH-2′), 6.09 (d, J = 7.5 Hz, 1H, H-1′), 6.14 (s, 1H, OH-3′), 7.68–7.72 (m, 2H, C-3$_{Phe}$, C-5$_{Phe}$), 8.85–8.89 (m, 2H, C-2$_{Phe}$, C-6$_{Phe}$), 8.97 (s, 1H, H-8), 9.04 (s, 1H, H-2). ^{13}C NMR (75 MHz,

DMSO-d_6) δ: 61.8 (C-5′), 72.8, 77.3, 78.0 (C-2′), 82.5, 86.2 (C-1′), 87.9 (C-4′), 128.9 (2C, C-3_{Phe}, C-5_{Phe}), 130.7 (C-5), 131.1 (2C, C-2_{Phe}, C-6_{Phe}), 134.0 (C-1_{Phe}), 136.1 (C-4_{Phe}), 145.4 (C-8), 151.7 (C-6), 152.0 (C-2), 152.6 (C-4). HRMS (ESI): calculated for $C_{18}H_{16}ClN_4O_4$ ($[M+H]^+$): 387.0855, found: 387.0869.

4.2.17. 6-(4-Fluorophenyl)-N9-(3′-C-ethynyl-β-D-ribofuranosyl)-purine (17)

17 was prepared according to General Procedure B. **12** (0.075 g, 0.151 mmol) gave rise to **17** (0.041 g, 0.112 mmol) as a white solid in 75% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 235 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.61 (s, 1H, ethynyl-H), 3.72–3.87 (m, 2H, H-5′, H-5″), 4.06 (dd, J = 4.8, 3.3 Hz, 1H, H-4′), 4.91 (t, J = 7.2 Hz, 1H, H-2′), 5.18 (t, J = 5.4 Hz, 1H, OH-5′), 6.00 (d, J = 6.9 Hz, 1H, OH-2′), 6.09 (d, J = 7.5 Hz, 1H, H-1′), 6.14 (s, 1H, OH-3′), 7.43–7.49 (m, 2H, H-3_{Phe}, H-5_{Phe}), 8.90–8.94 (m, 2H, H-2_{Phe}, H-6_{Phe}), 8.95 (s, 1H, H-8), 9.02 (s, 1H, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −109.01 (tt, J = 11.3, 5.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 77.3, 78.0 (C-2′), 82.5, 86.2 (C-1′), 87.9 (C-4′), 115.8 (d, J = 21.8 Hz, 2C, C-3_{Phe}, C-5_{Phe}), 130.6 (C-5), 131.7 (d, J = 2.3 Hz, 1C, C-1_{Phe}), 131.9 (d, J = 8.0 Hz, 2C, C-2_{Phe}, C-6_{Phe}), 145.3 (C-8), 151.9, 152.0, 152.5 (C-4), 164.0 (d, J = 248.4 Hz, 1C, C-4_{Phe}). HRMS (ESI): calculated for $C_{18}H_{16}FN_4O_4$ ($[M+H]^+$): 371.1150, found: 371.1163.

4.2.18. 4-chloro-5-fluoro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (19)

19 was prepared according to General Procedure C. **18** (0.63 g, 1.2 mmol) gave rise to 19 (0.246 g) as a white foam, containing some impurities. Therefore, 19 was immediately used in the next step.

4.2.19. 4,5-dichloro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (20)

20 was prepared according to General Procedure C. **18** (0.8 g, 1.5 mmol) gave rise to **20** (0.39 g) as a yellow foam, containing some impurities. Therefore, **20** was immediately used in the next step.

4.2.20. 4-chloro-5-iodo-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (22)

22 was prepared according to General Procedure C. **18** (1.69 g, 3.2 mmol) gave rise to **22** (1.25 g) as a yellow foam, containing some impurities. Therefore, **22** was immediately used in the next step.

4.2.21. 4-azido-5-fluoro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (23)

23 was prepared according to General Procedure D. **19** (0.246 g, 0.385 mmol) gave rise to **23** (0.14 g, 0.217 mmol) as a white solid in 56% yield. Purification: 0 → 30% EA/Hexanes. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.29 (s, 1H, ethynyl-H), 4.86 (dd, J = 12.0, 6.9 Hz, 1H, H-5″), 5.02 (dd, J = 12.0, 4.2 Hz, 1H, H-5′), 5.20 (dd, J = 6.6, 3.9 Hz, 1H, H-4′), 6.37 (d, J = 5.4 Hz, 1H, H-2′), 6.92 (dd, J = 5.4, 1.5 Hz, 1H, H-1′), 7.40–7.45 (m, 2H, OBz), 7.51–7.57 (m, 4H, OBz), 7.61–7.73 (m, 3H, OBz), 7.88–7.91 (m, 2H, OBz), 8.00–8.08 (m, 5H, OBz, H-6), 9.95 (s, 1H, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −163.54 (d, J = 2.0 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.6 (C-5′), 75.7, 76.4, 78.2 (C-2′), 80.4 (C-4′), 82.2, 85.7 (C-1′), 92.8 (d, J = 14.9 Hz, 1C, C-4a), 108.8 (d, J = 26.4 Hz, 1C, C-6), 127.6, 128.4, 128.9, 129.0, 129.2, 129.3, 129.4, 129.5, 133.7, 134.29, 134.34, 135.3 (C-2), 137.2 (d, J = 3.5 Hz, 1C, C-7a), 143.3 (d, J = 143.3 Hz, 1C, C-5), 144.4 (d, J = 3.5 Hz, 1C, C-4), 163.6 (C=O), 163.8 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{34}H_{24}FN_6O_7$ ($[M+H]^+$): 647.1685, found: 647.1686.

4.2.22. 4-azido-5-chloro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (24)

24 was prepared according to General Procedure D. **20** (0.38 g, 0.579 mmol) gave rise to **24** (0.16 g, 0.24 mmol) as a slightly yellow foam in 41% yield. Purification: 0 → 60% EA/Hexanes. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.30 (s, 1H, ethynyl-H), 4.87 (dd, J = 12.0, 6.9 Hz, 1H, H-5″), 5.04 (dd, J = 12.0, 4.2 Hz, 1H, H-5′), 5.22 (dd, J = 6.6, 4.2 Hz, 1H, H-4′), 6.42 (d, J = 5.4 Hz, 1H, H-2′), 6.89 (d, J = 5.4 Hz, 1H, H-1′), 7.41–7.46 (m, 2H, OBz), 7.51–7.58 (m, 4H, OBz), 7.61–7.76 (m, 3H, OBz), 7.89–7.92 (m, 2H, OBz), 8.01–8.08 (m, 4H, OBz), 8.22 (s, 1H, H-6), 9.97 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.6 (C-5′), 75.8, 76.3, 78.2 (C-2′), 80.6 (C-4′), 82.2, 85.7 (C-1′), 102.0 (C-4a), 106.5 (C-5), 122.4 (C-6), 127.7, 128.4, 128.9, 129.0, 129.2, 129.3, 129.4, 129.5, 133.7, 134.29, 134.34, 135.5 (C-2), 140.3 (C-7a), 145.1 (C-4), 163.5 (C=O), 163.8 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{34}H_{24}ClN_6O_7$: 663.1390 ($[M+H]^+$), found: 663.1398.

4.2.23. 4-azido-5-iodo-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (26)

26 was prepared according to General Procedure D. **22** (0.133 g, 0.178 mmol) gave rise to **26** (0.082 g, 0.109 mmol) as a slightly yellow foam in 61% yield. Purification: 25% EA/Hexanes. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.31 (s, 1H, ethynyl-H), 4.86 (dd, J = 12.0, 6.3 Hz, 1H, H-5″), 5.03 (dd, J = 12.0, 4.2 Hz, 1H, H-5′), 5.21 (dd, J = 6.3, 4.5 Hz, 1H, H-4′), 6.41 (d, J = 5.4 Hz, 1H, H-2′), 6.86 (d, J = 5.4 Hz, 1H, H-1′), 7.41–7.46 (m, 2H, OBz), 7.52–7.58 (m, 4H, OBz), 7.61–7.76 (m, 3H, OBz), 7.88–7.91 (m, 2H, OBz), 8.01–8.08 (m, 4H, OBZ), 8.19 (s, 1H, H-6), 9.93 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.6 (C-5), 63.6 (C-5′), 75.8, 76.4, 78.2 (C-2′), 80.6 (C-4′), 82.2, 85.6 (C-1′), 107.0 (C-4a), 127.6, 128.4, 128.8, 129.0, 129.2, 129.3, 129.4, 129.5, 129.6 (C-6), 133.7, 134.27, 134.32, 135.2 (C-2), 141.8 (C-7a), 146.0 (C-4), 163.5 (C=O), 163.8 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{34}H_{24}IN_6O_7$: 755.0746 ($[M+H]^+$), found: 755.0697.

4.2.24. 4-amino-5-fluoro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (27)

27 was prepared according to General Procedure E. **23** (0.13 g, 0.201 mmol) gave rise to **27** (0.111 g, 0.179 mmol) as a slightly yellow foam in 89% yield. Purification: 20 → 65% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.96 (s, 1H, ethynyl-H), 4.84–4.94 (m, 2H, H-4′, H-5″), 5.01 (dd, J = 10.8, 3.0 Hz, 1H, H-5′), 5.49 (br. s, 2H, NH_2), 6.30 (d, J = 5.1 Hz, 1H, H-2′), 6.79 (dd, J = 5.1, 2.1 Hz, 1H, H-1′), 7.15 (d, J = 2.4 Hz, 1H, H-6), 7.28–7.31 (m, 2H, OBz), 7.39–7.51 (m, 5H, OBz), 7.57–7.62 (m, 2H, OBz), 7.87–7.90 (m, 2H, OBz), 8.02–8.06 (m, 2H, OBz), 8.14–8.18 (m, 2H, OBz), 8.22 (s, 1H, H-2). ^{19}F NMR (282 MHz, CDCl3) δ: −166.34 (d, J = 2.0 Hz). HRMS (ESI): calculated for $C_{34}H_{26}FN_4O_7$ ($[M+H]^+$): 621.1780, found: 621.1788.

4.2.25. 4-amino-5-chloro-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (28)

28 was prepared according to General Procedure E. **24** (0.14 g, 0.211 mmol) gave rise to **28** (0.120 g, 0.188 mmol) as a white foam in 89% yield. Purification: 20 → 65% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.97 (s, 1H, ethynyl-H), 4.88 (dd, J = 11.4, 5.4 Hz, 1H, H-5″), 4.93–4.96 (m, 1H, H-4′), 5.02 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 5.70 (br. s, 2H, NH_2), 6.34 (d, J = 5.1 Hz, 1H, H-2′), 6.72 (d, J = 4.8 Hz, 1H, H-1′), 7.27–7.32 (m, 2H, OBz), 7.35 (s, 1H, H-6), 7.40–7.52 (m, 5H, OBz), 7.57–7.63 (m, 2H, OBz), 7.88–7.91 (m, 2H, OBz), 8.03–8.06 (m, 2H, OBz), 8.15–8.18 (m, 2H, OBz), 8.23 (s, 1H, H-2). HRMS (ESI): calculated for $C_{34}H_{26}ClN_4O_7$ ($[M+H]^+$): 637.1485, found: 637.1455.

*4.2.26. 4-amino-5-bromo-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(29)***

29 was prepared by employing a sequence of General Procedure C, D & E. As such, **18** (0.53 g, 1.0 mmol) gave rise to **29** (0.077 g, 0.113 mmol) as a white foam in 11% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.96 (s, 1H, ethynyl-H), 4.89 (dd, J = 11.1, 5.1 Hz, 1H, H-5″), 4.93–4.86 (m, 1H, H-4′), 5.01 (dd, J = 11.1, 3.0 Hz, 1H, H-5′), 5.76 (br. s, 2H, NH_2), 6.34 (d, J = 5.1 Hz, 1H, H-2′), 6.71 (d, J = 5.1 Hz, 1H, H-1′), 7.27–7.32 (m, 2H, OBz), 7.40–7.52 (m, 6H, OBz, H-6), 7.57–7.63 (m, 2H, OBz), 7.88–7.91 (m, 2H, OBz), 8.03–8.06 (m, 2H, OBz), 8.15–8.18 (m, 2H, OBz), 8.21 (s, 1H, H-2). ^{13}C NMR (75 MHz, CDCl3) δ: 63.8 (C-5′), 76.4, 76.9, 78.5 (C-2′), 72.2, 80.9 (C-4′), 85.4 (C-1′), 89.9 (C-5), 102.4 (C-4a), 120.9 (C-6), 128.5, 128.6, 128.8, 128.9, 129.7, 130.0, 130.1, 133.6, 133.9, 134.1, 150.9 (C-7a), 153.3 (C-2), 157.0 (C-4), 164.4 (C=O), 164.6 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{34}H_{26}BrN_4O_7$ ([M+H]$^+$): 681.0979, found: 681.1010.

*4.2.27. 4-amino-5-iodo-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(30)***

30 was prepared according to General Procedure E. **26** (0.075 g, 0.099 mmol) gave rise to **30** (0.065 g, 0.089 mmol) as a slightly yellow foam in 90% yield. Purification: 25 → 75% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.96 (s, 1H, ethynyl-H), 4.88 (dd, J = 11.1, 4.8 Hz, 1H, H-5″), 4.94–4.96 (m, 1H, H-4′), 5.02 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 5.68 (br. s, 2H, NH2), 6.36 (d, J = 5.1 Hz, H-2′), 6.69 (d, J = 5.4 Hz, 1H, H-1′), 7.27–7.32 (m, 2H, OBz), 7.40–7.53 (m, 5H, OBz), 7.50 (s, 1H, H-6), 7.58–7.64 (m, 2H, OBz), 7.88–7.91 (m, 2H, OBz), 8.03–8.06 (m, 2H, OBz), 8.15–8.18 (m, 2H, OBz), 8.22 (s, 1H, H-2). HRMS (ESI): calculated for $C_{34}H_{26}IN_4O_7$ ([M+H]$^+$): 729.0841, found: 729.0861.

*4.2.28. 4-amino-5-fluoro-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(31)***

31 was prepared according to General Procedure B. **27** (0.105 g, 0.169 mmol) gave rise to **31** (0.05 g, 0.16 mmol) as a white solid in 95% yield. Purification: 1 → 15% MeOH/DCM. Melting point: 235 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (s, 1H, ethynyl-H), 3.63–3.75 (m, 2H, H-5′, H-5″), 3.91 (t, J = 3.6 Hz, 1H, H-4′), 4.49 (t, J = 7.5 Hz, 1H, H-2′), 5.20 (t, J = 5.10 Hz, 1H, OH-5′), 5.78 (d, J = 7.2 Hz, 1H, OH-2′), 5.91 (s, 1H, OH-3′), 6.04 (dd, J = 7.5, 1.8 Hz, 1H, H-1′), 7.02 (br. s, 2H, NH_2), 7.38 (d, J = 2.1 Hz, 1H, H-6), 8.06 (s, 1H, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −167.55 to −167.54 (m, 1 F). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 76.8, 78.2 (C-2′), 83.1, 85.4 (C-1′), 86.7 (C-4′), 92.6 (d, J = 16.1 Hz, 1C, H-4a), 104.6 (d, J = 26.3 Hz, 1C, C-6), 142.6 (d, J = 243.8 Hz, 1C, C-5), 146.6 (C-7a), 152.8 (C-2), 155.9 (d, J = 3.4 Hz, 1C, C-4). HRMS (ESI): calculated for $C_{13}H_{14}FN_4O_4$ ([M+H]$^+$): 309.0994, found: 309.0993.

*4.2.29. 4-amino-5-chloro-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(32)***

32 was prepared according to General Procedure B. **28** (0.12 g, 0.188 mmol) gave rise to **32** (0.035 g, 0.109 mmol) as a white solid in 58% yield. Purification: 5 → 10% MeOH/DCM. Melting point: 254 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (s, 1H, ethynyl-H), 3.62–3.77 (m, 2H, H-5′, H-5″), 3.92 (t, J = 3.6 Hz, 1H, H-4′), 4.55 (t, J = 7.2 Hz, 1H, H-2′), 5.25 (t, J = 4.8 Hz, 1H, OH-5′), 5.80 (d, J = 7.2 Hz, 1H, OH-2′), 5.94 (s, 1H, OH-3′), 6.02 (d, J = 7.8 Hz, 1H, H-1′), 6.90 (br. s, 2H, NH_2), 7.62 (s, 1H, H-6), 8.09 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5′), 72.8, 76.9, 78.2 (C-2′), 83.0, 85.6 (C-1′), 86.9 (C-4′), 99.9 (C-4a), 102.8 (C-5), 119.5 (C-6), 149.5 (C-7a), 152.7 (C-2), 156.8 (C-4). HRMS (ESI): calculated for $C_{13}H_{14}ClN_4O_4$ ([M+H]$^+$): 325.0698, found: 325.0691.

*4.2.30. 4-amino-5-bromo-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(33)***

33 was prepared according to General Procedure B. **29** (0.075 g, 0.110 mmol) gave rise to **33** (0.03 g, 0.081 mmol) as a white solid in 95% yield. Purification: 1 → 15% MeOH/DCM. Melting point: 245 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (s, 1H, ethynyl-H), 3.63–3.77 (m, 2H, H-5″, H-5′), 3.93 (t, J = 3.6 Hz, 1H, H-5′), 4.56 (t, J = 7.2 Hz, 1H, H-2′), 5.25 (t, J = 5.1 Hz, 1H, OH-5′), 5.81 (d, J = 6.9 Hz, 1H, OH-2′), 5.95 (s, 1H, OH-3′), 6.03 (d, J = 7.5 Hz, 1H, H-1′), 6.82 (br. s, 2H, NH2), 7.68 (s, 1H, H-6), 8.10 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5′), 72.8, 76.9, 78.2 (C-2′), 82.9, 85.6 (C-1′), 86.9 (2C, C-4′, C-5), 101.1 (C-4a), 122.1 (C-6), 149.9 (C-7a), 152.4 (C-2), 157.0 (C-4). HRMS (ESI): calculated for $C_{13}H_{14}BrN_4O_4$ ([M+H]$^+$): 369.0193, found: 369.0199.

*4.2.31. 4-amino-5-iodo-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(34)***

34 was prepared according to General Procedure B. **30** (0.39 g, 0.535 mmol) gave rise to **34** (0.1 g, 0.24 mmol) as a white solid in 45% yield. Purification: 6% MeOH/DCM. Melting point: 230 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (s, 1H, ethynyl-H), 3.63–3.77 (m, 2H, H-5′, H-5″), 3.93 (dd, J = 3.9, 3.3 Hz, 1H, H-4′), 4.56 (t, J = 7.5 Hz, 1H, H-2′), 5.25 (dd, J = 5.7, 4.5 Hz, 1H, OH-5′), 5.78 (d, J = 7.2 Hz, 1H, OH-2′), 5.92 (s, 1H, OH-3′), 6.01 (d, J = 7.5 Hz, 1H, H-1′), 6.69 (br. s, 2H, NH2), 7.71 (s, 1H, H-6), 8.10 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 52.1 (C-5), 61.8 (C-5′), 72.8, 76.8, 78.2 (C-2′), 83.0, 85.6 (C-1′), 86.9 (C-4′), 103.3 (C-5), 127.5 (C-6), 150.4 (C-7a), 151.9 (C-2), 157.2 (C-4). HRMS (ESI): calculated for $C_{13}H_{14}IN_4O_4$ ([M+H]$^+$): 417.0054, found: 417.0056.

*4.2.32. 4-azido-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(35)***

26 (0.22 g, 0.292 mmol, 1 eq.) was co-evaporated with anhydrous toluene (10 mL) three times. Then, the resulting foam was dissolved in anhydrous toluene (2.5 mL, 8.5 mL/mmol SM) under argon and cooled to −65 °C. After stirring at this temperature for ~15 min, iPrMgCl.LiCl (1.3 M in THF, 0.45 mL, 0.583 mmol, 2 eq.) was added dropwise with the help of a syringe pump (70 μL/min). After complete addition, the mixture was stirred at −65 °C for ~30min, after which the cooling was removed, and aq. sat. NH_4Cl was added. Then, the mixture was diluted with EA and additional water was added. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. the residue was purified by column chromatography 0 → 10% Et_2O/Toluene to give **35** (0.14 g, 0.222 mmol) as a white foam in 76% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.30 (s, 1H, ethynyl-H), 4.87 (dd, J = 12.0, 6.0 Hz, 1H, H-5″), 5.01 (dd, J = 12.0, 4.2 Hz, 1H, H-5′), 5.21 (dd, J = 6.0, 4.2 Hz, 1H, H-4′), 6.41 (dd, J = 5.1, 0.9 Hz, 1H, H-2′), 6.89 (d, J = 5.1 Hz, 1H, H-1′), 7.32 (d, J = 3.6 Hz, 1H, H-5), 7.82–7.43 (m, 2H, OBz), 7.51–7.75 (m, 7H, OBz), 7.86–7.89 (m, 2H, OBz), 8.00–8.08 (m, 5H, OBz, H-6), 9.91 (s, 1H, H-2). HRMS (ESI): calculated for $C_{34}H_{25}N_6O_7$ ([M+H]$^+$): 629.1779, found: 629.1796.

*4.2.33. 4-amino-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(36)***

36 was prepared according to General Procedure E. **35** (0.193 g, 0.307 mmol) gave rise to **36** (0.160 g, 0.266 mmol) as a white foam in 87% yield. Purification: 50 → 70% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.93 (s, 1H, ethynyl-H), 4.88 (dd, J = 11.1, 5.1 Hz, 1H, H-5″), 4.96 (dd, J = 5.1, 3.3 Hz, 1H, H-4′), 5.02 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 5.29 (br. s, 2H, NH_2), 6.41 (d, J = 5.1 Hz, 1H, H-2′), 6.45 (d, J = 3.9 Hz, 1H, H-5), 6.74 (d, J = 5.1 Hz, 1H, H-1′), 7.13–7.26 (m, 2H, OBz), 7.40–7.52 (m, 6H, OBz, H-6), 7.56–7.63 (m, 2H, OBz), 7.88–7.91 (m, 2H, OBz), 8.04–8.07 (m, 2H, OBz), 8.15–8.18 (m, 2H,

OBz), 8.27 (s, 1H, H-2). HRMS (ESI): calculated for $C_{34}H_{27}N_4O_7$ ($[M+H]^+$): 603.1874, found: 603.1877.

4.2.34. 4-amino-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (37)

37 was prepared according to General Procedure B. **36** (0.15 g, 0.249 mmol) gave rise to **37** (0.021 g, 0.073 mmol) as a white solid in 30% yield. Purification: 5 → 20% MeOH/DCM. Melting point: 150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (s, 1H, ethynyl-H), 3.70–3.72 (m, 2H, H-5′, H-5″), 3.92 (t, J = 3.3 Hz, 1H, H-4′), 4.61 (t, J = 7.5 Hz, 1H, H-2′), 5.49 (dd, J = 6.3, 4.5 Hz, 1H, OH-5′), 5.75 (d, J = 7.2 Hz, 1H, OH-2′), 5.87 (s, 1H, OH-3′), 5.94 (d, J = 7.5 Hz, 1H, H-1′), 6.60 (d, J = 3.6 Hz, 1H, H-5), 7.07 (br. s, 2H, NH_2), 7.38 (d, J = 3.9 Hz, 1H, H-6), 8.04 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 62.0 (C-5′), 72.8, 76.7, 78.0 (C-2′), 83.3, 86.6 (C-1′), 86.7 (C-4′), 99.7 (C-5), 103.2 (C-4a), 122.7 (C-6), 150.1 (C-7a), 151.5 (C-2), 157.6 (C-4). HRMS (ESI): calculated for $C_{13}H_{15}N_4O_4$ ($[M+H]^+$): 291.1088, found: 291.1089.

4.2.35. 4-amino-5-(furan-2-yl)-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (38)

34 (0.086 g, 0.207 mmol, 1 eq.), furan-2-boronic acid (0.035 g, 0.31 mmol, 1.5 eq.), Na_2CO_3 (0.2 g, 1.86 mmol, 9 eq.), $Pd(OAc)_2$ (0.002 g, 0.01 mmol, 0.05 eq.) and TPPTS (0.018 g, 0.031 mmol, 0.15 eq.) were added to a 10 mL round-bottom flask, equipped with a stir bar. Next, the flask was evacuated and refilled with argon. This procedure was repeated three times in total. Next, degassed MeCN (0.75 mL) and H_2O (1.5 mL) were added to the solids under argon. After 5 min of stirring, the mixture was heated to 100 °C in a pre-heated oil bath. When the starting material was fully consumed (30 min), the mixture was cooled to ambient temperature, and neutralized (pH ~ 7) with 0.5 M aq. HCl. The mixture was evaporated till dryness, resuspended in MeOH and evaporated (three times). Next, the mixture was adsorbed onto Celite® (from MeOH) and eluted over a short silica pad (~5 cm) with 20% MeOH/DCM. The liquid was evaporated in vacuo and purified by column chromatography 1 → 10% MeOH/DCM, to give **38** (0.022 g, 0.061 mmol) as a white solid in 29% yield. Melting point: 208–210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (s, 1H, ethynyl-H), 3.66–3.78 (m, 2H, H-5′, H-5″), 3.95 (dd, J = 4.2, 3.3 Hz, 1H, H-4′), 4.63 (t, J = 7.5 Hz, 1H, H-2′), 5.59 (dd, J = 5.7, 4.8 Hz, 1H, OH-5′), 5.81 (d, J = 7.2 Hz, 1H, OH-2′), 5.94 (s, 1H, OH-3′), 6.07 (d, J = 7.8 Hz, 1H, H-1′), 6.62 (dd, J = 3.3, 1.8 Hz, 1H, H-4$_{furan}$), 6.68 (dd, J = 3.3, 0.6 Hz, 1H, H-3$_{furan}$), 6.93 (br. s, 2H, NH_2), 7.79 (dd, J = 1.8, 0.6 Hz, 1H, H-5$_{furan}$), 7.88 (s, 1H, H-6), 8.13 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 76.9, 78.1 (C-2′), 83.1, 85.8 (C-1′), 86.9 (C-4′), 99.4 (C-4a), 105.3 (C-3$_{furan}$), 106.3 (C-5), 111.9 (C-4$_{furan}$), 120.8 (C-6), 142.1 (C-5$_{furan}$), 148.2 (C-2$_{furan}$), 151.2 (C-7a), 152.1 (C-2), 157.3 (C-4). HRMS (ESI): calculated for $C_{17}H_{17}N_4O_5$ ($[M+H]^+$): 357.1193, found: 357.1196.

4.2.36. 4-chloro-6-trifluoromethyl-7H-pyrrolo[2,3-d]pyrimidine (39)[35]

A suspension of 6-chloro-7-deazapurine (0.31 g, 2 mmol, 1 eq.) and sodiumtrifluoromethylsulfinate (0.94 g, 6 mmol, 3 eq.) in a mixture of DCM/water (8 mL/3.2 mL; 2.5/1 ratio; 0.18 M concentration in total) was cooled in an ice bath to 0 °C. After stirring at that temperature for ~10 min, 70% aq. tBuOOH (1.4 mL, 10 mmol, 5 eq.) was added dropwise (0.1 mL/min). When the addition was complete, the ice bath was removed, and vigorous stirring continued for 3 days. Then, the mixture was partitioned between sat. aq. $NaHCO_3$ solution and DCM, and the layers separated. The water layer was extracted twice more with DCM. The combined organic layers were dried over Na_2SO_4, filtered and evaporated till dryness. Purification by column chromatography 16% EA/Hexanes gave **39** (0.05 g, 0.226 mmol) as a white solid in 11% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.10 (s, 1H, H-5), 8.83 (s, 1H, H-2), 13.29 (br. s, 1H, NH). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −61.6.^{13}C NMR (75 MHz, $CDCl_3$) δ: 101.6 (q, J = 3.5 Hz, 1C, C-5), 117.5 (C-4a), 120.5 (q, J = 267.9 Hz, 1H, CF_3), 128.5 (q, J = 40.1 Hz, 1C, C-6), 151.6, 152.4 (C-2), 155.7. Spectral data were in accordance with literature values [35].

4.2.37. t-butyl-4-chloro-5-iodo-7H-pyrrolo[2,3-d]pyrimidine-7-carboxylate (41)

40 [34] (0.84 g, 3 mmol, 1 eq.) was suspended in anhydrous 1,4-dioxane (12 mL, 4 mL/mmol SM). Then, DMAP (0.073 g, 0.6 mmol, 0.2 eq.) was added, followed by DBU (0.9 mL, 6 mmol, 2 eq.). Next, Boc_2O (2.07 mL, 9 mmol, 3 eq.) was added dropwise and the resulting mixture was stirred at ambient temperature overnight. Next, the mixture was added to sat. aq. NH_4Cl, and EA/water was added. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography (15% EA/Hexanes) to yield **41** (1.09 g, 2.87 mmol) as a slightly yellow solid in 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68 (s, 9H, t-Bu), 7.87 (s, 1H, H-6), 8.83 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (3C, t-Bu CH_3), 56.6 (C-5), 86.8 (t-Bu, C-$(CH_3)_3$), 119.0 (C-4a), 133.4 (C-6), 146.0 (C=O), 151.7 (C-7a), 153.2 (C-2), 153.6 (C-4). HRMS (ESI): calculated for $C_{11}H_{12}IN_3O_2$ ($[M+H]^+$): 379.9657; found: 379.9667.

4.2.38. 4-chloro-5-trifluoromethyl-7H-pyrrolo[2,3-d]pyrimidine (42)

In a flame dried culture flask, equipped with a stir bar, was added under argon: **41** (0.76 g, 2 mmol, 1 eq.), KF (0.349 g, 6 mmol, 3 eq.), CuI (0.076 g, 0.4 mmol, 0.2 eq.) and 1,10-phenanthroline (0.072 g, 0.4 mmol, 0.2 eq.). The flask was evacuated and refilled with argon three times. Then, anhydrous DMSO (4 mL, 2 mL/mmol SM) was added, followed by $B(OMe)_3$ and $TMSCF_3$. The mixture was submerged in a pre-heated oil bath at 60 °C for 24h. Then, the mixture was allowed to cool to ambient temperature and partitioned between water and Et_2O. The layers were separated, and the water layer extracted once more with Et_2O. The combined organic layers were then washed with diluted aq. ammonia solution (1×), followed by brine (1×), and dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography 3 → 4% acetone/DCM. The resulting product was found to be sufficiently pure for use in the next step (Vorbrüggen glycosylation). *[However, the slight amount of remaining iodoheterocycle (**40**; thus without Boc protecting group) could be efficiently removed by employing a Sonogashira reaction [$Pd(Ph_3P)_2Cl_2$ (0.05 eq.), CuI (0.1 eq.), Et_3N (0.2 mL/mmol), DMF (10 mL/mmol)] with butyn-1-ol (1 eq.) (reaction time 3H). As such, **42** was obtained as a slightly yellow solid (0.1 g, 0.45 mmol) in 23% yield.]* Melting point: 217–218 °C. ^{1}H NMR (300 MHz, **$CDCl_3$**) δ: 7.83 (d, J = 0.9 Hz, 1H, H-6), 8.80 (s, 1H, H-2), 10.47 (br. s, 1H, NH). ^{19}F NMR (282 MHz, **$CDCl_3$**) δ: −55.98.^{1}H NMR (300 MHz, **DMSO-d_6**) δ: 8.42 (br. s, 1H, H-6), 8.77 (s, 1H, H-2), 13.38 (br. s, 1H, NH). ^{19}F NMR (282 MHz, **DMSO-d_6**) δ: −53.62 (d, J = 2.0 Hz). ^{13}C NMR (75 MHz, **DMSO-d_6**) δ: 102.7 (q, J = 37.8 Hz, 1C, C-5), 112.1 (q, J = 2.4 Hz, 1C, C-4a), 122.7 (q, J = 264.5 Hz, 1C, CF_3), 130.6 (q, J = 5.78 Hz, 1C, C-6), 150.0, 151.9 (C-2), 152.9. HRMS (ESI): calculated for $C_7H_4ClF_3N_3$ ($[M+H]^+$): 222.0040, found: 222.0041.

4.2.39. 4-chloro-5-trifluoromethyl-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (43)

43 was prepared according to General Procedure C. **18** (0.550 g, 1.03 mmol) gave rise to **43** (0.309 g) as a slightly yellow foam, containing some impurities. Therefore, **43** was immediately used in the next steps (General Procedure D & General Procedure E).

*4.2.40. 4-chloro-6-trifluoromethyl-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(44)***

44 was prepared according to General Procedure C. **18** (0.634 g, 1.2 mmol) gave rise to **44** (0.436 g) as a slightly yellow foam, containing some impurities. Therefore, **44** was immediately used in the next step.

*4.2.41. 4-azido-6-trifluoromethyl-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(46)***

46 was prepared according to General Procedure D. **44** (0.436 g, 0.632 mmol) gave rise to **46** (0.191 g, 0.274 mmol) as a white foam in 43% yield. Purification: 5 → 25% EA/Hexanes. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.19 (s, 1H, ethynyl-H), 4.85 (dd, J = 12, 6.9 Hz, 1H, H-5″), 5.10 (d, J = 12, 4.2 Hz, 1H, H-5′), 5.32 (dd, J = 6.9, 4.5 Hz, 1H, H-4′), 6.58 (d, J = 6.9 Hz, 1H, H-1′), 6.95 (d, J = 7.2 Hz, 1H, H-2′), 7.46–7.56 (m, 6H, OBz), 7.64–7.75 (m, 3H, OBz), 7.87–7.90 (m, 2H, OBz), 7.96–8.06 (m, 5H, OBz, H-5), 10.06 (1H, s, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −56.73. HRMS (ESI): calculated for $C_{35}H_{24}F_3N_6O_7$ ([M+H]$^+$): 697.1653, found: 697.1677.

*4.2.42. 4-amino-5-trifluoromethyl-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(47)***

47 was prepared according to General Procedure D & General Procedure E. **43** (0.309 g, 0.448 mmol) was transformed into **47** (0.12 g, 0.179 mmol) as a white foam in 21% yield (over 3 steps). Purification: 25 → 65% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.97 (s, 1H, ethynyl-H), 4.87–4.95 (m, 1H, H-5″), 4.98–5.05 (m, 2H, H-4′, H-5′), 5.54 (br. s, 2H, NH_2), 6.37 (d, J = 4.8 Hz, 1H, H-2′), 6.75 (d, J = 4.8 Hz, 1H, H-1′), 7.28–7.33 (m, 2H, OBz), 7.40–7.54 (m, 5H, OBz), 7.58–7.64 (m, 2H, OBz), 7.85–7.92 (m, 3H, OBz, H-6), 8.04–8.07 (m, 2H, OBz), 8.14–8.17 (m, 2H, OBz), 8.30 (s, 1H, H-2). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −55.76 (d, J = 2.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.7 (C-5′), 76.4, 77.4, 78.7 (C-2′), 79.4, 81.1 (C-4′), 85.8 (C-1′), 99.3 (d, J = 2.3 Hz, 1C, C-4a), 106.9 (q, J = 37.8 Hz, 1C, C-5), 122.7 (q, J = 5.8 Hz, 1C, C-6), 123.2 (q, J = 265.6 Hz, 1C, CF_3), 128.3, 128.6, 128.76, 128.80, 129.5, 129.9, 130.1, 133.6, 134.0, 134.1, 152.3 (C-7a), 153.6 (C-2), 156.2 (C-4), 164.3 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{26}F_3N_4O_7$ ([M+H]$^+$): 671.1748, found: 671.1736.

*4.2.43. 4-amino-6-trifluoromethyl-N7-(3′-C-ethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(48)***

48 was prepared according to General Procedure E. **46** (0.13 g, 0.201 mmol) gave rise to **48** (0.144 g, 0.215 mmol) as a white foam in 55% yield. Purification: 40 → 65% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.88 (s, 1H, ethynyl-H), 5.09–5.24 (m, 3H, H-4′, H-5′, H-5″), 5.53 (br. s, 2H, NH_2), 6.32 (d, J = 7.8 Hz, 1H, H-1′), 6.78 (s, 1H, H-5), 7.38–7.64 (m, 10H, OBz, H-2′), 7.99–8.02 (m, 2H, OBz), 8.10–8.13 (m, 2H, OBz), 8.14–8.17 (m, 2H, OBz), 8.24 (s, 1H, H-2). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −58.34.^{13}C NMR (75 MHz, $CDCl_3$) δ: 64.5 (C-5′), 75.8 (C-2′), 76.3, 77.4, 79.0, 82.5 (C-4′), 86.4 (C-1′), 102.5 (C-4a), 103.3 (q, J = 3.8 Hz, 1C, C-5), 120.8 (q, J = 266.8 Hz, 1C, CF_3), 124.5 (q, J = 37.8 Hz, 1C, C-6), 128.5, 128.7, 128.8, 129.6, 129.98, 130.03, 130.1, 133.1, 133.9, 134.0, 153.0 (C-7a), 154.4 (C-2), 157.9 (C-4), 164.3 (C=O), 164.9 (C=O), 166.5 (C=O). HRMS (ESI): calculated for $C_{35}H_{25}F_3N_4O_7$ ([M+H]$^+$): 671.1748, found: 671.1807.

*4.2.44. 4-amino-5-trifluoromethyl-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(49)***

49 was prepared according to General Procedure B. **47** (0.12 g, 0.179 mmol) gave rise to **49** (0.051 g, 0.142 mmol) as a white solid in 80% yield. Purification: 0 → 10% MeOH/DCM. Melting point: 207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (s, 1H, ethynyl-H), 3.63–3.81 (m, 2H, H-5′, H-5″), 3.98 (t, J = 3.3 Hz, 1H, H-4′), 4.65 (t, J = 7.5 Hz, 1H, H-2′), 5.30 (dd, J = 5.4, 4.5 Hz, 1H, OH-5′), 5.84 (d, J = 6.9 Hz, 1H, OH-2′), 5.99 (s, 1H, OH-3′), 6.10 (d, J = 7.5 Hz, 1H, H-1′), 6.63 (br. s, 2H, NH_2), 8.21 (d, J = 0.9 Hz, 1H, H-6), 8.23 (s, 1H, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −53.83.^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.7 (C-5′), 72.9, 77.0, 78.4 (C-2′), 82.8, 85.9 (C-1′), 87.3 (C-4′), 98.0 (d, J = 2.3 Hz, 1C, C-4a), 103.6 (q, J = 36.6 Hz, 1C, C-5), 123.4 (q, J = 264.5 Hz, 1C, CF_3), 124.5 (q, J = 5.7 Hz, 1C, C-6), 151.7 (C-7a), 153.1 (C-2), 156.3 (C-4). HRMS (ESI): calculated for $C_{14}H_{14}F_3N_4O_4$ ([M+H]$^+$): 359.0962, found: 359.0962.

*4.2.45. 4-amino-6-trifluoromethyl-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(50)***

50 was prepared according to General Procedure B. **48** (0.116 g, 0.173 mmol) gave rise to **50** (0.054 g, 0.151 mmol) as a white solid in 87% yield. Purification: 6 → 10% MeOH/DCM. Melting point: 150–152 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (s, 1H, ethynyl-H), 3.68–3.85 (m, 2H, H-5′, H-5″), 4.01 (t, J = 3.0 Hz, 1H, H-4′), 5.35 (t, J = 7.5 Hz, 1H, H-2′), 5.63 (d, J = 8.4 Hz, 1H, H-1′), 5.87 (d, J = 6.6 Hz, 1H, OH-2′), 5.94 (s, 1H, OH-3′), 5.94 (dd, J = 9.0, 2.7 Hz, 1H, OH-5′), 7.33 (s, 1H, H-5), 7.68 (br. s, 2H, NH_2), 8.18 (s, 1H, H-2). ^{19}F NMR (282 MHz, DMSO-d_6) δ: −56.89.^{13}C NMR (75 MHz, DMSO-d_6) δ: 62.2 (C-5′), 72.6, 75.0 (C-2′), 76.7, 82.9, 88.17 (C-1′), 88.25 (C-4′), 101.9 (C-4a), 104.3 (q, J = 4.7 Hz, 1C, C-5), 120.8 (q, J = 265.7 Hz, 1C, CF_3), 122.5 (q, J = 37.8 Hz, 1C, C-6), 151.0 (C-7a), 154.0 (C-2), 159.0 (C-4). HRMS (ESI): calculated for $C_{14}H_{14}F_3N_4O_4$: 359.0962, found: 359.0977.

*4.2.46. 1-O-acetyl-2,3,5-tri-O-benzoyl-3-C-trimethylsilylethynyl-α,β-D-ribofuranose **(51)***

18 (1.32 g, 2.5 mmol, 1 eq.) was co-evaporated with anhydrous toluene (15 mL) three times. Next, the residue was dissolved in anhydrous toluene (25 mL, 10 mL/mmol SM) and cooled to −65 °C. After stirring at −65 °C for ~15 min, iPrMgCl.LiCl solution (1.3 M in THF, 2.11 mL, 2.75 mmol, 1.1 eq.) was added dropwise, and the resulting solution stirred at −65 °C for 30 min. Then, TMSCl (0.48 mL, 3.75 mmol, 1.5 eq.) was added in one portion and the cooling removed. The mixture was stirred another 30 min at ambient temperature and aq. sat. NH_4Cl solution was added, followed by EA and water. The layers were separated, and the water layer extracted twice more with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography 0 → 20% EA/Hexanes to give **51** (0.894 g, 1.49 mmol) as a sticky foam in 60% yield. (mixture of isomers in ~1:1.67 ratio (α/β)) ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.11 (s, 9H, TMS-$CH_{3,\alpha}$), 0.18 (s, 9H, TMS-$CH_{3,\beta}$), 1.98 (s, 3H, OAc_α), 2.14 (s, 3H, OAc_β), 4.74–5.03 (m, 2 × 3H, H-4, H-5, H-5′); 6.05 (d, J = 4.5 Hz, 1H, H-2$_\alpha$), 6.15 (d, J = 1.5 Hz, 1H, H-2$_\beta$), 6.35 (d, J = 1.2 Hz, 1H, H-1$_\beta$), 6.74 (d, J = 4.2 Hz, 1H, H-1$_\alpha$), 7.89–7.62 (m, 2 × 9H, OBz), 7.91–8.17 (m, 2 × 6H, OBz). HRMS (ESI): calculated for $C_{31}H_{29}O_7Si$ ([M-OAc]$^+$): 541.1677, found: 541.1682.

*4.2.47. 4-chloro-5-iodo-N7-(3′-C-trimethylsilylethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine **(52)***

52 was prepared according to General Procedure C. **51** (0.85 g, 1.42 mmol) gave rise to **52** (0.344 g, 0.42 mmol) as a slightly yellow foam, containing minor impurities. Yield: 30%. Purification: 12.5% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.27 (s, 9H, TMS-CH_3), 4.83 (dd, J = 11.4, 6.3 Hz, 1H, H-5″), 4.88–4.91 (m, 1H, H-4′), 5.03 (dd, J = 11.4, 3.0 Hz, 1H, H-5′), 6.25 (d, J = 3.3 Hz, 1H, H-2′), 6.70 (d, J = 3.9 Hz, 1H, H-1′), 7.22–7.28 (m, 2H, OBz), 7.40–7.51 (m, 5H, OBz), 7.57–7.63 (m, 2H, OBz), 7.80–7.83 (m, 2H, OBz), 8.00 (s, 1H, H-6), 8.01–8.06 (m, 2H, OBz), 8.12–8.17 (m, 2H, OBz), 8.58 (s, 1H, H-2). HRMS (ESI): calculated for $C_{37}H_{32}IN_4O_7Si$ ([M+H]$^+$): 820.0737, found: 820.0780.

4.2.48. 4-chloro-5-trimethylsilylethynyl-N7-(3′-C-trimethylsilylethynyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (53)

In a flame-dried 10 mL round bottom flask under argon was added: **52** (0.33 g, 0.402 mmol, 1 eq.), CuI (0.008 g, 0.0402 mmol, 0.1 eq.) and $Pd(Ph_3P)_2Cl_2$ (0.014 g, 0.0201 mmol, 0.05 eq.). The flask was evacuated and refilled with argon, three times. Then, anhydrous degassed DMF (2 mL, 4 mL/mmol SM) was added, followed by degassed Et_3N (0.16 mL, 0.4 mL/mmol SM) and ethynyltrimethylsilane (0.57 mL, 4.02 mmol, 10 eq.). Then, the mixture was stirred at ambient temperature overnight and subsequently evaporated till dryness. The residue was purified by column chromatography (0 → 25% EA/Hexanes; three sequences), to give **53** (0.125 g, 0.158 mmol) as a yellowish foam in 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.26 (s, 9H, TMS-CH_3), 0.29 (s, 9H, TMS-CH_3), 4.78–4.90 (m, 2H, H-4′, H-5″), 5.04 (dd, J = 11.4, 3.0 Hz, 1H, H-5′), 6.20 (d, J = 3.3 Hz, 1H, H-2′), 6.69 (d, J = 3.3 Hz, 1H, H-1′), 7.19–7.24 (m, 2H, OBz), 7.39–7.51 (m, 5H, OBz), 7.56–7.63 (m, 2H, OBz), 7.78–7.81 (m, 2H, OBz), 8.00–8.17 (m, 6H, OBz), 8.13 (s, 1H, H-6), 8.60 (s, 1H, H-2). HRMS (ESI): calculated for $C_{42}H_{41}ClN_3O_7Si_2$ ($[M+H]^+$): 790.2166, found: 790.2181.

4.2.49. 4-amino-5-ethynyl-N7-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (54)

54 was prepared by a sequential combination of General Procedure D, E and B. As such, **53** (0.125 g, 0.158 mmol) gave rise to **54** (0.023 g, 0.072 mmol) as a white solid in 46% yield. Purification: 0 → 10% MeOH/DCM. Melting point: 195 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (s, 1H, ethynyl-H_{purine}), 3.63–3.78 (m, 2H, H-5′, H-5″), 3.94 (t, J = 3.3 Hz, 1H, H-4′), 4.30 (s, 1H, ethynyl-H_{ribo}), 4.59 (t, J = 7.5 Hz, 1H, H-2′), 5.34 (dd, J = 6.0, 4.8 Hz, 1H, OH-5′), 5.83 (d, J = 7.2 Hz, 1H, OH-2′), 5.97 (s, 1H, OH-3′), 5.99 (d, J = 7.8 Hz, 1H, H-1′), 6.74 (br. s, 2H, NH_2), 7.85 (s, 1H, H-6), 8.12 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5′), 72.8 (C-3′), 76.9, 77.1, 78.2 (C-2′), 82.9, 83.2, 86.1 (C-1′), 87.1 (C-4′), 94.2 (C-5), 102.4 (C-4a), 127.8 (C-6), 149.8 (C-7a), 152.8 (C-2), 157.6 (C-4). HRMS (ESI): calculated for $C_{15}H_{15}N_4O_4$ ($[M+H]^+$): 315.1088, found: 315.1096.

4.2.50. 1-O-acetyl-2,3,5-tri-O-benzoyl-3-C-ethyl-α,β-D-ribofuranose (55)

18 (1.47 g, 2.78 mmol) was dissolved in EA (15 mL, 5 mL/mmol SM) under a nitrogen atmosphere. Then, a catalytic amount of Pd/C was added, and the reaction mixture was stirred under a hydrogen atmosphere (balloon) for approximately 7 h. Then, the mixture was flushed with nitrogen and filtered over a short pad of Celite®. The filtrate was evaporated till dryness and purified by column chromatography 15% EA/Hexanes to give **55** (1.37, 2.57 mmol) as white foam in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (t, J = 7.8 Hz, 3H, $CH_{3\beta}$), 1.26 (t, J = 7.2 Hz, 3H, $CH_{3\alpha}$), 1.91–2.06 (m, 1H, $CH_{2\alpha}$), 1.97 (s, 3H, OAc_α), 2.03 (s, 3H, OAc_β), 2.16–2.28 (m, 1H, $CH_{2\beta}$), 2.64–2.76 (m, 1H, $CH_{2\beta}$), 2.78–2.91 (m, 1H, $CH_{2\alpha}$), 4.60 (dd, J = 12.3, 4.8 Hz, 1H, H-5′$_\alpha$), 4.63 (dd, J = 12.0, 6.0 Hz, 1H, H-5′$_\beta$), 4.82 (dd, J = 12.3, 3.6 Hz, 1H, H-5$_\alpha$), 4.99 (dd, J = 12.0, 3.6 Hz, 1H, H-5$_\beta$), 5.08 (dd, J = 6.3, 3.6 Hz, 1H, H-4$_\beta$), 5.18 (dd, J = 4.2, 3.6 Hz, 1H, H-4$_\alpha$), 5.64 (d, J = 4.5 Hz, 1H, H-2$_\alpha$), 5.96 (d, J = 2.4 Hz, 1H, H-2$_\beta$), 6.40 (d, J = 2.4 Hz, 1H, H-1$_\beta$), 6.69 (d, J = 4.8 Hz, 1H, H-1$_\alpha$), 7.32–7.65 (m, 2 × 9H, OBz), 7.94–8.19 (m, 2 × 6H, OBz). HRMS (ESI): calculated for $C_{28}H_{25}O_7$ ($[M\text{-}OAc]^+$): 473.1595, found: 473.1606.

4.2.51. 4,5-dichloro-N7-(3′-C-ethyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (56)

56 was prepared according to General Procedure C. **55** (0.558 g, 1.05 mmol) gave rise to **56** (0.471 g) as a slightly yellow foam, containing minor impurities. Purification: 14% EA/Hexanes.

4.2.52. 4-azido-5-chloro-N7-(3′-C-ethyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (57)

57 was prepared according to General Procedure D. **56** (0.471 g, 0.71 mmol) gave rise to **57** (0.387 g, 0.581 mmol) as a white foam. Yield = 55%. Purification: 20 → 25% EA/Hexanes. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.91 (t, J = 7.2 Hz, 3H, CH_3), 2.31–2.43 (m, 1H, CH_2), 2.66–2.78 (m, 1H, CH_2), 4.79 (dd, J = 12.0, 6.6 Hz, 1H, H-5″), 4.96 (dd, J = 12.3, 4.2 Hz, 1H, H-5′), 5.28 (dd, J = 6.6, 4.2 Hz, 1H, H-4′), 6.38 (d, J = 7.2 Hz, 1H, H-2′), 6.86 (d, J = 7.2 Hz, 1H, H-1′), 7.45–7.79 (m, 9H, OBz), 7.89–7.92 (m, 2H, OBz), 8.01–8.04 (m, 2H, OBz), 8.14–8.17 (m, 2H, OBz), 8.35 (s, 1H, H-6), 9.90 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 7.4 (CH_3), 24.3 (CH_2), 63.5 (C-5′), 77.8 (C-2′), 81.8 (C-4′), 84.8 (C-1′), 86.5 (C-3′), 101.1 (C-4a), 106.0 (C-5), 122.9 (C-6), 128.1, 128.9, 128.96, 129.04, 129.2, 129.3, 129.4, 129.6, 133.6, 134.0, 134.2, 135.3 (C-2), 140.5 (C-7a), 145.1 (C-4), 164.3 (C=O), 164.6 (C=O), 165.4 (C=O). HRMS (ESI): calculated for $C_{34}H_{27}ClN_6O_7$ ($[M+H]^+$): 666.1630, found: 667.1703.

4.2.53. 4-amino-5-chloro-N7-(3′-C-ethyl-2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (58)

58 was prepared according to General Procedure E. **57** (0.374 g, 0.561 mmol) gave rise to **58** (0.32 g, 0.497 mmol) as a white foam. Yield = 88%. Purification: 40 → 75% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.95 (t, J = 7.5 Hz, 3H, CH_3), 2.16–2.28 (m, 1H, CH_2), 2.82–2.95 (m, 1H, CH_2), 4.80 (dd, J = 12.6, 3.9 Hz, 1H, H-5″), 4.94 (dd, J = 12.3, 3.9 Hz, 1H, H-5′), 5.24 (t, J = 3.6 Hz, 1H, H-4′), 5.64 (br. s, 2H, NH_2), 6.29 (d, J = 7.8 Hz, 1H, H-2′), 6.72 (d, J = 7.5 Hz, 1H, H-1′), 7.13 (s, 1H, H-6), 7.40–7.68 (m, 9H, OBz), 8.03–8.06 (m, 2H, OBz), 8.15 (s, 1H, H-2), 8.17–8.21 (m, 4H, OBz). HRMS (ESI): calculated for $C_{34}H_{30}ClN_4O_7$ ($[M+H]^+$): 641.1798, found: 641.1795.

4.2.54. 4-amino-5-chloro-N7-(3′-C-ethyl-β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (59)

59 was prepared according to General Procedure B. **58** (0.3 g, 0.468 mmol) gave rise to **59** (0.126 g, 0.383 mmol) as a white solid in 87% yield. Purification: precipitation from MeOH. Melting point: 272 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.96 (t, J = 7.2 Hz, 3H, CH_3), 1.57–1.75 (m, 2H, CH_2), 3.50–3.62 (m, 2H, H-5′, H-5″), 3.84 (t, J = 2.7 Hz, 1H, H-4′), 4.29 (t, J = 7.4 Hz, 1H, H-2′), 4.51 (s, 1H, OH-3′), 5.26 (d, J = 6.9 Hz, 1H, OH-2′), 5.42 (t, J = 4.8 Hz, 1H, OH-5′), 6.02 (d, J = 7.8 Hz, 1H, H-1′), 6.86 (br. s, 2H, NH_2), 7.64 (s, 1H, H-6), 8.08 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 7.8 (CH_3), 25.7 (CH_2), 61.2 (C-5′), 77.3 (C-2′), 78.2 (C-3′), 86.4 (C-1′), 87.0 (C-4′), 100.0 (C-4a), 102.4 (C-5), 120.0 (C-6), 149.4 (C-7a), 152.5 (C-2), 156.8 (C-4). HRMS (ESI): calculated for $C_{13}H_{18}ClN_3O_4$: 329.1011, found: 329.1034.

4.2.55. 3,4-dichloro-1H-pyrrolo[2,3-b]pyridine (60)

1*H*-4-chloro-pyrrolo[2,3-*b*]pyridine (0.763 g, 5.0 mmol, 1 eq.) was dissolved in DMF (7.5 mL, 1.5 mL/mmol SM) and NCS (0.701 g, 5.25 mmol, 1.05 eq.) was added. The resulting mixture was stirred at ambient temperature overnight, protected from light. Then, ice-cold water (25 mL, 5 mL/mmol SM) was added and the resulting precipitate filtered. The solids were washed four additional times with ice-cold water (4 × 10 mL, 2 mL/mmol SM). The solid was collected and dried under high vacuum to give **60** (0.861 g, 4.6 mmol) as an off-white solid in 92% yield. Melting point: 236 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.21 (d, J = 4.2 Hz, 1H, H-5), 7.77 (s, 1H, H-2), 8.20 (d, J = 4.2 Hz, 1H, H-6), 12.35 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 101.1 (C-3), 113.7 (C-3a), 117.1 (C-5), 125.1 (C-2), 133.8 (C-4), 144.4 (C-6), 147.6 (7a). HRMS (ESI): calculated for $C_7H_5Cl_2N_2$ ($[M+H]^+$): 186.9824, found: 186.9824.

4.2.56. 3-bromo-4-chloro-1H-pyrrolo[2,3-b]pyridine (61)

61 was prepared as has been described for **60**, except for the use of NBS instead of NCS. 1*H*-4-chloro-pyrrolo[2,3-*b*]pyridine (0.763 g,

5 mmol) gave rise to **61** (1.12 g, 4.8 mmol) as a yellow solid in 96% yield. Melting point: 210 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.23 (d, J = 5.1 Hz, 1H, H-5), 7.81 (d, J = 2.7 Hz, 1H, H-2), 8.21 (d, J = 5.1 Hz, 1H, H-6), 12.44 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 85.0 (C-3), 114.6 (C-3a), 117.1 (C-5), 127.7 (C-2), 134.2 (C-4), 144.2 (C-6), 148.0 (C-7a). HRMS (ESI): calculated for $C_7H_5BrClN_2$ ([M+H]$^+$): 230.9319, found: 230.9332.

*4.2.57. 3-iodo-4-chloro-1H-pyrrolo[2,3-b]pyridine **(62)***

62 was prepared as has been described for **60**, except for the use of NIS instead of NCS. 1*H*-4-chloro-pyrrolo[2,3-*b*]pyridine (0.763 g, 5 mmol, 1 eq.) gave rise to **62** (1.29 g, 4.6 mmol) as a yellow solid in 92% yield. Melting point: 222 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.19 (d, J = 5.1 Hz, 1H, H-5), 7.81 (d, J = 2.4 Hz, 1H, H-2), 8.18 (d, J = 5.1 Hz, 1H, H-6), 12.45 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 49.7 (C-3), 116.3 (C-3a), 116.9 (C-5), 133.1 (C-2), 134.8 (C-5), 143.7 (C-6), 148.4 (C-7a). HRMS (ESI): calculated for $C_7H_5ClIN_2$ ([M+H]$^+$): 278.9180, found: 278.9197.

*4.2.58. 3,4-dichloro-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(63)***

63 was prepared according to General Procedure C. **18** (1.11 g, 2.1 mmol) gave rise to **63** (0.519 g, 0.792 mmol) as a slight yellow foam in 39% yield. Reaction time: 2.5 h. Purification: 12 → 15% EA/cHex. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.98 (s, 1H, ethynyl-H), 4.89 (dd, J = 11.1, 5.4 Hz, 1H, H-5″), 4.96 (dd, J = 5.1, 3.3 Hz, 1H, H-4′), 5.03 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 6.39 (d, J = 5.1 Hz, H-2′), 6.85 (d, J = 5.1 Hz, 1H, H-1′), 7.11 (d, J = 5.1 Hz, 1H, H-5), 7.27–7.31 (m, 2H, OBz), 7.40–7.51 (m, 5H, OBz), 7.58–7.63 (m, 2H, OBz), 7.66 (s, 1H, H-2), 7.87–7.90 (m, 2H, OBz), 8.03–8.07 (m, 2H, OBz), 8.14 (d, J = 5.1 Hz, 1H, H-6), 8.15–8.18 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.8 (C-5′), 76.4, 76.9, 78.4 (C-2′), 79.3, 80.8 (C-4′), 85.8 (C-1′), 106.8 (C-3), 116.1 (C-3a), 119.0 (C-5), 123.1 (C-2), 128.5, 128.5, 128.7, 128.9, 129.7, 129.96, 130.06, 130.09, 133.5, 133.9, 134.0, 136.7 (C-4), 144.6 (C-6), 147.6 (C-7a), 164.3 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{25}Cl_2N_2O_7$ ([M+H]$^+$): 655.1033, found: 655.1045.

*4.2.59. 3-bromo-4-chloro-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(64)***

64 was prepared according to General Procedure C. **18** (0.666 g, 1.26 mmol) gave rise to **64** (0.275 g, 0.392 mmol) as a slight yellow foam in 32% yield. Reaction time: 3h. Purification: 15% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.98 (s, 1H, ethynyl-H), 4.90 (dd, J = 11.1, 4.5 Hz, 1H, H-5″), 4.96 (dd, J = 5.1, 3.3 Hz, 1H, H-4′), 5.03 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 6.40 (d, J = 5.1 Hz, 1H, H-2′), 6.85 (d, J = 5.1 Hz, 1H, H-1′), 7.12 (d, J = 5.1 Hz, 1H, H-5), 7.27–7.32 (m, 2H, OBz), 7.40–7.52 (m, 5H, OBz), 7.59–7.64 (m, 2H, OBz), 7.74 (s, 1H, H-2), 7.87–7.90 (m, 2H, OBz), 8.03–8.07 (m, 2H, OBz), 8.13 (d, J = 5.1 Hz, 1H, H-6), 8.15–8.18 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 63.8 (C-5′), 76.4, 76.9, 78.5 (C-2′), 79.3, 80.8 (C-4′), 85.9 (C-1′), 90.4 (C-3), 117.0 (C-3a), 119.0 (C-5), 125.9 (C-2), 128.47, 128.54, 128.7, 128.9, 129.7, 130.0, 130.1, 133.6, 133.9, 134.0, 137.2 (C-4), 144.3 (C-6), 147.8 (C-7a), 164.3 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{25}BrClN_2O_7$ ([M+H]$^+$): 699.0528, found: 699.0550.

*4.2.60. 3-iodo-4-chloro-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(65)***

65 was prepared according to General Procedure C. **18** (0.666 g, 1.26 mmol) gave rise to **65** (0.354 g, 0.474 mmol) as a slight yellow foam in 39% yield. Purification: 15% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.98 (s, 1H, ethynyl-H), 4.89 (dd, J = 11.1, 4.8 Hz, 1H, H-5″), 4.97 (dd, J = 4.8, 3.6 Hz, 1H, H-4′), 5.02 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 6.42 (d, J = 5.1 Hz, 1H, H-2′), 6.83 (d, J = 5.1 Hz, 1H, H-1′), 7.11 (d, J = 5.1 Hz, 1H, H-5), 7.26–7.52 (m, 2H, OBz), 7.40–7.52 (m, 5H, OBz), 7.58–7.63 (m, 2H, OBz), 7.84 (s, 1H, H-2), 7.87–7.91 (m, 2H, OBz), 8.03–8.07 (m, 2H, OBz), 8.13 (d, J = 5.1 Hz, 1H, H-6), 8.15–8.18 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 53.2 (C-3), 63.8 (C-5′), 76.5, 76.8, 78.5 (C-2′), 79.3, 80.9 (C-4′), 85.9 (C-1′), 118.5 (C-3a), 119.0 (C-5), 128.5, 128.6, 128.76, 128.82, 128.9, 129.7, 130.0, 130.1, 131.6 (C-2), 133.6, 133.9, 134.0, 137.8 (C-4), 144.0 (C-6), 147.9 (C-7a), 164.4 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{25}ICl_2N_2O_7$ ([M+H]$^+$): 747.0389, found: 747.0412.

*4.2.61. 3,4-dichloro-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(66)***

66 was prepared according to General Procedure B. **63** (0.32 g, 0.494 mmol) gave rise to **66** (0.130 g, 0.379 mmol) as a white solid in 77% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 86–88 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.58 (s, 1H, ethynyl-H), 3.65–3.80 (m, 2H, H-5′, H-5″), 3.96 (dd, J = 4.2, 3.0 Hz, 1H, H-4′), 4.60 (t, J = 7.2 Hz, 1H, H-2′), 5.13 (t, J = 4.8 Hz, 1H, OH-5′), 5.82 (d, J = 6.9 Hz, 1H, OH-2′), 5.99 (s, 1H, OH-3′), 6.27 (d, J = 7.5 Hz, 1H, H-1′), 7.35 (d, J = 5.1 Hz, 1H, H-5), 8.13 (s, 1H, H-2), 8.29 (d, J = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 63.8 (C-5′), 72.8, 77.0, 78.5 (C-2′), 82.9, 85.4 (C-1′), 87.0 (C-4′), 102.7 (C-3), 114.5 (C-3a), 118.2 (C-5), 125.2 (C-2), 134.4 (C-4), 144.6 (C-6), 147.3 (C-7a). HRMS (ESI): calculated for $C_{14}H_{12}Cl_2N_2O_4$: 343.0247, found: 343.0259.

*4.2.62. 3-bromo-4-chloro-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(67)***

67 was prepared according to General Procedure B. **64** (0.09 g, 0.129 mmol) gave rise to **67** (0.038 g, 0.098 mmol) as a white solid in 76% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 102–104 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.58 (s, 1H, ethynyl-H), 3.66–3.80 (m, 2H, H-5′, H-5″), 3.96 (dd, J = 3.9, 3.3 Hz, 1H, H-4′), 4.61 (t, J = 7.5 Hz, 1H, H-2′), 5.20 (t, J = 4.8 Hz, 1H, OH-5′), 5.83 (d, J = 7.2 Hz, 1H, OH-2′), 5.99 (s, 1H, OH-3′), 6.27 (d, J = 7.5 Hz, 1H, H-1′), 7.34 (d, J = 5.4 Hz, 1H, H-5), 8.16 (s, 1H, H-2), 8.27 (d, J = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.8 (C-5′), 72.8, 77.0, 78.5 (C-2′), 82.9, 85.5 (C-1′), 86.8 (C-3), 87.0 (C-4′), 115.4 (C-3a), 118.3 (C-5), 127.8 (C-2), 134.8 (C-4), 144.4 (C-6), 147.6 (C-7a). HRMS (ESI): calculated for $C_{14}H_{13}BrClN_2O_4$: 386.9742, found: 386.9756.

*4.2.63. 3-iodo-4-chloro-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(68)***

68 was prepared according to General Procedure B. **65** (0.10 g, 0.134 mmol) gave rise to **68** (0.040 g, 0.092 mmol) as a white solid in 70% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 176–178 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (s, 1H, ethynyl-H), 3.65–3.80 (m, 2H, H-5′, H-5″), 3.96 (dd, J = 3.9, 3.0 Hz, 1H, H-4′), 4.61 (t, J = 7.5 Hz, 1H, 2′-H), 5.14 (t, J = 4.8 Hz, 1H, OH-5′), 5.81 (d, J = 7.2 Hz, 1H, OH-2′), 5.97 (s, 1H, OH-3′), 6.24 (d, J = 7.8 Hz, 1H, H-1′), 7.30 (d, J = 5.1 Hz, 1H, H-5), 8.17 (s, 1H, H-2), 8.24 (d, J = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 51.9 (C-3), 61.8 (C-5′), 72.8, 77.0, 78.5 (C-2′), 82.9, 85.5 (C-1′), 87.0 (C-4′), 117.3 (C-4a), 118.1 (C-5), 133.2 (C-2), 135.5 (C-4), 143.9 (C-6), 148.0 (C-7a). HRMS (ESI): calculated for $C_{14}H_{13}ICl_2N_2O_4$: 434.9603, found: 434.9628. Purity: 91%.

*4.2.64. 4-chloro-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine **(69)***

69 was prepared as described for **35**.**65** (0.1 g, 0.134 mmol) gave rise to **69** (0.062 g, 0.0998 mmol) as a foam in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.95 (s, 1H, ethynyl-H), 4.89 (dd, J = 10.8, 4.8 Hz, 1H, H-5″), 4.94–5.05 (m, 2H, H-4′, H-5′), 6.48 (d, J = 5.1 Hz, 1H, H-2′), 6.68 (d, J = 3.9 Hz, 1H, H-3), 6.87 (d, J = 5.1 Hz, 1H, H-1′), 7.12 (d, J = 5.4 Hz, 1H, H-5), 7.26–7.31 (m, 2H, OBz), 7.40–7.52 (m, 5H, OBz), 7.57–7.64 (m, 2H, OBz), 7.71 (d, J = 3.9 Hz, 1H, H-6),

7.88–7.91 (m, 2H, OBz), 8.05–8.08 (m, 2H, OBz), 8.14–8.18 (m, 2H, OBz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 64.0 (C-5′), 76.5, 77.0, 78.5 (C-2′), 79.1, 80.72 (C-4′), 85.9 (C-1′), 101.7 (C-3), 117.4 (C-5), 120.8 (C-3a), 125.7 (C-2), 128.5, 128.6, 128.71, 128.74, 129.0, 129.8, 130.0, 130.09, 130.12, 133.5, 133.8, 134.0, 136.9 (C-4), 143.4 (C-6), 148.5 (C-7a), 164.4 (C=O), 164.6 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{26}ClN_2O_7$ ($[M+H]^+$): 621.1423, found: 621.1402.

4.2.65. 4-chloro-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (70)

70 was prepared according to General Procedure B. **69** (0.06 g, 0.0967 mmol) gave rise to **70** (0.024 g, 0.078 mmol) as a white solid in 80% yield. Purification: 0 → 8% MeOH/DCM. Melting point: 162 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.57 (s, 1H, ethynyl-H), 3.65–3.79 (m, 2H, H-5′, H-5″), 3.96 (t, J = 3.6 Hz, 1H, H-4′), 4.64 (t, J = 7.2 Hz, 1H, H-2′), 5.16 (t, J = 5.1 Hz, 1H, OH-5′), 5.81 (d, J = 6.9 Hz, 1H, OH-2′), 5.96 (s, 1H, OH-3′), 6.22 (d, J = 7.5 Hz, 1H, H-1′), 6.65 (d, J = 3.6 Hz, 1H, H-3), 7.30 (d, J = 5.1 Hz, 1H, H-5), 7.95 (d, J = 3.6 Hz, 1H, H-2), 8.23 (d, J = 5.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 76.9, 78.4 (C-2′), 83.1, 86.1 (C-1′), 86.7 (C-4′), 98.9 (C-3), 116.4 (C-5), 119.6 (C-3a), 128.1 (C-2), 134.5 (C-4), 143.3 (C-6), 1483.4 (C-7a). HRMS (ESI): calculated for $C_{14}H_{14}ClN_2O_4$($[M+H]^+$): 309.0637, found: 309.0644.

4.2.66. 4-azido-1H-pyrrolo[2,3-b]pyridine (71)[42]

*[**Caution:** this reaction employs large amounts of sodium azide in combination with mild acid as well as substantial heating, and can therefore be considered explosive (HN_3)! No accidents have occurred when performing this reaction (>10 runs), however reaction scale has never exceeded 10 mmol of heterocycle SM. Additional protection by means of a blast shield, and closed fume hood is strongly recommended]* 4-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (0.765 g, 5 mmol, 1 eq.) was dissolved in DMF (15 mL, 3 mL/mmol SM), and NH_4Cl (1.34 g, 25 mmol, 5 eq.) was added, followed by NaN_3 (1.63 g, 25 mmol, 5 eq.). the mixture was heated to 110 °C behind a blast shield. After 7 h, the mixture was allowed to cool to ambient temperature, diluted with EA, and poured into half-sat. aq. $NaHCO_3$ solution. The layers were separated, and the water layer washed twice with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated till dryness. The residue was purified by column chromatography (30% EA/PET) to give **71** (0.53 g, 3.32 mmol) as a white solid in 66% yield. Melting point: 180 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.46 (dd, J = 3.3, 1.8 Hz, 1H, H-3), 6.88 (d, J = 5.4 Hz, 1H, H-5), 7.46 (dd, J = 3.6, 2.4 Hz, 1H, H-2), 8.18 (d, J = 5.1 Hz, 1H, H-6), 11.86 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 96.6 (C-3), 105.0 (C-5), 112.0 (C-3a), 125.9 (C-2), 139.5 (C-7a), 143.7 (C-6), 149.9 (C-4). HRMS (ESI): calculated for $C_7H_6N_5$ ($[M+H]^+$): 160.0618, found: 160.0585.

4.2.67. 3-chloro-4-azido-1H-pyrrolo[2,3-b]pyridine (72)

*[**Caution:** See note for **71** with regard to additional safety measures when performing this reaction!]* **60** (0.53 g, 2.83 mmol, 1 eq.) and NH_4Cl (0.62 g, 14.17 mmol, 5 eq.) were suspended in DMF (10 mL, 3 mL/mmol SM). Then, NaN_3 (0.92 g, 14.17 mmol, 5 eq.) was added and the resulting mixture heated at 110 °C for 6 h behind a blast shield. After cooling to ambient temperature, the mixture was diluted with EA, and poured in to half-saturated aq. $NaHCO_3$ solution. The layers were separated, and the water layer extracted twice with EA. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography 30% EA/Hexanes to give **72** (0.39 g, 2.01 mmol) as a grey powder in 71% yield. Melting point: 205 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.05 (d, J = 5.4 Hz, 1H, H-5), 7.60 (d, J = 1.8 Hz, 1H, H-2), 8.24 (d, J = 5.4 Hz, 1H, H-6), 12.10 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 100.6 (C-3), 106.0 (C-5), 108.1 (C-3a), 123.6 (C-2), 140.3 (C-4), 144.9 (C-6), 148.24 (C-7a). HRMS (ESI): calculated for $C_7H_5ClN_5$ ($[M+H]^+$): 194.0228, found: 194.0210.

4.2.68. 3-iodo-4-azido-1H-pyrrolo[2,3-b]pyridine (73)

71 (0.32 g, 2 mmol, 1 eq.) was dissolved in DMF (3 mL, 1.5 mL/mmol SM) and NIS (0.472 g, 2.1 mmol, 1.05 eq.) was added. The mixture was stirred in the dark overnight. Then, ice-cold water (10 mL, 5 mL/mmol SM) was added and the resulting precipitate filtered. The solids were washed four additional times with ice-cold water (2 mL, 1 mL/mmol SM). The solid was collected and dried under high vacuum to give **73** (0.527 g, 1.85 mmol) as a yellow solid in 93% yield. Melting point: 192 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.03 (d, J = 5.1 Hz, 1H, H-5), 7.64 (d, J = 2.4 Hz, 1H, H-2), 8.22 (d, J = 5.4 Hz, 1H, H-6), 12.20 (br. s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 48.5 (C-3), 105.8 (C-5), 111.4 (C-3a), 131.40 (C-2), 140.4 (C-7a), 144.4 (C-6), 149.3 (C-4). HRMS (ESI): calculated for $C_7H_5IN_5$ ($[M+H]^+$): 285.9584, found: 285.9573.

4.2.69. 3-chloro-4-amino-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (76) & 3-chloro-4-amino-N7-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (77)

76 and 77 were prepared by employing General Procedure C (Reaction time: 3H) and General Procedure E. As such, **18** (0.805 g, 1.52 mmol) gave rise to azido nucleosides [General Procedure C] **74** (0.242 g, 0.366 mmol; R_f = 0.27, 25% EA/hexanes) and a lower running isomer **75** (0.213 g, 0.35 mmol; R_f = 0.19, 25% EA/hexanes) in 24% and 23% yield, respectively (both containing some impurities). Purification: 18 → 25% EA/hexanes. Reaction time: 3H. [Remark: The upper-running fraction, containing **74**, has the same R_f on TLC in a variety of solvent systems but can be identified via staining with *p*-anisaldehyde/sulfuric acid spray, which gives a characteristic reddish colour for **74**.]

Next, both isomeric fractions were subjected to General Procedure E.

4.2.70. N-1 isomer

74 (0.242 g, 0.366 mmol) gave rise to **76** (0.192 g, 0.302 mmol) as a yellowish foam in 82% yield. Purification: 0 → 40% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.95 (s, 1H, ethynyl-H), 4.86 (dd, J = 11.1, 5.4 Hz, 1H, H-5″), 4.92–5.00 (br. s, 3H, NH_2, H-4′), 5.01 (dd, J = 11.1, 3.3 Hz, 1H, H-5′), 6.21 (d, J = 5.7 Hz, 1H, H-5), 6.40 (d, J = 5.4 Hz, 1H, H-2′), 6.83 (d, J = 5.1 Hz, 1H, H-1′), 7.25–7.34 (m, 2H, OBz), 7.35 (s, 1H, H-2), 7.38–7.50 (m, 5H, OBz), 7.56–7.62 (m, 2H, OBz), 7.88–7.92 (m, 3H, OBz, H-6), 8.02–8.05 (m, 2H, OBz), 8.15–8.18 (m, 2H, OBz). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 63.9 (C-5′), 76.4, 77.1, 78.2 (C-2′), 79.0, 80.5 (C-4′), 85.2 (C-1′), 102.8 (C-5), 105.3 (C-3a), 105.8 (C-3), 118.3 (C-2), 128.5, 128.6, 128.7, 129.0, 129.8, 130.0, 130.1, 133.5, 133.7, 133.9, 145.7 (C-6), 148.1 (C-7a), 164.4 (C=O), 164.6 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{27}ClN_3O_7$ ($[M+H]^+$): 636.1532, found: 636.1587. *[Remark:* 1C*, namely C-4 could not be detected].*

4.2.71. N-7 isomer

75 (0.213 g, 0.322 mmol) gave rise to **77** (0.115 g, 0.181 mmol) as a yellow foam in 56% yield. Purification: 25 → 75% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.91 (s, 1H, ethynyl-H), 4.94–5.06 (m, 3H, H-4′, H-5′, H-5″), 5.93 (br. s, 2H, NH_2), 6.13 (d, J = 7.2 Hz, 1H, H-5), 6.29 (d, J = 4.8 Hz, 1H, H-2′), 7.17 (s, 1H, H-2), 7.23 (d, J = 4.8 Hz, 1H, H-1′), 7.25–7.31 (m, 2H, OBz), 7.37–7.51 (m, 5H, OBz), 7.55–7.62 (m, 2H, OBz), 7.89–7.93 (m, 3H, OBz, H-6), 8.00–8.03 (m, 2H, OBz), 8.12–8.16 (m, 2H, OBz). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 63.8 (C-5′), 76.2, 76.8, 78.9 (C-2′), 79.6, 81.4 (C-4′), 88.3 (C-1′), 99.0 (C-5), 101.3 (C-3), 106.8 (C-3a), 128.38, 128.42, 128.67, 128.70, 129.1 (C-6), 129.6, 129.9, 130.06, 130.09, 133.5, 133.8, 134.0, 134.3 (C-2), 145.7 (C-7a),

150.9 (C-4), 164.4 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{27}ClN_3O_7$ ([M+H]$^+$): 636.1532, found: 636.1541.

4.2.72. 3-iodo-4-azido-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (78)

78 was prepared according to General Procedure C. **18** (0.793 g, 1.5 mmol) gave rise to **78** (0.288 g, 0.382 mmol) as a slight yellow foam in 25% yield. Reaction time: 2.5 h. Purification: 15% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.97 (s, 1H, ethynyl-H), 4.89 (dd, J = 11.1, 4.8 Hz, 1H, H-5″), 4.95–4.97 (m, 1H, H-4′), 5.02 (dd, J = 11.1, 3.8 Hz, 1H, H-5′), 6.41 (d, J = 5.1 Hz, 1H, H-2′), 6.80 (d, J = 5.1 Hz, 1H, H-1′), 6.87 (d, J = 5.1 Hz, 1H, H-5), 7.27–7.32 (m, 2H, OBz), 7.40–7.52 (m, 5H, OBz), 7.57–7.64 (m, 2H, OBz), 7.71 (s, 1H, H-2), 7.87–7.91 (m, 2H, OBz), 8.03–8.06 (m, 2H, OBz), 8.15–8.18 (m, 2H, OBz), 8.20 (d, J = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 52.0 (C-3), 63.8 (C-5′), 76.4, 76.8, 78.4 (C-2′), 79.3 (C-4′), 80.8, 85.7 (C-1′), 107.0 (C-5), 113.2 (C-3a), 128.47, 128.53, 128.66, 128.73, 128.8, 128.9, 129.6, 130.0, 130.1, 130.2 (C-2), 133.6, 133.8, 134.0, 142.4 (C-4), 145.0 (C-6), 149.1 (C-7a), 164.3 (C=O), 164.5 (C=O), 166.4 (C=O). HRMS (ESI): calculated for $C_{35}H_{25}IN_5O_7$ ([M+H]$^+$): 754.0793, found: 754.0775.

4.2.73. 3-iodo-4-amino-N1-(3′-C-ethynyl-2′,3′-5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (79)

79 was prepared according to General Procedure E. **78** (0.28 g, 0.372 mmol) gave rise to **79** (0.2 g, 0.275 mmol) as a yellow foam in 74% yield. Purification: 5 → 40% EA/Hexanes. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.94 (s, 1H, ethynyl-H), 4.86 (dd, J = 11.4, 5.1 Hz, 1H, H-5″), 4.93–5.03 (br. s, 4H, NH_2, H-4′, H-5′), 6.23 (d, J = 5.7 Hz, 1H, H-5), 6.43 (d, J = 5.4 Hz, 1H, H-2′), 6.81 (d, J = 5.1 Hz, 1H, H-1′), 7.27–7.33 (m, 2H, OBz), 7.39–7.52 (6H, OBz, H-2), 7.56–7.64 (m, 2H, OBz), 7.90–7.94 (m, 3H, OBz, H-6), 8.03–8.06 (m, 2H, OBz), 8.16–8.20 (m, 2H, OBz). HRMS (ESI): calculated for $C_{35}H_{27}IN_3O_7$ ([M+H]$^+$): 728.0888, found: 728.0899.

4.2.74. 3-chloro-4-amino-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (80)

80 was prepared according to General Procedure B. **77** (0.19 g, 0.299 mmol) gave rise to **80** (0.070 g, 0.218 mmol) as a white solid in 73% yield. Purification: 0 → 20% MeOH/DCM. Melting point: 162 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (s, 1H, ethynyl-H), 3.60–3.75 (m, 2H, H-5′, H-5″), 3.92 (t, J = 3.3 Hz, 1H, H-4′), 4.62 (t, J = 7.5 Hz, 1H, H-2′), 5.65 (dd, J = 6.6, 4.5 Hz, 1H, OH-5′), 5.72 (d, J = 7.2 Hz, 1H, OH-2′), 5.85 (s, 1H, OH-3′), 5.99 (d, J = 7.8 Hz, 1H, H-1′), 6.21 (br. s, 2H, NH_2), 6.29 (d, J = 5.4 Hz, 1H, H-5), 7.52 (s, 1H, H-2), 7.76 (d, J = 5.7 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 61.9 (C-5′), 72.8, 76.7, 77.5 (C-2′), 83.2, 86.6 (C-1′), 86.8 (C-4′), 101.5, 102.1 (C-5), 104.3, 120.2, 144.6 (C-6), 147.3, 148.7. HRMS (ESI): calculated for $C_{14}H_{15}ClN_3O_4$ ([M+H]$^+$): 324.0746, found: 324.0753.

4.2.75. 3-iodo-4-amino-N1-(3′-C-ethynyl-β-D-ribofuranosyl)-pyrrolo[2,3-b]pyridine (81)

81 was prepared according to General Procedure B. **79** (0.19 g, 0.261 mmol) gave rise to **81** (0.08 g, 0.19 mmol) as a white solid in 74% yield. Purification: 0 → 5% MeOH/DCM. Melting point: 204 °C (decomposed). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.53 (s, 1H, ethynyl-H), 3.62–3.75 (m, 2H, H-5′, H-5″), 3.92 (t, J = 3.3 Hz, 1H, H-4′), 4.63 (t, J = 7.5 Hz, 1H, H-2′), 5.67–5.71 (m, 1H, OH-5′), 5.71 (d, J = 7.2 Hz, 1H, OH-2′), 5.84 (s, 1H, OH-3′), 5.98 (d, J = 7.8 Hz, 1H, H-1′), 6.12 (br. s, 2H, NH_2), 6.31 (d, J = 5.7 Hz, 1H, H-5), 7.61 (s, 1H, H-2), 7.76 (d, J = 5.4 Hz, 1H, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 50.1 (C-3), 61.9 (C-5′), 72.8, 76.7, 77.5 (C-2′), 83.3, 86.6 (C-1′), 86.9 (C-4′), 101.6 (C-5), 106.8 (C-3a), 128.3 (C-2), 144.0 (C-6), 148.0 (C-7a), 148.9 (C-4). HRMS (ESI): calculated for $C_{14}H_{15}IN_3O_4$ ([M+H]$^+$): 416.0102, found: 416.0113.

4.3. Biology

4.3.1. Cell proliferation

L1210, HeLa and CEM cells [21]: All assays were performed in 96-well microtiter plates. To each well were added (5–7.5) × 10^4 tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (Analis, Belgium). The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

NCI-60 evaluation: Assay protocols concerning NCI-60 tumor cell panel evaluation can be found in the following references: [43,44].

Endothelial cells (ECs). Primary human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HMVEC-d) were purchased from Lonza (Verviers, Belgium) and the human microvascular endothelial cell line HMEC-1 was obtained from the Centers for Disease Control and Prevention (CDC, Atalanta, GA, USA). The ECs were seeded in gelatin-coated 48-well plates at 20,000 cells/well in EC growth medium (EGM2, Lonza) and after an overnight incubation, 5-fold dilutions of the compounds were added. The ECs were allowed to proliferate for four days in the presence of the compounds, trypsinized and counted by means of a Coulter counter to determine the IC_{50} values.

4.3.2. In vivo evaluation of antitumor activity of 32

Female severe combined immunodeficient (SCID) mice were used at the age of 8 weeks. The animals were bred at the animal facility of the Rega Institute for Medical Research (KU Leuven, Belgium). The MDA-MB-231-LM2 lung metastatic cell line (clone 4715) was a kind gift of Prof. Massagué [46]. LM2 cells (10^6) were suspended in 50% matrigel (BD Bioscience) in PBS and orthotopically engrafted in the exposed left, fourth inguinal mammary fat pad of anesthetized SCID mice. Once the tumor was palpable (day 10), **32** was injected intratumorally (i.t.) at 0.3 mg/kg in PBS containing 1% DMSO, 3 times a week, for 2-consecutive weeks. Control mice received only PBS with 1% DMSO.

The growth of luciferase-positive LM2 cells was quantified with an IVIS Spectrum imaging system (Caliper Life Sciences, Hopkinton, MA, USA). Before imaging, mice were anesthetized and injected subcutaneously with 150 mg/kg D-luciferin (PerkinElmer). Images were acquired every 2 min and plateau radiance values (photons/sec) were retained. Lung metastasis was determined after shielding the primary tumor with a black paper. Tumor size was measured using a digital caliper and calculated with the following formula: tumor volume (mm^3) = 0.5 × a x b^2, where a is the longest diameter and b is the shortest diameter.

Ethics statement: All studies were done in compliance with the ethical guidelines for animal welfare of the KU Leuven (P277/2015).

4.3.3. Antiviral Evaluation [21]

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK$^-$) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) strain Oka, TK$^-$ VZV strain 07–1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, adenovirus-2, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Sindbis, reovirus-1, Punta Toro. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human

embryonic lung (Hel) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin-Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%. Cytotoxic concentration was expressed as the MCC or minimal cytotoxic concentration being the compound concentration that was required to afford a microscopically visible alteration of cell morphology.

Assays involving hCMV and VZV were performed as described in literature [47]. In short; the antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (Hel) fibroblasts. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of HCMV (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (VZV). After a 1–2 h, the residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity (hCMV) or plaque formation (VZV) was recorded as soon as it reached completion in the control virus-infected cell cultures (untreated controls). Antiviral activity was expressed as the EC_{50} or concentration required for reducing virus-induced cytopathicity or viral plaque formation by 50%.

Declaration of interests

Declaration of interests: none.

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Appendix A. Supplementary data

Copies of ^{1}H, ^{13}C and ^{19}F NMR spectra of compounds **13**–**17**, **23**, **24**, **26**, **31**–**34**, **37**, **37**–**39**, **42**, **47**–**50**, **54**, **57**, **59**, **63**–**68**, **70**, **76**–**78**, **80**, **81**; as well as $^{1}H-^{13}C$ gHSQC & gHMBC, and 2D NOESY spectra of compounds **23, 24, 26, 33, 47**–**49**, **54**, **57**, **63**–**65**, **76**–**78** can be found in the Supporting Information. Full NCI-60 assay data for compounds **31**–**34** and **37** can be found in the Supporting Information.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.07.062.

Abbreviations

BLI	Bioluminescence imaging
CMV	cytomegalovirus
EAdo	3′-*C*-ethynyladenosine;
ECyd	3′-*C*-ethynylcytidine
HMDS	hexamethyldisilazane
HMEC-1	human dermal microvascular endothelial cell line;
Hel	human embryonic lung fibroblasts
HMVEC	human microvascular endothelial cells
HUVEC	human umbilical vein endothelial cells
HSV-1	herpes simplex virus-1
HSV-2	herpes simplex virus-2
iPrMgCl.LiCl	isopropylmagnesium chloride.lithiumchloride complex
TMSOTf	trimethylsilyltriflate
TPPTS	trisodium 3-bis(3-sulfonatophenyl)phosphanyl benzenesulfonate
VZV	varicella zoster virus

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