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Synthesis and Cytostatic and Antiviral Activities of 2′-Deoxy-2′,2′-difluororibo- and 2′-Deoxy-2′-fluororibonucleosides Derived from 7-(Het)aryl-7-deazaadenines

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A series of sugar-modified derivatives of cytostatic 7-heteroaryl-7-deazaadenosines (2′-deoxy-2′-fluororibo- and 2′-deoxy-2′,2′-difluororibonucleosides) bearing an aryl or heteroaryl group at position 7 was prepared and screened for biological activity. The difluororibonucleosides were prepared by non-stereoselective glycosidation of 6-chloro-7-deazapurine with benzoyl-protected 2-deoxy-2,2-difluoro-D-*erythro*-pentofuranosyl-1-mesylate, followed by amination and aqueous Suzuki cross-couplings with (het)arylboronic acids. The fluororibo derivatives were prepared by aqueous palladium-catalyzed cross-coupling reactions of the corresponding 7-iodo-7-deazaadenine 2′-deoxy-2′-fluororibonucleoside **20** with (het)arylboronic acids. The key intermediate **20** was prepared by a six-step sequence from the corresponding arabinonucleoside by selective protection of 3′- and 5′-hydroxy groups with acid-labile groups, followed by stereoselective S_N2 fluorination and deprotection. Some of the title nucleosides and 7-iodo-7-deazaadenine intermediates showed micromolar cytostatic or anti-HCV activity. The most active were 7-iodo and 7-ethynyl derivatives. The corresponding 2′-deoxy-2′,2′-difluororibonucleoside 5′-*O*-triphosphates were found to be good substrates for bacterial DNA polymerases, but are inhibitors of human polymerase α.

Introduction

Within the framework of our long-term project on the synthesis and biological activity screening of modified purine and 7-deazapurine ribonucleosides,[1] we recently discovered a new group[2] of nucleoside cytostatics, 7-heteroaryl-7-deazaadenine ribonucleosides **2** (Figure 1), as derivatives of natural cytotoxic antibiotic tubercidin (**1**). Nucleosides **2** exerted a significant in vitro cytostatic effect against a broad spectrum of leukemia and tumor cell lines at nanomolar concentrations, as well as nonselective anti-HCV activities (probably caused by cytotoxicity). More recently,[3] we prepared their 2′-*C*-methylribonucleoside, arabinonucleoside, and 2′-fluoroarabinonucleoside derivatives **3** (Figure 1). These sugar-modified derivatives showed low micromolar cytostatic or anti-HCV activities, indicating that the *ribo* configuration of the sugar is not crucial for activity. To complement the structure–activity relationship of this class of biologically active nucleosides, herein we report sugar-modified derivatives containing a fluorine atom in the *ribo* configuration. The 2′,2′-difluororibose motif is inspired by cytostatic gemcitabine[4] (2′,2′-difluorodeoxycytidine, **4**) which is a drug in

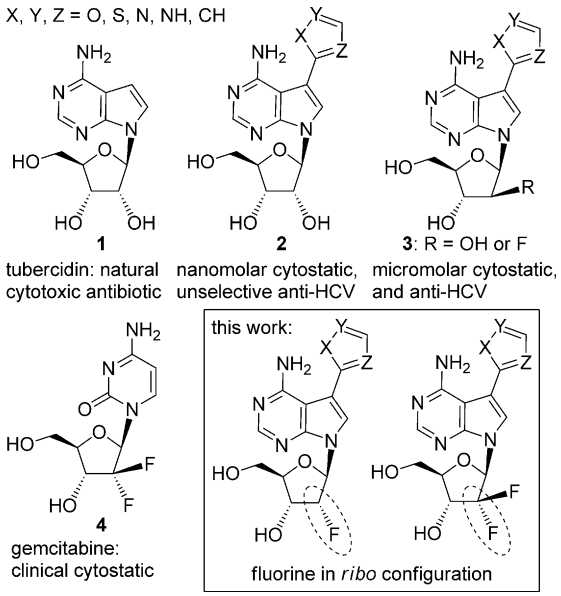

Figure 1. Biological activity of 7-deazaadenine nucleosides and gemcitabine.

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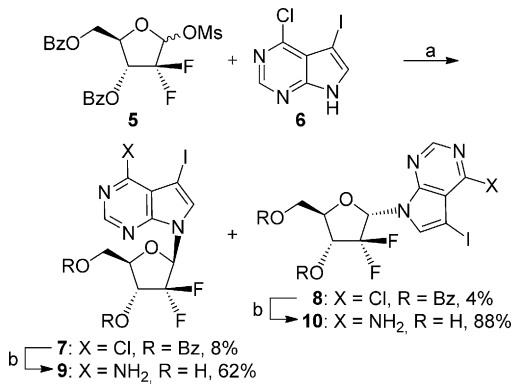
clinical use for the treatment of solid tumors (pancreas and lung cancer). Some 2′-deoxy-2′-fluororibonucleosides are also known cytostatics.[5]

Results and Discussion

Synthesis

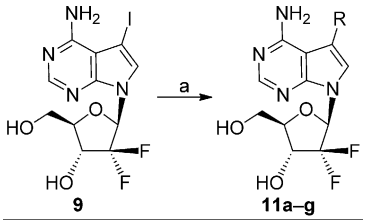
The synthetic approach to the target 7-heteroaryl-7-deazaadenine 2′-deoxy-2′,2′-difluoro-D-*erythro*-pentofuranosyl nucleosides relied on the glycosidation of 6-chloro-7-iodo-7-deazapurine (**6**) with commercially available difluorosugar mesylate **5**. However, the TMSOTf-catalyzed glycosylation of **6** with **5** according to a published procedure[6] failed. Therefore, we focused on nucleobase anion glycosylation, which was previously used for the synthesis of 7-deazapurine 2′-deoxy-2′,2′-difluororibonucleosides.[7] The reaction of **6** with mesylate **5** was performed in the presence of tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1; 1 equiv) and potassium hydroxide (2 equiv) in acetonitrile at 50 °C (Scheme 1). Under these conditions, the reaction



Scheme 1. *Reagents and conditions*: a) TDA-1, KOH, CH_3CN, 4 days, 50 °C; b) $NH_{3(aq)}$ (25% *w/w*), dioxane, 20 h, 120 °C.

proceeded with incomplete conversion to form both anomers of 2′-deoxy-2′,2′-difluoro-D-*erythro*-pentofuranosyl nucleosides **7** and **8** in low yields (18% total, β/α ratio ~1:1). At higher temperature (80 °C) the overall yield of nucleosides **7** and **8** was improved to 27%, but the β/α ratio increased to 1:3.6. On the other hand, a greater excess of KOH (3.5 equiv) led to the formation of anomers **7** and **8** in 2:1 β/α ratio, but the overall yield dropped to 6%. Nucleoside anomers **7** and **8** were separable only by two successive column chromatographic steps with very slow gradient. Therefore, the glycosylation reaction was performed on a larger scale (10 g), and the pure anomers **7** and **8** were isolated in low yields (8 and 4%, respectively), but in sufficient quantities for subsequent steps. Benzoylated anomeric 6-chloro-7-iodo-7-deazapurine nucleosides **7** and **8** were subjected to a simultaneous amination and deprotection reaction by treating with aqueous ammonia in dioxane in a steel pressure flask to obtain free nucleosides **9** and **10** in good yields (62 and 88%, respectively; Scheme 1).

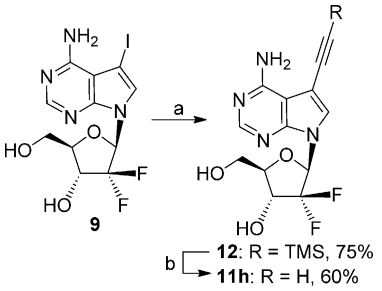
Unprotected nucleoside **9** was used as starting material for a series of palladium-catalyzed Suzuki cross-coupling reactions with various (het)arylboronic acids under aqueous-phase conditions[8] to synthesize 7-(het)aryl-7-deazaadenine 2′-deoxy-2′,2′-difluoro-β-D-*erythro*-pentofuranosyl nucleosides **11a–g** in moderate to good yields (39–71%; Scheme 2). The cross-couplings were performed in the presence of Na_2CO_3, triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt (TPPTS), $Pd(OAc)_2$ in water/acetonitrile 2:1 at 100 °C.



	R	yield [%]
11a	2-furyl	65
11b	3-furyl	55
11c	2-thienyl	53
11d	3-thienyl	60
11e	phenyl	71
11f	2-naphtyl	71
11g	2-benzofuryl	39

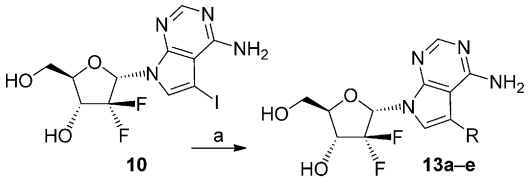
Scheme 2. *Reagents and conditions*: a) R-$B(OH)_2$, Na_2CO_3, TPPTS, $Pd(OAc)_2$, H_2O/CH_3CN (2:1), 2 h, 100 °C.

The (trimethylsilyl)ethynyl derivative **12** was prepared in 75% yield by the Sonogashira reaction of nucleoside **9** with (trimethylsilyl)acetylene in the presence of copper iodide, triethylamine, and $PdCl_2(PPh_3)_2$ in *N*,*N*-dimethylformamide (DMF). Subsequent desilylation using potassium carbonate in methanol provided ethynyl derivative **11h** in 60% yield (Scheme 3).



Scheme 3. *Reagents and conditions*: a) (trimethylsilyl)acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, DMF, 18 h, RT; b) K_2CO_3, CH_3OH, 1 h, RT.

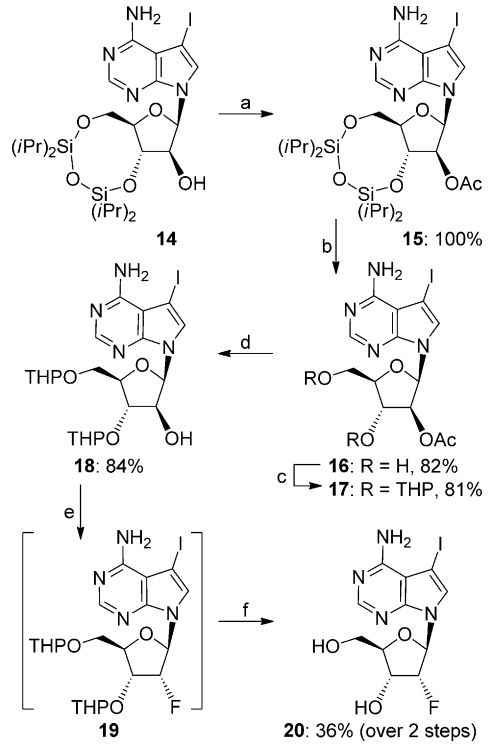
Analogously, the Suzuki cross-coupling reaction was also used for the introduction of (het)aryl groups into position 7 of α-anomeric 7-iodo-7-deazaadenine nucleoside **10**. The corresponding 7-(het)aryl-7-deazaadenine 2′-deoxy-2′,2′-difluoro-α-D-*erythro*-pentofuranosyl nucleosides **13a–e** were prepared in 38–65% yields (Scheme 4).



	R	yield [%]
13a	2-furyl	38
13b	3-furyl	61
13c	2-thienyl	58
13d	3-thienyl	65
13e	phenyl	65

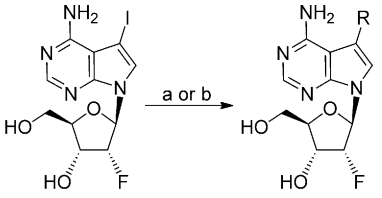
Scheme 4. Reagents and conditions: a) R-B(OH)$_2$, Na_2CO_3, TPPTS, Pd(OAc)$_2$, H_2O/CH_3CN (2:1), 2 h, 100 °C.

The synthetic approach to 7-heteroaryl-7-deazaadenine 2′-deoxy-2′-fluororibonucleosides **21** was based on S_N2 fluorination of the corresponding tetrahydropyran-2-yl (THP)-protected arabinonucleoside (Scheme 5). At first, the known[3] silylated 7-iodo-7-deazaadenine arabinonucleoside **14** was acetylated in quantitative yield. Subsequent desilylation of acetate **15** using $Et_3N{\cdot}3HF$ provided 3′,5′-diol **16** (82%). THP groups were then introduced at positions 3′ and 5′ to give the bis-THP-protected acetate **17** in 81% yield. Zemplén deacetylation gave 3′,5′-THP-protected arabinonucleoside **18** (84%) which was allowed to react with (diethylamino)sulfur trifluoride (DAST) in the presence of pyridine in dichloromethane at 0 °C. The resulting 2′-deoxy-2′-fluororibonucleoside **19** was directly deprotected by 90% trifluoroacetic acid (TFA) to give free 2′-deoxy-2′-fluororibonucleoside **20** (36% over two steps). The relative configuration of fluorine in compound **20** was established by analysis of H,H and H,F coupling constants in the ^{1}H NMR spectra; coupling constants were identical to those observed for 2′-deoxy-2′-fluororibonucleosides, reported previously.[7]



Scheme 5. *Reagents and conditions*: a) Ac_2O, Et_3N, DMAP, CH_3CN, RT, overnight; b) $Et_3N{\cdot}3HF$, THF, RT, overnight; c) DHP, TsOH, DMF, 0 °C→RT, overnight; d) MeONa, CH_3OH, RT, overnight; e) DAST, pyridine, CH_2Cl_2, 0 °C→RT, overnight; f) 90% TFA, RT, 2 h.

The 7-iodo-7-deazaadenine fluororiboside **20** was then used as a starting material for a series of Suzuki cross-coupling reactions with (het)aryl boronic acids under standard conditions. In this way, the title 7-heteroaryl-7-deazaadenosine 2′-deoxy-2′-fluororibonucleosides **21a–e** were synthesized in 53–69% yields (Scheme 6). The additional ethynyl derivative **21h** was prepared by Sonogashira reaction with (trimethylsilyl)acetylene (76%). The trimethylsilyl group was spontaneously cleaved during hydrolytic reaction workup (Scheme 6).



	R	conditions	yield [%]
21a	2-furyl	a	53
21b	3-furyl	a	63
21c	2-thienyl	a	53
21d	3-thienyl	a	61
21e	phenyl	a	69
21h	ethynyl	b	76

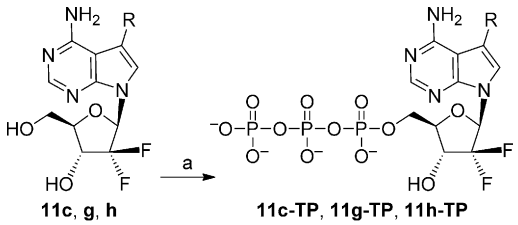
Scheme 6. *Reagents and conditions*: a) R-B(OH)$_2$, Na_2CO_3, TPPTS, Pd(OAc)$_2$, H_2O/CH_3CN (2:1), 2 h, 100 °C; b) (trimethylsilyl)acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N/DMF, overnight, RT, then hydrolytic workup.

To test substrate activities for DNA polymerases, the corresponding nucleoside 5′-*O*-triphosphates **11c-TP**, **11g-TP**, and **11h-TP** were synthesized from nucleosides **11c**,**g**,**h** by a standard phosphorylation method,[9] using phosphoryl chloride in trimethyl phosphate, followed by nucleophilic substitution with $(NHBu_3)_2H_2P_2O_7$ and finally hydrolysis with an aqueous solution of triethylammonium bicarbonate (TEAB). After ion exchange and lyophilization from water, the desired triphosphates **11c-TP**, **11g-TP**, and **11h-TP** were obtained as sodium salts (12–36%, Scheme 7). Analogously, the triphosphate of gemcitabine, **4-TP**, was prepared from commercial gemcitabine hydrochloride.

Biological activity profiling

Cytostatic activity

All the title nucleosides **9**, **10**, **11a–h**, **13a–e**, **20**, and **21a–h** were subjected to biological activity screening. The in vitro cytotoxic activity was studied on the following cell cultures: 1) human pro-myelocytic leukemia HL60 cells (ATCC CCL 240),



	R	yield [%]
11c-TP	2-thienyl	36
11g-TP	2-benzofuryl	13
11h-TP	ethynyl	12

Scheme 7. *Reagents and conditions*: a) 1. $POCl_3$, $PO(OMe)_3$, 2.5 h, 0 °C, 2. $(NHBu_3)_2H_2P_2O_7$, tri(*n*-butyl)amine, DMF, 2.5 h, −5 °C, 3. Workup with TEAB (2 M).

2) human cervical carcinoma HeLa S3 cells (ATCC CCL 2.2), 3) human T lymphoblastoid CCRF-CEM cells (ATCC CCL 119), and 4) hepatocellular carcinoma HepG2 cells (ATCC HB 8065). Cell viability was determined after incubation for three days using a metabolic 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT)-based method.[10] Table 1 summarizes the cytostatic activities. The most active (sub-micromolar) were both 7-iodo-7-deazaadenine fluoronucleosides **9** and **20**. The 2-furyl and 2-thienyl as well as bulky naphthyl and benzofuryl derivatives of 2′,2′-difluororibonucleosides **11a**,**c**,**g**,**f** showed micromolar cytostatic effects, whereas the 3-furyl, 3-thienyl, and phenyl derivatives **11b**,**d**,**e** were inactive. This finding is quite surprising, because in the parent ribonucleoside series the 7-deazaadenosines, bearing bulky substituents at position 7, were inactive.[2] Unexpectedly, one of the α-anomeric derivatives (**13c**) also exerted moderate activity. In the fluororibo series, the 7-(het)aryl derivatives **21a**–**e** were all inactive, whereas the 7-iodo- and 7-ethynyl-7-deazaadenine derivatives **20** and **21h** showed significant cytostatic effects. This indicates that the mechanism of action in each series may be distinct, and most likely differs from that of the parent ribonucleosides.

Table 1. Cytotoxic activities of fluoronucleosides **9**–**13**, **20**, and **21**.

Compd	IC_{50} [μM]			
	HL60	HeLa S3	CCRF-CEM	HepG2
9	0.42	0.64	0.42	0.35
10	5.46	6.94	7.38	3.76
11a	7.44	9.77	6.63	>10
11b	>10	>10	>10	>10
11c	6.33	9.71	3.44	>10
11d	>10	>10	>10	>10
11e	>10	>10	>10	>10
11f	6.12	8.56	8.81	>10
11g	3.69	5.57	7.07	>10
11h	1.41	2.62	1.67	0.40
13a	>10	>10	>10	>10
13b	>10	>10	>10	>10
13c	5.05	>10	4.42	>10
13d	>10	>10	>10	>10
13e	>10	>10	>10	>10
20	0.24	0.35	0.64	0.15
21a	>10	>10	>10	>10
21b	>10	>10	>10	>10
21c	>10	>10	>10	>10
21d	>10	>10	>10	>10
21e	>10	>10	>10	>10
21h	1.14	1.76	1.13	0.52

Antiviral activity

The title modified nucleosides were also tested up to 100 μM for antiviral activities: HIV, RSV, and HCV genotypes 1A, 1B, and 2A replicons.[11] No antiviral activity against HIV or RSV was observed for any of these derivatives. On the other hand, all of the title nucleosides showed micromolar inhibition in the HCV replicon assays, although usually accompanied by cytotoxicity (Table 2). The most promising were the 7-iodo and 7-ethynyl derivatives **9**, **11h**, and **21h** which exerted sub-micromolar EC_{50} values and cytotoxicities at concentrations higher by one to two orders of magnitude. These results suggest that, similarly to the previously reported ribonucleosides,[2] these sugar-modified deazapurine ribonucleosides interfere with critical cell-growth processes, and most of the activity toward HCV viral replication is nonspecific.

Table 2. Anti-HCV activities of fluoronucleosides **9**–**13**, **20**, and **21**.

Compd	Replicon 1A		Replicon 1B		Replicon 2A	
	EC_{50} [μM]	CC_{50} [μM]	EC_{50} [μM]	CC_{50} [μM]	EC_{50} [μM]	CC_{50} [μM]
9	1.10	30.66	0.06	4.02	1.12	9.6
10	2.48	23.60	0.70	14.15	4.67	23.76
11a	6.04	>44	4.93	25.47	5.62	37.06
11b	16.51	>44	13.18	20.05	10.30	>44
11c	5.30	32.71	2.93	9.84	4.82	26.36
11d	6.52	>44	5.74	8.91	7.38	>44
11e	8.97	>44	6.49	18.86	8.58	41.83
11f	1.43	10.36	1.21	2.55	0.81	5.99
11g	1.10	16.25	1.08	4.02	1.62	8.78
11h	0.44	17.10	0.50	10.94	3.29	15.62
13a	2.04	>44	1.63	40.61	13.76	>44
13b	4.56	>44	1.98	>44	14.45	>44
13c	1.52	>44	0.44	18.91	2.24	38.42
13d	4.71	>44	0.91	40.45	8.52	>44
13e	22.73	>44	2.64	43.14	10.75	>44
20	1.30	5.98	1.02	10.20	4.36	7.37
21a	1.98	>44	1.85	33.46	10.74	>44
21b	4.37	>44	5.75	37.44	14.17	>44
21c	2.00	>44	1.99	18.53	7.96	>44
21d	6.52	>44	5.98	27.55	13.21	>44
21e	33.13	>44	18.83	>44	20.73	>44
21h	0.26	28.92	0.47	12.38	1.86	21.90

Biochemistry

To verify the mechanism of action of these biologically active nucleosides, we decided to test the substrate activity and/or inhibition of polymerases by the corresponding nucleoside triphosphates (NTPs). The parent ribonucleosides **2** were previously shown[2] to inhibit RNA synthesis, but gemcitabine (**4**) is known[4] to inhibit DNA synthesis (its NTP is incorporated by polymerases but then terminates the chain). Therefore, three examples of NTPs derived from the title difluororibonucleo-

sides (**11 c-TP**, **11 g-TP**, and **11 h-TP**) were prepared and tested with bacterial DNA polymerases (as a simple model) and with human polymerase α.

Enzymatic incorporation of 11 c-TP, 11 g-TP, and 11 h-TP by bacterial polymerases

The enzymatic incorporation of adenosine derivatives **11 c-TP**, **11 g-TP**, and **11 h-TP** was tested using four different model bacterial DNA polymerases (Pwo, KOD XL, Vent(exo⁻), and DeepVent(exo⁻)) and a 31-base-long template ($Temp^{Prb4basII}$), allowing the incorporation of four modifications. The best substrate activities of these modified NTPs were observed with Vent(exo⁻) DNA polymerase, which readily and selectively incorporated all three modified nucleotides derived from **11 c**, **11 g**, and **11 h** (Figure 2). The formation of full-length oligonucleotides with Vent(exo⁻) DNA polymerase was also confirmed by MALDI-TOF analysis (Supporting Information). KOD XL DNA polymerase efficiently incorporated only 7-thienyl- and 7-ethynyl-7-deazaadenine nucleotides **11 c-TP** and **11 h-TP**, whereas the benzofuryl derivative **11 g-TP** was not incorporated. DeepVent(exo⁻) incorporated only **11 c-TP**, whereas Pwo DNA polymerase afforded only a mixture of full-length 31-mer products together with four-base-shorter oligonucleotide products (figure S1 in Supporting Information). The very good substrate activities of the 7-substituted-7-deazaadenine difluororibonucleoside triphosphates could be used for polymerase synthesis of fluorinated modified DNA.[12, 13]

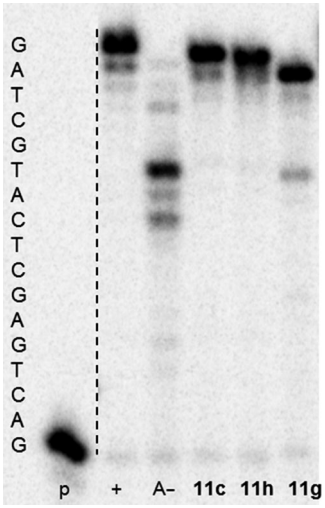


Figure 2. Denaturing PAGE analysis of PEX products with template $Temp^{Prb4basII}$, modified **11 c-TP**, **11 g-TP**, or **11 h-TP** and Vent(exo⁻) DNA polymerase. The experiments were supplemented with a ^{32}P-radiolabeled primer (p), positive control (+: all natural dNTPs), and negative control (A−: absence of dATP).

Enzymatic incorporation by human DNA polymerase α

Further studies focused on the incorporation of **11 c-TP**, **11 g-TP**, and **11 h-TP** into DNA by human polymerase α, which should be relevant to the cytostatic/cytotoxic effects. The radiolabeled primer was first annealed to a template ($Temp^{MonoT}$) and then subjected to primer extension experiment with human polymerase α (Figure 3). Triphosphates derived from gemcitabine (**4-TP**), tubercidin (**1-TP**), and 7-(2-thienyl)-7-deazaadenosine (**2 c-TP**)[2] were also used in the study to compare their effects on human polymerase α with the effect of **11 c-TP**, **11 g-TP**, and **11 h-TP**. While nucleotides derived from tubercidin (**1**) and 7-thienyltubercidin (**2 c**) were not incorporated into DNA (and did not show any significant inhibition of the polymerase), gemcitabine (**4**) nucleotide was partly incorporated, and its incorporation resulted in chain termination. The 7-substituted 7-deazaadenine difluororibonucleoside triphosphates **11 c-TP**, **11 g-TP**, and **11 h-TP** inhibited the polymerase, yet with varied potencies. The thienyl and ethynyl nucleotides **11 c** and **11 h** were incorporated to some extent; however, their incorporation led to a stop one base after them. Only traces of the full-length products were observed. The benzofuryl derivative **11 g-TP** fully inhibited the polymerase; only a primer band was observed on the gel.

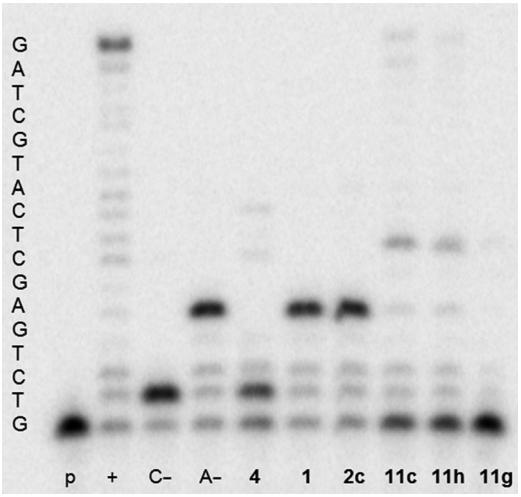


Figure 3. Denaturing PAGE analysis of PEX products with template $Temp^{MonoT}$, human DNA polymerase α, and modified nucleoside triphosphates **4-TP**, **1-TP**, **2 c-TP**, **11 c-TP**, **11 h-TP**, and **11 g-TP**. The experiments were supplemented with a ^{32}P-radiolabeled primer (p), positive control (+: all natural dNTPs), and two negative controls (C−: absence of dCTP; A−: absence of dATP).

Inhibition of DNA polymerase α activity by 11 c-TP, 11 g-TP, and 11 h-TP

To determine the effect of **11 c-TP**, **11 g-TP**, and **11 h-TP** on DNA polymerase α activity, the primer was extended in the presence of all four natural dNTPs (100 μM) and various concentrations of **11 c-TP**, **11 g-TP**, and **11 h-TP** (50–900 μM). The gel (Figure 4) demonstrates that polymerase α is not inhibited when **11 c-TP** and **11 h-TP** are present up to 100 μM. **11 g-TP** is a stronger inhibitor and inhibits the polymerase even at 50 μM. Therefore, a more detailed study was carried out with **11 g-TP**

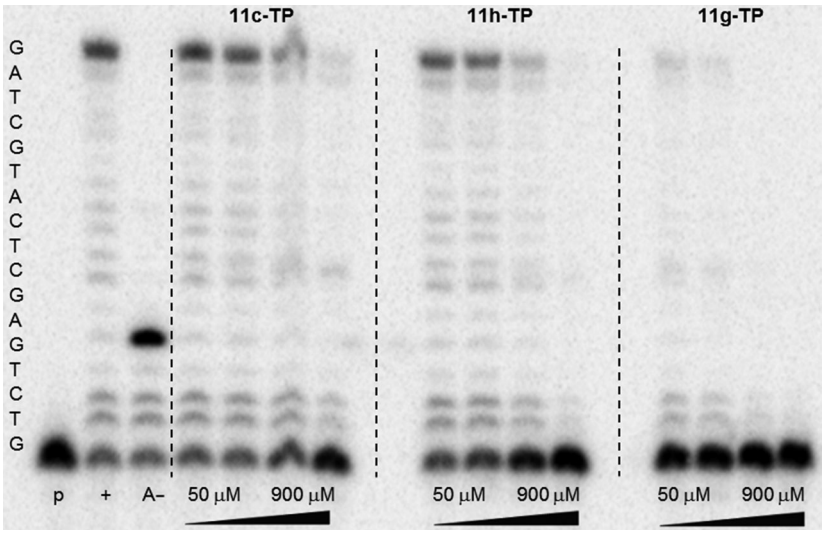


Figure 4. Inhibition of human polymerase α activity by **11 c-TP**, **11 h-TP**, and **11 g-TP**. The reaction mixtures contained all four natural dNTPs (100 μM) together with the modified nucleoside triphosphate **11 c-TP**, **11 h-TP**, or **11 g-TP** (50, 100, 300, or 900 μM). The experiments were supplemented with a ^{32}P-radiolabeled primer (p), positive control (+: all natural dNTPs) and negative control (A−: absence of dATP).

at concentrations between 5 and 900 μM (figure S2 in Supporting Information). The IC_{50} value, obtained from the plot (Figure 5) of primer intensities against the logarithm of **11 g-TP** concentrations, is 35 μM. We have not studied the intracellular

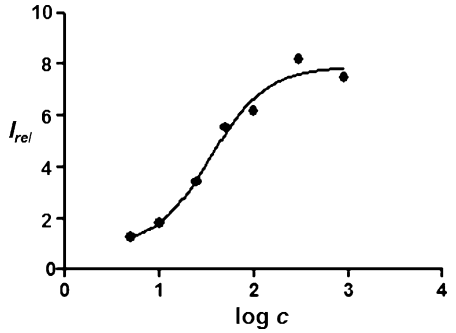


Figure 5. Inhibition of human polymerase α activity by **11 g-TP**. Plot of $\log(c_{\mathbf{11\,g\text{-}TP}})$ against the intensity of primer in the denaturing PAGE analysis of the PEX products with all four natural dNTPs and **11 g-TP** at an indicated concentration.

phosphorylation of nucleosides **11** (but we suppose that it should be feasible in analogy[2] to ribonucleosides **2**), and thus a plausible mechanism of action is phosphorylation to NTPs and inhibition of DNA polymerases. However, because we have not specifically studied the inhibition of other enzymes (nucleotide metabolism, kinases, etc.) by the nucleosides themselves, we cannot rule out other parallel modes of action.

Conclusions

In conclusion, we have designed and synthesized novel sugar-modified fluorinated derivatives of previously reported[2] 7-heteroaryl-7-deazaadenosines. The difluororibonucleosides were prepared by a low-yielding glycosidation of 6-chloro-7-iodo-7-deazapurine followed by amination and cross-couplings. The fluororibonucleosides were obtained by a longer sequence involving S_N2 fluorination of protected arabinonucleoside intermediates followed by cross-coupling arylations. Some of the title fluorinated nucleosides **11** and **21** exerted micromolar cytostatic and anti-HCV activities, significantly lower than the (nanomolar) activities[2] of the parent ribonucleosides **2**. The SAR and mechanism of action is different from the ribonucleosides as well. The NTPs derived from difluororibonucleosides are inhibitors of human DNA polymerase α (which is most likely the major mode of their action), whereas they are very good substrates for bacterial DNA polymerases (e.g., Vent(exo$^-$)) which makes them applicable for the enzymatic synthesis of modified fluorinated DNA.

Experimental Section

NMR spectra were recorded using a 500 MHz (^{1}H at 500 MHz, ^{13}C at 125.7 MHz, ^{19}F at 470.3 MHz) spectrometer, in $CDCl_3$ or [D_6]DMSO. Chemical shifts (δ) are given in ppm, and coupling constants (J) in Hz. Melting points were measured on a Kofler block and are uncorrected. Both low- and high-resolution MS data were collected using electrospray ionization. Optical rotations were measured at 25 °C; $[\alpha]_D$ data are given in 10^{-1} deg cm^2 g^{-1}. Reversed-phase high-performance flash chromatography (HPFC) purifications were done on a Biotage SP1 apparatus with C_{18} columns, using a H_2O/CH_3OH gradient (0→100 %). All final compounds were > 98 % pure according to elemental analysis. 3,5-Di-*O*-benzoyl-2-deoxy-2,2-difluoro-1-*O*-methanesulfonyl-D-*erythro*-pentofuranose (**5**) and 6-chloro-7-deazapurine (**6**) were purchased from Nucleo Chemistry Co. (Shenzhen, China). Gemcitabine hydrochloride (**4**) was purchased from Sigma–Aldrich.

Synthetic oligonucleotides ($Prim^{248short}$, $Temp^{Prb4basII}$, $Temp^{MonoT}$, Table 3) were purchased from Sigma–Aldrich. Human DNA polymerase α was purchased from Chimerx. DNA polymerases Vent(exo$^-$) and DeepVent(exo$^-$), as well as natural nucleoside triphosphates (dATP, dCTP, dGTP, dTTP) were purchased from New England Biolabs; KOD XL DNA polymerase was obtained from Merck, and Pwo polymerase was from Peqlab. Streptavidin magnetic particles were obtained from Roche. All solutions for the PEX experiments were prepared in Milli-Q water.

MALDI-TOF spectra were measured on a Reflex IV instrument (Bruker Daltonics, Germany) with a nitrogen UV laser (λ 337 nm). The measurements were done in reflectron mode by dried droplet technique, with the mass range up to 100 kDa and resolution > 25 000. The matrix consisted of 3-hydroxypicolinic acid (HPA)

Table 3. Sequences of the primer and templates used in PEX experiments.[a]

$Prim^{248short}$	5′-CATGGGCGGCATGGG-3′
$Temp^{Prb4basII}$	5′-(bio)-CTAGCATGAGCTCAGT**CCCATGCCGCCCATG**-3′
$Temp^{MonoT}$	5′-CTAGCATGAGCTCAGA**CCCATGCCGCCCATG**-3′

[a] Segments that form duplex with the primer are highlighted in bold; bio = biotin.

/picolinic acid (PA)/ammonium tartrate at a ratio of 9:1:1. The sample (0.5 μL) and matrix (2 μL) were mixed on target by use of anchor–chip desk. The crystallized spots were washed once with 0.1% formic acid and once with water.

4-Chloro-7-[3,5-di-*O*-benzoyl-2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-iodopyrrolo[2,3-*d*]pyrimidine (7) and 4-chloro-7-[3,5-di-*O*-benzoyl-2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-iodopyrrolo[2,3-*d*]pyrimidine (8): 6-Chloro-7-iodo-7-deazapurine (**6**; 10 g, 35.78 mmol) was dissolved in anhydrous CH_3CN (200 mL), and KOH (3.06 g, 53.68 mmol), TDA-1 (11.46 mL, 35.78 mmol), and mesylate **5** (32.67 g, 71.56 mmol) were added. The reaction mixture was stirred at 50 °C for 4 days. Then, the reaction mixture was diluted with EtOAc (150 mL). After adding aqueous HCl (1 M, 150 mL), a precipitate was formed. This precipitate was removed by filtration and washed several times with EtOAc (100 mL). The filtrate was then washed with H_2O (150 mL). Organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude reaction product was purified by column chromatography on silica (hexane/EtOAc 10:1) to obtain crude nucleosides **8** and **7** (2.74 g, 12%) as a mixture of anomers (α/β 1:2). The mixture was purified again by column chromatography on silica (hexane/EtOAc 12:1→10:1) to give **7** (1.65 g, 8%), and **8** (874 mg, 4%). Both were white solids. **Compound 7**: mp: 128–141 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.65 (dddd, 1H, $J_{4',3'}$ = 5.6 Hz, $J_{4',5'a}$ = 4.5 Hz, $J_{4',5'b}$ = 3.5 Hz, $J_{4',F}$ = 1.3 Hz, H-4′), 4.73 (dd, 1H, J_{gem} = 12.4 Hz, $J_{5'a,4'}$ = 4.5 Hz, H-5′a), 4.87 (dd, 1H, J_{gem} = 12.4 Hz, $J_{5'b,4'}$ = 3.6 Hz, H-5′b), 5.84 (ddd, 1H, $J_{3',F}$ = 13.4 Hz, $J_{3',4'}$ = 5.5 Hz, $J_{3',F}$ = 3.7 Hz, H-3′), 6.73 (dd, 1H, $J_{1',F}$ = 11.4 and 6.8 Hz, H-1′), 7.47–7.54 (m, 4H, H-*m*-Bz), 7.58 (d, 1H, $J_{6,F}$ = 2.8 Hz, H-6), 7.61 and 7.66 (2 m, 2×1H, H-*p*-Bz), 8.08–8.14 (m, 4H, H-*o*-Bz), 8.66 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 54.20 (C-5), 62.55 (CH_2-5′), 71.46 (dd, $J_{C,F}$ = 34.2 and 16.7 Hz, CH-3′), 78.25 (dd, $J_{C,F}$ = 4.8 and 2.4 Hz, CH-4′), 82.77 (dd, $J_{C,F}$ = 37.9 and 21.5 Hz, CH-1′), 117.43 (C-4a), 120.58 (dd, $J_{C,F}$ = 265.3 and 260.1 Hz, C-2′), 127.88 (C-*i*-Bz), 128.73 and 128.74 (CH-*m*-Bz), 129.12 (C-*i*-Bz), 129.74 and 130.15 (CH-*o*-Bz), 132.48 (d, $J_{C,F}$ = 4.1 Hz, CH-6), 133.61 and 134.28 (CH-*p*-Bz), 151.38 (C-7a), 151.47 (CH-2), 153.24 (C-4), 164.74 and 166.00 ppm (CO); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): δ = −116.57 and −110.53 ppm (2×d, 2×1F, J_{gem} = 246.1 Hz); IR (ATR): $\tilde{\nu}$ = 3146, 2922, 2853, 1718, 1450, 1262, 1221, 1106, 1094, 1061, 1033 cm^{-1}; MS (ESI) *m/z* (%): 662 (100) [*M*+Na], 640 (25) [*M*+H]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{25}H_{18}F_2N_3O_5ICl$: 639.99422, found: 639.99420. **Compound 8**: mp: 158–165 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.67–4.74 (m, 2H, H-5′), 5.00 (m, 1H, H-4′), 5.89 (bdt, 1H, $J_{3',F}$ = 12.0 Hz, $J_{3',F}$ = $J_{3',4'}$ = 3.8 Hz, H-3′), 6.94 (dd, 1H, $J_{1',F}$ = 9.0 and 4.2 Hz, H-1′), 7.44–7.57 (m, 4H, H-*m*-Bz), 7.60 and 7.67 (2 m, 2×1H, H-*p*-Bz), 7.74 (d, 1H, $J_{6,F}$ = 1.9 Hz, H-6), 8.04–8.11 (m, 4H, H-*o*-Bz), 8.67 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 53.87 (C-5), 63.15 (d, $J_{C,F}$ = 1.2 Hz, CH_2-5′), 72.20 (dd, $J_{C,F}$ = 34.4 and 17.3 Hz, CH-3′), 81.46 (dd, $J_{C,F}$ = 3.8 and 2.6 Hz, CH-4′), 84.22 (dd, $J_{C,F}$ = 42.6 and 22.3 Hz, CH-1′), 117.22 (C-4a), 122.02 (dd, $J_{C,F}$ = 270.4 and 255.3 Hz, C-2′), 127.76 (C-*i*-Bz), 128.56 and 128.99 (CH-*m*-Bz), 129.07 (C-*i*-Bz), 129.83 and 130.07 (CH-*o*-Bz), 131.98 (d, $J_{C,F}$ = 2.4 Hz, CH-6), 133.55 and 134.35 (CH-*p*-Bz), 151.38 (C-7a), 151.60 (CH-2), 153.16 (C-4), 164.52 and 165.98 ppm (CO); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): δ = −116.72 and −103.35 ppm (2×d, 2×1F, J_{gem} = 252.4 Hz); IR (ATR): $\tilde{\nu}$ = 2922, 2852, 1740, 1715, 1276, 1254, 1111, 1085, 1061, 1022 cm^{-1}; MS (ESI) *m/z* (%): 662 (80) [*M*+Na], 640 (20) [*M*+H]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{25}H_{18}F_2N_3O_5ICl$: 639.99422, found: 639.99382.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-iodopyrrolo[2,3-*d*]pyrimidine (9): Benzoylated nucleoside **7** (1.98 g, 3.09 mmol) in dioxane (13 mL) and aqueous NH_3 (25% *w/w*, 12 mL) was stirred in a steel bomb at 120 °C for 20 h. After cooling, the volatiles were removed under reduced pressure and several times co-evaporated with EtOH. The residual solid was triturated with a boiling solution H_2O/CH_3OH 4:1 (2 mL) and then cooled to 4 °C for 24 h. Solids was filtered off and washed several times with H_2O/CH_3OH 4:1 (30 mL) to give a white solid **9** (786 mg, 62%); mp: 247–255 °C; $[\alpha]_D^{25}$ = +67.0 cm^3 g^{-1} dm^{-1} (*c* = 0.106, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.66 (dm, 1H, J_{gem} = 12.6 Hz, H-5′a), 3.78 (dm, 1H, J_{gem} = 12.6 Hz, H-5′b), 3.88 (dm, 1H, $J_{4',3'}$ = 8.2 Hz, H-4′), 4.39 (m, 1H, H-3′), 5.25 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6 Hz, OH-5′), 6.31 (d, 1H, $J_{OH,3'}$ = 6.5 Hz, OH-3′), 6.38 (dd, 1H, $J_{1',F}$ = 10.9 and 5.0 Hz, H-1′), 6.78 (bs, 2H, NH_2), 7.65 (s, 1H, H-6), 8.14 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 53.56 (C-5), 59.56 (CH_2-5′), 68.64 (dd, $J_{C,F}$ = 25.7 and 18.6 Hz, CH-3′), 81.07 (d, $J_{C,F}$ = 8.4 Hz, CH-4′), 82.43 (dd, $J_{C,F}$ = 39.8 and 24.3 Hz, CH-1′), 103.11 (C-4a), 123.14 (dd, $J_{C,F}$ = 260.0 and 255.5 Hz, C-2′), 126.76 (CH-6), 150.27 (C-7a), 152.70 (CH-2), 157.51 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): δ = −113.98 and −113.00 ppm (2×d, 2×1F, J_{gem} = 234.8 Hz); IR (ATR): $\tilde{\nu}$ = 3495, 3394, 2926, 1630, 1558, 1480, 1443, 1305, 1203, 1055, 1033 cm^{-1}; MS (ESI) *m/z* (%): 413 (100) [*M*+H], 435 (50) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{11}H_{12}F_2N_4O_3I$: 412.99167, found: 412.99170; Anal. calcd for $C_{11}H_{11}F_2N_4O_3I$: C 32.06, H 2.69, N 13.59, found: C 31.80, H 2.61, N 13.22.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-iodopyrrolo[2,3-*d*]pyrimidine (10): Compound **10** was prepared as described for compound **9** from benzoylated nucleoside **8** (838 mg, 1.16 mmol) in dioxane (7 mL) and aqueous NH_3 (25% *w/w*, 7 mL), to give white solid **6** (420 mg, 88%); mp: 246–251 °C; $[\alpha]_D^{25}$ = +43.6 cm^3 g^{-1} dm^{-1} (*c* = 0.211, DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 3.57 (ddd, 1H, J_{gem} = 12.4 Hz, $J_{5'a,OH}$ = 6.3 Hz, $J_{5'a,4'}$ = 4.5 Hz, H-5′a), 3.65 (bdt, 1H, J_{gem} = 12.4 Hz, $J_{5'b,4'}$ = $J_{5'b,OH}$ = 4.3 Hz, H-5′b), 4.28 (m, 1H, H-4′), 4.43 (m, 1H, H-3′), 5.08 (dd, 1H, $J_{OH,5'a}$ = 6.3 Hz, $J_{OH,5'b}$ = 5.3 Hz, OH-5′), 6.45 (d, 1H, $J_{OH,3'}$ = 3.7 Hz, OH-3′), 6.57 (dd, 1H, $J_{1',F}$ = 9.4 and 7.6 Hz, H-1′), 6.78 (bs, 2H, NH_2), 7.54 (d, 1H, $J_{6,F}$ = 2.6 Hz, H-6), 8.14 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 53.56 (C-5), 60.27 (CH_2-5′), 69.82 (dd, $J_{C,F}$ = 26.5 and 17.6 Hz, CH-3′), 82.28 (dd, $J_{C,F}$ = 40.5 and 20.5 Hz, CH-1′), 83.99 (d, $J_{C,F}$ = 7.1 Hz, CH-4′), 102.88 (C-4a), 123.12 (dd, $J_{C,F}$ = 262.3 and 254.8 Hz, C-2′), 127.45 (d, $J_{C,F}$ = 2.9 Hz, CH-6), 150.52 (C-7a), 152.68 (CH-2), 157.50 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): δ = −118.42 and −109.56 ppm (2×d, 2×1F, J_{gem} = 234.3 Hz); IR (ATR): $\tilde{\nu}$ = 3473, 3354, 2919, 1630, 1561, 1478, 1446, 1342, 1244, 1057, 1030 cm^{-1}; MS (ESI) *m/z* (%): 413 (100) [*M*+H], 435 (40) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{11}H_{12}F_2N_4O_3I$: 412.99167, found: 412.99171; Anal. calcd for $C_{11}H_{11}F_2N_4O_3I$: C 32.06, H 2.69, N 13.59, found: C 31.91, H 2.55, N 13.30.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(furan-2-yl)pyrrolo[2,3-*d*]pyrimidine (11 a): An argon-purged mixture of compound **9** (150 mg, 0.36 mmol), furan-2-boronic acid (61.1 mg, 0.55 mmol), Na_2CO_3 (115.7 mg, 1.09 mmol), $Pd(OAc)_2$ (4.09 mg, 0.018 mmol), and TPPTS (31.0 mg, 0.55 mmol) in H_2O/CH_3CN (2:1, 3 mL) was stirred at 100 °C for 2 h. After cooling, vola-

tiles were removed under reduced pressure, and the residue was purified by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O) to give compound **11a** (84 mg, 65%) as a beige solid after recrystallization (H_2O/CH_3OH 4:1); mp: 189–191 °C; $[\alpha]_D^{25} = -3.8\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.236$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.69$ (ddd, 1H, $J_{gem} = 12.6$ Hz, $J_{5'a,OH} = 6.0$ Hz, $J_{5'a,4'} = 4.2$ Hz, H-5′a), 3.81 (dm, 1H, $J_{gem} = 12.6$ Hz, H-5′b), 3.91 (ddd, 1H, $J_{4',3'} = 8.1$ Hz, $J_{4',5'a} = 4.2$ Hz, $J_{4',5'b} = 2.8$ Hz, H-4′), 4.43 (m, 1H, H-3′), 5.27 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.7$ Hz, OH-5′), 6.33 (d, 1H, $J_{OH,3'} = 6.5$ Hz, OH-3′), 6.47 (dd, 1H, $J_{1',F} = 10.9$ and 5.2 Hz, H-1′), 6.62 (dd, 1H, $J_{4,3} = 3.3$ Hz, $J_{4,5} = 1.9$ Hz, H-4-furyl), 6.70 (dd, 1H, $J_{3,4} = 3.3$ Hz, $J_{3,5} = 0.9$ Hz, H-3-furyl), 7.00 (bs, 2H, NH_2), 7.80 (dd, 1H, $J_{5,4} = 1.9$ Hz, $J_{5,3} = 0.8$ Hz, H-5-furyl), 7.81 (bs, 1H, H-6), 8.18 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 59.71$ (CH_2-5′), 68.84 (dd, $J_{C,F} = 25.3$ and 19.0 Hz, CH-3′), 81.13 (d, $J_{C,F} = 8.0$ Hz, CH-4′), 82.44 (dd, $J_{C,F} = 40.0$ and 24.4 Hz, CH-1′), 99.11 (C-4a), 105.92 (CH-3-furyl), 107.36 (C-5), 112.13 (CH-4-furyl), 119.92 (CH-6), 123.20 (dd, $J_{C,F} = 260.2$ and 255.1 Hz, C-2′), 142.49 (CH-5-furyl), 148.25 (C-2-furyl), 151.10 (C-7a), 152.85 (CH-2), 157.54 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -113.95$ and −112.95 ppm (2×d, 2×1F, $J_{gem} = 234.1$ Hz); IR (ATR): $\tilde{\nu} = 3487$, 3383, 3128, 1634, 1557, 1487, 1457, 1318, 1203, 1071, 1041 cm^{-1}; MS (ESI) *m/z* (%): 353 (100) [*M*+H], 375 (40) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{15}F_2N_4O_4$: 353.10559, found: 353.10559; Anal. calcd for $C_{15}H_{14}F_2N_4O_4 \cdot 0.75\,H_2O$: C 49.25, H 4.27, N 15.32, found: C 49.58, H 4.15, N 14.95.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(furan-3-yl)pyrrolo[2,3-*d*]pyrimidine (11b): Compound **11b** was prepared as described for compound **11a** from compound **9** (150 mg, 0.36 mmol) and furan-3-boronic acid (61.1 mg, 0.55 mmol), to give compound **11b** (71 mg, 55%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 106–110 °C; $[\alpha]_D^{25} = 0\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.157$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.67$ (ddd, 1H, $J_{gem} = 12.6$ Hz, $J_{5'a,OH} = 6.0$ Hz, $J_{5'a,4'} = 4.2$ Hz, H-5′a), 3.79 (dm, 1H, $J_{gem} = 12.6$ Hz, H-5′b), 3.89 (m, 1H, H-4′), 4.43 (m, 1H, H-3′), 5.23 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.6$ Hz, OH-5′), 6.32 (d, 1H, $J_{OH,3'} = 6.5$ Hz, OH-3′), 6.37 (bs, 2H, NH_2), 6.45 (dd, 1H, $J_{1',F} = 11.1$ and 5.3 Hz, H-1′), 6.71 (dd, 1H, $J_{4,5} = 1.8$ Hz, $J_{4,2} = 0.9$ Hz, H-4-furyl), 7.48 (s, 1H, H-6), 7.82 (t, 1H, $J_{5,2} = J_{5,4} = 1.7$ Hz, H-5-furyl), 7.86 (dd, 1H, $J_{2,5} = 1.6$ Hz, $J_{2,4} = 0.9$ Hz, H-2-furyl), 8.17 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 59.71$ (CH_2-5′), 68.87 (dd, $J_{C,F} = 25.4$ and 18.6 Hz, CH-3′), 81.02 (d, $J_{C,F} = 8.3$ Hz, CH-4′), 82.38 (dd, $J_{C,F} = 39.3$ and 24.3 Hz, CH-1′), 100.82 (C-4a), 107.55 (C-5), 111.66 (CH-4-furyl), 118.34 (C-3-furyl), 120.49 (CH-6), 123.27 (dd, $J_{C,F} = 260.0$ and 255.6 Hz, C-2′), 140.12 (CH-2-furyl), 144.50 (CH-5-furyl), 151.01 (C-7a), 152.55 (CH-2), 157.77 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -113.91$ and −112.89 ppm (2×d, 2×1F, $J_{gem} = 233.5$ Hz); IR (ATR): $\tilde{\nu} = 3490$, 3378, 3163, 1626, 1563, 1475, 1457, 1307, 1210, 1086, 1035 cm^{-1}; MS (ESI) *m/z* (%): 353 (100) [*M*+H], 375 (25) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{15}F_2N_4O_4$: 353.10559, found: 353.10554; Anal. calcd for $C_{15}H_{14}F_2N_4O_4$: C 51.14, H 4.01, N 15.90, found: C 51.03, H 4.10, N 15.76.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(thiophen-2-yl)pyrrolo[2,3-*d*]pyrimidine (11c): Compound **11c** was prepared as described for compound **11a** from compound **9** (150 mg, 0.36 mmol) and thiophene-2-boronic acid (69.9 mg, 0.55 mmol), to give compound **11c** (71 mg, 53%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 112–114 °C; $[\alpha]_D^{25} = -6.7\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.105$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.67$ (bddd, 1H, $J_{gem} = 12.7$ Hz, $J_{5'a,OH} = 5.9$ Hz, $J_{5'a,4'} = 4.0$ Hz, H-5′a), 3.79 (bdm, 1H, $J_{gem} = 12.7$ Hz, H-5′b), 3.90 (m, 1H, H-4′), 4.45 (m, 1H, H-3′), 5.27 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.6$ Hz, OH-5′), 6.32 (bd, 1H, $J_{OH,3'} = 6.0$ Hz, OH-3′), 6.41 (bs, 2H, NH_2), 6.47 (bdd, 1H, $J_{1',F} = 10.7$ and 5.2 Hz, H-1′), 7.17–7.21 (m, 2H, H-3,4-thienyl), 7.59 (m, 1H, H-5-thienyl), 7.60 (s, 1H, H-6), 8.20 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 59.51$ (CH_2-5′), 68.63 (dd, $J_{C,F} = 25.1$ and 19.2 Hz, CH-3′), 81.05 (d, $J_{C,F} = 7.7$ Hz, CH-4′), 82.44 (dd, $J_{C,F} = 39.3$ and 24.6 Hz, CH-1′), 100.43 (C-4a), 109.71 (C-5), 121.47 (CH-6), 123.27 (bdd, $J_{C,F} = 259.6$ and 256.4 Hz, C-2′), 126.27 (CH-5-thienyl), 126.87 (CH-3-thienyl), 128.55 (CH-4-thienyl), 135.07 (C-2-thienyl), 150.83 (C-7a), 152.77 (CH-2), 157.56 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -113.92$ and −113.05 ppm (2×d, 2×1F, $J_{gem} = 233.7$ Hz); IR (ATR): $\tilde{\nu} = 3337$, 3139, 1632, 1588, 1546, 1464, 1311, 1207, 1077, 1057 cm^{-1}; MS (ESI) *m/z* (%): 369 (100) [*M*+H], 391 (23) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{15}F_2N_4O_3S$: 369.08274, found: 369.08274; Anal. calcd for $C_{15}H_{14}F_2N_4O_3S \cdot 0.4\,CH_3OH \cdot 0.8\,H_2O$: C 46.76, H 4.38, N 14.16, found: C 46.63, H 4.15, N 13.94.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(thiophen-3-yl)pyrrolo[2,3-*d*]pyrimidine (11d): Compound **11d** was prepared as described for compound **11a** from compound **9** (150 mg, 0.36 mmol) and thiophene-3-boronic acid (69.9 mg, 0.55 mmol), to give compound **11d** (81 mg, 60%) as a light-brown solid after recrystallization (H_2O/CH_3OH 4:1); mp: 116–122 °C; $[\alpha]_D^{25} = +1.6\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.189$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.67$ (ddd, 1H, $J_{gem} = 12.6$ Hz, $J_{5'a,OH} = 5.9$ Hz, $J_{5'a,4'} = 4.2$ Hz, H-5′a), 3.79 (bdm, 1H, $J_{gem} = 12.6$ Hz, H-5′b), 3.90 (m, 1H, H-4′), 4.44 (m, 1H, H-3′), 5.23 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.6$ Hz, OH-5′), 6.31 (bs, 2H, NH_2), 6.32 (d, 1H, $J_{OH,3'} = 6.3$ Hz, OH-3′), 6.47 (dd, 1H, $J_{1',F} = 11.1$ and 5.2 Hz, H-1′), 7.27 (dd, 1H, $J_{4,5} = 4.9$ Hz, $J_{4,2} = 1.3$ Hz, H-4-thienyl), 7.53 (s, 1H, H-6), 7.56 (dd, 1H, $J_{2,5} = 2.9$ Hz, $J_{2,4} = 1.3$ Hz, H-2-thienyl), 7.72 (dd, 1H, $J_{5,4} = 4.9$ Hz, $J_{5,2} = 2.9$ Hz, H-5-thienyl), 8.18 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 59.66$ (CH_2-5′), 68.82 (dd, $J_{C,F} = 24.9$ and 19.3 Hz, CH-3′), 81.01 (d, $J_{C,F} = 7.8$ Hz, CH-4′), 82.39 (dd, $J_{C,F} = 39.4$ and 24.6 Hz, CH-1′), 100.61 (C-4a), 112.18 (C-5), 120.56 (CH-6), 122.66 (CH-2-thienyl), 123.29 (dd, $J_{C,F} = 259.4$ and 255.7 Hz, C-2′), 127.73 (CH-5-thienyl), 128.58 (CH-4-thienyl), 134.39 (C-3-thienyl), 150.85 (C-7a), 152.50 (CH-2), 157.68 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -113.85$ and −112.94 ppm (2×d, 2×1F, $J_{gem} = 233.6$ Hz); IR (ATR): $\tilde{\nu} = 3419$, 3327, 3113, 1632, 1592, 1549, 1463, 1311, 1210, 1066, 1032 cm^{-1}. (ESI) *m/z* (%): 369 (100) [*M*+H], 391 (40) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{15}F_2N_4O_3S$: 369.08274, found: 369.08272; Anal. calcd for $C_{15}H_{14}F_2N_4O_3S$: C 48.91, H 3.83, N 15.21, found: C 48.66, H 3.82, N 14.94.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-phenyl-pyrrolo[2,3-*d*]pyrimidine (11e): Compound **11e** was prepared as described for compound **11a** from compound **9** (140 mg, 0.34 mmol) and phenylboronic acid (62.2 mg, 0.51 mmol), to give compound **11e** (87 mg, 71%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 122–124 °C; $[\alpha]_D^{25} = -1.8\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.224$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.67$ (ddd, 1H, $J_{gem} = 12.6$ Hz, $J_{5'a,OH} = 6.0$ Hz, $J_{5'a,4'} = 4.1$ Hz, H-5′a), 3.79 (bdm, 1H, $J_{gem} = 12.6$ Hz, H-5′b), 3.91 (bddd, 1H, $J_{4',3'} = 8.3$ Hz, $J_{4',5'a} = 4.0$ Hz, $J_{4',5'b} = 2.6$ Hz, H-4′), 4.46 (m, 1H, H-3′), 5.24 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.6$ Hz, OH-5′), 6.25 (bs, 2H, NH_2), 6.32 (d, 1H, $J_{OH,3'} = 5.4$ Hz, OH-3′), 6.49 (dd, 1H, $J_{1',F} = 10.9$ and 5.3 Hz, H-1′), 7.39 (m, 1H, H-*p*-Ph), 7.47–7.52 (m, 4H, H-*o*,*m*-Ph), 7.53 (s, 1H, H-6), 8.20 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 59.66$ (CH_2-5′), 68.81 (dd, $J_{C,F} = 25.0$ and 19.2 Hz, CH-3′), 81.03 (d, $J_{C,F} = 8.1$ Hz, CH-4′), 82.44 (dd, $J_{C,F} = 39.0$ and 24.9 Hz, CH-1′), 100.31 (C-4a), 117.53 (C-5), 120.67 (CH-6), 123.35 (dd, $J_{C,F} = 259.7$ and 255.4 Hz, C-2′), 127.39 (CH-*p*-Ph), 128.66 and 129.27 (CH-*o*,*m*-Ph), 134.20 (C-*i*-Ph), 151.14 (C-7a), 152.49 (CH-2), 157.62 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -113.81$ and −112.92 ppm (2×d, 2×1F, $J_{gem} =$

233.9 Hz); IR (ATR): $\tilde{\nu}$ = 3437, 3348, 3156, 1630, 1582, 1537, 1462, 1307, 1211, 1062, 1035 cm^{-1}; MS (ESI) *m/z* (%): 363 (100) [*M*+H], 385 (20) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{17}H_{17}F_2N_4O_3$: 363.12632, found: 363.12628; Anal. calcd for $C_{17}H_{16}F_2N_4O_3 \cdot 1.2\,H_2O$: C 53.18, H 4.83, N 14.59, found: C 53.35, H 4.69, N 14.3.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(naphtalen-2-yl)pyrrolo[2,3-*d*]pyrimidine (11 f): Compound **11 f** was prepared as described for compound **11 a** from compound **9** (150 mg, 0.36 mmol) and naphtalene-2-boronic acid (93.9 mg, 0.55 mmol). Crude product was purified by column chromatography on silica in 3% CH_3OH in $CHCl_3$, and by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O) to give compound **11 f** (110 mg, 71%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 143–146 °C; $[\alpha]_D^{25}$ = 0 $cm^3\,g^{-1}\,dm^{-1}$ (*c* = 0.211, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): δ = 3.68 (ddd, 1H, J_{gem} = 12.7 Hz, $J_{5'a,OH}$ = 6.0 Hz, $J_{5'a,4'}$ = 4.1 Hz, H-5′a), 3.81 (dm, 1H, J_{gem} = 12.6 Hz, H-5′b), 3.92 (b ddd, 1H, $J_{4',3'}$ = 8.1 Hz, $J_{4',5'a}$ = 4.0 Hz, $J_{4',5'b}$ = 2.7 Hz, H-4′), 4.49 (m, 1H, H-3′), 5.25 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6 Hz, OH-5′), 6.32 (b s, 2H, NH_2), 6.34 (d, 1H, $J_{OH,3'}$ = 6.3 Hz, OH-3′), 6.53 (dd, 1H, $J_{1',F}$ = 11.0 and 5.5 Hz, H-1′), 7.51–7.59 (m, 2H, H-6,7-naphthyl), 7.642 (s, 1H, H-6), 7.644 (dd, 1H, $J_{3,4}$ = 8.4 Hz, $J_{3,1}$ = 1.8 Hz, H-3-naphthyl), 7.95–8.00 (m, 2H, H-5,8-naphthyl), 8.00 (b d, 1H, $J_{1,3}$ = 1.9 Hz, H-1-naphthyl), 8.04 (b d, 1H, $J_{4,3}$ = 8.5 Hz, H-4-naphthyl), 8.22 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): δ = 59.69 (CH_2-5′), 68.88 (dd, $J_{C,F}$ = 25.4 and 19.4 Hz, CH-3′), 81.04 (d, $J_{C,F}$ = 7.9 Hz, CH-4′), 82.47 (dd, $J_{C,F}$ = 39.8 and 24.4 Hz, CH-1′), 100.41 (C-4a), 117.56 (C-5), 121.00 (CH-6), 123.35 (b dd, $J_{C,F}$ = 259.6 and 255.2 Hz, C-2′), 126.22 (CH-6-naphthyl), 126.77 (CH-7-naphthyl), 126.99 (CH-1-naphthyl), 127.07 (CH-3-naphthyl), 127.86 and 128.07 (CH-5,8-naphthyl), 128.76 (CH-4-naphthyl), 131.64 (C-2-naphthyl), 132.14 (C-4a-naphthyl), 133.41 (C-8a-naphthyl), 151.29 (C-7a), 152.54 (CH-2), 157.72 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): δ = −113.77 and −112.80 ppm (2×d, 2×1F, J_{gem} = 233.3 Hz); IR (ATR): $\tilde{\nu}$ = 3441, 3311, 3201, 1627, 1583, 1564, 1472, 1308, 1202, 1066 cm^{-1}; MS (ESI) *m/z* (%): 413 (100) [*M*+H], 435 (13) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{21}H_{19}F_2N_4O_3$: 413.14197, found: 413.14195; Anal. calcd for $C_{21}H_{18}F_2N_4O_3 \cdot 1.15\,H_2O$: C 58.24, H 4.72, N 12.94, found: C 58.47, H 4.57, N 12.61.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(benzofuran-2-yl)pyrrolo[2,3-*d*]pyrimidine (11 g): Compound **11 g** was prepared as described for compound **11 a** from compound **9** (150 mg, 0.36 mmol) and 2-benzofurylboronic acid (88.4 mg, 0.55 mmol). The crude product was purified by column chromatography on silica in 3% CH_3OH in $CHCl_3$, and by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O), to give compound **11 g** (57 mg, 39%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 223–226 °C; $[\alpha]_D^{25}$ = −6.8 $cm^3\,g^{-1}\,dm^{-1}$ (*c* = 0.222, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): δ = 3.72 (m, 1H, H-5′a), 3.84 (m, 1H, H-5′b), 3.95 (m, 1H, H-4′), 4.48 (m, 1H, H-3′), 5.32 (b t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6 Hz, OH-5′), 6.37 (d, 1H, $J_{OH,3'}$ = 6.5 Hz, OH-3′), 6.52 (b dd, 1H, $J_{1',F}$ = 10.6 and 5.0 Hz, H-1′), 7.10 (b s, 2H, NH_2), 7.16 (s, 1H, H-3-benzofuryl), 7.26–7.34 (m, 2H, H-5,6-benzofuryl), 7.64–7.71 (m, 2H, H-3,4-benzofuryl), 8.07 (s, 1H, H-6), 8.23 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): δ = 59.73 (CH_2-5′), 68.83 (dd, $J_{C,F}$ = 25.8 and 18.9 Hz, CH-3′), 81.24 (d, $J_{C,F}$ = 8.2 Hz, CH-4′), 82.56 (dd, $J_{C,F}$ = 40.1 and 24.2 Hz, CH-1′), 99.23 (C-4a), 102.39 (CH-3-benzofuryl), 106.79 (C-5), 111.37 (CH-7-benzofuryl), 120.95 (CH-4-benzofuryl), 122.16 (CH-6), 123.24 (dd, $J_{C,F}$ = 260.4 and 255.7 Hz, C-2′), 123.71 (CH-5-benzofuryl), 124.25 (CH-6-benzofuryl), 128.91 (C-3a-benzofuryl), 150.71 (C-2-benzofuryl), 151.34 (C-7a), 153.07 (CH-2), 154.07 (C-7a-benzofuryl), 157.60 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): δ = −113.93 and −112.89 ppm (2×d, 2×1F, J_{gem} = 233.9 Hz); IR (ATR): $\tilde{\nu}$ = 3453, 3301, 3138, 1650, 1573, 1560, 1454, 1318, 1209, 1098, 1076 cm^{-1}; MS (ESI) *m/z* (%): 403 (100) [*M*+H], 425 (60) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{19}H_{17}F_2N_4O_4$: 403.12124, found: 413.12109; Anal. calcd for $C_{19}H_{16}F_2N_4O_4 \cdot 0.35\,H_2O$: C 55.84, H 4.12, N 13.71, found: C 56.06, H 4.05, N 13.37.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-[(trimethylsilyl)ethynyl]-pyrrolo[2,3-*d*]pyrimidine (12): An argon-purged mixture of derivative **9** (200 mg, 0.49 mmol), $PdCl_2(PPh_3)_2$ (17 mg, 0.024 mmol), CuI (9.22 mg, 0.049 mmol), trimethylsilylacetylene (0.68 mL, 4.9 mmol) and triethylamine (200 μL) was stirred in DMF (800 μL) at room temperature for 18 h. Volatiles were removed under reduced pressure and crude product is purified twice by column chromatography on silica in 3% CH_3OH in $CHCl_3$. The product was then purified by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O) to give trimethylsilylethynyl derivative **12** (139 mg, 75%) as a white crystalline solid; mp: 188–186 °C; ^1H NMR (500 MHz, [D_6]DMSO): δ = 0.25 (s, 3×3H, CH_3Si), 3.67 (ddd, 1H, J_{gem} = 12.7 Hz, $J_{5'a,OH}$ = 6.1 Hz, $J_{5'a,4'}$ = 4.0 Hz, H-5′a), 3.79 (b ddd, 1H, J_{gem} = 12.7 Hz, $J_{5'b,OH}$ = 5.0 Hz, $J_{5'b,4'}$ = 2.5 Hz, H-5′b), 3.89 (ddd, 1H, $J_{4',3'}$ = 8.3 Hz, $J_{4',5'a}$ = 4.0 Hz, $J_{4',5'b}$ = 2.5 Hz, H-4′), 4.40 (m, 1H, H-3′), 5.26 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6 Hz, OH-5′), 6.33 (d, 1H, $J_{OH,3'}$ = 6.3 Hz, OH-3′), 6.39 (b dd, 1H, $J_{1',F}$ = 10.1 and 5.4 Hz, H-1′), 7.81 (s, 1H, H-6), 8.17 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): δ = −0.04 (CH_3-Si), 59.57 (CH_2-5′), 68.57 (dd, $J_{C,F}$ = 24.1 and 20.0 Hz, CH-3′), 81.19 (d, $J_{C,F}$ = 7.7 Hz, CH-4′), 82.61 (dd, $J_{C,F}$ = 37.9 and 26.5 Hz, CH-1′), 95.95 (C-5), 97.60 (C≡**C**Si), 98.64 (**C**≡CSi), 102.08 (C-4a), 123.14 (b t, $J_{C,F}$ = 257.6 Hz, C-2′), 126.90 (CH-6), 149.70 (C-7a), 153.63 (CH-2), 157.85 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): δ = −113.91 and −113.24 ppm (2×d, 2×1F, J_{gem} = 234.2 Hz); IR (ATR): $\tilde{\nu}$ = 3505, 3395, 2959, 2159, 1636, 1577, 1248, 1204, 1070, 1042, 1019 cm^{-1}; MS (ESI) *m/z* (%): 383 (100) [*M*+H], 405 (40) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{16}H_{21}F_2N_4O_3Si$: 383.13455, found: 383.13459.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-ethynyl-pyrrolo[2,3-*d*]pyrimidine (11 h): A mixture of the silylated compound **12** (120 mg, 0.31 mmol) and K_2CO_3 (43.4 mg, 0.31 mmol) in CH_3OH (5 mL) was stirred at room temperature for 1 h. The crude product was purified by column chromatography on silica in 3% CH_3OH in $CHCl_3$. Then, the product was purified by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O) to give compound **11 h** (58.1 mg, 60%) as a white crystalline solid after recrystallization (H_2O/CH_3OH 4:1); mp: 219–225 °C; $[\alpha]_D^{25}$ = +2.7 $cm^3\,g^{-1}\,dm^{-1}$ (*c* = 0.219, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): δ = 3.67 (ddd, 1H, J_{gem} = 12.7 Hz, $J_{5'a,OH}$ = 6.0 Hz, $J_{5'a,4'}$ = 4.0 Hz, H-5′a), 3.79 (dm, 1H, J_{gem} = 12.7 Hz, H-5′b), 3.89 (ddd, 1H, $J_{4',3'}$ = 8.3 Hz, $J_{4',5'a}$ = 3.9 Hz, $J_{4',5'b}$ = 2.5 Hz, H-4′), 4.34 (s, 1H, CH), 4.41 (m, 1H, H-3′), 5.30 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.5 Hz, OH-5′), 6.35 (d, 1H, $J_{OH,3'}$ = 6.3 Hz, OH-3′), 6.39 (dd, 1H, $J_{1',F}$ = 9.9 and 5.6 Hz, H-1′), 6.77 (b s, 2H, NH_2), 7.81 (s, 1H, H-6), 8.16 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): δ = 59.54 (CH_2-5′), 68.53 (dd, $J_{C,F}$ = 23.5 and 20.5 Hz, CH-3′), 76.91 (**C**≡CH), 81.17 (d, $J_{C,F}$ = 7.0 Hz, CH-4′), 82.61 (dd, $J_{C,F}$ = 37.7 and 26.5 Hz, CH-1′), 83.88 (C≡**C**H), 95.35 (C-5), 102.12 (C-4a), 123.18 (b dd, $J_{C,F}$ = 258.8 and 256.8 Hz, C-2′), 126.95 (CH-6), 149.76 (C-7a), 153.59 (CH-2), 157.80 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): δ = −113.99 and −113.33 ppm (2×d, 2×1F, J_{gem} = 233.8 Hz); IR (ATR): $\tilde{\nu}$ = 3495, 3448, 3394, 3330, 3165, 1632, 1576, 1450, 1303, 1180, 1029, 1012 cm^{-1}; MS (ESI) *m/z* (%): 311 (100) [*M*+H], 333 (50) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{13}H_{13}F_2N_4O_3$: 311.09502, found: 311.09497; Anal. calcd for $C_{13}H_{13}F_2N_4O_3$: C 50.33, H 3.90, N 18.06, found: C 50.26, H 3.82, N 17.74.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-(furan-2-yl)pyrrolo[2,3-*d*]pyrimidine (13a): Compound **13a** was prepared as described for compound **11a** from compound **10** (146 mg, 0.36 mmol) and furan-2-boronic acid (61.1 mg, 0.55 mmol), to give compound **13a** (49 mg, 38%) as a beige solid after recrystallization (H_2O/CH_3OH 4:1); mp: 243–248 °C; $[\alpha]_D^{25} = +55.3\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.170$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.60$ (ddd, 1H, $J_{gem} = 12.4$ Hz, $J_{5'a,OH} = 6.2$ Hz, $J_{5'a,4'} = 4.4$ Hz, H-5′a), 3.69 (dm, 1H, $J_{gem} = 12.4$ Hz, H-5′b), 4.33 (m, 1H, H-4′), 4.48 (m, 1H, H-3′), 5.12 (dd, 1H, $J_{OH,5'a} = 6.2$ Hz, $J_{OH,5'b} = 5.2$ Hz, OH-5′), 6.44 (d, 1H, $J_{OH,3'} = 5.4$ Hz, OH-3′), 6.62 (dd, 1H, $J_{4,3} = 3.4$ Hz, $J_{4,5} = 1.9$ Hz, H-4-furyl), 6.66 (dd, 1H, $J_{1',F} = 10.4$ and 7.0 Hz, H-1′), 6.78 (dd, 1H, $J_{3,4} = 3.3$ Hz, $J_{3,5} = 0.8$ Hz, H-3-furyl), 7.02 (bs, 2H, NH_2), 7.73 (d, 1H, $J_{6,F} = 2.8$ Hz, H-6), 7.80 (dd, 1H, $J_{5,4} = 1.9$ Hz, $J_{5,3} = 0.8$ Hz, H-5-furyl), 8.17 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 60.31$ (CH_2-5′), 69.76 (dd, $J_{C,F} = 26.0$ and 18.1 Hz, CH-3′), 82.19 (dd, $J_{C,F} = 40.0$ and 19.9 Hz, CH-1′), 83.78 (d, $J_{C,F} = 7.6$ Hz, CH-4′), 98.89 (C-4a), 105.93 (CH-3-furyl), 107.41 (C-5), 112.16 (CH-4-furyl), 120.50 (bd, $J_{C,F} = 2.0$ Hz, CH-6), 123.09 (dd, $J_{C,F} = 261.8$ and 255.8 Hz, C-2′), 142.38 (CH-5-furyl), 148.26 (C-2-furyl), 151.33 (C-7a), 152.80 (CH-2), 157.52 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -119.08$ and -110.75 ppm (2×d, 2×1F, $J_{gem} = 232.7$ Hz); IR (ATR): $\tilde{\nu} = 3470$, 3345, 3186, 1621, 1571, 1557, 1456, 1300, 1229, 1067, 1055 cm^{-1}; MS (ESI) *m/z* (%): 353 (100) [*M*+H], 375 (20) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{15}H_{15}F_2N_4O_4$: 353.10559, found: 353.10558; Anal. calcd for $C_{15}H_{14}F_2N_4O_4.0.5\ CH_3OH$: C 50.55, H 4.38, N 15.21, found: C 50.38, H 4.12, N 14.96.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-(furan-3-yl)pyrrolo[2,3-*d*]pyrimidine (13b): Compound **13b** was prepared as described for compound **11a** from compound **10** (144 mg, 0.35 mmol) and furan-3-boronic acid (63.9 mg, 0.52 mmol), to give compound **13b** (75 mg, 61%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 102–105 °C; $[\alpha]_D^{25} = +62.8\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.121$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.59$ (ddd, 1H, $J_{gem} = 12.4$ Hz, $J_{5'a,OH} = 6.3$ Hz, $J_{5'a,4'} = 4.4$ Hz, H-5′a), 3.67 (bdt, 1H, $J_{gem} = 12.4$ Hz, $J_{5'b,4'} = J_{5'b,OH} = 4.3$ Hz, H-5′b), 4.27 (bdddd, 1H, $J_{4',3'} = 7.3$ Hz, $J_{4',5'a} = 4.3$ Hz, $J_{4',5'a} = 3.2$ Hz, $J_{4',F} = 1.3$ Hz, H-4′), 4.46 (m, 1H, H-3′), 5.10 (dd, 1H, $J_{OH,5'a} = 6.2$ Hz, $J_{OH,5'b} = 5.4$ Hz, OH-5′), 6.37 (bs, 2H, NH_2), 6.44 (d, 1H, $J_{OH,3'} = 6.2$ Hz, OH-3′), 6.65 (dd, 1H, $J_{1',F} = 10.1$ and 7.4 Hz, H-1′), 6.74 (dd, 1H, $J_{4,5} = 1.8$ Hz, $J_{4,2} = 0.9$ Hz, H-4-furyl), 7.38 (d, 1H, $J_{6,F} = 2.9$ Hz, H-6), 7.82 (t, 1H, $J_{5,2} = J_{5,4} = 1.7$ Hz, H-5-furyl), 7.88 (dd, 1H, $J_{2,5} = 1.6$ Hz, $J_{2,4} = 0.9$ Hz, H-2-furyl), 8.17 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 60.37$ (CH_2-5′), 69.86 (dd, $J_{C,F} = 26.4$ and 17.9 Hz, CH-3′), 82.12 (dd, $J_{C,F} = 40.2$ and 20.1 Hz, CH-1′), 83.80 (d, $J_{C,F} = 7.5$ Hz, CH-4′), 100.61 (C-4a), 107.59 (C-5), 111.63 (CH-4-furyl), 118.29 (C-3-furyl), 121.08 (bd, $J_{C,F} = 2.6$ Hz, CH-6), 123.21 (dd, $J_{C,F} = 261.8$ and 255.0 Hz, C-2′), 140.13 (CH-2-furyl), 144.47 (CH-5-furyl), 151.27 (C-7a), 152.52 (CH-2), 157.75 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -118.70$ and -109.93 ppm (2×d, 2×1F, $J_{gem} = 233.5$ Hz); IR (ATR): $\tilde{\nu} = 3499$, 3390, 3135, 1622, 1561, 1463, 1303, 1241, 1059, 1040 cm^{-1}; MS (ESI) *m/z* (%): 353 (100) [*M*+H], 375 (25) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{15}H_{15}F_2N_4O_4$: 353.10559, found: 353.10557; Anal. calcd for $C_{15}H_{14}F_2N_4O_4{\cdot}1.7\,H_2O$: C 47.05, H 4.58, N 14.63, found: C 47.23, H 4.41, N 14.43.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-(thiophen-2-yl)pyrrolo[2,3-*d*]pyrimidine (13c): Compound **13c** was prepared as described for compound **11a** from compound **10** (141 mg, 0.34 mmol) and thiophene-2-boronic acid (67.5 mg, 0.51 mmol), to give compound **13c** (73 mg, 58%) as a white lyophilizate (*t*BuOH-benzene 2:1); mp: 87–96 °C; $[\alpha]_D^{25} = +58.7\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.288$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.59$ (dd, 1H, $J_{gem} = 12.5$ Hz, $J_{5'a,4'} = 4.6$ Hz, H-5′a), 3.67 (bdd, 1H, $J_{gem} = 12.5$ Hz, $J_{5'b,4'} = 3.1$ Hz, H-5′b), 4.30 (m, 1H, H-4′), 4.46 (m, 1H, H-3′), 5.12 (bs, 1H, OH-5′), 6.20–6.80 (m, 3H, NH_2, OH-3′), 6.66 (bt, 1H, $J_{1',F} = 8.6$ Hz, H-1′), 7.17–7.21 (m, 2H, H-3,4-thienyl), 7.46 (d, 1H, $J_{6,F} = 2.7$ Hz, H-6), 7.59 (m, 1H, H-5-thienyl), 8.20 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 60.34$ (CH_2-5′), 69.91 (dd, $J_{C,F} = 26.7$ and 18.1 Hz, CH-3′), 82.28 (dd, $J_{C,F} = 40.6$ and 20.3 Hz, CH-1′), 84.05 (d, $J_{C,F} = 7.0$ Hz, CH-4′), 100.23 (C-4a), 109.81 (C-5), 122.11 (d, $J_{C,F} = 2.6$ Hz, CH-6), 123.27 (dd, $J_{C,F} = 262.2$ and 254.5 Hz, C-2′), 126.30 (CH-5-thienyl), 126.92 (CH-3-thienyl), 128.59 (CH-4-thienyl), 135.02 (C-2-thienyl), 151.14 (C-7a), 152.81 (CH-2), 157.59 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -118.34$ and -109.22 ppm (2×d, 2×1F, $J_{gem} = 234.6$ Hz); IR (ATR): $\tilde{\nu} = 3482$, 3372, 3242, 1616, 1549, 1459, 1190, 1065, 1041 cm^{-1}; MS (ESI) *m/z* (%): 369 (100) [*M*+H], 391 (60) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{15}H_{15}F_2N_4O_3S$: 369.08274, found: 369.08278; Anal. calcd for $C_{15}H_{14}F_2N_4O_3S$: C 48.91, H 3.83, N 15.21, found: C 48.55, H 3.86, N 14.99.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-(thiophen-3-yl)pyrrolo[2,3-*d*]pyrimidine (13d): Compound **13d** was prepared as described for compound **11a** from compound **10** (140 mg, 0.34 mmol) and thiophene-3-boronic acid (65.3 mg, 0.51 mmol). The crude product was purified by column chromatography on silica in 3% CH_3OH in $CHCl_3$, and by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O), to give compound **13d** (81 mg, 65%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 154–155 °C; $[\alpha]_D^{25} = +76.6\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.197$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.59$ (ddd, 1H, $J_{gem} = 12.4$ Hz, $J_{5'a,OH} = 6.3$ Hz, $J_{5'a,4'} = 4.4$ Hz, H-5′a), 3.68 (bdt, 1H, $J_{gem} = 12.4$ Hz, $J_{5'b,OH} = J_{5'b,4'} = 4.3$ Hz, H-5′b), 4.29 (bdddd, 1H, $J_{4',3'} = 7.3$ Hz, $J_{4',5'a} = 4.4$ Hz, $J_{4',5'b} = 3.4$ Hz, $J_{4',F} = 1.3$ Hz, H-4′), 4.46 (m, 1H, H-3′), 5.11 (dd, 1H, $J_{OH,5'a} = 6.2$ Hz, $J_{OH,5'b} = 5.3$ Hz, OH-5′), 6.32 (bs, 2H, NH_2), 6.46 (d, 1H, $J_{OH,3'} = 3.6$ Hz, OH-3′), 6.67 (dd, 1H, $J_{1',F} = 9.6$ and 7.6 Hz, H-1′), 7.30 (dd, 1H, $J_{4,5} = 4.9$ Hz, $J_{4,2} = 1.3$ Hz, H-4-thienyl), 7.43 (d, 1H, $J_{6,F} = 2.9$ Hz, H-6), 7.58 (dd, 1H, $J_{2,5} = 2.9$ Hz, $J_{2,4} = 1.3$ Hz, H-2-thienyl), 7.72 (dd, 1H, $J_{5,4} = 4.9$ Hz, $J_{5,2} = 2.9$ Hz, H-5-thienyl), 8.18 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): $\delta = 60.36$ (CH_2-5′), 69.90 (dd, $J_{C,F} = 26.5$ and 17.9 Hz, CH-3′), 81.18 (dd, $J_{C,F} = 40.6$ and 20.3 Hz, CH-1′), 83.88 (d, $J_{C,F} = 7.3$ Hz, CH-4′), 100.43 (C-4a), 112.26 (C-5), 121.21 (d, $J_{C,F} = 2.9$ Hz, CH-6), 122.70 (CH-2-thienyl), 123.27 (dd, $J_{C,F} = 261.6$ and 254.6 Hz, C-2′), 127.74 (CH-5-thienyl), 128.61 (CH-4-thienyl), 134.34 (C-3-thienyl), 151.13 (C-7a), 152.51 (CH-2), 157.70 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): $\delta = -118.46$ and -109.47 ppm (2×d, 2×1F, $J_{gem} = 234.1$ Hz); IR (ATR): $\tilde{\nu} = 3456$, 3355, 3122, 1626, 1578, 1552, 1449, 1225, 1053, 1026 cm^{-1}; MS (ESI) *m/z* (%): 369 (100) [*M*+H], 391 (20) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{15}H_{15}F_2N_4O_3S$: 369.08274, found: 369.08272; Anal. calcd for $C_{15}H_{14}F_2N_4O_3S$: C 48.91,H 3.83, N 15.21, found: C 48.69, H 3.79, N 15.11.

4-Amino-7-[2-deoxy-2,2-difluoro-α-D-*erythro*-pentofuranosyl]-5-phenyl-pyrrolo[2,3-*d*]pyrimidine (13e): Compound **13e** was prepared as described for compound **11a** from compound **10** (140 mg, 0.34 mmol) and phenylboronic acid (62.2 mg, 0.51 mmol). The crude product was purified by column chromatography on silica in 3% CH_3OH in $CHCl_3$, and by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O), to give compound **13e** (80 mg, 65%) as a white solid after recrystallization (H_2O/CH_3OH 4:1); mp: 114–116 °C; $[\alpha]_D^{25} = +13.2\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.170$, DMSO); ^1H NMR (500 MHz, [D$_6$]DMSO): $\delta = 3.59$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 6.3$ Hz, $J_{5'a,4'} = 4.5$ Hz, H-5′a), 3.68 (bdm, 1H, $J_{gem} = 12.3$ Hz, H-5′b), 4.30 (m, 1H, H-4′), 4.46 (m, 1H, H-3′), 5.11 (dd, 1H, $J_{OH,5'a} = 6.3$ Hz, $J_{OH,5'b} = 5.2$ Hz, OH-5′), 6.24 (bs, 2H, NH_2), 6.47 (d, 1H, $J_{OH,3'} = 4.7$ Hz,

OH-3′), 6.68 (dd, 1H, $J_{1',F}$ = 9.3 and 7.9 Hz, H-1′), 7.39 (m, 1H, H-*p*-Ph), 7.42 (d, 1H, $J_{6,F}$ = 2.9 Hz, H-6), 7.47–7.52 (m, 4H, H-*o*,*m*-Ph), 8.20 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 60.36 (CH_2-5′), 69.94 (dd, $J_{C,F}$ = 26.6 and 18.1 Hz, CH-3′), 82.25 (dd, $J_{C,F}$ = 40.4 and 20.5 Hz, CH-1′), 83.94 (d, $J_{C,F}$ = 7.3 Hz, CH-4′), 100.12 (C-4a), 117.56 (C-5), 121.30 (d, $J_{C,F}$ = 2.4 Hz, CH-6), 123.33 (dd, $J_{C,F}$ = 262.7 and 252.4 Hz, C-2′), 127.40 (CH-*p*-Ph), 128.67 and 129.27 (CH-*o*,*m*-Ph), 134.14 (C-*i*-Ph), 151.41 (C-7a), 152.48 (CH-2), 157.62 ppm (C-4); ^{19}F NMR (470.3 MHz, [D$_6$]DMSO): δ = −118.29 and −109.16 ppm (2×d, 2×1F, J_{gem} = 234.3 Hz); IR (ATR): $\tilde{\nu}$ = 3464, 3145, 1636, 1571, 1459, 1303, 1211, 1073, 1041 cm^{-1}; MS (ESI) *m*/*z* (%): 363 (100) [*M*+H], 385 (18) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{17}H_{17}F_2N_4O_3$: 363.12632, found: 363.12631; Anal. calcd for $C_{17}H_{16}F_2N_4O_3 \cdot 0.7H_2O$: C 54.46, H 4.68, N 14.94, found: C 54.28, H 4.41, N 14.70.

4-Amino-7-[2-*O*-acetyl-3,5-*O*-(tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl]-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (15): To mixture of arabinoside **14** (2.80 g, 4.41 mmol), Et_3N (1.5 mL, 10.6 mmol) and DMAP (54 mg, 0.44 mmol) in anhydrous CH_3CN (150 mL), acetic anhydride (1.0 mL, 10.6 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h. Then the reaction mixture was diluted with EtOAc (100 mL) and extracted with saturated aqueous solution of $NaHCO_3$ and H_2O. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Acetate **15** (2.98 g, 100%) was obtained as a colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.95–1.25 (m, 28H, $(CH_3)_2CH$), 1.69 (s, 3H, CH_3CO), 3.88 (dt, 1H, $J_{4',3'}$ = 8.6 Hz, $J_{4',5'a}$ = $J_{4',5'b}$ = 2.8 Hz, H-4′), 4.05 (dd, 1H, J_{gem} = 13.1 Hz, $J_{5'a,4'}$ = 3.0 Hz, H-5′a), 4.16 (dd, 1H, J_{gem} = 13.1 Hz, $J_{5'b,4'}$ = 2.6 Hz, H-5′b), 4.69 (t, 1H, $J_{3',4'}$ = $J_{3',2'}$ = 8.5 Hz, H-3′), 5.58 (dd, 1H, $J_{2',3'}$ = 8.4 Hz, $J_{2',1'}$ = 6.5 Hz, H-2′), 6.07 (bs, 2H, NH_2), 6.59 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-1′), 7.42 (s, 1H, H-6), 8.22 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 12.34, 12.88, 12.98 and 13.41 (($CH_3)_2$**C**H), 16.73, 16.76, 16.88, 16.94, 17.30, 17.48, 17.55 and 17.71 (($\mathbf{C}H_3)_2$CH), 20.13 (**C**H_3CO), 50.66 (C-5), 60.53 (CH_2-5′), 70.94 (CH-3′), 76.42 (CH-2′), 80.27 (CH-4′), 80.90 (CH-1′), 103.77 (C-4a), 127.70 (CH-6), 149.90 (C-7a), 150.49 (CH-2), 156.00 (C-4), 169.51 ppm (CO); MS (ESI) *m*/*z* (%): 677 (50) [*M*+H], 699 (100) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{25}H_{42}O_6N_4ISi_2$: 677.16821, found: 677.16796.

4-Amino-7-[2-*O*-acetyl-β-D-arabinofuranosyl]-5-iodo-7*H*-pyrrolo-[2,3-*d*]pyrimidine (16): Silylated acetate **15** (2.98 g, 4.40 mmol) was dissolved in CH_3CN (150 mL) and $Et_3N \cdot 3HF$ (1.44 mL, 8.81 mmol) was added. The reaction mixture was stirred overnight at room temperature The crude product was purified by column chromatography (SiO_2, 2% CH_3OH in CH_2Cl_2) to afford acetate **16** (1.56 g, 82%) as a white crystalline solid; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.68 (s, 3H, CH_3CO), 3.63 (ddd, 1H, J_{gem} = 12.1 Hz, $J_{5'a,OH}$ = 5.8 Hz, $J_{5'a,4'}$ = 4.8 Hz, H-5′a), 3.71 (ddd, 1H, J_{gem} = 12.1 Hz, $J_{5'b,OH}$ = 5.5 Hz, $J_{5'b,4'}$ = 3.6 Hz, H-5′b), 3.80 (ddd, 1H, $J_{4',3'}$ = 6.3 Hz, $J_{4',5'a}$ = 4.8 Hz, $J_{4',5'b}$ = 3.6 Hz, H-4′), 4.30 (dt, 1H, $J_{3',4'}$ = 6.3 Hz, $J_{3',OH}$ = $J_{3',2'}$ = 5.4 Hz, H-3′), 5.07 (t, 1H, $J_{OH,5'a}$ = $J_{OH,5'b}$ = 5.6 Hz, OH-5′), 5.20 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.5 Hz, H-2′), 5.77 (d, 1H, $J_{OH,3'}$ = 5.3 Hz, OH-3′), 6.54 (d, 1H, $J_{1',2'}$ = 5.6 Hz, H-1′), 6.66 (bs, 2H, NH_2), 7.55 (s, 1H, H-6), 8.08 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO): δ = 20.17 (CH_3), 51.69 (C-5), 60.29 (CH_2-5′), 71.82 (CH-3′), 77.69 (CH-2′), 81.25 (CH-1′), 82.89 (CH-4′), 102.79 (C-4a), 127.91 (CH-6), 149.95 (C-7a), 152.19 (CH-2), 157.27 (C-4), 169.08 ppm (CO); MS (ESI) *m*/*z* (%): 435 (100) [*M*+H], 457 (73) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{13}H_{16}O_5N_4I$: 435.01599, found: 435.01598.

4-Amino-7-[2-*O*-acetyl-3,5-di-*O*-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (17): Acetate **16** (1.50 g, 1.84 mmol) and TsOH·H_2O (1.31 g, 3.68 mmol) were dissolved in DMF (50 mL) and cooled to 0 °C. Then, 3,4-dihydro-2*H*-pyran (4.73 mL, 27.6 mmol) was added and the reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction mixture was diluted by EtOAc (100 mL) and extracted with saturated $NaHCO_3$ solution and brine. The organic layer was dried over $MgSO_4$ and volatiles were thoroughly removed in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/EtOAc 1:1) to afford compound **17** (1.68 g, 81%) as a pale-yellow foam (mixture of four diastereomers 1:1:1:1); ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 1.45–1.95 (m, 48H, H-3,4,5-*THP*), 1.71, 1.75, 1.76 and 1.77 (4s, 4×3H, CH_3), 3.42–3.56 (m, 8H, H-6a-*THP*), 3.69–3.79 (m, 4H, H-5′a), 3.78–3.96 (m, 8H, H-6b-*THP*), 3.97–4.08 (m, 4H, H-5′b), 4.16–4.23 (m, 4H, H-4′), 4.55 (bdd, 1H, $J_{3',4'}$ = 5.9 Hz, $J_{3',2'}$ = 4.8 Hz, H-3′), 4.60 (m, 1H, H-3′), 4.63 (bdd, 1H, $J_{3',4'}$ = 5.7 Hz, $J_{3',2'}$ = 4.5 Hz, H-3′), 4.69 (bdd, 1H, $J_{3',4'}$ = 5.9 Hz, $J_{3',2'}$ = 4.7 Hz, H-3′), 4.72–4.77 (m, 4H, H-2-*THP*), 4.79–4.84 and 4.87–4.91 (2 m, 2×2H, H-2-*THP*), 5.44 (dd, 1H, $J_{2',1'}$ = 5.3 Hz, $J_{2',3'}$ = 4.4 Hz, H-2′), 5.46 (dd, 1H, $J_{2',1'}$ = 5.5 Hz, $J_{2',3'}$ = 4.6 Hz, H-2′), 5.58 (dd, 1H, $J_{2',1'}$ = 5.5 Hz, $J_{2',3'}$ = 4.8 Hz, H-2′), 5.62 (t, 1H, $J_{2',1'}$ = $J_{2',3'}$ = 5.7 Hz, H-2′), 6.30 (m, 8H, NH_2), 6.68–6.70 (m, 2H, H-1′), 6.70 and 6.71 (2×d, 2H, 2 $J_{1',2'}$ = 5.5 Hz, H-1′), 7.62, 7.64, 7.65 and 7.67 (4 s, 4H, H-6), 8.10, 8.111, 8.113 and 8.115 ppm (4 s, 4H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]acetone): δ = 19.76, 19.81, 19.89, 19.90, 20.07, 20.10, 20.12 and 20.29 (CH_2-4-*THP*), 20.27, 20.34, 20.35 (CH_3), 26.02, 26.08, 26.10, 26.18, 26.21, 26.22 and 26.28 (CH_2-5-*THP*), 31.13, 31.17, 31.23, 31.28, 31.31, 31.34, 31.37 and 31.40 (CH_2-3-*THP*), 50.60, 50.62 and 50.68 (C-5), 62.00, 62.08, 62.35, 62.66, 62.72, 62.75 and 62.76 (CH_2-6-*THP*), 66.56, 66.64, 66.76 and 66.80 (CH_2-5′), 76.84, 76.91, 77.08 and 77.35 (CH-2′), 78.1, 78.05, 79.01 and 79.47 (CH-3′), 80.85, 81.04, 81.36 and 81.41 (CH-4′), 82.72, 82.74, 82.90 and 82.99 (CH-1′), 98.48, 98.52, 99.27, 99.30, 99.31, 99.37, 99.46 and 99.48 (CH-2-*THP*), 104.12, 104.15, 104.18 and 104.19 (C-4a), 128.61, 128.68, 128.78 and 128.82 (CH-6), 151.38, 151.41 and 151.44 (C-7a), 153.27, 153.28 and 153.29 (CH-2), 158.18 and 158.24 (C-4), 169.24, 169.26 and 169.45 ppm (CO); MS (ESI) *m*/*z* (%): 603 (15) [*M*+H], 625 (100) [*M*+Na]; HRMS-ESI [*M*+H]$^+$ calcd for $C_{23}H_{32}O_7N_4I$: 603.13102, found: 603.13118.

4-Amino-7-[3,5-di-*O*-(tetrahydropyran-2-yl)-β-D-arabinofuranosyl]-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (18): Acetate **17** (1.60 g, 2.66 mmol) was dissolved in anhydrous CH_3OH (30 mL) and methanolic solution of sodium methoxide (1 M, 2.7 mL, 2.7 mmol) was added. The reaction mixture was stirred at room temperature overnight and then it was neutralized using aqueous HCl (1 M) and evaporated under reduced pressure. The residue was chromatographed on silica (2% CH_3OH in $CHCl_3$) to give compound **18** (1.25 g, 84%) as a pale-yellow foam; ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 1.47–1.92 (m, 48H, H-3,4,5-*THP*), 3.47–3.57 (m, 8H, H-6a-*THP*), 3.67–3.76 (m, 4H, H-5′a), 3.81–3.99 (m, 8H, H-6b-*THP*), 3.96–4.07 (m, 4H, H-5′b), 4.12–4.17 (m, 4H, H-4′), 4.38–4.54, 4.60–5.00, (2 m, 20H, H-2′,3′,2-*THP*,OH), 6.26 (m, 8H, NH_2), 6.53 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′), 6.54 (d, 1H, $J_{1',2'}$ = 5.0 Hz, H-1′), 6.58 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′), 6.60 (d, 1H, $J_{1',2'}$ = 5.0 Hz, H-1′), 7.65, 7.66, 7.677 and 7.683 (4 s, 4H, H-6), 8.106, 8.109, 8.114 and 8.12 ppm (4 s, 4H, H-2); ^{13}C NMR (125.7 MHz, [D$_6$]acetone): δ = 19.99, 20.03, 20.04, 20.07, 20.08, 20.18 and 20.30 (CH_2-4-*THP*), 26.05, 26.07, 26.16, 26.17, 26.18, 26.20 and 26.27 (CH_2-5-*THP*), 31.14, 31.15, 31.21, 31.26, 31.42, 31.50 and 31.58 (CH_2-3-*THP*), 49.74, 49.76, 49.80 and 49.81 (C-5), 61.95, 62.08, 62.14, 62.27, 62.75, 62.81, 63.37 and 63.41 (CH_2-6-*THP*), 67.27, 67.37, 67.39 and 67.57 (CH_2-5′), 76.01, 76.15, 76.23, 76.28, 80.91, 80.99, 81.53, 81.75, 81.84, 81.85, 82.77 and 83.05 (CH-2′,3′,4′), 84.95, 84.97, 85.10 and 85.26 (CH-1′), 98.63, 98.68, 99.21, 99.32, 99.43, 99.44, 99.48 and 99.67 (CH-2-*THP*), 104.27 and 104.32

(C-4a), 129.77, 129.82, 129.85 and 129.87 (CH-6), 151.40, 151.41, 151.45 and 151.48 (C-7a), 152.96, 152.97, 152.98 and 153.01 (CH-2), 158.12, 158.15, 158.16 and 158.18 ppm (C-4); MS (ESI) *m/z* (%): 561 (11) $[M+H]$, 583 (100) $[M+Na]$; HRMS-ESI $[M+H]^+$ calcd for $C_{21}H_{30}O_6N_4I$: 561.12045, found: 561.12049.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (20): Nucleoside **18** (650 mg, 1.16 mmol) was dissolved in anhydrous CH_2Cl_2 (16 mL). Pyridine (700 μL, 8.70 mmol) was added and the solution was cooled to 0 °C. Then DAST (766 μL, 5.80 mmol) was added. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction was neutralized with aqueous $NaHCO_3$ (saturated), diluted with chloroform (30 mL) and extracted with H_2O. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product **19** was chromatographed on silica (hexane/EtOAc 2:1) and directly dissolved in 90% aqueous TFA (5 mL). The solution was stirred for 2 h and then it was repeatedly co-evaporated with CH_3OH. The final product was purified by column chromatography (silica, 2.5% CH_3OH in $CHCl_3$) to afford fluoronucleoside **20** (163 mg, 36% over two steps) as a white crystalline solid after recrystallization (H_2O/CH_3OH 3:1); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 3.57$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.7$ Hz, $J_{5'a,4'} = 3.7$ Hz, H-5′a), 3.71 (ddd, 1H, $J_{gem} = 12.2$ Hz, $J_{5'b,OH} = 5.2$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.92 (m, 1H, H-4′), 4.35 (dtd, 1H, $J_{3',F} = 16.1$ Hz, $J_{3',4'} = J_{3',OH} = 6.0$ Hz, $J_{3',2'} = 4.7$ Hz, H-3′), 5.19 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.4$ Hz, OH-5′), 5.22 (ddd, 1H, $J_{2',F} = 53.1$ Hz, $J_{2',3'} = 4.7$ Hz, $J_{2',1'} = 3.5$ Hz, H-2′), 5.67 (d, 1H, $J_{OH,3'} = 6.0$ Hz, OH-3′), 6.32 (dd, 1H, $J_{1',F} = 16.5$ Hz, $J_{1',2'} = 3.5$ Hz, H-1′), 6.73 (bs, 2H, NH_2), 7.69 (s, 1H, H-6), 8.12 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): $\delta = 52.67$ (C-5), 60.52 (CH_2-5′), 68.45 (d, $J_{C,F} = 15.8$ Hz, CH-3′), 83.99 (d, $J_{C,F} = 2.0$ Hz, CH-4′), 85.24 (d, $J_{C,F} = 32.6$ Hz, CH-1′), 93.78 (d, $J_{C,F} = 187.3$ Hz, C-2′), 103.41 (C-4a), 126.92 (CH-6), 149.89 (C-7a), 152.39 (CH-2), 157.45 ppm (C-4); ^{19}F NMR (470.3 MHz, $[D_6]$DMSO): $\delta = -200.75$ ppm (s, 1F, F-2′); MS (ESI) *m/z* (%): 395 (100) $[M+H]$, 417 (70) $[M+Na]$; HRMS-ESI $[M+H]^+$ calcd for $C_{11}H_{13}O_3N_4FI$: 395.00109, found: 395.00114.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-(furan-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (21a): Nucleoside **21a** was prepared as described for compound **11a** from nucleoside **20** (120 mg, 0.30 mmol) and furan-2-boronic acid (43 mg, 0.38 mmol). The crude product was purified by reversed-phase HPFC on C_{18} column (0→100% CH_3OH in H_2O). Nucleoside **21a** (54 mg, 53%) was obtained as a white crystalline solid after recrystallization (H_2O/CH_3OH 4:1); mp: 103–105 °C; $[\alpha]_D^{25} = -45.0\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.251$, DMSO); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 3.60$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.7$ Hz, $J_{5'a,4'} = 3.9$ Hz, H-5′a), 3.75 (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,OH} = 5.3$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.96 (m, 1H, H-4′), 4.40 (dtd, 1H, $J_{3',F} = 16.6$ Hz, $J_{3',4'} = J_{3',OH} = 6.0$ Hz, $J_{3',2'} = 4.6$ Hz, H-3′), 5.22 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.5$ Hz, OH-5′), 5.27 (ddd, 1H, $J_{2',F} = 53.1$ Hz, $J_{2',3'} = 4.6$ Hz, $J_{2',1'} = 3.4$ Hz, H-2′), 5.70 (d, 1H, $J_{OH,3'} = 6.0$ Hz, OH-3′), 6.39 (dd, 1H, $J_{1',F} = 16.4$ Hz, $J_{1',2'} = 3.4$ Hz, H-1′), 6.62 (dd, 1H, $J_{4,3} = 3.3$ Hz, $J_{4,5} = 1.9$ Hz, H-4-furyl), 6.66 (dd, 1H, $J_{3,4} = 3.3$ Hz, $J_{3,5} = 0.8$ Hz, H-3-furyl), 6.95 (bs, 2H, NH_2), 7.79 (dd, 1H, $J_{5,4} = 1.9$ Hz, $J_{5,3} = 0.8$ Hz, H-5-furyl), 7.86 (s, 1H, H-6), 8.16 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): $\delta = 60.62$ (CH_2-5′), 68.54 (d, $J_{C,F} = 15.8$ Hz, CH-3′), 83.95 (d, $J_{C,F} = 1.8$ Hz, CH-4′), 85.34 (d, $J_{C,F} = 32.4$ Hz, CH-1′), 93.83 (d, $J_{C,F} = 187.3$ Hz, C-2′), 99.45 (C-4a), 105.67 (CH-3-furyl), 106.79 (C-5), 112.10 (CH-4-furyl), 120.20 (CH-6), 142.33 (CH-5-furyl), 148.55 (C-2-furyl), 150.63 (C-7a), 152.57 (CH-2), 157.51 ppm (C-4); ^{19}F NMR (470.3 MHz, $[D_6]$DMSO): $\delta = -200.38$ ppm (s, 1F, F-2′); IR (ATR): $\tilde{\nu} = 3455$, 3128, 1635, 1561, 1482, 1460, 1297, 1234, 1109, 1067, 1043, 1017; MS (ESI) *m/z* (%): 335 (98) $[M+H]$, 357 (100) $[M+Na]$; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{16}O_4N_4F$: 335.11501, found: 335.11502; Anal. calcd for $C_{15}H_{15}O_4N_4F{\cdot}2H_2O$: C 48.65, H 5.17, N 15.13, found: C 48.42, H 4.81, N 14.84.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-(furan-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (21b): Nucleoside **21b** was prepared as described for compound **11a**. Nucleoside **20** (120 mg, 0.30 mmol) and furan-3-boronic acid (43 mg, 0.38 mmol) were used. Nucleoside **21b** (65 mg, 64%) was obtained as an off-white cotton after recrystallization (H_2O/CH_3OH 4:1); mp: 98–100 °C; $[\alpha]_D^{25} = -39.2\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.194$, DMSO); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 3.58$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.8$ Hz, $J_{5'a,4'} = 4.0$ Hz, H-5′a), 3.71 (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,OH} = 5.3$ Hz, $J_{5'b,4'} = 3.0$ Hz, H-5′b), 3.94 (m, 1H, H-4′), 4.39 (dtd, 1H, $J_{3',F} = 15.5$ Hz, $J_{3',4'} = J_{3',OH} = 5.9$ Hz, $J_{3',2'} = 4.7$ Hz, H-3′), 5.18 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.5$ Hz, OH-5′), 5.28 (ddd, 1H, $J_{2',F} = 53.1$ Hz, $J_{2',3'} = 4.6$ Hz, $J_{2',1'} = 3.7$ Hz, H-2′), 5.68 (d, 1H, $J_{OH,3'} = 5.9$ Hz, OH-3′), 6.32 (bs, 2H, NH_2), 6.39 (dd, 1H, $J_{1',F} = 16.6$ Hz, $J_{1',2'} = 3.7$ Hz, H-1′), 6.70 (dd, 1H, $J_{4,5} = 1.8$ Hz, $J_{4,2} = 0.9$ Hz, H-4-furyl), 7.52 (s, 1H, H-6), 7.81 (t, 1H, $J_{5,4} = J_{5,2} = 1.7$ Hz, H-5-furyl), 7.74 (dd, 1H, $J_{2,5} = 1.6$ Hz, $J_{2,4} = 0.9$ Hz, H-2-furyl), 8.15 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): $\delta = 60.75$ (CH_2-5′), 68.66 (d, $J_{C,F} = 15.7$ Hz, CH-3′), 83.97 (d, $J_{C,F} = 1.9$ Hz, CH-4′), 85.16 (d, $J_{C,F} = 32.3$ Hz, CH-1′), 93.75 (d, $J_{C,F} = 187.5$ Hz, C-2′), 101.11 (C-4a), 106.94 (C-5), 111.66 (CH-4-furyl), 118.54 (C-3-furyl), 120.72 (CH-6), 139.93 (CH-2-furyl), 144.39 (CH-5-furyl), 150.53 (C-7a), 152.23 (CH-2), 157.71 ppm (C-4); ^{19}F NMR (470.3 MHz, $[D_6]$DMSO): $\delta = -200.82$ ppm (s, 1F, F-2′); IR (ATR): $\tilde{\nu} = 3367$, 3139, 1626, 1566, 1470, 1454, 1096, 1022 cm^{-1}; MS (ESI) *m/z* (%): 335 (100) $[M+H]$, 357 (27) $[M+Na]$; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{16}O_4N_4F$: 335.11501, found: 335.11505; Anal. calcd for $C_{15}H_{15}O_4N_4F{\cdot}1.5H_2O$: C 49.86, H 5.02, N 15.51, found: C 50.22, H 4.65, N 15.35.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-(thiophen-2-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (21c): Nucleoside **21c** was prepared as described for compound **11a**. Nucleoside **20** (120 mg, 0.30 mmol) and thiophene-2-boronic acid (49 mg, 0.38 mmol) were used. Nucleoside **21c** (57 mg, 53%) was obtained as a white lyophilizate (*t*BuOH-benzene 3:1); mp: 96–101 °C; $[\alpha]_D^{25} = -40.2\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.293$, DMSO); ^1H NMR (500 MHz, $[D_6]$DMSO): $\delta = 3.58$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.5$ Hz, $J_{5'a,4'} = 3.9$ Hz, H-5′a), 3.73 (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,OH} = 5.0$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.95 (m, 1H, H-4′), 4.40 (bdt, 1H, $J_{3',F} = 16.1$ Hz, $J_{3',2'} = J_{3',4'} = 5.3$ Hz, H-3′), 5.21 (bt, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.3$ Hz, OH-5′), 5.30 (ddd, 1H, $J_{2',F} = 53.1$ Hz, $J_{2',3'} = 4.7$ Hz, $J_{2',1'} = 3.5$ Hz, H-2′), 5.70 (bs, 1H, OH-3′), 6.37 (bs, 2H, NH_2), 6.40 (dd, 1H, $J_{1',F} = 16.5$ Hz, $J_{1',2'} = 3.5$ Hz, H-1′), 7.16 (dd, 1H, $J_{3,4} = 3.5$ Hz, $J_{3,5} = 1.3$ Hz, H-3-thienyl), 7.18 (dd, 1H, $J_{4,5} = 5.1$ Hz, $J_{4,3} = 3.5$ Hz, H-4-thienyl), 7.58 (dd, 1H, $J_{5,4} = 5.1$ Hz, $J_{5,3} = 1.3$ Hz, H-5-thienyl), 7.66 (s, 1H, H-6), 8.18 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO): $\delta = 60.54$ (CH_2-5′), 68.51 (d, $J_{C,F} = 15.7$ Hz, CH-3′), 83.99 (d, $J_{C,F} = 2.0$ Hz, CH-4′), 85.33 (d, $J_{C,F} = 32.3$ Hz, CH-1′), 93.83 (d, $J_{C,F} = 187.7$ Hz, C-2′), 100.76 (C-4a), 109.12 (C-5), 121.82 (CH-6), 126.15 (CH-5-thienyl), 126.69 (CH-3-thienyl), 128.48 (CH-4-thienyl), 135.42 (C-2-thienyl), 150.39 (C-7a), 152.48 (CH-2), 157.52 ppm (C-4); ^{19}F NMR (470.3 MHz, $[D_6]$DMSO): $\delta = -200.58$ ppm (s, 1F, F-2′); IR (ATR): $\tilde{\nu} = 3340$, 3194, 1622, 1588, 1551, 1465, 1290, 1190, 1096, 1063; MS (ESI) *m/z* (%): 351 (100) $[M+H]$, 373 (40) $[M+Na]$; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{16}O_3N_4FS$: 351.09217, found: 351.09234; Anal. calcd for $C_{15}H_{16}O_3N_4FS$: C 51.42, H 4.32, N 15.99, found: C 51.02, H 4.68, N 15.73.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-(thiophen-3-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (21d): Nucleoside **21d** was prepared as described for compound **11a**. Nucleoside **20** (120 mg, 0.30 mmol) and thiophene-3-boronic acid (49 mg, 0.38 mmol) were

used. Nucleoside **21 d** (65 mg, 61%) was obtained as a white crystalline solid (H_2O/CH_3OH 4:1); mp: 106–108 °C; $[\alpha]_D^{25} = -34.7\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.285$, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 3.58$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.8$ Hz, $J_{5'a,4'} = 4.0$ Hz, H-5′a), 3.72 (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,OH} = 5.2$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.95 (m, 1H, H-4′), 4.40 (b dtd, 1H, $J_{3',F} = 15.6$ Hz, $J_{3',OH} = J_{3',4'} = 5.8$ Hz, $J_{3',2'} = 4.5$ Hz, H-3′), 5.19 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.5$ Hz, OH-5′), 5.30 (ddd, 1H, $J_{2',F} = 53.0$ Hz, $J_{2',3'} = 4.5$ Hz, $J_{2',1'} = 3.7$ Hz, H-2′), 5.69 (d, 1H, $J_{OH,3'} = 5.8$ Hz, OH-3′), 6.26 (b s, 2H, NH_2), 6.40 (dd, 1H, $J_{1',F} = 16.7$ Hz, $J_{1',2'} = 3.7$ Hz, H-1′), 7.27 (dd, 1H, $J_{4,5} = 4.9$ Hz, $J_{4,2} = 1.3$ Hz, H-4-thienyl), 7.53 (dd, 1H, $J_{2,5} = 2.9$ Hz, $J_{2,4} = 1.3$ Hz, H-2-thienyl), 7.57 (s, 1H, H-6), 7.72 (dd, 1H, $J_{5,4} = 4.9$ Hz, $J_{5,2} = 2.9$ Hz, H-5-thienyl), 8.16 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): $\delta = 60.73$ (CH_2-5′), 68.66 (d, $J_{C,F} = 15.7$ Hz, CH-3′), 83.99 (d, $J_{C,F} = 1.9$ Hz, CH-4′), 85.21 (d, $J_{C,F} = 32.3$ Hz, CH-1′), 93.81 (d, $J_{C,F} = 187.3$ Hz, C-2′), 100.92 (C-4a), 111.66 (C-5), 120.84 (CH-6), 122.41 (CH-2-thienyl), 127.64 (CH-5-thienyl), 128.63 (CH-4-thienyl), 134.67 (C-3-thienyl), 150.40 (C-7a), 152.22 (CH-2), 157.65 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): $\delta = -200.72$ ppm (s, 1F, F-2′); IR (ATR): $\tilde{\nu} = 3373$, 3106, 1620, 1550, 1463, 1300, 1127, 1097, 1041; MS (ESI) *m/z* (%): 351 (100) [*M*+H], 373 (75) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{15}H_{16}O_3N_4FS$: 351.09217, found: 351.09214; Anal. calcd for $C_{15}H_{16}O_3N_4FS{\cdot}2H_2O$: C 46.63, H 4.96, N 14.50, found: C 47.00, H 4.68, N 14.42.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (21 e): Nucleoside **21 e** was prepared as described for compound **11 a**. Nucleoside **20** (150 mg, 0.38 mmol) and phenylboronic acid (58 mg, 0.47 mmol) were used. Nucleoside **21 e** (91 mg, 69%) was obtained as a white lyophilizate (*t*BuOH-benzene 2:1); mp: 93–95 °C; $[\alpha]_D^{25} = -40.8\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.446$, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 3.58$ (dd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,4'} = 3.9$ Hz, H-5′a), 3.72 (dd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.95 (m, 1H, H-4′), 4.41 (ddd, 1H, $J_{3',F} = 15.9$ Hz, $J_{3',4'} = 6.0$ Hz, $J_{3',2'} = 4.7$ Hz, H-3′), 5.32 (ddd, 1H, $J_{2',F} = 53.1$ Hz, $J_{2',3'} = 4.7$ Hz, $J_{2',1'} = 3.6$ Hz, H-2′), 6.42 (dd, 1H, $J_{1',F} = 16.7$ Hz, $J_{1',2'} = 3.6$ Hz, H-1′), 7.38 (m, 1H, H-*p*-Ph), 7.46–7.52 (m, 4H, H-*o*,*m*-Ph), 7.58 (s, 1H, H-6), 8.18 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): $\delta = 60.70$ (CH_2-5′), 68.65 (d, $J_{C,F} = 15.8$ Hz, CH-3′), 83.99 (d, $J_{C,F} = 1.9$ Hz, CH-4′), 85.27 (d, $J_{C,F} = 32.4$ Hz, CH-1′), 93.88 (d, $J_{C,F} = 187.2$ Hz, C-2′), 100.61 (C-4a), 116.99 (C-5), 120.94 (CH-6), 127.24 (CH-*p*-Ph), 128.65 and 129.22 (CH-*o*,*m*-Ph), 134.46 (C-*i*-Ph), 150.68 (C-7a), 152.20 (CH-2), 157.58 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): $\delta = -200.56$ ppm (s, 1F, F-2′); IR (KBr): $\tilde{\nu} = 3351$, 3199, 1623, 1584, 1540, 1466, 1297, 1104, 1065; MS (ESI) *m/z* (%): 345 (100) [*M*+H], 367 (30) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{17}H_{18}O_3N_4F$: 345.13575, found: 345.13569; Anal. calcd for $C_{17}H_{17}O_3N_4F$: C 59.30, H 4.98, N 16.27, found: C 59.00, H 4.98, N 16.21.

4-Amino-7-[2-deoxy-2-fluoro-β-D-ribofuranosyl]-5-ethynyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (21 h): An argon-purged mixture of derivative **20** (130 mg, 0.33 mmol), $PdCl_2(PPh_3)_2$ (12 mg, 16 μmol), CuI (6.3 mg, 33 μmol), trimethylsilylacetylene (470 μL, 3.3 mmol) and triethylamine (150 μL) was stirred in DMF (600 μL) at room temperature overnight. Volatiles were removed under reduced pressure and the crude product was purified by column chromatography on silica in 2.5% CH_3OH in $CHCl_3$. Then, the product was purified by reversed-phase HPFC on C_{18} (0→100% CH_3OH in H_2O) to give ethynyl derivative **21 h** (73 mg, 76%) as a beige crystalline solid after recrystallization (CH_3OH/H_2O 1:4); mp: 243–248 °C; $[\alpha]_D^{25} = -42.7\ cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.446$, DMSO); ^1H NMR (500 MHz, [D_6]DMSO): $\delta = 3.58$ (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'a,OH} = 5.7$ Hz, $J_{5'a,4'} = 3.7$ Hz, H-5′a), 3.73 (ddd, 1H, $J_{gem} = 12.3$ Hz, $J_{5'b,OH} = 5.2$ Hz, $J_{5'b,4'} = 2.9$ Hz, H-5′b), 3.94 (m, 1H, H-4′), 4.30 (s, 1H, C≡CH), 4.37 (dtd, 1H, $J_{3',F} = 16.8$ Hz, $J_{3',OH} = J_{3',4'} = 6.2$ Hz, $J_{3',2'} = 4.6$ Hz, H-3′), 5.23 (t, 1H, $J_{OH,5'a} = J_{OH,5'b} = 5.4$ Hz, OH-5′), 5.24 (ddd, 1H, $J_{2',F} = 53.0$ Hz, $J_{2',3'} = 4.6$ Hz, $J_{2',1'} = 3.2$ Hz, H-2′), 5.70 (d, 1H, $J_{OH,3'} = 6.0$ Hz, OH-3′), 6.32 (dd, 1H, $J_{1',F} = 16.5$ Hz, $J_{1',2'} = 3.3$ Hz, H-1′), 7.84 (s, 1H, H-6), 8.15 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, [D_6]DMSO): $\delta = 60.45$ (CH_2-5′), 68.42 (d, $J_{C,F} = 15.9$ Hz, CH-3′), 77.24 (**C**≡CH), 83.54 (C≡**C**H), 84.03 (d, $J_{C,F} = 1.8$ Hz, CH-4′), 85.54 (d, $J_{C,F} = 32.6$ Hz, CH-1′), 93.90 (d, $J_{C,F} = 187.5$ Hz, C-2′), 94.62 (C-5), 102.52 (C-4a), 127.21 (CH-6), 149.30 (C-7a), 153.27 (CH-2), 157.77 ppm (C-4); ^{19}F NMR (470.3 MHz, [D_6]DMSO): $\delta = -200.42$ ppm (s, 1F, F-2′); IR (KBr): $\tilde{\nu} = 3508$, 3467, 3368, 3167, 1641, 1599, 1573, 1530, 1448, 1367, 1314, 1199, 1059, 1033, 1017; MS (ESI) *m/z* (%): 293 (100) [*M*+H], 315 (95) [*M*+Na]; HRMS-ESI $[M+H]^+$ calcd for $C_{13}H_{14}O_3N_4F$: 293.10445, found: 293.10459; Anal. calcd for $C_{13}H_{13}O_3N_4F{\cdot}0.5\ CH_3OH$: C 52.60, H 4.90, N 18.17, found: C 52.47, H 4.67, N 17.93.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(thiophene-2-yl)pyrrolo[2,3-*d*]pyrimidine-5′-*O*-triphosphate sodium salt (11 c-TP): Compound **11 c** (30 mg, 0.081 mmol) was dissolved in trimethyl phosphate (350 μL) in argon-purged vial and the suspension was cooled to 0 °C. Then $POCl_3$ (9.5 μL, 0.102 mmol) was added. The reaction mixture was stirred at 0 °C for 2.5 h and then ice-cold solution of $(NHBu_3)_2H_2P_2O_7$ (234.3 mg, 0.427 mmol) and tri(*n*-butyl)amine (92 μL, 0.387 mmol) in anhydrous DMF (1 mL) was added. The reaction mixture was stirred at −5 °C for 2.5 h. Aqueous solution of TEAB (2 M, 3 mL, 6 mmol) was added and the mixture was evaporated under reduced pressure. The residue was co-evaporated several times with H_2O. The product was purified on DEAE Sephadex column (0–1.2 M TEAB gradient), co-evaporated several times with H_2O and converted into a sodium salt form (Dowex 50WX8 in Na^+ cycle). Triphosphate **11 c-TP** was obtained as a white lyophilizate (H_2O; 19 mg, 36%); ^1H NMR (500 MHz, D_2O): $\delta = 4.27$ (m, 1H, H-4′), 4.35 (ddd, 1H, $J_{gem} = 12.0$ Hz, $J_{5'a,P} = 6.5$ Hz, $J_{5'a,4'} = 3.8$ Hz, H-5′a), 4.39 (m, 1H, H-5′b), 4.66 (ddd, 1H, $J_{3',F} = 12.6$ and 11.6 Hz, $J_{3',4'} = 8.3$ Hz, H-3′), 6.45 (dd, 1H, $J_{1',F} = 10.9$ and 5.1 Hz, H-1′), 7.12 (dd, H, $J_{3,4} = 3.5$ Hz, $J_{3,5} = 1.2$ Hz, H-3-thienyl), 7.17 (dd, 1H, $J_{4,5} = 5.2$ Hz, $J_{4,3} = 3.5$ Hz, H-4-thienyl), 7.49 (dd, 1H, $J_{5,4} = 5.2$ Hz, $J_{5,3} = 1.2$ Hz, H-5-thienyl), 7.50 (b s, 1H, H-6), 8.16 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, D_2O): $\delta = 61.18$ (d, $J_{C,P} = 5.1$ Hz, CH_2-5′), 69.61 (dd, $J_{C,F} = 27.5$ and 17.8 Hz, CH-3′), 79.51 (t, $J_{C,F} = J_{C,P} = 8.9$ Hz, CH-4′), 83.44 (dd, $J_{C,F} = 40.8$ and 23.6 Hz, CH-1′), 101.46 (C-4a), 111.87 (C-5), 122.45 (CH-6), 122.76 (dd, $J_{C,F} = 261.8$ and 255.4 Hz, C-2′), 127.22 (CH-5-thienyl), 128.01 (CH-3-thienyl), 128.87 (CH-4-thienyl), 134.31 (C-2-thienyl), 149.99 (C-7a), 151.39 (CH-2), 156.97 ppm (C-4); ^{19}F NMR (470.3 MHz, D_2O): $\delta = -114.64$ and −113.23 ppm (2×d, 2×1F, $J_{gem} = 238.2$ Hz); ^{31}P NMR (202.3 MHz, D_2O): $\delta = -21.54$ (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma} = 19.0$ Hz, P_β), −10.25 (d, 1P, $J_{\alpha,\beta} = 19.2$ Hz, P_α), −8.38 ppm (d, 1P, $J_{\gamma,\beta} = 18.7$ Hz, P_γ); MS (ESI−) *m/z* (%): 650 (10) [*M*+2Na], 628 (5) [*M*+Na+H], 549 (45) [*M*−PO_3+Na+H], 527 (100) [*M*−PO_3+2H], 447 (35) [*M*−2PO_3+H]; HRMS-ESI $[M+Na+H]^-$ calcd for $C_{15}H_{15}F_2N_4NaO_{12}P_3S$: 628.94913, found: 628.94963.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-(benzofuran-2-yl)pyrrolo[2,3-*d*]pyrimidine-5′-O-triphosphate sodium salt (11 g-TP): Compound **11 g-TP** was prepared as described for compound **11 c-TP** from compound **11 g** (28 mg, 0.070 mmol), with $POCl_3$ (8.17 μL, 0.087 mmol), $(NHBu_3)_2H_2P_2O_7$ (200.5 mg, 0.365 mmol), and tri(*n*-butyl)amine (78.8 μL, 0.331 mmol) to give the triphosphate **11 g-TP** (6.2 mg, 13%) as a white lyophlizate (H_2O); ^1H NMR (500 MHz, D_2O): $\delta = 4.24$ (m, 1H, H-4′), 4.38–4.49 (m, 2H, H-5′), 4.76 (m, 1H, H-3′), 6.09 (b dd, 1H, $J_{1',F} = 10.8$ and 4.3 Hz, H-1′), 6.86 (s, 1H, H-3-benzofuryl), 7.15–7.21 (m, 2H, H-5,6-benzofuryl), 7.29 (m, 1H, H-7-benzofuryl), 7.43 (m,

1H, H-4-benzofuryl), 7.71 (s, 1H, H-2), 7.79 ppm (s, 1H, H-6); ^{13}C NMR (125.7 MHz, D_2O): $\delta = 63.92$ (d, $J_{C,P} = 5.0$ Hz, CH_2-5′), 69.26 (dd, $J_{C,F} = 27.6$ and 17.5 Hz, CH-3′), 79.62 (t, $J_{C,F} = J_{C,P} = 8.9$ Hz, CH-4′), 83.22 (dd, $J_{C,F} = 40.7$ and 23.7 Hz, CH-1′), 99.71 (C-4a), 102.29 (CH-3-benzofuryl), 108.48 (C-5), 111.22 (CH-7-benzofuryl), 121.31 (CH-4-benzofuryl), 121.54 (CH-6), 122.90 (dd, $J_{C,F} = 261.9$ and 255.5 Hz, C-2′), 123.93 (CH-5-benzofuryl), 124.46 (CH-6-benzofuryl), 128.97 (C-3a-benzofuryl), 149.88 (C-2-benzofuryl), 149.94 (C-7a), 151.52 (CH-2), 153.90 (C-7a-benzofuryl), 157.04 ppm (C-4); ^{19}F NMR (470.3 MHz, D_2O): $\delta = -114.40$ and -112.86 ppm (2×d, 2×1F, $J_{gem} = 238.0$ Hz); ^{31}P NMR (202.3 MHz, D_2O): $\delta = -20.60$ (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma} = 19.8$ Hz, P_β), -10.04 (d, 1P, $J_{\alpha,\beta} = 19.3$ Hz, P_α), -5.20 ppm (d, 1P, $J_{\gamma'\beta} = 20.2$ Hz, P_γ); MS (ESI−) *m/z* (%): 685 (20) [*M* + 2Na + H], 663 (15) [*M* + Na + H], 583 (100) [*M* − PO_3 + Na + H], 561 (98) [*M* − PO_3 + 2H], 481 (80) [*M* − 2PO_3 + H]; HRMS-ESI [*M* + Na + H]$^-$ calcd for $C_{19}H_{15}F_2N_4NaO_{13}P_3$: 662.98762, found: 662.98747.

4-Amino-7-[2-deoxy-2,2-difluoro-β-D-*erythro*-pentofuranosyl]-5-ethynyl-pyrrolo[2,3-*d*]pyrimidine-5′-O-triphosphate sodium salt (11h-TP): Compound **11h-TP** was prepared as described for compound **11c-TP** from compound **11h** (30 mg, 0.097 mmol), with $POCl_3$ (11.27 μL, 0.121 mmol), $(NHBu_3)_2H_2P_2O_7$ (278.5 mg, 0.508 mmol), and tri(*n*-butyl)amine (109.4 μL, 0.459 mmol) to give the triphosphate **11h-TP** (6.8 mg, 12%) as a white lyophlizate (H_2O); ^{1}H NMR (500 MHz, D_2O): $\delta = 3.72$ (s, 1H, CH≡C), 4.28 (m, 1H, H-4′), 4.36 (ddd, 1H, $J_{gem} = 12.2$ Hz, $J_{5'a,P} = 6.5$ Hz, $J_{5'a,4'} = 3.5$ Hz, H-5′a), 4.44 (bdm, 1H, $J_{gem} = 12.2$ Hz, H-5′b), 4.66 (m, 1H, H-3′), 6.41 (dd, 1H, $J_{1',F} = 10.6$ and 4.3 Hz, H-1′), 7.71 (d, 1H, $J_{6,1'} = 1.1$ Hz, H-6), 8.14 ppm (s, 1H, H-2); ^{13}C NMR (125.7 MHz, D_2O): $\delta = 63.98$ (d, $J_{C,P} = 5.4$ Hz, CH_2-5′), 69.10 (dd, $J_{C,F} = 26.7$ and 18.3 Hz, CH-3′), 75.86 (CH≡**C**), 79.70 (bt, $J_{C,F} = J_{C,P} = 8.6$ Hz, CH-4′), 83.54 (dd, $J_{C,F} = 40.5$ and 24.1 Hz, CH-1′), 97.43 (C-5), 100.67 (**C**H≡C), 103.20 (C-4a), 122.67 (dd, $J_{C,F} = 261.7$ and 255.4 Hz, C-2′), 128.43 (CH-6), 148.52 (C-7a), 151.11 (CH-2), 156.29 ppm (C-4); ^{19}F NMR (470.3 MHz, D_2O): $\delta = -115.13$ and -114.10 ppm (2×d, 2×1F, $J_{gem} = 238.4$ Hz); ^{31}P NMR (202.3 MHz, D_2O): $\delta = -21.75$ (t, 1P, $J_{\beta,\alpha} = J_{\beta,\gamma} = 19.4$ Hz, P_β), -10.27 (d, 1P, $J_{\alpha,\beta} = 19.4$ Hz, P_α), -9.20 ppm (d, 1P, $J_{\gamma'\beta} = 19.4$ Hz, P_γ); MS (ESI−) *m/z* (%): 571 (15) [*M* + Na − H], 549 (100) [*M* − H]; HRMS-ESI [*M* − H]$^-$ calcd for $C_{13}H_{14}F_2N_4O_{12}P_3$: 548.97946, found: 548.97967.

Gemcitabine triphosphate sodium salt (4-TP): Dry $POCl_3$ (12 μL, 0.12 mmol) was added to a solution of gemcitabine hydrochloride (**4**, 19 mg, 0.06 mmol) and proton sponge (13 mg, 0.06 mmol) in dry trimethyl phosphate (1 mL) at 0 °C under argon atmosphere. The reaction mixture was stirred at 0 °C for 24 h. Next, an ice-cold solution of $(NHBu_3)_2H_2P_2O_7$ (173 mg, 0.32 mmol) and tri(*n*-butyl)amine (100 μL, 0.42 mmol) in anhydrous DMF (1 mL) was added. The reaction mixture was stirred at 0 °C for 45 min. The reaction was quenched with aqueous solution of TEAB (2 M, 1 mL) and the mixture was concentrated under reduced pressure. The residue was co-evaporated five times with H_2O. The product was purified on DEAE Sephadex column (0–1.2 M TEAB gradient). The obtained triphosphate was converted into the sodium salt using Ionex (Dowex 50WX8 in Na^+ cycle) and freeze-dried from H_2O. Compound **4-TP** was obtained as white solid (14 mg, 37%). NMR data are in accord with published values.[14] ^{1}H NMR (500 MHz, D_2O): 4.15–4.20 (m, 1H), $\delta = 4.34$–4.38 (m, 2H), 4.53–4.63 (m, 1H), 6.14 (d, 1H, $J = 7.7$ Hz), 6.25 (t, 1H, $J = 7.2$ Hz), 7.90 ppm (d, 1H, $J = 7.6$ Hz); ^{31}P NMR (202.3 MHz, D_2O): $\delta = -21.54$ (t, 1P, $J = 19.5$ Hz), -11.10 (d, 1P, $J = 19.5$ Hz), -6.21 ppm (d, 1P, $J = 20.1$ Hz).

Primer extension experiments with bacterial DNA polymerases: The reaction mixture for PEX (20 μL) contained DNA polymerase (Deep Vent(exo$^-$) (2 U μL^{-1}, 0.109 μL), Vent(exo$^-$) (2 U μL^{-1}, 0.109 μL), KOD XL (2.5 U μL^{-1}, 0.087 μL), or Pwo (1 U μL^{-1}, 2.0 μL)), natural dNTPs (4 mM, 0.5 μL), functionalized triphosphate **11c-TP**, **11g-TP** (4 mM, 1 μL), or **11h-TP** (4 mM, 2 μL), 5′-^{32}P-labeled primer (3 μM, 0.72 μL, Prim^{248short}: 3′-GGG TAC GGC GGG TAC-5′), and 31-mer template (3 μM, 1.09 μL, Temp^{Prb4basII}: 5′-CTA GCA TGA GCT CAG TCC CAT GCC GCC CAT G-3′) in the buffer (2 μL) supplied by the manufacturer with the enzyme (ThermoPol buffer for DeepVent(exo$^-$) and Vent(exo$^-$), KOD XL buffer, or Pwo buffer). The primer was labeled using [γ^{32}P]ATP according to standard techniques.[15] Reaction mixtures were incubated at 60 °C for 20 min (for KOD XL the incubation time was only 10 min). The reactions were stopped by the addition of PAGE stop solution (40 μL, 80% *v/v* formamide, 20 mM EDTA, 0.025% *w/v* bromophenol blue, 0.025% *w/v* xylene cyanol) and heated at 95 °C for 5 min. Aliquots (4 μL) were subjected to vertical electrophoresis in 12.5% denaturing polyacrylamide gel containing 1× TBE buffer (pH 8) and 7 M urea at 45 mA for 50 min. The gels were dried (85 °C 70 min), audioradiographed and visualized by phosphorimager (Typhoon 9410, Amersham Biosciences).

Primer extension experiments with bacterial DNA polymerases for MALDI-TOF analysis: The reaction mixture (100 μL) contained Vent(exo$^-$) DNA polymerase (2 U μL^{-1}, 1 μL), primer Prim$^{248\ short}$ (10 μM, 10 μL), 5′-biotinylated template Temp$^{Prb4basII-bio}$ (10 μM, 15 μL), natural dNTPs (4 mM, 5 μL) and the modified dNTP **11c-TP**, **11g-TP** or **11h-TP** (4 mM, 10 μL) in ThermoPol reaction buffer (10 μL). The reaction mixture was incubated at 60 °C for 20 min in a thermal cycler. The reaction was stopped by cooling to 4 °C. Streptavidin magnetic particles (Roche, 500 μL) were washed with binding buffer TEN_{100} (10 mM Tris, 1 mM EDTA, 100 mM NaCl, pH 7.5; 3× 750 μL). The reaction mixture after PEX was diluted with the binding buffer TEN_{100} (100 μL), the solution was added to the prewashed magnetic beads and incubated for 20 min at 18 °C and 1200 rpm. After the incubation, the magnetic beads were collected on a magnet (PureProteome Magnetic Stand, Merck) and the solution was discarded. The beads were washed successively with wash buffer TEN_{1000} (10 mM Tris, 1 mM EDTA, 1 M NaCl, pH 7.5; 2× 750 μL), and water (3×750 μL). Then water (50 μL) was added and the sample was denatured for 2 min at 65 °C and 900 rpm. The beads were collected on a magnet and the solution was transferred into a clean vial. The product was analyzed by MALDI-TOF mass spectrometry.

Primer annealing: The radiolabeled primer (Prim^{248short}: 3′-GGG TAC GGC GGG TAC-5′) was mixed with the template (Temp^{MonoT}: 5′-CTA GCA TGA GCT CAG ACC CAT GCC GCC CAT G-3′, 1.5-fold excess) in aqueous Tris·HCl (pH 7.5, 50 mM), DTT (5 mM), $MgCl_2$ (5 mM), so that the final primer concentration was 1.1 μM. The annealing was performed in a thermal cycler. The sample was first heated at 95 °C for 5 min and then slowly cooled to 25 °C over 60 min. Thus prepared primed template was stored at −20 °C.

Primer extension experiments using human DNA polymerase α: The reaction mixture (10 μL) contained primed template (0.1 pM primer, 0.15 pM template), human DNA polymerase α (4 U μL^{-1}, 0.5 U), BSA (0.1 mg mL^{-1}), glycerol (10%), Tris·HCl (pH 7.5, 50 mM), DTT (5 mM), $MgCl_2$ (5 mM), natural dNTPs (100 μM) and/or modified dNTP (200 μM). The reactions were carried out at 37 °C for 60 min and were stopped by the addition of 20 μL PAGE stop solution (80% *v/v* formamide, 20 mM EDTA, 0.025% *w/v* bromophenol blue, 0.025% *w/v* xylene cyanol) and heated at 95 °C for 5 min. Aliquots (4 μL) were subjected to vertical electrophoresis in 12.5% denaturing polyacrylamide gel containing 1× TBE buffer (pH 8) and 7 M urea at 45 mA for 50 min. The gels were dried (85 °C 70 min),

audioradiographed and visualized by phosphorimager (Typhoon 9410, Amersham Biosciences).

*Inhibition of human polymerase α with **11 c-TP, 11 g-TP** and **11 h-TP***: The reaction mixture (10 μL) contained primed template (0.1 pM primer, 0.15 pM template), human DNA polymerase α (4 U μL^{-1}, 0.5 U), BSA (0.1 mg mL^{-1}), glycerol (10%), Tris·HCl (pH 7.5, 50 mM), DTT (5 mM), $MgCl_2$ (5 mM), all four natural dNTPs (100 μM) and the modified dNTP (**11 c-TP**, **11 g-TP** or **11 h-TP**) at the given concentration (5–900 μM). The reactions were carried out at 37 °C for 60 min and were stopped by adding 20 μL PAGE stop solution and heating at 95 °C for 5 min. Aliquots (4 μL) were subjected to electrophoresis in 12.5% denaturing polyacrylamide gel.

IC_{50} evaluation: After autoradiography, the band intensities in the sequencing gels were quantified using ImageJ program.[16] Because of low intensities of the full-length products, the intensities of the primer bands were considered. The primer intensities obtained with varied concentrations of **11 g-TP** in the reactions were plotted against log(*c*) of the triphosphate in GraphPad program.[17]

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