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Synthesis of Purine and 7-Deazapurine Nucleoside Analogues of 6-*N*-(4-Nitrobenzyl)adenosine; Inhibition of Nucleoside Transport and Proliferation of Cancer Cells

Ramanjaneyulu Rayala^[a], Patricia Theard^[a], Heysell Ortiz^[a], Sylvia Yao^[b], James D. Young^[b], Jan Balzarini^[c], Morris J. Robins^[d], and Stanislaw F. Wnuk^[a]

Stanislaw F. Wnuk: wnuk@fiu.edu

^[a]Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199

^[b]Department of Physiology, The University of Alberta, Edmonton, Alberta, Canada T6G 2H7

^[c]Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium

^[d]Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602

Abstract

The 7-deazapurine nucleoside antibiotic tubercidin was converted into its 4-*N*-benzyl and 4-*N*-(4-nitrobenzyl) derivatives by alkylation at N3 followed by Dimroth rearrangement to the 4-*N*-isomer or by fluoro-diazotization followed by S_NAr displacement of the 4-fluoro group by a benzylamine. The 4-*N*-(4-nitrobenzyl) derivatives of sangivamycin and toyocamycin antibiotics were prepared by the alkylation approach. Cross-membrane transport of labeled uridine by hENT1 was inhibited to a weaker extent by the 4-nitrobenzylated tubercidin and sangivamycin analogues than was observed with 6-*N*-(4-nitrobenzyl)adenosine. Type-specific inhibition of cancer cell proliferation was observed at μM concentrations with the 4-*N*-(4-nitrobenzyl) derivatives of sangivamycin and toyocamycin, and also with 4-*N*-benzyltubercidin. Treatment of 2',3',5'-*O*-acetyladenosine with aryl isocyanates gave the 6-ureido derivatives but none of them exhibited inhibitory activity against cancer cell proliferation or hENT1.

Keywords

Antiproliferation; 7-deazapurine nucleosides; nitrogen heterocycles; nucleosides; nucleoside transporter inhibition

Introduction

Analogues and derivatives of 7-deazapurine nucleoside antibiotics have been synthesized and subjected to extensive biological testing.^[1] A sizeable number of active nucleoside compounds with such a pyrrolo[2,3-*d*]pyrimidine base has been prepared by chemical modifications of the parent antibiotics as well as by coupling base derivatives with protected

sugars.^[2] Noteworthy examples of such molecules include: (a) 5-iodotubercidin,^[3] an up-field activator of the p53 pathway; (b) sangivamycin analogues such as 6-hydrazinosangivamycin^[4] and xylocidine,^[5] *in vitro* down-field inhibitors of PKC and CDK in cancer cell lines; (c) a methyl-substituted tubercidin,^[6] which acts against the replication of polio and dengue viruses; (d) the anti-HSV agent xylobutercidin;^[7] (e) substituted toyocamycin analogues^[8] and 2'- β -C-methyl derivative of toyocamycin,^[9] sangivamycin,^[9] and tubercidin^[10] that have activity against HCV; (f) 2'-deoxy-2'-fluoroarabinotubercidin^[11] and 2-amino-2'-deoxy-2'-fluoroarabinotubercidin,^[12] which exhibit antiviral activity; (g) 4*N*,5-diaryltubercidin derivatives, which act as adenosine kinase inhibitors;^[12, 13] and (h) tubercidin derivatives such as 4-(het)aryl,^[14] 5-(het)aryl^[15] and some 4-substituted-5-(het)aryl^[16] compounds with nanomolar cytostatic activity against several cancer cell lines.

Direct alkylation of exocyclic amino groups on 7-deazapurine nucleosides has not been noted. Treatment of tubercidin with methyl iodide followed by sodium hydroxide produced 4-*N*-methyltubercidin^[2b] (6-*N*-methyl-7-deazaadenosine) *via* Dimroth rearrangement. Several other 4-*N*-substituted tubercidin derivatives have been prepared by S_NAr displacement of chloride from the 4-chloro analogue^[2b, 16] of tubercidin or by displacement of 1,2,4-triazole from 4-*N*-(1,2,4-triazol-4-yl) intermediates.^[17] Such 4-*N*-substituted toyocamycin derivatives also were prepared from the corresponding 4-chloro compounds.^[8] The 7-chloro derivative of the *C*-nucleoside antibiotic formycin (3- β -D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine) was prepared and then converted into 7-*N*-benzylformycin.^[18]

Purine and pyrimidine nucleosides and their derivatives play crucial roles in human physiology and pharmacology.^[19] These “salvage” metabolites can be converted into nucleotides, which in turn serve as the energy-rich currency of intermediary metabolism and as precursors of nucleic acid biosynthesis. Adenosine functions as a key signalling molecule and modulates diverse physiological processes through interactions with cell surface P1 purinergic receptors.

Many types of cancer and viral infections are treated with nucleoside drugs,^[20] but drug availabilities within cells and in the extracellular environment are determined primarily by specific nucleoside transporter (NT) proteins that facilitate their movement across plasma membranes and some organellar membranes. Because of the hydrophilic nature of nucleosides, NTs play key roles in the physiology, pathophysiology, and therapeutic actions of many nucleoside drugs.^[19] The human equilibrative nucleoside transporter 1 (hENT1) is the prototypical NT present in the outer plasma membrane and some intracellular membranes of most, if not all, human cells.^[21]

Nitrobenzylmercaptapurine ribonucleoside (NBMPR) and structurally related hENT1 probes such as 6-*N*-(4-nitrobenzyl)adenosine^[22] and 5'-S-{2-(6-[3-(fluorescein-5-yl)thioureido-1-yl]hexanamido)}ethyl-6-*N*-(4-nitrobenzyl)-5'-thioadenosine (FITC-SAHEMTA)^[23] bind with nM affinity within, or closely associated with, the outward-facing permeant-binding site of hENT1. Development of such probes has greatly aided investigations on the structure, function, and cell surface abundances of hENT1.^[19, 23–24] We now report synthesis of

analogues of these transport inhibitors with purine and 7-deazapurine bases that exhibit pronounced cytotoxicity in several cancer cell lines as well as moderate inhibition of hENT1 transport activity.

Results and Discussion

Chemistry

The *N*-benzylated 7-deazaadenosine analogues (**2a–d**) (Scheme 1) were prepared by two procedures. One involved alkylation of the 7-deazaadenosine antibiotics (**1a–c**) at N3 with a benzyl bromide followed by base-promoted Dimroth rearrangement.^[18, 25] The second route employed diazotization-fluorodediazoniation followed by S_NAr displacement of fluoride with a benzyl amine.^[26] Alkylation of adenosine occurs at N1, and the resulting cation undergoes rearrangement in basic solution to give the N6-alkylated product.^[27] Alkylation of tubercidin (**1a**) with benzyl bromide in DMF occurred at N3 (same as ring nitrogen N1 in adenosine), and treatment of the 3-benzyl intermediate with Me₂NH/THF in MeOH resulted in rearrangement to give 4-*N*-benzyltubercidin (**2a**, 67%).

Because the 6-*N*-(4-nitrobenzyl) derivatives of adenosine and other adenine nucleosides are more potent inhibitors of nucleoside transport,^[22] we also synthesized 4-nitrobenzyl derivatives of the 7-deaza antibiotics. Alkylation of **1a** with 4-nitrobenzyl bromide followed by treatment with Me₂NH/MeOH produced 4-*N*-(4-nitrobenzyl)tubercidin (**2b**, 56%). Analogous treatment of sangivamycin (**1b**) with 4-nitrobenzyl bromide and then Me₂NH/MeOH gave 4-*N*-(4-nitrobenzyl)sangivamycin (**2c**, 46%).

Treatment of toyocamycin (**1c**) with 4-nitrobenzyl bromide unexpectedly gave 4-*N*-(4-nitrobenzyl)toyocamycin (**2d**) directly. Formation of an intermediate alkylated at N3 apparently did not occur because TLC of the reaction mixture showed only a more rapidly migrating material corresponding to **2d** without indication of a more polar (cationic) product. Therefore, successive treatment with Me₂NH/MeOH was not required. Although direct alkylation on the exocyclic amino group of adenine and guanine with quinone methides is known,^[28] the observed direct alkylation on the exocyclic amine group of toyocamycin with 4-nitrobenzyl bromide has not been previously noted. This might have resulted from alteration of the nucleophilicity of the endocyclic N3 of toyocamycin relative to that of the exocyclic 4-amino nitrogen by the electron withdrawing pull of the cyano group at C5. A possible accompanying stereoelectronic effect might result from interference of the cylindrical cyano group with a synperiplanar orientation of the exocyclic amino group relative to the planar heterocyclic ring that would diminish lone-pair donation from the amino nitrogen toward N3 of that amidine system.^[29]

Alternatively, tubercidin (**1a**, Scheme 2) was acetylated and then subjected to diazotative-fluorodeamination^[23, 30] followed by S_NAr displacement of fluoride with benzylamines.^[23, 26] Thus, 2',3',5'-tri-*O*-acetyltubercidin (**3**) was treated with NaNO₂ in freshly prepared ~55% HF-pyridine at –10 °C^[31] for 15 min to yield the protected 4-fluorotubercidin **4** (82%). The concentration of HF-pyridine as well as the temperature are crucial.^[31b] Treatment of **4** with 4-nitrobenzylamine hydrochloride/Et₃N/MeOH^[23] gave 2',

3',5'-tri-*O*-acetyl-4-*N*-(4-nitrobenzyl)tubercidin (**5**, 47%), which was deacetylated (NH₃/MeOH) to give **2b** (85%).

Some 2',3'-bis-*O*-silylated adenosine derivatives with ureido substituents at the 5'- and 6-positions exhibit anti-proliferative activity.^[32] We prepared the 6-*N*-[*N*-arylcarbamoyl]adenosine derivatives (**9a–c**) (Scheme 3) with phenyl, 4-methoxyphenyl, and 4-nitrophenyl moieties to probe for binding to hENT1 and inhibition of nucleoside transport as observed with 6-*N*-(4-nitrobenzyl)adenosine.^[22] The 2',3',5'-tri-*O*-acetyl derivative **7** of adenosine (**6**) was treated with phenyl isocyanate to give **8a**. Deacetylation gave 6-*N*-[*N*-phenylcarbamoyl]adenosine (**9a**), and the analogous sequential treatment of **7** with the respective isocyanates produced the 4-methoxyphenyl and 4-nitrophenyl analogues **9b** and **9c**.

Nucleoside Transport

Inhibition of the initial rate of ³H-uridine (20 μM) uptake by recombinant hENT1 produced in the *Xenopus* oocyte heterologous expression system was determined as described previously.^[21] Minimal inhibition (*italic*>25%) of labelled uridine uptake was observed at a 100-μM concentration of each of the 6-ureidoadenosine compounds **9a**, **9b**, and **9c**. The 4-*N*-benzyltubercidin (**2a**) and 4-*N*-(4-nitrobenzyl)toyocamycin (**2d**) analogues also exhibited weak inhibition (*bold*>45%) but both 4-*N*-(4-nitrobenzyl)tubercidin (**2b**) and 4-*N*-(4-nitrobenzyl)sangivamycin (**2c**) fully inhibited labelled uridine uptake at that concentration. Dose-response evaluations indicated that **2c** (IC₅₀ = 123 ± 31 nM) was a better inhibitor of uridine transport by hENT1 than **2b** (IC₅₀ > 1000 nM). However, the 7-deaza analogue **2c** was a weaker inhibitor of hENT1-mediated transport than 6-*N*-(4-nitrobenzyl)adenosine, NBMPR, and other derivatives^[22] containing a nitrogen atom at the 7-position in the adenine ring.

Inhibition of Cell Proliferation

Inhibition of the proliferation of murine leukemia (L1210) cells and human T-lymphocyte (CEM), cervix carcinoma (HeLa), prostate cancer (PC-3), and kidney carcinoma (Caki-1) cells by the adenine and 7-deazaadenine nucleoside analogues was evaluated. No pronounced inhibitory effect was observed with the 6-ureido compounds **9a**, **9b**, and **9c** in any of the tumor cell lines (Table 1). Marked inhibition of the proliferation of PC-3 cells (IC₅₀: 0.92 μM) and HeLa cells (IC₅₀: 7.4 μM) by 4-*N*-benzyltubercidin (**2a**) was observed but no significant activity was found with its nitrobenzyl analogue **2b** (IC₅₀: >50 μM). The 4-*N*-(4-nitrobenzyl)sangivamycin (**2c**) and 4-*N*-(4-nitrobenzyl)toyocamycin (**2d**) analogues inhibited proliferation of L1210, HeLa, and PC-3 cells at ~0.9–9.4 μM with **2c** showing more potent effects (0.92–3.4 μM). It is intriguing that such striking differences in cytostatic activity were dependent on the nature of the tumor cell lines. HeLa and PC-3 tumor cells were highly susceptible to the cytostatic activity of **2a**, **2c**, and **2d** whereas the L1210 cells were sensitive only to **2c** and **2d**, and the CEM and Caki-1 cells were weakly sensitive to the antiproliferative effects of any of the **2a–2d** compounds.

Conclusions

Alkylation at N3 of tubercidin with 4-nitrobenzyl bromide and Dimroth rearrangement gave 4-*N*-(4-nitrobenzyl)tubercidin, which also was prepared by fluoro-diazotization of tubercidin and S_NAr displacement of the 4-fluoro group by 4-nitrobenzylamine. The alkylation-rearrangement sequence was employed to convert sangivamycin (the 5-carboxamidotubercidin antibiotic) into its 4-*N*-(4-nitrobenzyl) derivative. However, no evidence of formation of an initial N3-alkylated intermediate was observed upon treatment of toyocamycin (the 5-cyanotubercidin antibiotic) with 4-nitrobenzyl bromide. Alkylation occurred directly on the amino group to give 4-*N*-(4-nitrobenzyl)toyocamycin. The 4-*N*-(4-nitrobenzyl) derivatives of tubercidin and sangivamycin inhibited cross-membrane transport of labelled uridine by the human equilibrative nucleoside transporter hENT1 at >1 μM and ~120 nM, respectively. Inhibition of the proliferation of L1210, HeLa, and PC-3 tumor cells in culture was observed with 4-*N*-benzyltubercidin and the 4-*N*-(4-nitrobenzyl) derivatives of sangivamycin and toyocamycin at 0.92–9.4 μM concentrations.

Experimental Section

¹H (400 MHz), ¹³C (100.6 MHz), and ¹⁹F (376 MHz) NMR spectra were recorded at ambient temperature in solutions of CDCl₃ or DMSO-*d*₆. Reaction progress was monitored by TLC on Merck Kieselgel 60-F₂₅₄ sheets with product detection by 254-nm light. Products were purified by column chromatography using Merck Kieselgel 60 (230–400 mesh) or by automated flash chromatography using a CombiFlash system. UV spectra were recorded with a Varian Cary 100 Bio UV-visible spectrophotometer. Reagent grade chemicals were used and solvents were dried by reflux and distillation from CaH₂ under N₂ unless otherwise specified, and an atmosphere of N₂ was used for reactions. Purity of the synthesized compounds was determined to be 95% by elemental analysis (C, H, N) and/or HPLC.

4-Benzylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2a)

BnBr (345 μL, 496 mg, 2.9 mmol) was added to a stirred solution of tubercidin (**1a**; 266 mg, 1 mmol) in dried DMF (5 mL). After stirring for 48 hours at 40 °C, TLC showed almost complete conversion to a more polar product. Volatiles were evaporated to ~1 mL (< 40 °C, vacuum pump) and the resulting syrup was added dropwise to dried acetone (30 mL) with vigorous stirring. Et₂O (60 mL) was added to the suspension, which was chilled for 20 min at 0 °C and vacuum filtered. The hygroscopic precipitate was quickly dissolved in MeOH (10 mL) and Me₂NH/THF (2 M, 8 mL) was added. The resulting solution was stirred at reflux (65 °C, oil bath) for 20 h. TLC showed ~80% conversion to less polar spot, and volatiles were evaporated and coevaporated with MeOH (2 ×). The residue was dissolved in warm MeOH (10 mL), H₂O (60 mL) was added, and the solution was extracted with EtOAc (5 × 20 mL). The combined organic phase was dried (Na₂SO₄), concentrated *in vacuo*, coevaporated (2 × EtOH) and flash chromatographed (EtOAc) to give **2a** (238 mg, 67%) as a colorless oil: UV (MeOH) λ_{max} 276 nm, λ_{min} 244 nm; ¹H NMR (DMSO-*d*₆) δ 8.09–8.13 (m, 2 H, NH, H2), 7.38 (d, *J* = 3.7 Hz, 1 H, H6), 7.29–7.35 (m, 4 H, Ph), 7.22–7.25 (m, 1 H, Ph), 6.67 (d, *J* = 3.4 Hz, 1 H, H5), 6.01 (d, *J* = 5.9 Hz, 1 H, H1'), 5.27–5.32 (m, 2 H, 2'-OH, 5'-OH), 5.12 (d, *J* = 4.3 Hz, 1 H, 3'-OH), 4.70–4.78 (m, 2 H, CH₂), 4.44 (q, *J* = 5.7 Hz, 1 H,

H2'), 4.09 ("q", $J = 4.1$ Hz, 1 H, H3'), 3.90 (q, $J = 3.4$ Hz, 1 H, H4'), 3.60–3.65 (m, 1 H, H5'), 3.50–3.56 (m, 1 H, H5''); ^{13}C NMR (DMSO- d_6) δ 156.1, 151.4, 149.5, 140.1, 128.2 (Ph), 127.1 (Ph), 126.6, 122.3 (C6), 103.4, 99.2 (C5), 87.6 (C1'), 85.1 (C4'), 73.7 (C2'), 70.7 (C3'), 61.8 (C5'), 43.1 (CH₂); MS (ESI) m/z 357 (100%, MH⁺), HRMS (ESI) m/z 357.1581 (MH⁺), calcd for C₁₈H₂₉N₄O₄ 357.1563.

4-(4-Nitrobenzylamino)-7-(β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2b**)

Method A—Tubercidin (**1a**; 266 mg, 1.0 mmol) was treated with 4-nitrobenzyl bromide (648 mg, 3.0 mmol) at 80 °C for 24 h as described above for **2a** followed by addition of MeOH (15 mL) and Me₂NH/THF (2 M, 8 mL). The reaction mixture was stirred at reflux for 20 h; TLC showed ~85% conversion to a less polar product **2b** (244 mg, 56%, yellow oil): UV (MeOH) λ_{max} 278 nm, λ_{min} 240 nm; ^1H NMR (DMSO- d_6) δ 8.30 (t, $J = 6.1$ Hz, 1 H, NH), 8.19 ("d", $J = 8.8$ Hz, 2 H, Ph), 8.12 (s, 1 H, H2), 7.58 (d, $J = 8.8$ Hz, 2 H, Ph), 7.43 (d, $J = 3.7$ Hz, 1 H, H6), 6.69 (d, $J = 3.5$ Hz, 1 H, H5), 6.04 (d, $J = 6.3$ Hz, 1 H, H1'), 5.28–5.30 (m, 2 H, 2 $\times$ OH), 5.14 (s, 1 H, OH), 4.80–4.91 (m, 2 H, CH₂), 4.45 ("d", $J = 4.4$ Hz, 1 H, H2'), 4.11–4.13 (m, 1 H, H3'), 3.92 (q, $J = 3.5$ Hz, 1 H, H4'), 3.64 (dt, $J = 3.9, 11.8$ Hz, 1 H, H5'), 3.52–3.57 (m, 1 H, H5''); ^{13}C NMR (DMSO- d_6 -D₂O) δ 155.8, 151.0, 148.8, 147.9, 146.3, 127.9 (Ph), 123.4 (Ph), 122.8 (C6), 103.6, 99.3 (C5), 87.5 (C1'), 84.9 (C4'), 73.5 (C2'), 70.4 (C3'), 61.6 (C5'), 42.7 (CH₂); MS (ESI): m/z 402 (100%, MH⁺). Anal. Calcd for C₁₈H₁₉N₅O₆·1.25 H₂O (423.89): C, 51.00; H, 5.11; N, 16.52. Found: C, 51.05; H, 5.06; N, 16.03.

Method B

Step a: Ac₂O (377 μL , 408 mg, 4 mmol) was added to a stirred suspension of **1a** (266 mg, 1 mmol) in dried pyridine (5 mL) at 0 °C (ice bath) and stirring was continued at 0 °C for 12 h and then at ambient temperature for 9 h (total reaction time: 21 h). MeOH was added, the reaction mixture was stirred at ambient temperature for 30 min, and volatiles were evaporated (vacuum pump, <25 °C). MeOH was added and evaporated, and the resulting gum was partitioned between CHCl₃ (50 mL) and 2% AcOH/H₂O (50 mL). The aqueous layer was extracted with CHCl₃, and the combined organic phase was washed with NaHCO₃/H₂O, brine, and dried (MgSO₄). Volatiles were evaporated *in vacuo* and the residue was column chromatographed (EtOAc) to give 2',3',5'-tri-*O*-acetyltubercidin (**3**; 337 mg, 86%) as a colorless foam with data as reported.^[33]

Step b: NaNO₂ (66 mg, 0.95 mmol) was added to a solution of **3** (150 mg, 0.38 mmol) in freshly prepared ~55% HF-pyridine^[31b] (1.9 mL) at –10 °C in a sealed polypropylene vessel. The mixture was stirred at –10 °C for 15 min, and TLC showed almost complete conversion to a less polar spot. Ice/H₂O was added and the mixture was extracted with CH₂Cl₂. The combined organic phase was washed with NaHCO₃/H₂O, brine, and dried (Na₂SO₄). Volatiles were removed *in vacuo*, and the resulting brown oil was column chromatographed (30% EtOAc in hexanes) to give 7-(2,3,5-tri-*O*-acetyl- β -D-ribofuranosyl)-4-fluoropyrrolo[2,3-*d*]pyrimidine (**4**; 124 mg, 82%) as a colorless oil: UV (MeOH) λ_{max} 258 nm, λ_{min} 233 nm; ^1H NMR (CDCl₃) δ 8.53 (d, $^4J_{\text{H-F}} = 0.5$ Hz, 1 H, H2), 7.37 (d, $J = 3.8$ Hz, 1 H, H8), 6.66 (d, $J = 3.8$ Hz, 1 H, H7), 6.45 (d, $J = 6.0$ Hz, 1 H, H1'), 5.74 (t, $J = 5.8$ Hz, 1 H, H2'), 5.55 (dd or m, $J = 4.1, 5.6$ Hz, 1 H, H3'), 4.32–4.42 (m, 3 H,

H4', H5', H5''), 2.12 (s, 3 H, CH₃), 2.13 (s, 3 H, CH₃), 2.02 (s, 3 H, CH₃); ¹⁹F NMR (CDCl₃): δ -64.81 ppm (s); ¹³C NMR (CDCl₃) δ 170.2, 169.6, 169.4 (3 × C=O), 162.3 (d, ¹J_{C-F} = 253.8 Hz, C4), 155.1 (d, ³J_{C-F} = 11.9 Hz, C7a), 151.1 (d, ³J_{C-F} = 14.5 Hz, C2), 125.7 (d, ⁴J_{C-F} = 2.6 Hz, C6), 105.4 (d, ²J_{C-F} = 33.4 Hz, C4a), 99.6 (d, ³J_{C-F} = 4.9 Hz, C5), 86.1 (C1'), 79.9 (C4'), 73.1 (C2'), 70.1 (C3'), 63.3 (C5'), 20.7, 20.5, 20.3 (3 × s, 3 × CH₃); MS (ESI): *m/z* 396 (100%, MH⁺).

Step c: Freshly distilled Et₃N (113 μL, 82 mg, 0.81 mmol) was added to a stirred suspension of **4** (93 mg, 0.23 mmol) and 4-nitrobenzylamine hydrochloride (67 mg, 0.35 mmol) in MeOH (3 mL) and stirring was continued for 5 h at ambient temperature. Volatiles were evaporated *in vacuo* and the residue was column chromatographed (50% EtOAc in hexanes) to give 2',3',5'-tri-*O*-acetyl-4-*N*-(4-nitrobenzyl)tubercidin (**5**; 58 mg, 47%) as a colorless oil: UV (MeOH) λ_{max} 277 nm, λ_{min} 238 nm. ¹H NMR (CDCl₃) δ 8.36 (s, 1 H, H2), 8.17 (d, *J* = 8.7 Hz, 2 H, Ph), 7.51 (d, *J* = 8.7 Hz, 2 H, Ph), 7.12 (d, *J* = 3.768 Hz, 1 H, H6), 6.42–6.46 (dd, 3.8, 6.0 Hz, 2 H, H1', H5), 5.73 (t, *J* = 5.8 Hz, 1 H, H2'), 5.54–5.59 (m, 2 H, H3', NH), 4.95 (d, *J* = 6.1 Hz, 2 H, CH₂), 4.31–4.40 (m, 3 H, H4', H5', H5''), 2.14 (s, 6 H, 2 × CH₃), 2.04 (s, 3 H, CH₃); ¹³C NMR (CDCl₃) δ 170.4, 169.7, 169.5 (3 × C=O), 156.0 (C4), 152.3 (C2), 150.8 (C7a), 147.3 (Ph), 146.7 (Ph), 128.0 (Ph), 123.9 (Ph), 121.5 (C6), 103.8 (C4a), 99.4 (C5), 85.4 (C1'), 79.5 (C4'), 73.1 (C2'), 70.8 (C3'), 63.5 (C5'), 44.2 (CH₂), 20.8, 20.6, 20.4 (3 × s, 3 × CH₃). MS (ESI): *m/z* 528 (100%, MH⁺).

Step d: NH₃/MeOH (5 mL) was added to a stirred solution of **5** (54 mg, 0.1 mmol) in MeOH (1 mL) and stirring was continued at ambient temperature for 20 h. Volatiles were evaporated and the residue was column chromatographed with a 2 → 4% gradient of the upper phase of EtOAc/*i*-PrOH/H₂O (4:1:2) in EtOAc to give **2b** (35 mg, 85%) as a yellow oil.

5-Carboxamido-4-(4-nitrobenzylamino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2c**)

Sangivamycin (**1b**; 155 mg, 0.5 mmol) was treated with 4-nitrobenzyl bromide (162 mg, 1.5 mmol) for 26 h at 40 °C as described for **1a** → **2a** and the alkylated intermediate was then stirred with Me₂NH/THF (2 M, 6 mL) and MeOH (12 mL) for 45 h at ambient temperature. Me₂NH/THF (2 M, 2 mL) was added and the mixture was stirred at reflux for 32 h (total reaction time: 77 h). TLC showed ~80% conversion to the less polar **2c**, which was obtained as off-white crystals (101 mg, 45%) after recrystallization from EtOH: mp 154–158 °C (dec.); UV (MeOH) 286 nm (ε 23 100), λ_{min} 229 nm (ε 8700); ¹H NMR (DMSO-*d*₆) δ 10.29 (t, *J* = 6.0 Hz, 1 H, NH), 8.17–8.21 (m, 4 H, Ph, H2, H6), 8.11 and 7.47 (2 × s, 2 × 1 H, CONH₂), 7.59 (brd, *J* = 8.8 Hz, 2 H, Ph), 6.06 (d, *J* = 6.0 Hz, 1 H, H1'), 5.43 (d, *J* = 6.3 Hz, 1 H, 2'-OH), 5.20 (d, *J* = 5.0 Hz, 1 H, 3'-OH), 5.09 (t, *J* = 5.8 Hz, 1 H, 5'-OH), 4.90 ("d", *J* = 6.2 Hz, 2 H, CH₂), 4.37 (q, *J* = 5.8 Hz, 1 H, H2'), 4.10 ("q", *J* = 4.5 Hz, 1 H, H3'), 3.93 (q, *J* = 4.0 Hz, 1 H, H4'), 3.61–3.67 (m, 1 H, H5'), 3.53–3.58 (m, 1 H, H5''); ¹³C NMR (DMSO-*d*₆) δ 166.5, 156.5, 152.6, 150.4, 148.0, 146.4, 128.0 (Ph), 125.7, 123.6, 110.9, 101.7, 87.2 (C1'), 85.3 (C4'), 73.9 (C2'), 70.5 (C3'), 61.9 (C5'), 42.9 (CH₂); MS (ESI) *m/z* 445 (100%, MH⁺). Anal. Calcd for C₁₉H₂₀N₆O₇•1.5 H₂O (471.42): C, 48.41; H, 4.92; N, 17.83. Found: C, 48.59; H, 4.66; N, 17.65.

5-Cyano-4-(4-nitrobenzylamino)-7-(β -D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2d**)

Toyocamycin (**1c**; 146 mg, 0.5 mmol) was heated with 4-nitrobenzyl bromide (364 mg, 1.5 mmol) in dried DMF (3 mL) at 40 °C for 63 h (TLC showed ~85% conversion to a less polar product). Volatiles were evaporated to ~1 mL (< 40 °C, vacuum pump) and this material was added dropwise to 20 mL of vigorously stirred dried acetone. Et₂O (40 mL) was added to the stirred suspension, which was chilled at 0 °C for 20 minutes and the precipitate was collected by vacuum filtration. Recrystallization from MeOH gave **2d** (99 mg, 46%) as colorless crystals: mp 214–216 °C; UV (MeOH) λ_{max} 274, 236 nm (ϵ 22 100, 18 900), λ_{min} 250, 228 nm (ϵ 12 800, 18 000); ¹H NMR (DMSO-*d*₆) δ 8.35 (s, 1 H, H₂), 8.19–8.23 (m, 3 H, H₆, Ph), 7.59 (d, *J* = 8.6 Hz, 2 H, Ph), 6.87 (s, 1 H, NH), 5.93 (d, *J* = 5.6 Hz, 1 H, H1'), 5.47 (d, *J* = 6.0 Hz, 1 H, 2'-OH), 5.35 ("s", 2 H, CH₂), 5.21 (d, *J* = 5.0 Hz, 1 H, 3'-OH), 5.09 (t, *J* = 5.4 Hz, 1 H, 5'-OH), 4.31 (q, *J* = 5.5 Hz, 1 H, H2'), 4.08 (q, *J* = 4.6 Hz, 1 H, H3'), 3.92 (q, *J* = 3.7 Hz, 1 H, H4'), 3.62–3.68 (m, 1 H, H5'), 3.53–3.58 (m, 1 H, H5''); ¹³C NMR (DMSO-*d*₆-D₂O) δ 152.6, 149.4, 146.7, 144.9, 142.7, 129.4, 128.5 (Ph), 123.5 (Ph), 115.2, 105.0, 87.7 (C1'), 85.8 (C4'), 85.5, 74.6 (C2'), 70.1 (C3'), 61.1 (C5'), 48.8 (CH₂); MS (ESI): *m/z* 427 (100%, MH⁺). Anal. Calcd for C₁₉H₁₈N₆O₆•0.5 H₂O (435.39): C, 52.41; H, 4.40; N, 19.30. Found: C, 52.17; H, 4.29; N, 19.30.

6-*N*-[*N*-phenylcarbamoyl]adenosine (**9a**)

Step a—A solution of phenyl isocyanate (35 μ L, 39 mg, 0.32 mmol) and 2',3',5'-tri-*O*-acetyladenosine (**7**; 106 mg, 0.27 mmol) in CH₂Cl₂ (5 mL) was stirred at ambient temperature under N₂. An additional portion of phenyl isocyanate (35 μ L, 39 mg, 0.324 mmol) was added after 48 h, and stirring was continued for 20 h (total reaction time: 68 h). The reaction mixture was partitioned (NaHCO₃/H₂O//CHCl₃) and the organic layer was dried (Na₂SO₄), concentrated, and column chromatographed (70% EtOAc/hexanes) to give 2',3',5'-tri-*O*-acetyl-6-*N*-[*N*-phenylcarbamoyl]adenosine (**8a**; 111 mg, 80%): ¹H NMR (CDCl₃) δ 11.89 (s, 1 H, NH), 9.40 (s, 1 H, NH), 8.62 (s, 1 H, H₂), 8.56 (s, 1 H, H₈), 7.63 ("d", *J* = 7.6 Hz, 2 H, Ph), 7.35 ("t", *J* = 7.9 Hz, 2 H, Ph), 7.11 ("t", *J* = 7.4 Hz, 1 H, Ph), 6.25 (d, *J* = 5.4 Hz, 1 H, H1'), 6.03 (t, *J* = 5.5 Hz, 1 H, H2'), 5.69 ("dd", *J* = 4.4, 5.4 Hz, 1 H, H3'), 4.37–4.48 (m, 3 H, H4', H5', H5''), 2.15 (s, 3 H, 1 $\times$ CH₃), 2.08 (s, 3 H, CH₃), 2.07 (s, 3 H, CH₃).

Step b—NH₃/MeOH (6 mL) was added to **8a** (103 mg, 0.2 mmol) in MeOH (3 mL) and the mixture was stirred at ambient temperature for 2 h. Volatiles were evaporated and the residual solid was recrystallized from MeOH to give **9a** (66 mg, 85%): mp 193–195 °C; UV (MeOH) λ_{max} 279 nm (ϵ 28 250), λ_{min} 241 nm (ϵ 9200); ¹H NMR (DMSO-*d*₆) δ 11.76 (s, 1 H, NH), 10.17 (s, 1 H, NH), 8.72 (s, 1 H, H₂), 8.69 (s, 1 H, H₈), 7.63 (d, *J* = 7.7 Hz, 2 H, Ph), 7.36 (t, *J* = 7.9 Hz, 2 H, Ph), 7.10 (t, *J* = 7.4 Hz, 1 H, Ph), 6.01 (d, *J* = 5.6 Hz, 1 H, H1'), 5.54 (d, *J* = 6.0 Hz, 1 H, 2'-OH), 5.24 (d, *J* = 5.0 Hz, 1 H, 3'-OH), 5.14 (t, *J* = 5.5 Hz, 1 H, 5'-OH), 4.61 (q, *J* = 5.6 Hz, 1 H, H2'), 4.19 (q, *J* = 4.7 Hz, 1 H, H3'), 3.99 (q, *J* = 3.8 Hz, 1 H, H4'), 3.67–3.73 (m, 1 H, H5'), 3.55–3.61 (m, 1 H, H5''); ¹³C NMR (DMSO-*d*₆) δ 150.83 (C=O), 150.81 (C2), 150.6, 150.0, 142.4 (C8), 138.4, 128.9 (Ph), 123.2 (Ph), 120.6, 119.4 (Ph), 87.7 (C1'), 85.7 (C4'), 73.8 (C2'), 70.3 (C3'), 61.3 (C5'); MS (ESI) *m/z* 387

(100%, MH⁺). Anal. Calcd for C₁₇H₁₈N₆O₅•0.5 H₂O (395.37): C, 51.64; H, 4.84; N, 21.26. Found: C, 51.38; H, 4.77; N, 21.02.

6-*N*-[*N*-(4-Methoxyphenyl)carbamoyl]adenosine (9b)

Step a—A solution of **7** (197 mg, 0.5 mmol) and 4-methoxyphenyl isocyanate (78 μL, 90 mg, 0.6 mmol) in CH₂Cl₂ (5 mL) was stirred at ambient temperature under N₂. Additional 4-methoxyphenyl isocyanate (78 μL, 90 mg, 0.6 mmol) was added after 12 h, and stirring was continued for 12 h (total reaction time: 24 h). The mixture was partitioned (NaHCO₃/H₂O/CHCl₃) and the organic layer was dried (Na₂SO₄) and evaporated to give residual **2'**, **3'**, **5'**-tri-*O*-acetyl-6-*N*-[*N*-(4-methoxyphenyl)carbamoyl]adenosine (**8b**; 260 mg, 96%) of sufficient purity for NMR characterization and use in the next step: ¹H NMR (CDCl₃) δ 11.45 (s, 1 H, NH), 8.62 (s, 1 H, H2), 8.59 (s, 1 H, H8), 8.12 (s, 1 H, NH), 7.53 (“d”, *J* = 8.9 Hz, 2 H, Ph), 6.91 (“d”, *J* = 9.0 Hz, 2 H, Ph), 6.21 (d, *J* = 5.3 Hz, 1 H, H1'), 5.97 (t, *J* = 5.4 Hz, 1 H, H2'), 5.66 (“t”, *J* = 4.8 Hz, 1 H, H3'), 4.38–4.48 (m, 3 H, H4', H5', H5''), 2.16 (s, 3 H, CH₃), 2.14 (s, 3 H, CH₃), 2.10 (s, 3 H, CH₃).

Step b—NH₃/MeOH (6 mL) was added to crude **8b** (260 mg, 0.5 mmol) in MeOH (3 mL) and the mixture was stirred at ambient temperature for 2 h. Volatiles were evaporated and the solid residue was recrystallized from MeOH to give **9b** (170 mg, 80%): mp 181–182 °C; UV (MeOH) λ_{max} 280 nm (ε 21 300), λ_{min} 247 nm (ε 11 100); ¹H NMR (DMSO-*d*₆) δ 11.59 (s, 1 H, NH), 10.07 (s, 1 H, NH), 8.70 (s, 1 H, H2), 8.67 (s, 1 H, H8), 7.53 (“d”, *J* = 4.5 Hz, 2 H, Ph), 6.94 (“d”, *J* = 4.5 Hz, 2 H, Ph), 6.01 (d, *J* = 5.7 Hz, 1 H, H1'), 5.54 (d, *J* = 6.0 Hz, 1 H, 2'-OH), 5.24 (d, *J* = 5.0 Hz, 1 H, 3'-OH), 5.14 (t, *J* = 5.6 Hz, 1 H, 5'-OH), 4.61 (q, *J* = 5.5 Hz, 1 H, H2'), 4.18 (q, *J* = 4.8 Hz, 1 H, H3'), 3.98 (q, *J* = 3.8 Hz, 1 H, H4'), 3.75 (s, 3 H, OCH₃), 3.67–3.72 (m, 1 H, H5'), 3.55–3.61 (m, 1 H, H5''); ¹³C NMR (DMSO-*d*₆) δ 155.4 (C=O), 150.9 (C2), 150.8, 150.4, 149.8, 142.3 (C8), 131.1, 121.2 (Ph), 120.3, 114.1 (Ph), 87.7 (C1'), 85.6 (C4'), 73.7 (C2'), 70.1 (C3'), 61.1 (C5'), 55.2 (OCH₃); MS (ESI) *m/z* 417 (100%, MH⁺). Anal. Calcd for C₁₈H₂₀N₆O₆•1.5 H₂O (443.41): C, 48.76; H, 5.23; N, 18.95. Found: C, 48.54; H, 4.97; N, 18.85.

6-*N*-[*N*-(4-Nitrophenyl)carbamoyl]adenosine (9c)

Step a—A solution of 4-nitrophenyl isocyanate (54 mg, 0.33 mmol) and **7** (117 mg, 0.30 mmol) in CH₂Cl₂ (20 mL) was stirred at ambient temperature under N₂ for 17 h (TLC showed ~85 % conversion to a less polar product) and then was heated at reflux for 24 h (total reaction time: 41 h). The mixture was partitioned (NaHCO₃/H₂O/CHCl₃) and the organic layer was dried (Na₂SO₄) and evaporated to give solid **2'**, **3'**, **5'**-tri-*O*-acetyl-6-*N*-[*N*-(4-nitrophenyl)carbamoyl]adenosine (**8c**; 160 mg, 96%) of sufficient purity for NMR characterization and use in the next step: ¹H NMR (DMSO-*d*₆) δ 12.13 (s, 1 H, NH), 10.62 (s, 1 H, NH), 8.75 (s, 1 H, H2), 8.71 (s, 1 H, H8), 8.27 (d, *J* = 9.2 Hz, 2 H, Ph), 7.90 (d, *J* = 9.2 Hz, 2 H, Ph), 6.33 (d, *J* = 5.3 Hz, 1 H, H1'), 6.05 (d, *J* = 5.7 Hz, 1 H, H2'), 5.65 (t, *J* = 5.4 Hz, 1 H, H3'), 4.42–4.47 (m, 1 H, H4', H5'), 4.25–4.3 (m, 1 H, H5''), 2.13 (s, 3 H, CH₃), 2.05 (s, 3 H, CH₃), 2.03 (s, 3 H, CH₃).

Step b—NH₃/MeOH (6 mL) was added to crude **8c** (160 mg, 0.29 mmol) in MeOH (3 mL) and the mixture was stirred at ambient temperature for 2 h. Volatiles were evaporated and

the residual solid was recrystallized from MeOH to give **9c** (80 mg, 65%): mp 202–208 °C; UV (MeOH) λ_{max} 317, 278 nm (ϵ 16 200, 11 300), λ_{min} 286, 241 nm (ϵ 10 300, 5600). ^1H NMR (DMSO- d_6) δ 12.27 (s, 1 H, NH), 10.61 (s, 1 H, NH), 8.73 (s, 1 H, H2), 8.72 (s, 1 H, H8), 8.27 (“d”, J = 9.2 Hz, 2 H, Ph), 8.0 (“d”, J = 9.2 Hz, 2 H, Ph), 6.01 (d, J = 5.7 Hz, 1 H, H1’), 5.52 (“s”, J = 22.7 Hz, 1 H, 2’-OH), 5.24 (d, J = 14.1 Hz, 1 H, 3’-OH), 5.14 (“s”, 1 H, 5’-OH), 4.61 (“s”, 1 H, H2’), 4.18 (“s”, 1 H, H3’), 3.98 (q, J = 3.8 Hz, 1 H, H4’), 3.68–3.71 (m, 1 H, H5’), 3.55–3.61 (m, 1 H, H5’); ^1H NMR (D₂O/DMSO- d_6) δ 8.71 (s, 1 H, H2), 8.68 (s, 1 H, H8), 8.24 (“d”, J = 9.2 Hz, 2 H, Ph), 7.88 (“d”, J = 9.2 Hz, 2 H, Ph), 6.00 (d, J = 5.7 Hz, 1 H, H1’), 4.58 (t, J = 5.4 Hz 1 H, H2’), 4.15 (“t”, J = 4.3 Hz 1 H, H3’), 3.99 (q, J = 3.7 Hz, 1 H, H4’), 3.68 (dd, J = 3.8, 12.1 Hz 1 H, H5’), 3.57 (dd, J = 3.9, 12.2 Hz 1 H, H5’); ^{13}C NMR (DMSO- d_6) δ 150.9, 150.72, 150.66, 149.4, 144.5, 142.6 (C8), 142.2, 125.0, 120.5, 119.1, 87.7 (C1’), 85.6 (C4’), 73.7 (C2’), 70.1 (C3’), 61.1 (C5’); MS (ESI) m/z 432 (100%, MH⁺). Anal. Calcd for C₁₇H₁₇N₇O₇•H₂O (449.37): C, 45.44; H, 4.26; N, 21.82. Found: C, 45.10; H, 3.96; N, 21.59.

Cytostatic activity assays

All assays were performed in 96-well microtiter plates. To each well of 200 μL were added $(5\text{--}7.5) \times 10^4$ tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210), 72 h (human lymphocytic CEM), 96 h (human cervix carcinoma HeLa), 144 h (human prostate cancer PC-3), or 168 h (human kidney Caki-1) at 37 °C in a humidified CO₂-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC₅₀ (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

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References

1. a) Suhadolnik, R.J. Nucleoside Antibiotics. Wiley-Interscience; 1970. b) Suhadolnik, R.J. Nucleosides as Biological Probes. Wiley; 1979. c) Seela F, Peng X. Curr Top Med Chem. 2006; 6:867–892. [PubMed: 16787281] d) Seela F, Peng X, Budow S. Curr Org Chem. 2007; 11:427–462. e) Seela F, Budow S, Peng X. Curr Org Chem. 2012; 16:161–223.
2. a) Gerster JF, Carpenter B, Robins RK, Townsend LB. J Med Chem. 1967; 10:326–331. [PubMed: 22185124] b) Tolman RL, Robins RK, Townsend LB. J Heterocycl Chem. 1967; 4:230–238. c) Tolman RL, Robins RK, Townsend LB. J Amer Chem Soc. 1969; 91:2102–2108. [PubMed: 5805382] d) Hinshaw BC, Gerster JF, Robins RK, Townsend LB. J Heterocycl Chem. 1969; 6:215–221. e) Uematsu T, Suhadolnik R.J. J Med Chem. 1973; 16:1405–1407. [PubMed: 4587857] f) Maruyama T, Wotring LL, Townsend LB. J Med Chem. 1983; 26:25–29. [PubMed: 6827526] g) Bergstrom DE, Brattesani AJ, Ogawa MK, Reddy PA, Schweickert MJ, Balzarini J, De Clercq E. J Med Chem. 1984; 27:285–292. [PubMed: 6699874] h) De Clercq E, Balzarini J, Madej D, Hansske F, Robins MJ. J Med Chem. 1987; 30:481–486. [PubMed: 3820218] i) Turk SR, Shipman C, Nassiri R, Genzlinger G, Krawczyk SH, Townsend LB, Drach JC. Antimicrob Agents Chemother. 1987; 31:544–550. [PubMed: 3037998] j) Gupta PK, Daunert S, Nassiri MR, Wotring LL, Drach JC, Townsend LB. J Med Chem. 1989; 32:402–408. [PubMed: 2913300] k) Robins MJ, Wilson JS, Madej D, Low NH, Hansske F, Wnuk SF. J Org Chem. 1995; 60:7902–7908. l) Bookser BC,

- Ugarkar BG, Matelich MC, Lemus RH, Allan M, Tsuchiya M, Nakane M, Nagahisa A, Wiesner JB, Erion MD. *J Med Chem.* 2005; 48:7808–7820. [PubMed: 16302820]
3. Zhang X, Jia D, Liu H, Zhu N, Zhang W, Feng J, Yin J, Hao B, Cui D, Deng Y, Xie D, He L, Li B. *PLoS ONE.* 2013; 8:1–11.
 4. Stockwin L, Yu S, Stotler H, Hollingshead M, Newton D. *BMC Cancer.* 2009; 9:63–75. [PubMed: 19232100]
 5. Choi BY, Lee CH. *Bioorg Med Chem Lett.* 2010; 20:3880–3884. [PubMed: 20627562]
 6. Wu R, Smidansky ED, Oh HS, Takhampunya R, Padmanabhan R, Cameron CE, Peterson BR. *J Med Chem.* 2010; 53:7958–7966. [PubMed: 20964406]
 7. De Clercq E, Robins MJ. *Antimicrob Agents Chemother.* 1986; 30:719–724. [PubMed: 3800347]
 8. Varaprasad CVNS, Ramasamy KS, Girardet JL, Gunic E, Lai V, Zhong W, An H, Hong Z. *Bioorg Chem.* 2007; 35:25–34. [PubMed: 16945403]
 9. Ding Y, An H, Hong Z, Girardet JL. *Bioorg Med Chem Lett.* 2005; 15:725–727. [PubMed: 15664845]
 10. a) Eldrup AB, Prhave M, Brooks J, Bhat B, Prakash TP, Song Q, Bera S, Bhat N, Dande P, Cook PD, Bennett CF, Carroll SS, Ball RG, Bosserman M, Burlein C, Colwell LF, Fay JF, Flores OA, Getty K, LaFemina RL, Leone J, MacCoss M, McMasters DR, Tomassini JE, von Langen D, Wolanski B, Olsen DB. *J Med Chem.* 2004; 47:5284–5297. [PubMed: 15456273] b) Olsen DB, Eldrup AB, Bartholomew L, Bhat B, Bosserman MR, Ceccacci A, Colwell LF, Fay JF, Flores OA, Getty KL, Grobler JA, LaFemina RL, Markel EJ, Migliaccio G, Prhave M, Stahlhut MW, Tomassini JE, MacCoss M, Hazuda DJ, Carroll SS. *Antimicrob Agents Chemother.* 2004; 48:3944–3953. [PubMed: 15388457]
 11. Bhattacharya BK, Rao TS, Revankar GR. *J Chem Soc Perkin Trans.* 1995; 1:1543–1550.
 12. a) Bhattacharya BK, Ojwang JO, Rando RF, Huffman JH, Revankar GR. *J Med Chem.* 1995; 38:3957–3966. [PubMed: 7562929] b) Ojwang JO, Bhattacharya BK, Marshall HB, Korba BE, Revankar GR, Rando RF. *Antimicrob Agents Chemother.* 1995; 39:2570–2573. [PubMed: 8585748]
 13. a) Ugarkar BG, Castellino AJ, DaRe JM, Kopcho JJ, Wiesner JB, Schanzer JM, Erion MD. *J Med Chem.* 2000; 43:2894–2905. [PubMed: 10956197] b) Boyer SH, Ugarkar BG, Solbach J, Kopcho J, Matelich MC, Ollis K, Gomez-Galeno JE, Mendonca R, Tsuchiya M, Nagahisa A, Nakane M, Wiesner JB, Erion MD. *J Med Chem.* 2005; 48:6430–6441. [PubMed: 16190769]
 14. Naus P, Pohl R, Votruba I, Dzubak P, Hajdich M, Ameral R, Birkus G, Wang T, Ray AS, Mackman R, Cihlar T, Hocek M. *J Med Chem.* 2010; 53:460–470. [PubMed: 19929004]
 15. Bourderioux A, Naus P, Perlikova P, Pohl R, Pichova I, Votruba I, Dzubak P, Konecny P, Hajdich M, Stray KM, Wang T, Ray AS, Feng JY, Birkus G, Cihlar T, Hocek M. *J Med Chem.* 2011; 54:5498–5507. [PubMed: 21711054]
 16. Naus P, Caletkova O, Konecny P, Dzubak P, Bogdanova K, Kolar M, Vrbkova J, Slavetinska L, Tloust'ova E, Perlikova P, Hajdich M, Hocek M. *J Med Chem.* 2014; 57:1097–1110. [PubMed: 24397620]
 17. Miles RW, Samano V, Robins MJ. *J Am Chem Soc.* 1995; 117:5951–5957.
 18. Robins MJ, Trip EM. *Biochemistry.* 1973; 12:2179–2187. [PubMed: 4710578]
 19. Young JD, Yao SYM, Baldwin JM, Cass CE, Baldwin SA. *Mol Aspects Med.* 2013; 34:529–547. [PubMed: 23506887]
 20. Jordheim LP, Durantel D, Zoulim F, Dumontet C. *Nat Rev Drug Discov.* 2013; 12:447–464. [PubMed: 23722347]
 21. Griffiths M, Beaumont N, Yao SYM, Sundaram M, Boumah CE, Davies A, Kwong FYP, Coe I, Cass CE, Young JD, Baldwin SA. *Nat Med.* 1997; 3:89–93. [PubMed: 8986748]
 22. Robins MJ, Asakura J-i, Kaneko M, Shibuya S, Jakobs ES, Agbanyo FR, Cass CE, Paterson ARP. *Nucleosides and Nucleotides.* 1994; 13:1627–1646.
 23. Robins MJ, Peng Y, Damaraju VL, Mowles D, Barron G, Tackaberry T, Young JD, Cass CE. *J Med Chem.* 2010; 53:6040–6053. [PubMed: 20718495]
 24. Young, JD.; Yao, SYM.; Cass, CE.; Baldwin, SA. *Red Cell Membrane Transport in Health and Disease.* Bernhardt, I.; Ellory, JC., editors. Springer-Verlag; Berlin: 2003. p. 321–337.

25. Dimroth O. Justus Liebigs Ann Chem. 1909; 364:183–226.
26. Liu J, Robins MJ. J Am Chem Soc. 2007; 129:5962–5968. [PubMed: 17439120]
27. a) Engel JD. Biochem Biophys Res Commun. 1975; 64:581–586. [PubMed: 1147943] b) Fujii T, Itaya T. Heterocycles. 1998; 48:359–390.
28. Pande P, Shearer J, Yang J, Greenberg WA, Rokita SE. J Am Chem Soc. 1999; 121:6773–6779.
29. Zhong M, Robins MJ. J Org Chem. 2006; 71:8901–8906. [PubMed: 17081021]
30. a) Robins MJ, Uzna ski B. Can J Chem. 1981; 59:2608–2611. b) Liu J, Robins MJ. Org Lett. 2005; 7:1149–1151. [PubMed: 15760161]
31. Olah GA, Welch JT, Vankar YD, Nojima M, Kerekes I, Olah JA. J Org Chem. 1979; 44:3872–3881. b) Typical procedure for the preparation of ~55% HF-pyridine: 1.8 mL of 70% HF-pyridine was added to 0.6 mL of anhydrous pyridine at –78 °C to generate 2.4 mL of ~55% HF-pyridine. The mixture was allowed to warm gradually to –10 °C and used directly.
32. a) Peterson MA, Oliveira M, Christiansen MA. Nucleosides Nucleotides Nucleic Acids. 2009; 28:394–407. [PubMed: 20183591] b) Shelton JR, Cutler CE, Browning MS, Balzarini J, Peterson MA. Bioorg Med Chem Lett. 2012; 22:6067–6071. [PubMed: 22959517]
33. Nowak I, Conda-Sheridan M, Robins MJ. J Org Chem. 2005; 70:7455–7458. [PubMed: 16122277]

Reagents and Conditions: (a) ArCH₂Br/DMF; (b) Me₂NH (2 M THF)/MeOH

Scheme 1.

Synthesis of 4-*N*-benzylated nucleosides by alkylation

Reagents and Conditions: (a) ArCH₂Br/DMF; (b) Me₂NH (2 M THF)/MeOH

Scheme 2.

$\text{S}_{\text{N}}\text{Ar}$ -based synthesis of 4-*N*-(4-nitrobenzyl)tubercidin (**2b**)

Synthesis of 6-ureidoadenosine analogues

For compounds **8** and **9**: **a**, R = H; **b**, R = OCH₃; **c**, R = NO₂

Table 1

Inhibitory effects on the proliferation of cells in culture

Compd	IC ₅₀ (μM) *				
	L1210	CEM	HeLa	PC-3	Caki-1
2a	172 ± 47	182 ± 20	7.4 ± 2.2	0.92 ± 0.67	116 ± 23
2b	125 ± 28	250	143 ± 4	76 ± 10	132 ± 30
2c	0.92 ± 0.04	115 ± 28	1.8 ± 0.2	3.4 ± 0.9	116 ± 23
2d	5.5 ± 1.5	109 ± 16	9.4 ± 3.0	5.6 ± 3.3	98 ± 10
9a	>250	221 ± 0	201 ± 44	115 ± 20	150 ± 33
9b	>250	>250	161 ± 10	110 ± 31	250
9c	>250	>250	>250	45 ± 26	>250

* 50% inhibitory concentration