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FULL PAPER

Synthesis and biological evaluation of pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides with a ribose, 2'-deoxyribose and 2',3'-dideoxyribose sugar moiety

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Abstract: The synthesis of hitherto unknown pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides is described. Structural variations (chlorine, bromine, iodine, and cyano) were introduced at position 7 of 4-aza-7,9-dideazaadenine. In addition, pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides bearing a 2'-deoxy-, 2',3'-dideoxy-, and 2',3'-dehydrodideoxyribose moiety were also prepared. Among these analogues, the pyrrolo[2,1-f][1,2,4]triazine *C*-ribonucleosides with either a proton or cyano at position 7 of the nucleobase displayed potent cytotoxic activity in a panel of different cancer cell lines.

Introduction

The nucleoside analogues currently marketed for the treatment of different cancers and viral infections are all *N*-nucleosides, in which the heterocyclic aglycone and the carbohydrate moiety are joined together by a carbon–nitrogen bond.[1] However, *N*-nucleosides are liable to enzymatic and acid-catalyzed hydrolysis of their glycosidic bond. In contrast, *C*-nucleosides, having the sugar and heterocyclic aglycon connected by a C–C rather than a C–N bond, are resistant to hydrolytic and enzymatic cleavage. Since the discovery of the first *C*-nucleoside pseudouridine, a number of biologically interesting *C*-nucleosides have been described in the literature, as summarized in a recent review paper.[2]

In recent years, much efforts have been devoted to the synthesis of *C*-nucleosides featuring 4-aza-7,9-dideazaadenine (or pyrrolo[2,1-f][1,2,4]triazine-4-amine) as nucleobase. This particular heterocycle has been coupled to a variety of sugars giving rise to novel *C*-nucleosides (Figure 1). The ribose containing congener, also known as 4-aza-7,9-dideazaadenosine (compound **1**), exhibited a pronounced growth inhibitory activity against several mouse and human neoplastic cell lines.[3] Compound **2** with a 2'*C*-Me ribose carbohydrate moiety displayed excellent *in vitro* activity against different genotypes of HCV, but was not selected as clinical candidate due to its adverse effects in rat safety studies.[4] 2'-Deoxy-2'-α-fluoro-4'-α-cyano-4-aza-7,9-dideazaadenosine (compound **3**) has been shown to be a potent inhibitor of RSV replication.[5] A double phosphoramidate and ester prodrug of 1′-cyano-2′-*C*-methyl-4-aza-7,9-dideazaadenosine (GS-6620, compound **4**) demonstrated excellent activity against HCV, eventually reaching phase I clinical trials.[6] A *C*-nucleoside with a 1′-cyano-ribose moiety and 4-aza-7,9-dideazaadenine as nucleobase (GS-5734, compound **5**) has been evaluated in a rhesus monkey model of ebolavirus infection. Parental treatment with GS-5734 yielded a substantial antiviral effect along with 100% survival. Safety and pharmacokinetics of GS-5734 have been evaluated in phase I clinical trials.[7]

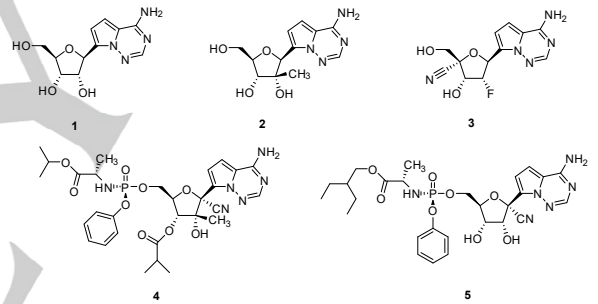

Figure 1. Biologically active *C*-nucleosides with 4-aza-7,9-dideazaadenine as nucleobase.

Overall, 4-aza-7,9-dideazaadenine seems to be a privileged nucleobase in the design of novel *C*-nucleosides with promising biological activity. However, structural variations of the 4-aza-7,9-dideazaadenine moiety (e.g., substitution at position 7[8]) or coupling with other sugars (such as 2'-deoxyribose[9] and 2',3'-dideoxyribose[10]) that are known to deliver biologically active nucleosides have not been carried out. In this study, the synthesis as well as the biological evaluation in relevant assays (either antitumoral or antiviral) of novel pyrrolo[2,1-f][1,2,4]triazine-4-amine *C*-nucleosides is described.

Results and Discussion

Chemistry

4-Aza-7,9-dideazaadenosine (compound **1**) has been synthesized before, using a linear sequence strategy that builds up the heterocycle on a protected D-ribose derivative.[3] Herein, we opted for a convergent approach, whereby the nucleobase is synthesized separately and then coupled with the desired sugar moiety. In initial attempts, 4-aza-7-deaza-9-bromo-adenine **8**, which was synthesized according to a literature procedure,[11] was selected as coupling partner (Scheme 1). The hydroxyl groups of

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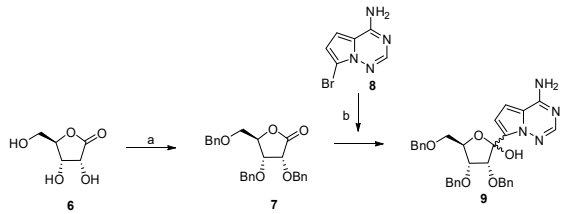
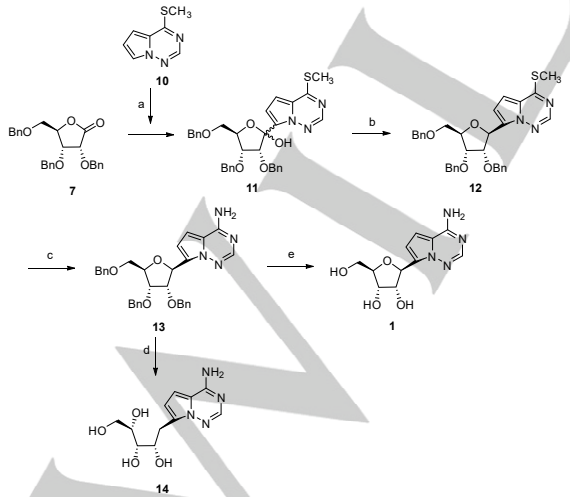
ribolactone **6** were fully protected as benzyl ethers using benzyl 2,2,2-trichloroacetimidate under acidic conditions.[12] Nucleophilic addition of the lithium salt of heterocycle **8** to tribenzyl lactone **7** afforded compound **9** as an anomeric mixture. In our hands, this reaction proceeded with yields between 10 and 30%, which are lower than the reported yield of 38%. A literature report indicates that the transient protection of the exocyclic amino group as its stabase adduct led to an improvement of the yield to 60%.[12]



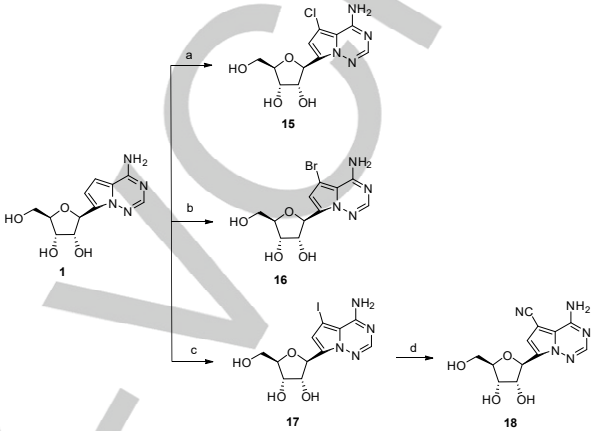
Scheme 1. Synthesis of *C*-nucleoside **9**. *Reagents and conditions*: a) $CCl_3C(NH)OBn$, CF_3SO_3H, dioxane, 0 °C, 3 h, 80%; b) *n*BuLi, THF, -78 °C, 3 h, 10-30%.

However, we decided to switch to an alternative nucleobase rather than to protect the exocyclic amino group. Thus, 4-(methylthio)pyrrolo[2,1-fl[1,2,4]triazine **10** was synthesized according to a literature procedure.[13] Formation of the corresponding lithium species by treatment with lithium diisopropylamide was followed by coupling with ribolactone **7**, which afforded lactol **11** as a mixture of anomers in 80% yield. Anomeric reduction of hemiacetal **11** using triethylsilane and boron trifluoride etherate afforded stereoselectively the β-anomer **12**.[12] Amine displacement of the thiomethyl group furnished the benzyl protected *C*-nucleoside **13**. In the last step of the synthesis, *O*-debenzylation was achieved by hydrogenolysis catalyzed by palladium hydroxide on carbon ($Pd(OH)_2/C$). The acid used turned out to play a crucial role in the outcome of this reaction. When a concentrated aqueous hydrochloric acid solution was used, the ring-opened, debenzylated analogue **14** was isolated in 95% yield. On the other hand, when using acetic acid as solvent, only debenzylation of the sugar hydroxyl groups was observed, leaving the ribose ring intact and furnishing the desired *C*-nucleoside **1** in 96% yield.



Scheme 2. Synthesis of 4-aza-7,9-dideazaadenosine. *Reagents and conditions*: a) LDA, THF, -78 °C, 3 h, 60%; b) $BF_3.OEt_2$, triethylsilane, CH_2Cl_2, 0 °C, 40 min, 73%; c) 7 M NH_3 in MeOH, 100 °C, 24 h, 90%; d) $Pd(OH)_2/C$, H_2, conc. HCl, MeOH/EtOAc, rt, 48 h, 95%; e) $Pd(OH)_2/C$, H_2, CH_3COOH, rt, 48 h, 96%.

C-nucleoside **1** was used to introduce different structural modifications at position 7 (purine numbering) of the nucleobase (Scheme 3). Halogens (chlorine, bromine, and iodine) were easily introduced by treatment with a suitable *N*-halo-succinimide affording 7-halo *C*-nucleosides **15**-**17** in 20-43% yields. The rather moderate yields and sometimes long reaction times are in agreement with the reaction conditions for similar substrates.[14] The 7-iodo derivative **17** was submitted to a palladium-catalyzed cross-coupling reaction with zinc cyanide, affording the 7-cyano derivative **18**.



Scheme 3. Structural variation at position 7. *Reagents and conditions*: a) NCS, DMF, 0 °C to rt, 2 days, 20 %; b) NBS, DMF, 0 °C to rt, 1 h, 25%; c) NIS, DMF, 0 °C to rt, 4 days, 43%; d) (i) Zn(0), $Zn(CN)_2$, $Pd(P(tBu_3))_2$, DMA, 150 °C, 1 h; (ii) PS-TPP, 21 h, 13% over 2 steps.

Pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides with a 2'-deoxy-ribose, 2',3'-dideoxyribose and 2',3'-dehydro-dideoxyribose carbohydrate moiety are hitherto unknown (Scheme 4). The Heck-type coupling between an anomeric carbon center and a nucleobase is the most popular method for the synthesis of 2'-deoxy-*C*-nucleosides owing to its excellent regio- and stereoselectivity.[15] Bis(dibenzylideneacetone)Pd(0) is normally used as a source of palladium, while Ph_3As is the ligand of choice and usually an organic base is used (e.g., tri-*n*-butylamine). The palladium-catalyzed Heck coupling of pyrrolo[2,1-f][1,2,4]triazines with glycals has not been reported before in the literature. The protected, iodinated pyrrolo[2,1-f][1,2,4]triazine **20** was obtained by electrophilic iodination of **19**,[16] followed by reaction with benzoyl chloride. Heck coupling between glycal **22**, obtained in turn from thymine **21** by the Pedersen's method,[17] and nucleobase **20** gave the desired compound **23** in a stereoselective and regioselective way. Sodium triacetoxyborohydride mediated reduction of the 3'-keto moiety afforded the 2'-deoxyribofuranosyl *C*-nucleoside **24**. Finally, alkaline deprotection of the benzamide moiety furnished the 2'-deoxy-*C*-nucleoside **25**. At this stage of the synthesis, the β-configuration of the nucleobase was determined by NMR analysis of the NOE interaction of H-2' to the H-8 of the nucleobase and H-5' of the sugar moiety. The observed vicinal coupling constant couplings of 10.8 and 5.6 Hz for H-1' (at 5.66 ppm) to H-2' and H-2", respectively, are characteristic for a predominant C2' endo conformation of the five-membered sugar ring. The correct stereochemistry of the 3'-hydroxyl group was confirmed by the vicinal couplings of 1.8 Hz for H-3' (at 4.45 ppm) to H-2" and H-4'. In order to have access to the 2',3'-dideoxy derivative **29**, the primary hydroxyl group of compound **24** was protected as a TBDPS group. Mesylation of the secondary hydroxyl group of **26**

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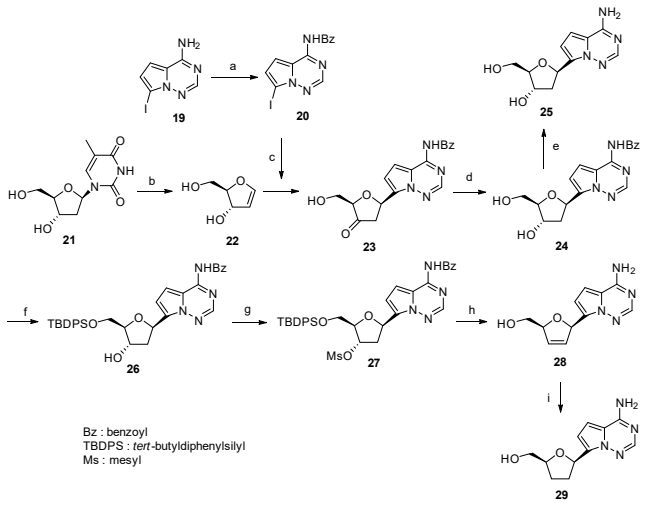
afforded compound **27**. Base promoted elimination, with concomitant deprotection of the TBDPS and benzoyl groups, yielded the 2',3'-dehydrodideoxy-*C*-nucleoside analogue **28**. Finally, catalytic hydrogenation of the double bond gave rise to the 2',3'-dideoxy analogue **29**.



Scheme 4. Synthesis of 2'-deoxy- and 2',3'-dideoxyribosyl *C*-nucleosides. *Reagents and conditions*: a) (i) NIS, DMF, -20 °C, 3 h; (ii) BzCl, pyridine, rt, 12 h, 70% over 2 steps; b) HMDS, $(NH_4)_2SO_4$, 130 °C, 2 h, 50%; c) bis(dibenzylideneacetone)Pd(0), Ph_3As, tri-*n*-butylamine, DMF, 100 °C, 7 h, 60%; d) sodium triacetoxyborohydride, CH_3CN, rt, 3 h, 75%; e) 7 M NH_3 in MeOH, rt, 12 h, 90%; f) TBDPSCl, imidazole, pyridine, rt, 12 h, 60%; g) MsCl, pyridine, rt, overnight, 75%; h) (i) KO*t*Bu, DMSO, 2 h; (ii) 7 M NH_3 in MeOH, rt, overnight, 35% over 2 steps; e) Pd/C, H_2, MeOH, rt, 24 h, 50%.

Antiviral and antitumoral screening

4-Aza-7,9-dideazaadenosine **1** is a known cytotoxic nucleoside analogue, that has been profiled before against two mouse tumor cell lines (L1210, a leukemia cell line, and S-180, a sarcoma cell line) and one human promyelocytic cell line (HL-60). It displayed low nanomolar activity against these three cell lines.[3] In the current study, in order to explore the potential of compound **1** as anticancer drug, it was tested against a panel of eight different human cancer lines, representing solid tumor types including pancreatic adenocarcinoma (Capan-1), colorectal carcinoma (HCT-116), and lung carcinoma (NCI-H460), as well as hematological tumors such as HL-60 (acute myeloid leukemia), K-562 (chronic myeloid leukemia), Z-138 (non-Hodgkin lymphoma), MM.1S (multiple myeloma), and DND-41 (acute lymphoblastic leukemia). The inhibition of cell proliferation of these different cancer cell lines was monitored in real-time over a period of 3 days using the IncuCyte live-cell imager that uses automated imaging to determine cellular confluence as a measure of cellular viability. The growth curves clearly demonstrate a differential effect of the compounds on the growth of the 8 cancer cell lines, with especially compounds **1** and **18** displaying potent cytotoxic activity (Figure 2). The compound concentrations required to inhibit tumor cell viability by 50% (EC_{50} values) after 72 h of incubation were calculated (Table 1).

These results confirm the potent cytotoxicity of **1** against the HL-60 cell line. In addition, compound **1** also displayed low nanomolar activity against the other hematological cancers as well as the solid tumors. Among the 7-substituted analogues, the 7-cyano derivative **18** emerged as the most active. It is endowed with EC_{50} values ranging from 8 to 300 nM and possessed a similar profile as its 7-unsubstituted congener **1**. The 7-iodo congener **17** exhibited a different antitumoral profile than **1**. It had very potent antitumoral activity against the Z-138 and MM.1S, cell lines, whereas it resulted less active in the other cell lines. The 7-chloro derivative **15** was the least active among the 7-substituted congeners. It is 30- to 460-fold less potent (depending on the cell line studied), when compared to the unsubstituted analogue **1**. The 7-bromo analogue **16** is endowed with intermediate anticancer activity, displaying EC_{50} values of less than 1 µM against five of the cancer cell lines (Capan-1, HCT-116, Z-138, MM.1S, and DND-41) (IC_{50} values around 0.8 µM), whereas its activity against the other three cell lines is in the range of 1.7–3.7 µM.

Different marketed antitumoral nucleosides contain a 2'-deoxyribose sugar moiety. Examples include cladribine, floxuridine, and decitabine.[9] These compounds share a similar mechanism of action. They are intracellularly converted to their respective nucleotide analogues, which interfere with DNA synthesis by inhibition of crucial enzymes involved in DNA replication (such as DNA polymerase, ribonucleotide reductase, thymidylate synthase, and DNA methyltransferase). In order to exploit this mechanism of action, 2'-deoxy-*C*-nucleoside **25** was prepared and investigated for its antitumoral activity. Compound **25** completely lacks activity, displaying EC_{50} values of more than 10 µM, against all tumor cell lines tested, except in the MM.1S cell line, where it is endowed with low µM activity. This suggests that either compound **25** is not phosphorylated or alternatively, once phosphorylated, it does not inhibit one of the enzymes involved in DNA metabolism.

2',3'-Dideoxynucleosides (e.g., didanosine, zalcitabine) and 2',3'-dehydro-dideoxynucleosides (e.g., abacavir and stavudine) are established anti-HIV agents.[10] Therefore, 2',3'-dideoxy-*C*-nucleoside **29** and its 2',3'-unsaturated analogue **28** were also assayed for anti-HIV activity, but were found to completely lack activity against HIV-1 (EC_{50} > 203 µM for compound **28**, and EC_{50} > 534 µM for compound **29**).

Conclusions

In this study, the synthesis of two novel series of pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides was described. The first set focused on structural variations at position 7 of the 4-aza-7,9-dideazaadenine nucleobase coupled to a D-ribose as sugar moiety. The unsubstituted congener **1** as well as the 7-cyano analogue **18** displayed potent cytotoxic activity against a panel of human hematological and solid cancer cell lines. These compounds deserve to be further evaluated as anticancer agents by testing in appropriate animal models. The exact molecular target of these compounds is currently unknown and should be the subject of future biochemical studies. The second series encompassed the structural modification of the carbohydrate moiety. The 2'-deoxyribose, 2',3'-dideoxyribose, and 2',3'-dehydrodideoxynucleoside derivatives completely lacked antitumoral or antiretroviral activity.

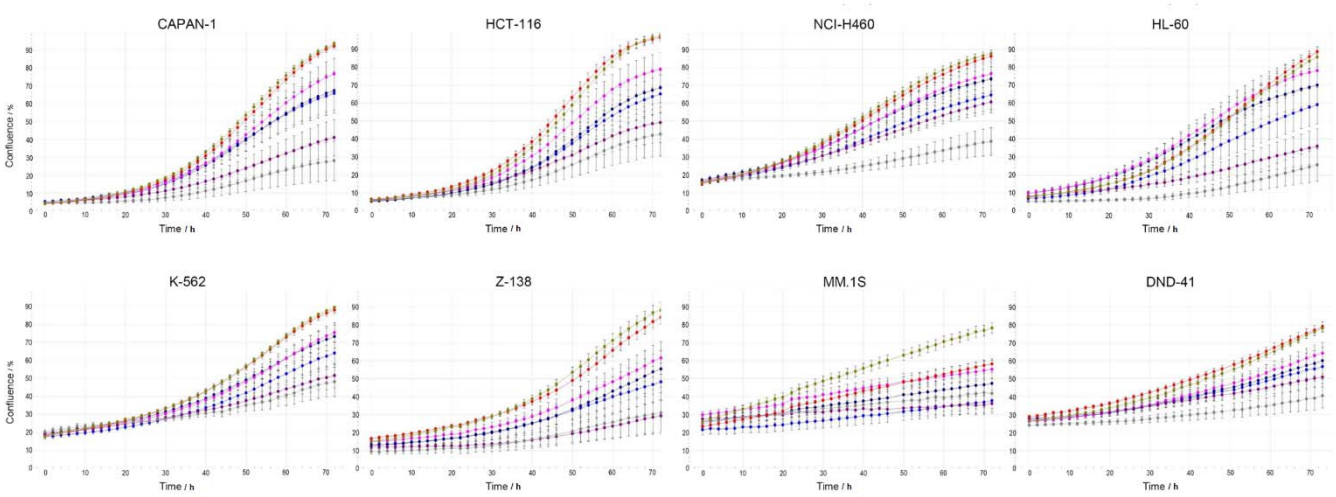


Figure 2. Effect of *C*-nucleosides **1**, **15-18**, and **25** on the proliferation of different cancer cell lines, as measured by real-time imaging. Data are expressed as the mean percent cell confluence over the different compound concentrations at each time point (error bars are sd). Treatments are: compound **1** (grey), compound **15** (pink), compound **16** (dark blue), compound **17** (light blue), compound **18** (purple), compound **25** (red), and DMSO as negative control (brown).

Table 1. Cytotoxic activity of *C*-nucleosides **1**, **15-18**, and **25** against tumor cell lines

Cmpd	EC_{50} (μM)[a]							
	Capan-1	**HCT-116**	**NCI-H460**	**HL-60**	**K-562**	**Z-138**	**MM.1S**	**DND-41**
1	0.008 ± 0.0007	0.031 ± 0.006	0.028 ± 0.003	0.010 ± 0.0007	0.042 ± 0.004	0.009 ± 0.0002	0.019 ± 0.007	0.018 ± 0.005
15	3.699 ± 0.351	2.491 ± 0.763	4.493 ± 0.202	4.580 ± 0.209	3.650 ± 0.284	0.458 ± 0.113	2.576 ± 0.064	2.287 ± 0.076
16	0.875 ± 0.057	0.796 ± 0.133	3.659 ± 0.124	3.096 ± 1.268	1.75 ± 0.564	0.199 ± 0.114	0.484 ± 0.079	0.832 ± 0.025
17	0.753 ± 0.007	0.766 ± 0.010	0.810 ± 0.469	0.863 ± 0.018	1.239 ± 0.074	0.048 ± 0.033	0.038 ± 0.037	0.747 ± 0.010
18	0.023 ± 0.003	0.043 ± 0.004	0.327 ± 0.088	0.023 ±0.002	0.035 ± 0.003	0.008 ± 0	0.014 ± 0.004	0.073 ± 0.002
25	>10	>10	>10	>10	>10	>10	3.219 ± 1.972	>10

[a] EC_{50} is the compound concentration required to inhibit tumor cell viability by 50%.

Experimental Section

General

All reagents and solvents were purchased from commercial sources and used as obtained. Moisture sensitive reactions were carried out using oven-dried glassware under a nitrogen or argon atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or 500 MHz spectrometer with tetramethylsilane as internal standard or referenced to the residual solvent signal. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets. Coupling constants are expressed in Hz. A 2D [^{1}H,^{1}H] NOESY (500 MHz, MeOD) spectrum was recorded with a sweep width of 5000 Hz in both dimensions and a mixing time of 300 ms. The spectrum was acquired with 8 scans, 4096 data points in t2, and 512 FIDs in t1. The data were apodized with a shifted sine-bell square function in both dimensions and processed to a 4kx1k matrix. High-resolution mass spectra (HRMS) were obtained on a quadruple orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min, and spectra were obtained in positive (or negative) ionization mode with a resolution of 15 000 (fwhm) using leucine enkephalin as the lock mass. Pre-coated aluminum sheets (254 nm) were used for TLC and spots were visualized with UV light. The products were purified by flash column chromatography on silica gel (60 Å, 0.035−0.070 mm, Acros Organics).

2,3,5-Tri-*O*-benzyl-D-ribono-1,4-lactone (7). Benzyl 2,2,2-trichloroacetimidate (17 mL, 91.15 mmol) and trifluoromethanesulfonic acid (0.178 mL, 2.03 mmol) were added at 0 °C to a solution of D-(+)-ribono-1,4-lactone **6** (3 g, 20.25 mmol) in dry dioxane (50 mL) under an argon atmosphere. The solution was stirred for 3 h, and then quenched with an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered, and evaporated. Flash column chromatography of the crude residue (heptane/EtOAc = 15:1) afforded the title compound as a yellow oil (6.5 g, 80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.37-7.28 (m, 13H), 7.18-7.12 (m, 2H), 4.92 (d, *J* = 11.8 Hz, 1H), 4.69 (t, *J* = 11.8 Hz, 2H), 4.56-4.35 (m, 5H), 4.08 (dd, *J* = 5.5, 1.7 Hz, 1H), 3.64 (dd, *J* = 11.1, 2.8 Hz, 1H), 3.53 (dd, *J* = 11.1, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 174.1, 137.5, 137.4, 137.2, 129.0, 128.8, 128.7, 128.7, 128.6, 128.4, 128.4, 128.3, 128.0, 127.9, 82.1, 75.7, 74.1, 73.9, 73.1, 72.7, 69.0.

(3*R*,4*R*,5*R*)-2-(4-Aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (9). To a solution of compound **8** (150 mg, 0.704 mmol) and compound **7** in THF (100 mL) was slowly added n-Buli (1.6 M in hexane, 1.45 mL, 2.23 mmol) at -78 °C under an argon atmosphere. The resulting mixture was stirred for 3 h at -78 °C. The reaction was quenched by adding a saturated NH_4Cl

solution and then extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 100:1) to give the title compound as a mixture of anomers (110 mg, 28% yield). HR-MS: calcd for $C_{32}H_{32}N_4O_5$ ([M+H]$^+$) 575.2265, found 575.2272.

(3*R*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)-2-(4-(methylthio)pyrrolo[2,1-f] [1,2,4]triazin-7-yl)tetradrofuran-2-ol (11). To a solution of 4-(methylthio)pyrrolo[2,1-f][1,2,4]triazine **10** (100 mg, 0.605 mmol) in THF (5 mL) was added lithium diisopropylamide (2 M in THF, 0.45 mL, 0.907 mmol) at -78 °C under an argon atmosphere. The resulting mixture was stirred for 30 min at -78 °C. Then, a solution of compound **7** (253 mg, 0.605 mmol) in THF (4 mL) was added at -78 °C and the resulting reaction mixture was stirred for 3 h at -78 °C. The reaction was quenched by adding a saturated NH_4Cl solution and then extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by silica gel flash chromatography (heptane/EtOAc = 5:1) to give the title compound as a yellow oil (219 mg, 60% yield).

7-((2*S*,3*S*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)-tetrahydrofuran-2-yl)-4-(methylthio)pyrrolo[2,1-f][1,2,4]triazine (12). To a solution of compound **11** (130 mg, 0.222 mmol) in dichloromethane (5 mL) was added triethylsilane (0.14 mL, 0.890 mmol) and trifluoroborane (0.056 mL, 0.445 mmol) at 0 °C under an argon atmosphere. The resulting solution was stirred for 40 min at 0 °C and then quenched by a saturated aqueous Na_2CO_3 solution. The mixture was extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by flash chromatography (heptane/EtOAc = 10:1) to give the title compound as a yellow oil (110 mg, 87% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.18 (s, 1H), 7.43-7.21 (m, 15H), 6.80 (d, *J* = 4.7 Hz, 1H), 6.68 (d, *J* = 4.7 Hz, 1H), 5.70 (d, *J* = 4.1 Hz, 1H), 4.72 (s, 2H), 4.65-4.37 (m, 5H), 4.25 (t, *J* = 4.7 Hz, 1H), 4.12 (t, *J* = 4.9 Hz, 1H), 3.78 (dd, *J* = 10.5, 2.9 Hz, 1H), 3.66 (dd, *J* = 10.5, 3.5 Hz, 1H), 2.65 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 165.1, 145.4, 138.6, 138.2, 138.1, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 126.2, 112.5, 102.2, 81.0, 79.2, 76.3, 73.7, 72.3, 71.9, 70.0, 11.7; HR-MS: calcd for $C_{33}H_{33}N_3O_4S_1$ ([M+H]$^+$) 568.6624, found 568.2270.

7-((2*S*,3*S*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)-tetrahydrofuran-2-yl)pyrrolo[2,1-f][1,2,4]triazin-4-amine (13). A solution of compound **12** (73 mg, 0.128 mmol) in a 7 N NH_3 in methanol solution (20 mL) was stirred for 24 h at 100 °C. The solvent was evaporated under vacuum. The crude residue was purified by flash chromatography (heptane/ EtOAc = 5:1) to afford the title compound as yellow oil (62 mg, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.87 (s, 1H), 7.43-7.08 (m, 15H), 6.64 (d, *J* = 3.8 Hz, 1H), 6.47 (d, *J* = 4.5 Hz, 1H), 5.85 (br, 2H), 5.66 (d, *J* = 3.8 Hz, 1H), 4.71 (s, 2H), 4.62-4.35 (m, 5H), 4.26 (t, *J* = 4.2 Hz, 1H), 4.11 (t, *J* = 5.1 Hz, 1H), 3.76 (dd, *J* = 10.5, 3.0 Hz, 1H), 3.64 (dd, *J* = 10.5, 3.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 155.6, 147.2, 138.6, 138.3, 138.2, 129.9, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 115.0, 110.9, 100.3, 80.8, 79.25, 77.5, 76.5, 73.7, 72.3, 71.9, 70.1; HRMS: calcd for $C_{32}H_{32}N_4O_4$ ([M+H]$^+$) 537.2496, found 537.2491.

(2*S*,3*R*,4*S*,5*R*)-2-(4-Aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5(hydroxylmethyl)tetrahydrofuran-3,4-diol (1). To a solution of compound **13** (100 mg, 0.186 mmol) in acetic acid (10 mL) was added $Pd(OH)_2$ (10%) (100 mg) and the reaction mixture was stirred under a hydrogen atmosphere at 25 °C for 48 h. The mixture was filtered through a Celite pad and the filtrate was concentrated. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH = 8:1) affording the title compound as a white solid (46 mg, 95% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.82 (s, 1H), 7.69 (br, 2H), 6.84 (d, *J* = 4.4 Hz, 1H), 6.68 (d, *J* = 4.1 Hz, 1H), 5.11 (d, *J* = 6.3 Hz, 1H), 5.04-4.73 (m, 3H), 4.23 (t, *J* = 5.5 Hz, 1H), 3.95 (t, *J* = 4.2 Hz, 1H), 3.79 (q, *J* = 4.6 Hz, 1H), 3.60-3.40 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 155.7, 147.8, 129.1, 115.0, 109.8, 100.9, 84.6, 75.5, 73.9, 71.4, 62.2; HRMS: calcd for $C_{11}H_{14}N_4O_4$ ([M+H]$^+$) 267.1087, found 267.1094.

(3*S*,4*S*)-5-(4-Aminopyrrolo[1,2-f][1,2,4]triazin-6-yl)pentane-1,2,3,4-tetraol (14). To a solution of compound **13** (1 g, 1.86 mmol) in a mixture of methanol and ethyl acetate (4:1, 10 mL) was added $Pd(OH)_2$ (10%) (1 g) and a concentrated HCl solution (2 mL). The reaction mixture was stirred under a hydrogen atmosphere at 25 °C for 48 h. The mixture was filtered through a Celite pad and the filtrate was concentrated. The crude residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 8:1) to give the title compound (490 mg, 96% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.78 (s, 1H), 7.58 (br, 2H), 6.84 (d, *J* = 4.0 Hz, 1H), 6.50 (d, *J* = 4.4 Hz, 1H), 4.82 (d, *J* = 5.1 Hz, 1H), 4.70 (t, *J* = 6.8 Hz, 2H), 4.50 (t, *J* = 5.5 Hz, 1H), 4.01-3.91 (m, 1H), 3.64-3.50 (m, 2H), 3.47-3.41 (m, 2H), 3.18 (dd, *J* = 14.2, 5.0 Hz, 1H), 2.88 (q, *J* = 9.6 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 155.6, 147.5, 129.3, 113.6, 110.1, 100.9, 74.8, 73.0, 70.8, 63.4, 28.4; HR-MS: calcd for $C_{11}H_{16}N_4O_4$ ([M+H]$^+$) 269.1244, found 269.1252.

(2*R*,3*R*,4*S*,5*R*)-2-(4-Amino-5-chloropyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (15). A solution of compound **1** (110 mg, 0.413 mmol) in anhydrous DMF (6 mL) was cooled to 0 °C. *N*-Chlorosuccinimide (60.68 mg, 0.454 mmol) was added and the reaction mixture was stirred for 2 days at room temperature. The reaction mixture was concentrated and the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH = 10:1) furnishing the title compound as a white solid (25 mg, 20% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.84 (s, 1H), 6.83 (s, 1H), 5.12 (d, *J* = 6.4 Hz, 1H), 5.06 (d, *J* = 8.1 Hz, 1H), 4.91 (d, *J* = 4.8 Hz, 1H), 4.78 (t, *J* = 5.6 Hz, 1H), 4.15 (q, *J* = 5.6 Hz, 1H), 3.94 (q, *J* = 5.6 Hz, 1H), 3.79-3.77 (m, 1H), 3.57-3.43 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 155.1, 148.3, 129.5, 110.6, 109.8, 103.0, 84.6, 75.1, 74.3, 71.2, 61.9; HRMS: calcd for $C_{11}H_{13}ClN_4O_4$ ([M+H]$^+$) 301.0698, found 301.0706.

(2*R*,3*R*,4*S*,5*R*)-2-(4-Amino-5-bromopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (16). A solution of compound **1** (153 mg, 0.574 mmol) in anhydrous DMF (5 mL) was cooled to 0 °C. *N*-Bromosuccinimide (112.50 mg, 0.623 mmol) was added and the reaction was stirred for 1 h at room temperature. The reaction mixture was concentrated and purified by flash chromatography (CH_2Cl_2/MeOH = 10:1) to give the title compound as a white solid (50 mg, 25% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.81 (s, 1H), 6.90 (s, 1H), 5.12 (d, *J* = 7.2 Hz, 2H), 4.96 (d, *J* = 7.1 Hz, 1H), 4.80 (t, *J* = 5.6 Hz, 1H), 4.19 (q, *J* = 5.6 Hz, 1H), 3.96 (q, *J* = 4.9 Hz, 1H), 3.81-3.79 (m, 1H), 3.59-3.46 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 155.3, 148.2, 130.6, 112.6, 111.8, 87.0, 84.6, 75.1, 74.3, 71.2, 61.9; HRMS: calcd for $C_{11}H_{13}BrN_4O_4$ ([M+H]$^+$) 345.0193, found 345.0192.

(2*R*,3*R*,4*S*,5*R*)-2-(4-Amino-5-iodopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (17). A solution of compound **1** (630 mg, 2.37 mmol) in anhydrous DMF (15 mL) was cooled to 0 °C. *N*-Iodosuccinimide was added (612 mg, 2.74 mmol) and the reaction mixture was stirred for 4 days at room temperature. The reaction mixture was concentrated and the crude residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 10:1) affording the pure title compound as a slight yellow solid (400 mg, 43% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.88 (s, 1H), 6.97 (s, 1H), 5.10 (d, *J* = 4.8 Hz, 1H), 5.04 (d, *J* = 3.2 Hz, 1H), 4.90 (d, *J* = 4.8 Hz, 1H), 4.78 (t, *J* = 5.5 Hz, 1H), 4.18-4.14 (m, 1H), 3.94-3.78 (m, 1H), 3.78-3.77 (m, 1H), 3.48-3.40 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 155.6, 147.9, 132.1, 118.2, 114.1, 84.6, 75.1, 74.2, 71.2, 61.9, 52.4; HRMS: calcd for $C_{11}H_{13}IN_4O_4$ ([M+H]$^+$) 393.0056, found 393.0061.

4-Amino-7-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrrolo[2,1-f][1,2,4]triazine-5-carbonitrile (18). A solution of compound **17** (100 mg, 0.255 mmol) in anhydrous dimethylacetamide (3 mL) was evaporated and purged with argon. Zinc power (5.84 mg, 0.0892 mmol), bis(tri-*tert*-

butylphosphine)palladium(0) (17 mg, 0.0331 mmol), and zinc cyanide (93 mg, 0.102 mmol) were added. The resulting reaction mixture was stirred at 150 °C under an argon atmosphere for 1 h. The mixture was cooled to room temperature. Solid-phase bound triphenylphoshine (300 mg) was added and the mixture was stirred at room temperature for 21 h. The mixture was filtered through Celite and washed with EtOH. The filtrate was concentrated under reduced pressure and the crude residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 10:1) to give the title compound as a white solid (10 mg, 13% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 8.12 (s, 1H), 7.34 (s, 1H), 5.12 (d, *J* = 7.2 Hz, 2H), 4.96 (d, *J* = 7.1 Hz, 1H), 4.80 (t, *J* = 5.6 Hz, 1H), 4.19 (q, *J* = 5.6 Hz, 1H), 3.96 (q, *J* = 5.0 Hz, 1H), 3.81-3.79 (m, 1H), 3.59-3.46 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 154.9, 149.6, 131.5, 118.3, 115.7, 114.6, 84.6, 82.4, 75.2, 74.1, 71.0, 61.7; HRMS: calcd for $C_{12}H_{13}N_5O_4$ ([M+H]$^+$) 292.1040, found 292.1043.

***N*-(7-Iodopyrrolo[2,1-f][1,2,4]triazin-4-yl)benzamide (20).** To a solution of compound **19** (1.8 g, 6.92 mmol) in pyridine (30 mL) was added benzoyl chloride (0.964 mL, 8.31 mmol) at 0 °C. After stirring for 2 h at room temperature, the reaction was quenched by addition of methanol (10 mL). The solvent was evaporated and the residual pyridine was co-evaporated with toluene. After removal of all the volatiles, methanol (50 mL) was added, and a yellow solid precipitated, which was filtered and washed with methanol 5 times (each time 10 mL) to afford a yellow powder (2.3 g, 91%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 8.07 (s, 1H), 7.95 (dd, *J* = 8.2 and 7.8 Hz, 1H), 7.66-7.47 (m, 4H), 7.30 (dd, *J* = 3.9 and 4.6 Hz, 1H), 7.11 (s, 1H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 172.07, 146.90, 133.97, 133.13, 132.98, 129.39, 129.06, 128.69, 128.56, 123.75, 121.00, 106.76; HRMS: calcd for $C_{13}H_9I_1N_4O_1$ ([M+H]$^+$) 364.9895, found 364.9895.

1,4-Anhydro-2-deoxy-D-erythro-pent-1-enitol (22). A solution of thymidine **21** (4.48 g, 18.5 mmol), hexamethyldisilazane (25 mL), and ammonium sulfate (489 mg, 307 mmol) was heated at reflux for 2 h. The volatiles were evaporated in vacuo, and a mixture of ice and water was added to the residue until precipitation of a gummy solid. Cold dichloromethane was added, and the solid was filtered off and washed with cold dichloromethane. The organic layer was recovered after a rapid and cold extraction. The extraction was performed two more times using cold CH_2Cl_2. The organic layers were combined, washed with cold brine, dried over Na_2SO_4, and concentrated under reduced pressure. The brown oily residue (containing the silylated intermediate) was used without further purification. The crude residue was dissolved in THF (10 mL) and a TBAF solution (1 M in THF, 12.25 mL, 12.25 mmol) was added. The solution was stirred at room temperature for 3 h. The solvent was evaporated and the crude residue was purified by chromatography (eluent Et_2O/acetone = 5:1) to afford the title compound as a colorless oil (900 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 6.55 (s, 1H), 5.05-4.96 (m, 2H), 4.85 (t, *J* = 10.9 Hz, 1H), 4.56 (s, 1H), 4.13-4.05 (m, 1H), 3.37-3.26 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 148.5, 104.1, 89.3, 73.8, 61.6.

***N*-(6-((2*S*,5*R*)-5-(Hydroxymethyl)-4-oxotetrahydrofuran-2-yl)pyrrolo[1,2-f][1,2,4]triazin-4-yl)benzamide (23).** Bis(dibenzylideneacetone)palladium(0) (16 mg, 0.028 mmol) and triphenylarsine (17 mg, 0.057 mmol) were both dissolved in DMF (3 mL) and stirred under a nitrogen atmosphere at 25 °C for 15 min. This solution was then transferred by a syringe to another solution of compound **22** (207 mg, 1.79 mmol), compound **20** (130 mg, 0.357 mmol), and tri-*n*-butylamine (0.099 mL, 0.417 mmol) in DMF (10 mL). The reaction mixture was stirred under a nitrogen atmosphere at 100 °C for 18 h. After cooling to room temperature, the solution was extracted with ethyl acetate. The organic layer was washed with water (3 times), brine (3 times), and dried over Na_2SO_4. The crude residue was purified by silica gel column chromatography (EtOAc/heptane = 1:2 to 1:1) to yield the title compound as a yellow solid (75 mg, 60% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.28 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.69-7.50 (m, 3H), 7.18 (d, *J* = 4.6 Hz, 1H), 7.12 (d, *J* = 4.6 Hz, 1H), 5.81 (t, *J* = 8.6 Hz, 1H), 4.93 (br, 1H), 4.07 (t, *J* = 5.3 Hz, 1H), 3.65 (s, 2H), 2.93-2.87 (m, 2H), 2.52-2.48 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 214.0, 151.3, 134.8, 133.0, 131.2, 129.1, 128.6, 117.6, 111.8, 106.3, 82.6, 68.0, 60.7, 41.6; HRMS: calcd for $C_{18}H_{16}N_4O_4$ ([M+H]$^+$) 353.1244, found 353.1239.

***N*-(6-((2*S*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrrolo[1,2-f][1,2,4]triazin-4-yl)benzamide (24).** To a solution of compound **23** (75 mg, 0.212 mmol) in CH_3CN (6 mL) was added portionwise sodium tri(acetoxy)borohydride (224 mg, 1.06 mmol). After stirring for 5 h at room temperature, the solvent was evaporated. The crude residue was purified by silica gel flash chromatography (eluent CH_2Cl_2/MeOH 20:1) to afford the title compound as a yellow solid (52 mg, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.04 (d, *J* = 6.8 Hz, 2H), 7.87 (s, 1H), 7.55-7.36 (m, 3H), 7.15 (d, *J* = 4.3 Hz, 1H), 6.17 (d, *J* = 4.3 Hz, 1H), 5.65-5.57 (m, 1H), 4.51 (s, 1H), 4.08 (s, 1H), 3.70 (q, *J* = 10.6 Hz, 2H), 2.54-2.12 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 151.2, 133.1, 132.3, 129.0, 128.84, 118.3, 112.7, 108.3, 88.2, 73.9, 63.5, 39.9; HRMS: calcd for $C_{18}H_{18}N_4O_4$ ([M+H]$^+$) 355.1400, found 355.1396.

(2*R*,3*S*,5*S*)-5-(4-Aminopyrrolo[1,2-f][1,2,4]triazin-6-yl)-2-(hydroxymethyl)tetrahydrofuran-3-ol (25). A solution of compound **24** (150 mg, 0.423 mmol) in 7 N NH_3 in methanol (25 mL) was stirred for 24 h at room temperature. The solvent was evaporated under vacuum. The crude residue was purified by silica gel flash chromatography (CH_2Cl_2/EtOAc = 10:1) to afford the title compound as a foam (90 mg, 90% yield). ^{1}H NMR (500 MHz, MeOD) δ (ppm): 7.79 (s, 1H), 6.87 (d, *J* = 6.8 Hz, 2H), 6.72 (d, *J* = 4.3 Hz, 1H), 5.66 (dd, *J* = 10.8, 5.6 Hz, 1H), 4.45 (dt, *J* = 5.8, 1.8 Hz, 1H), 4.02 (dt, *J* = 4.4, 1.8 Hz, 1H), 3.73-3.70 (m, 2H), 2.48 (ddd, *J* = 13.0, 10.8, 5.8 Hz, 1H), 2.22 (ddd, *J* = 13.0, 5.6, 1.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 155.7, 147.8, 130.4, 114.8, 108.7, 100.9, 87.6, 72.4, 70.8, 62.6, 39.8; HRMS: calcd for $C_{11}H_{14}N_4O_3$ ([M+H]$^+$) 251.1138, found 251.1140.

***N*-(6-((2*S*,4*S*,5*R*)-5-((*tert*-Butyldiphenylsilyloxy)methyl)-4-hydroxytetrahydrofuran-2-yl)pyrrolo[1,2-f][1,2,4]triazin-4-yl)benzamide (26).** *tert*-Butyldiphenylchlorosilane (0.26 mL, 1.02 mmol) was added to a solution of **24** (300 mg, 0.846 mmol) and imidazole (288 mg, 4.23 mmol) in dry pyridine (10 mL). The resulting reaction mixture was stirred for 18 h at room temperature under a nitrogen atmosphere. The solvents were evaporated and the residue was partitioned between water and ethyl acetate. The organic phase was washed with water and brine, then dried over Na_2SO_4. The volatiles were evaporated, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100:1) to afford the title compound as a yellow oil (300 mg, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.17 (s, 1H), 7.94-7.78 (m, 1H), 7.71-7.29 (m, 15H), 6.77 (d, *J* = 4.3 Hz, 1H), 5.74 (q, *J* = 4.2 Hz, 1H), 4.11-4.05 (m, 1H), 3.89-3.82 (m, 1H), 3.74-3.62 (m, 1H), 2.42-2.31 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 151.1, 135.9, 133.4, 133.0, 130.1, 130.0, 128.9, 128.8, 128.1, 111.3, 108.7, 87.3, 74.5, 71.8, 64.9, 40.0, 29.9, 27.2, 19.5; HRMS: calcd for $C_{34}H_{36}N_4O_4Si$ ([M+H]$^+$) 593.2578, found 593.2571.

(2*R*,3*S*,5*S*)-5-(4-Benzamidopyrrolo[1,2-f][1,2,4]triazin-6-yl)-2-((*tert*-butyldiphenylsilyloxy)methyl)tetrahydrofuran-3-yl methanesulfonate (27). Compound **26** (700 mg, 1.18 mmol) was dissolved in a mixture of dichloromethane and pyridine (4:1, 8 mL:4 mL). Then, mesyl chloride (1.83 mL, 23.62 mmol) was added at 0 °C. The mixture was stirred at room temperature overnight. The reaction was quenched by adding methanol. The solvents were evaporated. The residue was dissolved in dichloromethane and washed with water, brine, and dried over Na_2SO_4. The solvent was evaporated under vacuum. The residue was purified by silica gel flash chromatography (CH_2Cl_2/EtOAc = 300:1) to afford the title compound as a yellow oil (600 mg, 75% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.14 (s, 2H), 7.89 (s, 1H), 7.73-7.28 (m, 14H), 6.76 (d, *J* = 4.7 Hz, 1H), 5.71 (q, *J* = 5.3 Hz, 1H), 5.49 (d, *J* = 5.5 Hz), 4.39-4.33 (m, 1H), 3.88 (dd, *J* = 11.0, 3.5 Hz, 1H), 3.73 (dd, *J* = 11.0, 5.2 Hz, 1H), 3.05 (s, 3H), 2.71 (dd, *J* = 13.8, 5.0 Hz, 1H), 2.53-2.41 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 151.3, 135.9, 133.1, 130.3, 130.2, 129.2, 128.9, 128.2, 128.1, 111.4, 108.6, 85.3, 82.8, 72.2, 63.9, 43.9, 38.9, 38.3, 27.2, 19.5; HRMS: calcd for $C_{35}H_{38}N_4O_6Si$ ([M+H]$^+$) 671.2353, found 671.2367.

((2S,5S)-5-(4-aminopyrrolo[1,2-f][1,2,4]triazin-6-yl)-2,5-dihydrofuran-2-yl)methanol (28). To a solution of compound **27** (500 mg, 0.745 mmol) in anhydrous DMSO (5 ml) was added potassium *tert*-butoxide (1.02 g). The reaction was allowed to react for 2 h at room temperature, and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in 7 N NH_3 in methanol (6 mL) and stirred for 24 h at room temperature. The solvents were removed under reduced pressure and the crude residue was purified by silica gel flash chromatography (CH_2Cl_2/EtOAc 50:1 to 20:1) to afford the title compound as a white solid (60 mg, 35% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.80 (s, 1H), 6.87 (d, *J* = 4.2 Hz, 1H), 6.64 (d, *J* = 4.6 Hz, 1H), 6.35 (d, *J* = 3.9 Hz, 1H), 6.12 (s, 2H), 4.96 (d, *J* = 4.6 Hz, 1H), 3.68-3.62 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 155.3, 145.9, 130.7, 128.2, 128.02, 114.2, 109.5, 101.3, 87.1, 78.6, 64.5; HRMS: calcd for $C_{11}H_{12}N_4O_2$ ([M+H]$^+$) 233.1032, found 233.1040.

((2S,5S)-5-(4-Aminopyrrolo[1,2-f][1,2,4]triazin-6-yl)tetrahydrofuran-2-yl)methanol (29). To a solution of compound **28** (20 mg, 0.086 mmol) in methanol was added Pd/C (10%) (20 mg). The reaction was stirred under a hydrogen atmosphere at 25 °C for 24 h. The mixture was filtered through a Celite pad and the filtrate was concentrated. The crude residue was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 20:1) furnishing the title compound as a white solid (10 mg, 50% yield). ^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 7.78 (s, 1H), 6.85 (d, *J* = 4.5 Hz, 1H), 6.71 (d, *J* = 4.7 Hz, 1H), 5.42 (t, *J* = 6.5 Hz, 1H), 4.22-4.14 (m, 1H), 3.72-3.57 (m, 2H), 2.37-1.91 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 155.5, 146.2, 130.9, 114.4, 108.8, 100.9, 79.9, 73.3, 64.2, 30.0, 27.1; HRMS: calcd for $C_{11}H_{14}N_4O_2$ ([M+H]$^+$) 235.1189, found 235.1182.

Antitumoral assays

All tumor cell lines were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA), except for the DND-41 cell line which was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ Leibniz-Institut, Germany). Capan-1, HL-60, K-562, Z-138, MM.1S, and DND-41 were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco Life Technologies, USA), HCT-116 were grown in McCoy's 5A Medium (Gibco Life Technologies, USA), and NCI-H460 were cultured in RPMI (Gibco Life Technologies, USA). All media were supplemented with 10% fetal bovine serum (HyClone, GE Healthcare Life Sciences, USA).

Adherent cell lines HCT-116, NCI-H460, and Capan-1 cells were seeded at a density between 400 and 1,250 cells per well, in 384-well, black-walled, clear-bottomed tissue culture plates (Greiner). After overnight incubation, cells were treated with the test compounds at 7 different concentrations ranging from 10 to 6.4 x 10^{-4} µM. Suspension cell lines HL-60, K-562, Z-138, MM.1S, and DND-41 were seeded at densities ranging from 3,000 to 10,000 cells per well in 384-well, black-walled, clear-bottomed tissue culture plates containing the test compounds at the same 7 concentration points. The plates were incubated and monitored at 37 °C for 72 h in an IncuCyte (Essen BioScience Inc., Ann Arbor, MI, USA) for real-time imaging. Images were taken every 2 h, with one field imaged per well under 10x magnification. All compounds were tested with duplicate data points and averaged.

HIV Antiviral Assay

Evaluation of the antiviral activity of the compounds against the HIV-1 strain IIIB was performed using the MTT assay as previously described.[18]

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Keywords: Antitumor agents • Antiviral agents • Drug discovery • Nucleosides

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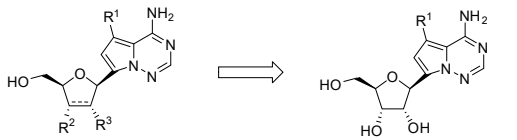

R^1 = H, Cl, Br, I, CN ; R^2 = H, OH; R^3 = H, OH R^1 = H or CN : potent cytotoxicity

The synthesis of pyrrolo[2,1-f][1,2,4]triazine *C*-nucleosides with structural variations of the 4-aza-7,9-dideazaadenine and the sugar moiety is described. Among these analogues, the pyrrolo[2,1-f][1,2,4]triazine *C*-ribonucleosides with either a proton or cyano at position 7 of the nucleobase displayed potent cytotoxic activity in different cancer cell lines.