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Synthesis and antiviral properties of aza-analogues of *ganciclovir* derived from 5,5-bis(hydroxymethyl)-pyrrolidin-2-one

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Abstract—Novel aza-analogues of *ganciclovir* were obtained from 1,5,5-tris(pivaloyloxymethyl)pyrrolidin-2-one by a one-pot base silylation/nucleoside coupling procedure. The compounds were evaluated for, but found to be devoid of, antiviral activity in vitro.

1. Introduction

Nucleoside analogues are used in the therapy of the contemporary plagues—cancer and viral diseases.[1,2] They are structurally very diverse compounds modified at the sugar, nucleobase, or both. The replacement of the furanose ring by different heterocycles is one of the approaches developed to obtain novel therapeutic agents.[3,4] Recently, the synthesis of analogues containing an azaheterocycle as the sugar mimic (i.e., azanucleosides) has attracted considerable attention because of their interesting biological properties.[3] Most of them are derivatives of pyrrolidine or pyrrolidin-2-one, although analogues possessing an azaheterocycle different from the five-membered ring have been also described.[3,5] Taking into account the way of linking the nucleobase with the sugar mimic, azanucleosides can be classified into two major categories: (a) derivatives with the nucleobase directly connected to the sugar mimic,[6–8] and (b) derivatives having a carbon spacer between the sugar mimic and the nucleobase.[9,10] Among azanucleosides belonging to the latter category, compounds with the carbon spacer between the nucleobase and the pyrrolidin-2-one nitrogen atom are known (Fig. 1, compounds **A**),[9] but there is much less work on their synthesis than on compounds **B**.[10] Azanucleosides **A** have been prepared by alkylation of a silylated nucleobase with the corresponding chloromethyl precursor. To the best of our knowledge, in contrast to the derivatives **B**,[10h] the azanucleosides **A** have not been examined for antiviral activity.

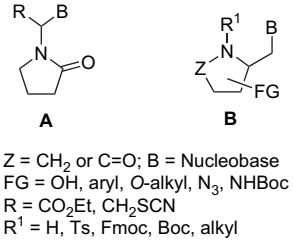

Figure 1. Pyrrolidine or pyrrolidin-2-one derived azanucleosides with a carbon spacer between a sugar mimic and a nucleobase.

Since nucleoside analogues with bis(hydroxymethyl) branched sugars (including azanucleosides[3c]) display antiviral activities, their synthesis has attracted particular attention.[11] Thus, continuing our studies on azanucleosides,[12] we became interested in the synthesis of azanucleosides **C** (Fig. 2), in which the nucleobase is connected to the nitrogen

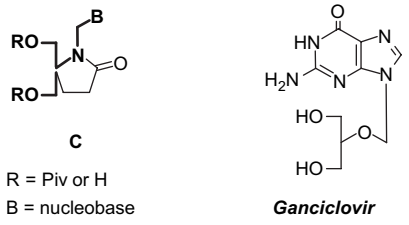

Figure 2. Aza-analogues of *ganciclovir* derived from 5,5-bis(hydroxymethyl)pyrrolidin-2-one.

Keywords: Azanucleosides; Nucleoside analogues; Pyrrolidinone; Antiviral agents; *Ganciclovir*; *N*-(Pivaloyloxymethyl)amide.
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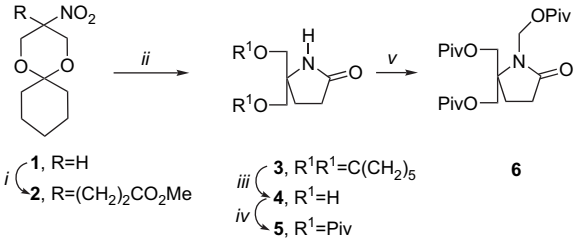
atom of 5,5-bis(hydroxymethyl)pyrrolidin-2-one via the methylene spacer; these compounds can be considered as aza-analogues of *ganciclovir*. We were also influenced by a report that the derivatives of 1-(4-carboxyphenyl)-5,5-bis(hydroxymethyl)pyrrolidin-2-one are active as inhibitors of influenza A sialidase.[13] Considering the fact that the lipophilicity of nucleoside analogues is one of the major factors in determining their physiological activity,[14] we synthesized azanucleosides **C** in the form of the pivaloyl esters and the corresponding hydroxyl derivatives.

2. Results and discussion

It is known that, regardless of the molar ratio of the reactants, the amidoalkylation of silylated pyrimidine nucleobases with *N*-(chloromethyl)pyrrolidin-2-one affords 1,3-disubstituted pyrimidine-2,4-diones as the only products.[9a] Therefore, based on our preliminary studies on the amidoalkylation of silylated nucleobases with *N*-(pivaloyloxymethyl)amides in the presence of a Lewis acid,[15] we decided to employ 1,5,5-tris(pivaloyloxymethyl)pyrrolidin-2-one **6** (Scheme 1) as the substrate in the synthesis of azanucleosides of the type **C** (Schemes 2 and 3, compounds **7** and **9**). Synthesis of **6** from the readily available nitrodioxane **1**[16] is shown in Scheme 1.



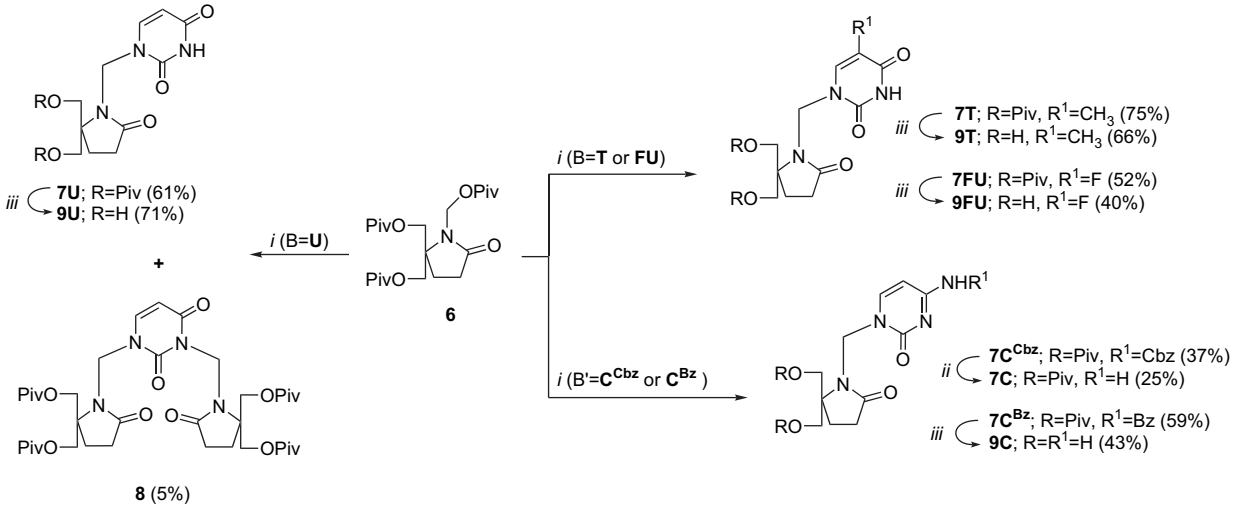
Scheme 1. *Reagents and conditions*: (i) CH_2=CH–C(O)OMe, TMG, MeOH, 70 °C, 4 h, 68%; (ii) H_2, 10% Pd/C, MeOH, 50 atm, 70 °C, 1 day, 57%; (iii) Dowex-50 (H^+), MeOH, rt, 1 day; (iv) PivCl, Py, rt, 1 day, 65% (from **3**); (v) NaH, $PivOCH_2Cl$, DMF, rt, 3 days, 75%.

Michael addition of **1** to methyl acrylate in the presence of tetramethylguanidine (TMG) afforded nitroester **2**. Catalytic reduction of the nitro group in **2** followed by the closure of the pyrrolidin-2-one ring gave 5,5-bis(hydroxymethyl)pyrrolidin-2-one in the form of the cyclohexylidene acetal **3**. This methodology allowed us to obtain **3** on multigram scale without chromatographic purification. Our approach to the synthesis of **3** was much more efficient than the six-step procedure reported for the preparation of isopropylidene acetal of 5,5-bis(hydroxymethyl)-pyrrolidin-2-one from 2-amino-2-(hydroxymethyl)propane-1,3-diol.[17] Deprotection of **3** under acidic conditions (Dowex-50 H^+) followed by esterification of **4** with pivaloyl chloride (PivCl) in pyridine furnished 5,5-bis(pivaloyloxymethyl)pyrrolidin-2-one **5**. *N*-Alkylation of **5** with chloromethyl pivaloate ($PivOCH_2Cl$) in the presence of sodium hydride completed the synthesis of **6**.

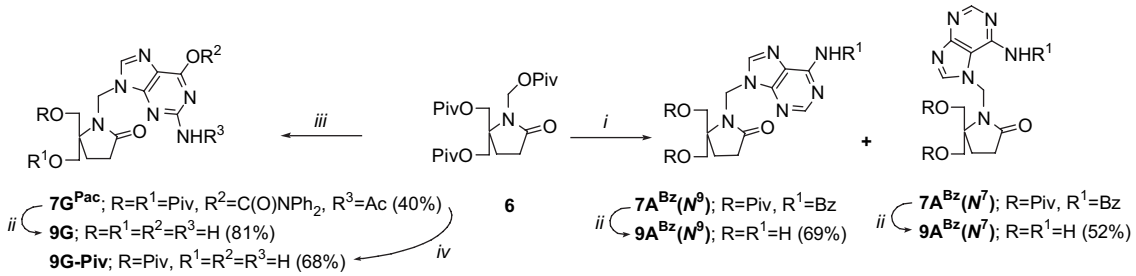
The test reaction of **6** with silylated uracil (**U**) in the presence of trimethylsilyl triflate (TMSOTf), performed by the one-pot base silylation/nucleoside coupling methodology,[18] gave azanucleoside **7U** as the major product (Scheme 2, 61% yield). The minor product, 1,3-disubstituted uracil **8** (5%), was readily separated by column chromatography.

Following the same procedure, the condensation of **6** with the silylated thymine (**T**), 5-fluorouracil (**FU**), N^4-Cbz-cytosine[19] ($\mathbf{C^{Cbz}}$), or N^4-Bz-cytosine ($\mathbf{C^{Bz}}$) was conducted (Scheme 2, conditions (i)) to give azanucleosides **7T**, **7FU**, $\mathbf{7C^{Cbz}}$, or $\mathbf{7C^{Bz}}$, respectively, as the only products. The palladium-catalyzed hydrogenolysis of $\mathbf{7C^{Cbz}}$ gave the cytosine derivative **7C** possessing the pivaloyloxymethyl groups at the azasugar moiety (Scheme 2, conditions (ii)). Whereas, the treatment of derivatives $\mathbf{7C^{Bz}}$, **7T**, **7U**, and **7FU** with aqueous ammonia in methanol at 70 °C afforded the corresponding hydroxyl azanucleosides **9C**, **9T**, **9U**, and **9FU** in 40–71% yields (Scheme 2, conditions (iii)). The N^1-substitution pattern of the pyrimidine azanucleosides was confirmed by the ^1H–^{13}C HMBC correlations observed for the thymine derivative **7T** (Fig. 3).

The coupling of **6** with N^6-Bz-adenine ($\mathbf{A^{Bz}}$) was carried out in the presence of tin(IV) chloride as the catalyst, instead of TMSOTf, by the same methodology that was described for the coupling of **6** with the pyrimidine nucleobases (Scheme 3, conditions (i)). The mixture of $\mathbf{7A^{Bz}}(\boldsymbol{N^9})$ (N^9-isomer) and



Scheme 2. *Reagents and conditions*: (i) (a) pyrimidine nucleobase (**B** or **B′**), BSA, acetonitrile, rt, 1 h; (b) **6**, TMSOTf, acetonitrile, rt, 2 days; (ii) H_2 (ballon), 10% Pd/C, MeOH, rt, 1 day; (iii) NH_3 (aq), MeOH, sealed tube, 70 °C, 1 day.



Scheme 3. *Reagents and conditions*: (i) (a) $\mathbf{A^{Bz}}$, BSA, acetonitrile, rt, 1 h; (b) **6**, $SnCl_4$, acetonitrile, 2 days, rt, 68% summary yield, ($\mathbf{7A^{Bz}}(N^9)$/$\mathbf{7A^{Bz}}(N^7)$=1.0:1.2); (ii) NH_3 (aq), MeOH, sealed tube, 70 °C, 1 day; (iii) (a) $\mathbf{G^{Pac}}$, BSA, 1,2-dichloroethane, rt, 1 h; (b) **6**, TMSOTf, toluene, 80 °C, 1 h; (iv) NH_3 (aq), MeOH, rt, 1 day.

$\mathbf{7A^{Bz}}(N^7)$ (N^7-isomer) was obtained in 68% combined yield. The ratio of the regioisomers (N^9/N^7=1.0:1.2) was estimated from the intensities of signals corresponding to the exocyclic methylene protons (–N–CH_2–N–): δ_H 5.51 ppm [$\mathbf{7A^{Bz}}(N^9)$] and δ_H 5.76 ppm [$\mathbf{7A^{Bz}}(N^7)$]. The separation of these regioisomers was very difficult; chromatographic purification furnished pure $\mathbf{7A^{Bz}}(N^9)$ (13% calculated with respect to **6**) and $\mathbf{7A^{Bz}}(N^7)$ (7% calculated with respect to **6**), and the $\mathbf{7A^{Bz}}(N^9)$/$\mathbf{7A^{Bz}}(N^7)$ mixture in the ratio of 1.7:1.0, respectively (^{1}H NMR). Treatment of $\mathbf{7A^{Bz}}(N^9)$ or $\mathbf{7A^{Bz}}(N^7)$ with aqueous ammonia in methanol at 70 °C gave azanucleosides $\mathbf{9A}(N^9)$ or $\mathbf{9A}(N^7)$ in 69 or 52% yield, respectively (Scheme 3). The structure of these isomers was determined by the examination of the ^{1}H–^{13}C HMBC correlations (Fig. 4).[20]

The condensation of **6** with N^2-Ac-O^6-(diphenylcarbamoyl)-guanine ($\mathbf{G^{Pac}}$) by the Robins' methodology[21] furnished $\mathbf{7G^{Pac}}$ in 40% yield as a single N^9-isomer (Scheme 3, conditions (*iii*)). The deprotection of $\mathbf{7G^{Pac}}$ with aqueous ammonia in methanol at room temperature afforded azanucleoside **9G-Piv** in 68% yield, whereas the same reaction performed at 70 °C gave **9G** in 81% yield (Scheme 3). The N^9-substitution pattern of guanine was confirmed by the ^{1}H–^{13}C HMBC correlations for **9G** (Fig. 4).

2.1. Antiviral activity

The antiviral activities of compounds **7T**, **7U**, **7FU**, **7C**, **9T**, **9U**, **9FU**, **9C**, $\mathbf{9A}(N^9)$, $\mathbf{9A}(N^7)$, **9G**, and **9G-Piv** were evaluated in vitro against a variety of viruses. The following viruses and host cells were used for the evaluation:

(a) Vero cell cultures: parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus, and Punta Toro virus;
(b) HeLa cell cultures: vesicular stomatitis virus, Coxsackie B4 virus, and respiratory syncytial virus;
(c) HEL cell cultures: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK^- KOS ACV^r), vaccinia virus, and vesicular stomatitis virus;

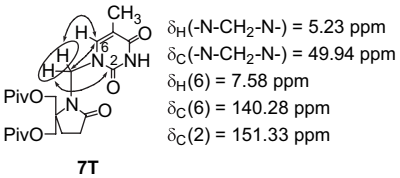


Figure 3. The principal ^{1}H–^{13}C HMBC correlations observed for **7T**.

(d) HEL cell cultures: cytomegalovirus (strains AD-169 and Davis), varicella-zoster virus (TK^+ VZV strain Oka, and TK^- VZV strain 07/1).

Brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], *ribavirin*, *acyclovir*, *cidofovir* and *ganciclovir* were used as the reference compounds. In the tests with viruses described in (a), (b), and (c) antiviral activity[22] and, in parallel, cytotoxicity[23] were monitored with the compound concentrations up to 200 μM. Some of the azanucleosides showed very low antiviral activity in the following tests: the thymine derivative **7T** against Punta Toro virus in Vero cell cultures (IC_{50}=120 μM);[24] the cytosine derivative **9C** and the guanine derivative **9G** against herpes simplex virus-2 (G) in HEL cell cultures (IC_{50}=120 μM).[25] No specific antiviral effects were noted for the other compounds tested against any of the viruses evaluated. The following minimum cytotoxic concentration (MCC) values were estimated for all compounds tested: (i) Vero cell cultures, >200 μM; (ii) HEL cell cultures, >200 μM; (iii) HeLa cell cultures, >200 μM.[26] In the tests with cytomegalovirus and varicella-zoster virus (described in (d)), antiviral activity[27] and, in parallel, cytotoxicity[28] were monitored with the compound concentrations up to 100 μM. No specific antiviral effects were noted for any of the compounds against the CMV or VZV strains tested. The estimated values of MCC and the cytotoxic concentration (CC_{50}) for all compounds tested were as follows: MCC>100 μM, CC_{50}>100 μM.[29]

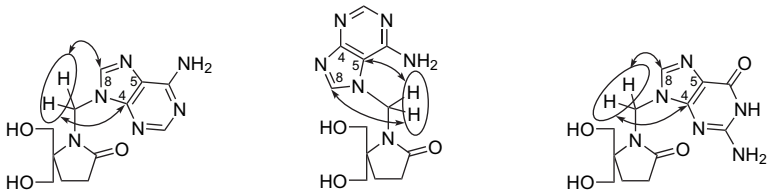
3. Conclusion

We synthesized aza-analogues of *ganciclovir* by the coupling of silylated nucleobases with the readily available 1,5,5-tris(pivaloyloxymethyl)pyrrolidin-2-one in the presence of a Lewis acid. Generally, amidoalkylation of silylated nucleobases with the readily available and stable *N*-(pivaloyloxymethyl)amides may be of help in synthesizing many nucleoside amidomethyl analogues from amino acids or peptides, for example.

4. Experimental

4.1. Materials and methods

Precoated Merck silica gel 60 F_{254} (0.2 mm) plates were used for thin-layer chromatography (TLC), and the spots



9A(N^9)

δ_H(-N-CH$_2$-N-) = 5.51 ppm
δ_C(4) = 148.83 ppm
δ_H(8) = 141.17 ppm

9A(N^7)

δ_H(-N-CH$_2$-N-) = 5.76 ppm
δ_C(5) = 110.71 ppm
δ_C(8) = 146.52 ppm

9G

δ_H(-N-CH$_2$-N-) = 5.28 ppm
δ_C(4) = 150.59 ppm
δ_C(8) = 137.24 ppm

Figure 4. The principal ^{1}H–^{13}C HMBC correlations observed for **9A(N^9)**, **9A(N^7)**, and **9G**.

were detected under UV light (254 nm). Column chromatography was performed using silica gel (200–400 mesh, Merck). *H*igh *R*esolution *M*ass *S*pectra (Electrospray Ionisation, ESI) were performed on a Mariner™ spectrometer in positive ionization mode. The IR spectra were recorded on a Specord M80 (Carl-Zeiss Jena) spectrometer in KBr disc; absorption maxima (ν_{max}) are given in cm^{-1} and quoted as 's' strong, 'm' medium, 'br' broad. The ^{1}H NMR spectra were measured on a Varian Gemini 200 at 200 MHz or on a Varian Gemini 400 spectrometer at 400 MHz. The ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer at 50 MHz. ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent signals: $CDCl_3$, δ_H (residual $CHCl_3$) 7.26 ppm, δ_C 77.16 ppm; or DMSO-d_6, δ_H (residual DMSO-d_5) 2.50 ppm, δ_C 39.52 ppm; signals are quoted as 's' (singlet), 'd' (doublet), 't' (triplet), 'm' (multiplet), and 'br s' (broad singlet). Coupling constants *J* are reported in hertz. ^{1}H–^{13}C HMBC (*H*eteronuclear *M*ultiple *B*ond *C*orrelation) spectra were measured on a Varian Gemini 400 spectrometer in DMSO-d_6. The anhydrous $MgSO_4$ was employed as a drying agent. Solvents were distilled off under reduced pressure on a rotating evaporator.

The methodology used for measuring the antiviral activity has been described previously.[30]

4.2. 3-(3-Nitro-1,5-dioxaspiro[5.5]undec-3-yl)propionic acid methyl ester (2)

A mixture of **1** (3.0 g, 15 mmol), tetramethylguanidine (TMG) (0.52 g, 5 mmol, 0.6 mL), and methanol (25 mL) was refluxed under an argon atmosphere for 15 min. Methyl acrylate (1.93 g, 22.5 mmol, 2 mL) was added at 70 °C in four equal portions within 1 h, and the reaction mixture was then refluxed for 4 h. Methanol was distilled off and the residue dissolved in methylene chloride (35 mL). This solution was washed with 5% aqueous hydrochloric acid, brine, dried, and passed through a silica gel pad. Solvent was distilled off from the eluate to give **2** (2.92 g, 68%) as a white, amorphous powder; mp 54–56 °C. ν_{max}/cm^{-1} 1736 (C═O). δ_H (200 MHz; $CDCl_3$) 1.50–1.68 (m, 8H), 1.74–1.82 (m, 2H), 2.12–2.21 (m, 2H), 2.28–2.36 (m, 2H), 3.38 (s, 3H), 3.96 and 4.45 (AB quartet, $^2J_{A–B}$ 12.9, 4H, 2×–C*H*$_2$–O–). δ_C (50 MHz; $CDCl_3$) 22.50, 22.61, 25.51, 27.67, 28.92, 29.62, 34.75, 52.19, 63.17, 85.60, 99.37, 172.10. HRMS *m/z* calcd for $C_{13}H_{21}NO_6Na$ $(M+Na)^+$ 310.1261, found 310.1262.

4.3. 1-Aza-7,14-dioxadispiro[4.2.5.2]pentadecan-2-one (3)

A mixture of **2** (5.2 g, 18 mmol) and 10% Pd/C (1.5 g) in methanol (100 mL) was hydrogenated under 50 bar pressure at 70 °C for 24 h. Then the mixture was filtered through a Celite pad and the solvent was distilled off. The residue was crystallized from hexane/ethyl acetate mixture (1:8, v/v) to afford **3** (2.33 g, 57%) as white crystals; mp 184–186 °C (from hexane/ethyl acetate). ν_{max}/cm^{-1} 3160 (NH), 1708 (C═O). δ_H (200 MHz; $CDCl_3$) 1.42–1.82 (m, 12H), 2.33–2.42 (m, 2H), 3.62 and 3.78 (AB quartet, $^2J_{A–B}$ 11.5, 4H, 2×–C*H*$_2$–O–), 7.02 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 22.63, 25.67, 26.07, 28.64, 29.27, 36.15, 56.13, 67.86, 98.67, 176.29. HRMS *m/z* calcd for $C_{12}H_{19}NO_3Na$ $(M+Na)^+$ 248.1257, found 248.1246.

4.4. 5,5-Bis(hydroxymethyl)pyrrolidin-2-one (4)

A mixture of **3** (1.81 g, 8.0 mmol), ion exchange resin Dowex-50 (H^+) (5.5 g) and methanol (45 mL) was shaken at room temperature for 3 days. The resin was filtered off and the solvent was distilled off from the filtrate. The residue was dried in a vacuum desiccator over P_2O_5 overnight to afford the crude **4** (0.8 g) as a white, amorphous powder. An analytical sample was obtained by crystallization from methanol; mp 169–170 °C (from methanol). δ_H (400 MHz; DMSO-d_6) 1.73–1.77 (m, 2H), 2.09–2.13 (m, 2H), 3.29 (d, $^3J_{H–H}$ 5.6, 4H,), 4.72 (t, $^3J_{H–H}$ 5.6, 2H), 7.29 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 25.03, 30.41, 63.95, 64.78, 177.10. Anal. Calcd for $C_6H_{11}NO_3$: C 49.65, H 7.64, N 9.65. Found C 49.76, H 7.55, N 9.64.

4.5. 5,5-Bis(pivaloyloxymethyl)pyrrolidin-2-one (5)

A mixture of the crude **4** (0.8 g, 5.5 mmol), pivaloyl chloride (2.55 g, 21 mmol, 2.6 mL), and pyridine (45 mL) was stirred at room temperature for 1 day. The solvent was distilled off and cold water (100 mL) was added to the residue. The mixture was extracted with ethyl acetate (2×50 mL). The combined extracts were washed with diluted hydrochloric acid (5%), brine, saturated aqueous solution of sodium bicarbonate, brine, and dried. The solvent was distilled off; the traces of pyridine were removed by co-distillation with toluene. The residue was dried in vacuum desiccator over P_2O_5 overnight to afford the crude **5** (1.62 g, 65% from **3**) as a white, amorphous powder. An analytical sample was obtained by

crystallization from ethyl acetate; mp 133–135 °C (from ethyl acetate). ν_{max}/cm^{-1} 3189 (NH), 3094 (NH), 1740 (C=O), 1723 (C=O), 1696 (C=O). δ_H (200 MHz; $CDCl_3$) 1.21 (s, 18H), 2.00–2.08 (m, 2H), 2.41–2.48 (m, 2H), 3.93 and 4.22 (AB quartet, $^2J_{A-B}$ 11.4, 4H, 2× –C*H*$_2$OPiv), 5.79 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 26.62, 27.26, 29.45, 39.04, 60.64, 66.36, 177.08, 178.09. HRMS *m*/*z* calcd for $C_{16}H_{27}NO_5Na$ $(M+Na)^+$ 336.1781, found 336.1776.

4.6. 1,5,5-Tris(pivaloyloxymethyl)pyrrolidin-2-one (6)

A mixture of the crude **5** (2.73 g, 8.71 mmol), sodium hydride (60% suspension in mineral oil, 585 mg, 17.42 mmol), and dry DMF (30 mL) was stirred at room temperature for 1 h, then it was cooled in an ice/water bath, and chloromethyl pivaloate (3.94 g, 26 mmol, 3.8 mL) was added. The mixture was stirred at room temperature for 3 days and poured into cold water (120 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (4×60 mL). Extracts and the organic phase were combined and washed with brine and dried. The solvent was distilled off. The residue was purified by column chromatography (chloroform) to give **6** (2.78 g, 75%) as a white, amorphous powder; mp 92–93 °C. ν_{max}/cm^{-1} 1726 (C=O), 1687 (C=O). δ_H (200 MHz; $CDCl_3$) 1.19 (s, 18H), 1.20 (s, 9H), 1.97–2.05 (m, 2H), 2.49–2.57 (m, 2H), 4.11 and 4.24 (AB quartet, $^2J_{A-B}$ 11.7, 4H, 2× –C*H*$_2$OPiv), 5.42 (s, 2H). δ_C (50 MHz; $CDCl_3$) 25.56, 27.15, 27.23, 29.07, 38.82, 39.00, 63.66, 65.48, 65.73, 175.60, 177.81, 177.90. HRMS *m*/*z* calcd for $C_{22}H_{37}NO_7Na$ $(M+Na)^+$ 450.2462, found 450.2442.

4.7. Coupling of 6 with uracil (U)

A mixture of uracil (**U**) (224 mg, 2.0 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (815 mg, 4.0 mmol, 1.0 mL) in dry acetonitrile (10 mL) was stirred at room temperature for 1 h under an argon atmosphere, and then a solution of **6** (428 mg, 1.0 mmol) in dry acetonitrile (1 mL) and trimethylsilyl triflate (TMSOTf) (370 mg, 1.7 mmol, 0.3 mL) were added. The reaction mixture was kept for 24 h at room temperature. Then ethyl acetate (50 mL) and a saturated aqueous solution of sodium bicarbonate (1 mL) were added. The mixture was stirred for 1 h and filtered through a Celite pad. The organic phase was separated, washed with brine, and dried. The solvent was distilled off. The residue was purified by column chromatography (chloroform/acetone, 9:1, v/v) to give **7U** (265 mg, 61%) as a white, amorphous powder (mp 184–186 °C), and **8** (38 mg, 5%) as a foam.

4.7.1. 1-{[5′,5′-Bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*,3*H*-pyrimidin-2,4-dione (7U). ν_{max}/cm^{-1} 1737s, 1727s, 1697s, 1680s, 1481m, 1462m, 1399m, 1357s, 1249m, 1146s. δ_H (200 MHz; $CDCl_3$) 1.16 (s, 18H), 2.01–2.10 (m, 2H), 2.47–2.55 (m, 2H), 4.14 and 4.37 (AB quartet, $^2J_{A-B}$ 12.1, 4H, 2×–C*H*$_2$OPiv), 5.27 (s, 2H), 5.69 (dd, $^4J_{H-H}$ 2.4, $^3J_{H-H}$ 8.0, 1H), 7.85 (d, $^4J_{H-H}$ 8.0, 1H), 8.71 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 25.56, 27.26, 29.39, 39.04, 50.46, 64.45, 64.93, 10.40, 144.90, 151.00, 162.91, 177.68, 177.98. HRMS *m*/*z* calcd for $C_{21}H_{31}N_3O_7Na$ $(M+Na)^+$ 460.2054, found 460.2044.

4.7.2. 1,3-Bis-{[5′,5′-Bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*,3*H*-pyrimidin-2,4-dione (8). ν_{max}/cm^{-1} 1738s, 1703s, 1673s, 1482m, 1461m, 1399m, 1369m, 1283m, 1145s. δ_H (200 MHz; $CDCl_3$) 1.13 (s, 18H), 1.16 (s, 18H), 1.95–2.10 (m, 4H), 2.41–2.53 (m, 4H), 4.06 and 4.34 (AB quartet, $^2J_{A-B}$ 11.7, 4H, 2×–C*H*$_2$O-Piv), 4.09 and 4.39 (AB quartet, $^2J_{A-B}$ 12.0, 4H, 2×–C*H*$_2$O-Piv), 5.25 (s, 2H), 5.54 (s, 2H), 5.69 (d, $^3J_{H-H}$ 8.0, 1H), 7.79 (d, $^3J_{H-H}$ 8.0, 1H). δ_C (50 MHz; $CDCl_3$) 25.15, 25.49, 27.06, 27.08, 29.20, 29.3, 38.81, 46.41, 51.06, 64.22, 64.58, 64.76, 102.68, 143.16, 151.60, 162.34, 175.48, 177.35, 177.69, 177.83. HRMS *m*/*z* calcd for $C_{38}H_{59}N_4O_{12}$ $(M+H)^+$ 763.4124, found 763.4121.

4.8. General procedure for the coupling of 6 with thymine (T), 5-fluorouracil (FU) and N^4-Bz-cytosine (C^{Bz})

A solution of a silylated nucleobase in dry acetonitrile (10 mL) was prepared from the corresponding nucleobase [thymine (**T**), 5-fluorouracil (**FU**), or N^4-Bz-cytosine ($\mathbf{C^{Bz}}$)] (2.0 mmol) and BSA (815 mg, 4.0 mmol, 1.0 mL) and then treated with a solution of **6** (428 mg, 1.0 mmol) in dry acetonitrile (1 mL) and TMSOTf (370 mg, 1.7 mmol, 0.3 mL) according to the procedure used for the coupling of **6** with uracil (**U**). Purification by column chromatography provided **7T**, **7FU** or $\mathbf{7C^{Bz}}$.

4.8.1. 1-{[5′,5′-Bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]-methyl}-5-methyl-1*H*,3*H*-pyrimidin-2,4-dione (7T). According to the general procedure, **7T** was synthesized from **6** and thymine (**T**). Chromatographic purification (chloroform/acetone, 9:1, v/v) afforded **7T** (339 mg, 75%) as a white, amorphous powder; mp 176–177 °C. ν_{max}/cm^{-1} 1744s, 1735s, 1698s, 1657m, 1482m, 1463m, 1412m, 1356m, 1282m, 1260m, 1136s. δ_H (200 MHz; $CDCl_3$) 1.10 [s, 18H, 2×(C*H*$_3$)$_3$C–], 1.83 (d, $^4J_{H-H}$ 1.2, 3H, –C*H*$_3$), 1.96–2.04 (m, 2H, H-4′), 2.43–2.52 (m, 2H, H-3′), 4.11 and 4.33 (AB quartet, $^2J_{A-B}$ 12.0, 4H, 2×–C*H*$_2$O-Piv), 5.23 (s, 2H, –N–C*H*$_2$–N–), 7.58 (q, $^4J_{H-H}$ 1.2, 1H, H-5), 9.62 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 12.39 (*C*H$_3$), 25.33 (C-4′), 27.08 [2×(*C*H$_3$)$_3$C–], 29.38 (C-3′), 38.87 [2×(CH$_3$)$_3$*C*–], 49.94 (–N–*C*H$_2$–N–), 64.26 (2×–*C*H$_2$O-Piv), 64.87 (C-5′), 111.92 (C-5), 140.28 (C-6), 151.33 (C-2), 164.05 (C-4), 177.54 [2×–*C*(O)C(CH$_3$)$_3$], 177.80 (C-2′). HRMS *m*/*z* calcd for $C_{22}H_{33}N_3O_7Na$ $(M+Na)^+$ 474.2211, found 474.2228.

4.8.2. 1-{[5′,5′-Bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-5-fluoro-1*H*,3*H*-pyrimidin-2,4-dione (7FU). According to the general procedure, **7FU** was synthesized from **6** and 5-fluorouracil (**FU**). Chromatographic purification (chloroform/methanol, 98:2, v/v) afforded **7FU** (237 mg, 52%) as a white, amorphous powder; mp 229–231 °C. ν_{max}/cm^{-1} 1733s, 1730s, 1703s, 1664m, 1481m, 1363w, 1363m, 1340m, 1285m, 1143s. δ_H (200 MHz; DMSO-d_6) 1.09 (s, 18H), 1.98–2.06 (m, 2H), 2.39–2.47 (m, 2H), 4.12 and 4.25 (AB quartet, $^2J_{A-B}$ 11.8, 4H, 2×–C*H*$_2$OPiv), 5.15 (s, 2H), 7.88 (d, $^3J_{H-F}$ 6.8, 1H), 12.00 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 24.72, 26.54, 28.77, 50.41, 63.74, 64.17, 128.81 (d, $^2J_{C-F}$ 30.5), 139.34 (d, $^1J_{C-F}$ 230.5), 149.46, 156.89 (d, $^2J_{C-F}$ 22.0), 176.69, 177.25. HRMS *m*/*z* calcd for $C_{21}H_{30}N_3O_7FNa$ $(M+Na)^+$ 478.1960, found 478.1968.

4.8.3. 4-(Benzoylamino)-1-{[5′,5′-bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*-pyrimidin-2-one (7C^{Bz}). According to the general procedure, **7C^{Bz}** was synthesized from **6** and N^4-Bz-cytosine (**C^{Bz}**). Chromatographic purification (chloroform/acetone, 9:1, v/v) afforded **7C^{Bz}** (321 mg, 59%) as a foam. ν_{max}/cm^{-1} 1732s, 1716s, 1683s, 1656m, 1637m, 1511m, 1412s, 1346m, 1139s. δ_H (200 MHz; $CDCl_3$) 1.10 (s, 18H), 1.95–2.04 (m, 2H), 2.43–2.52 (m, 2H), 4.17 and 4.37 (AB quartet, $^2J_{A–B}$ 11.8, 4H, 2×–CH_2OPiv), 5.43 (s, 2H), 7.42–7.59 (m, 4H), 7.89–7.92 (m, 2H), 8.22 (d, $^3J_{H–H}$ 7.4, 1H), 9.93 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 25.34, 27.10, 29.33, 38.86, 64.33, 64.97, 97.80, 127.92, 128.91, 132.94, 133.23. 149.65, 155.43, 162.99, 166.99, 177.47, 177.86. HRMS *m*/*z* calcd for $C_{28}H_{37}N_4O_7$ (M+H)$^+$ 541.2657, found 541.2635.

4.9. 4-Amino-1-{[5′,5′-bis(pivaloyloxymethyl)-pyrrolidin-2′-on-1′-yl]methyl}-1*H*-pyrimidin-2-one (7C)

A solution of silylated N^4-Cbz-cytosine in dry acetonitrile (10 mL) was prepared from N^4-Cbz-cytosine (**C^{Cbz}**) (245 mg, 1.0 mmol) and BSA (408 mg, 2.0 mmol, 0.5 mL), and then treated with a solution of **6** (214 mg, 0.5 mmol) in dry acetonitrile (1 mL) and TMSOTf (187 mg, 0.8 mmol, 0.15 mL) according to the procedure used for the coupling of **6** with uracil (**U**). Purification by column chromatography (chloroform/acetone, 9:1, v/v) afforded crude **7C^{Cbz}** (106 mg, 37%) as a foam; an analytical sample was purified by column chromatography (chloroform/acetone, 95:5, v/v). ν_{max}/cm^{-1} 1736s, 1720s, 1679s, 1651m, 1633m, 1510m, 1412s, 1344m, 1133s. δ_H (200 MHz; $CDCl_3$) 1.12 (s, 18H), 1.95–2.04 (m, 2H), 2.43–2.52 (m, 2H), 4.18 and 4.37 (AB quartet, $^2J_{A–B}$ 12.0, 4H, 2×–CH_2OPiv), 5.21 (s, 2H), 5.41 (s, 2H), 7.21 (d, $^3J_{H–H}$ 7.4, 1H), 7.36 (m, 5H), 8.18 (d, $^3J_{H–H}$ 7.4, 1H). δ_C (50 MHz; $CDCl_3$) 25.37, 27.18, 29.37, 38.95, 51.69, 64.35, 65.08, 67.99, 95.99, 128.23, 128.76, 135.12, 149.52, 152.40, 155.48, 163.11, 177.54, 177.92. HRMS *m*/*z* calcd for $C_{29}H_{38}N_4O_8Na$ (M+Na)$^+$ 593.2582, found 593.2578.

A mixture of the crude **7C^{Cbz}** (102 mg, 0.18 mmol), 10% Pd on charcoal (10 mg) and methanol (10 mL) was hydrogenated under balloon pressure at room temperature for 24 h. The mixture was filtered through a Celite pad. The filtrate was evaporated to dryness. The residue was purified by flash chromatography (chloroform/methanol, 9:1, v/v) to afford **7C** (19 mg, 25%) as a white, amorphous powder; mp 169–177 °C. ν_{max}/cm^{-1} 3303m, 3088m, 1742s, 1725s, 1693s, 1660s, 1644s, 1503m, 1400m, 1359m, 1279m, 1141s. δ_H (200 MHz; DMSO-d_6) 1.08 (s, 18H), 1.94–2.01 (m, 2H), 2.36–2.45 (m, 2H), 4.11 and 4.22 (AB quartet, $^2J_{A–B}$ 11.5, 4H, 2×–CH_2OPiv), 5.14 (s, 2H), 5.69 (d, $^3J_{H–H}$ 7.3, 1H), 7.12 (br s, 1H, N*H*), 7.19 (br s, 1H, N*H*), 7.62 (d, $^3J_{H–H}$ 7.3, 1H). δ_C (50 MHz; DMSO-d_6) 24.83, 26.75, 29.07, 50.48, 63.93, 64.36, 94.71, 145.07, 155.70, 165.83, 176.88. HRMS *m*/*z* calcd for $C_{21}H_{33}N_4O_6$ (M+H)$^+$ 437.2395, found 437.2377.

4.10. Coupling of 6 with N^6-Bz-adenine (A^{Bz})

A solution of silylated N^6-Bz-adenine in dry acetonitrile (10 mL) was prepared from N^6-Bz-adenine (**A^{Bz}**) (957 mg, 4.0 mmol) and BSA (1.63 g, 8.0 mmol, 2.0 mL), and then treated with a solution of **6** (855 mg, 1.0 mmol) in dry acetonitrile (1 mL) and 1 M solution of tin(IV) chloride in dichloromethane (3.5 mL) according to the procedure used for the coupling of **6** with uracil (**U**). Purification by column chromatography (chloroform/acetone, 7:3, v/v) furnished a mixture of **7A^{Bz}(N^7)** and **7A^{Bz}(N^9)** (765 mg, 68%) as a foam; the ratio of the regioisomers was 1.2:1.0, respectively. The column chromatography of this mixture (chloroform/acetone, 9:1 to 6:4, v/v) gave **7A^{Bz}(N^9)** (146 mg, 13%) as a foam, **7A^{Bz}(N^7)/7A^{Bz}(N^9)** mixture in the ratio of 1.7:1.0, respectively, and **7A^{Bz}(N^7)** (76 mg, 7%) as a foam.

4.10.1. 6-(Benzoylamino)-9-{[5′,5′-bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-9*H*-purine [7A^{Bz}(N^9)]. ν_{max}/cm^{-1} 1737s, 1704s, 1610m, 1583m, 1481m, 1459m, 1365m, 1281s, 1139s. δ_H (200 MHz; $CDCl_3$) 1.07 (s, 18H), 2.00–2.09 (m, 2H), 2.48–2.56 (m, 2H), 4.15 and 4.43 (AB quartet, $^2J_{A–B}$ 12.1, 4H, 2×–CH_2O-Piv), 5.74 (s, 2H), 7.48–7.61 (m, 3H), 7.99–8.03 (m, 2H), 8.52 (s, 1H), 8.79 (s, 1H), 9.09 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 25.47, 27.07, 29.18, 38.88, 46.79, 64.73, 121.95, 127.98, 128.92, 132.86, 133.70, 144.71, 149.68, 151.46, 152.88, 164.62, 177.16, 177.56. HRMS *m*/*z* calcd for $C_{29}H_{36}N_6O_6Na$ (M+Na)$^+$ 587.2589, found 587.2576.

4.10.2. 6-(Benzoylamino)-7-{[5′,5′-bis(pivaloyloxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-7*H*-purine [7A^{Bz}(N^7)]. ν_{max}/cm^{-1} 1736s, 1705s, 1638s, 1599m, 1559m, 1481m, 1316s, 1281s, 1138s. δ_H (200 MHz; $CDCl_3$) 1.04 (s, 18H), 2.01–2.10 (m, 2H), 2.50–2.59 (m, 2H), 3.86 and 4.42 (AB quartet, $^2J_{A–B}$ 12.0, 4H, 2×–CH_2O-Piv), 6.42 (s, 2H), 7.42–7.55 (m, 3H), 8.24–8.32 (m, 3H), 8.66 (s, 1H). δ_C (50 MHz; $CDCl_3$) 25.36, 27.07, 29.36, 38.84, 50.73, 63.87, 65.39, 114.15, 128.42, 129.25, 132.40, 136.99, 142.20, 148.48, 150.85, 157.82, 165.86, 177.27, 177.49. LRMS *m*/*z* calcd for $C_{29}H_{37}N_6O_6$ (M+H)$^+$ 565.3, found 565.3.

4.11. General procedure for the deprotection of azanucleosides 7T, 7U, 7C, 7FU, 7A(N^7), and 7A(N^9)

A mixture of **7**, concentrated ammonium hydroxide, and methanol (the ratio of **7**/NH_3 aq/MeOH was 1.0 mmol:5 mL:10 mL, respectively) was heated in a sealed tube at 70 °C for 24 h. The volatiles were evaporated to dryness under reduced pressure. The residue was purified by column chromatography; the eluting solvents are given in the parentheses below. After removal of the solvent from the combined fractions, the residue was dissolved in a minimal amount of methanol and azanucleoside **9** was precipitated with diethyl ether.

4.11.1. 1-{[5′,5′-Bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-5-methyl-1*H*,3*H*-pyrimidin-2,4-dione (9T). According to the general procedure for the deprotection, **9T** was synthesized from **7T**. Chromatographic purification (chloroform/methanol, 9:1, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9T** (83 mg, 66%) as a white, amorphous powder; mp 228–230 °C (from diethyl ether/methanol). ν_{max}/cm^{-1} 3395s (OH), 1717s (C=O), 1679s (C=O), 1656s (C=O), 1384s, 1349m, 1264m, 1129m. δ_H (200 MHz; DMSO-d_6) 1.72 (d, $^4J_{H–H}$ 1.4, 3H), 1.88–1.96 (m, 2H), 2.27–2.35 (m,

2H), 3.29 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{A-X}$ 5.4, 2H, 2× –C*H*HOH), 3.44 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{B-X}$ 5.0, 2H, 2×–CH*H*OH), 4.87 (ABX triplet, 2H, 2×O*H*), 5.10 (s, 2H), 7.41 (q, $^4J_{H-H}$ 1.4, 1H), 11.27 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 12.24, 23.76, 29.50, 49.27, 61.98, 67.78, 109.11, 139.88, 150.89, 164.00, 177.63. HRMS *m/z* calcd for $C_{12}H_{17}N_3O_5Na$ (M+Na)$^+$ 306.1060, found 306.1073.

4.11.2. 1-{[5′,5′-Bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*,3*H*-pyrimidin-2,4-dione (9U). According to the general procedure for the deprotection, **9U** was synthesized from **7U**. Chromatographic purification (chloroform/methanol, 9:1, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9U** (87 mg, 71%) as a white, amorphous powder; mp 171–172 °C (from diethyl ether/methanol). ν_{max}/cm^{-1} 3431s (OH), 1725s (C═O), 1687s (C═O), 1662s (C═O), 1465m, 1355s, 1264s, 1160m. δ_H (200 MHz; DMSO-d_6) 1.88–1.96 (m, 2H), 2.27–2.35 (m, 2H), 3.29 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{A-X}$ 5.4, 2H, 2×–C*H*HOH), 3.44 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{B-X}$ 5.0, 2H, 2×–CH*H*OH), 4.89 (ABX triplet, 2H, 2×O*H*), 5.12 (s, 2H), 5.59 (d, $^3J_{H-H}$ 8.0, 1H), 7.55 (d, $^3J_{H-H}$ 8.0, 1H), 11.26 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 23.76, 29.43, 49.35, 61.97, 67.71, 101.72, 144.21, 150.86, 163.36, 177.61. HRMS *m/z* calcd for $C_{11}H_{15}N_3O_5Na$ (M+Na)$^+$ 292.0904, found 292.0917.

4.11.3. 1-{[5′,5′-Bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-5-fluoro-1*H*,3*H*-pyrimidin-2,4-dione (9FU). According to the general procedure for the deprotection, **9FU** was synthesized from **7FU**. Chromatographic purification (chloroform/methanol, 9:1, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9FU** (35 mg, 40%) as a white, amorphous powder; mp 171–172 °C (from diethyl ether/methanol). ν_{max}/cm^{-1} 3462s (OH), 1712s (C═O), 1648s (C═O), 1415m, 1389m, 1330m, 1261m. δ_H (200 MHz; DMSO-d_6) 1.88–1.96 (m, 2H), 2.28–2.37 (m, 2H), 3.28–3.48 (m, 4H), 4.92 (br s, 2H, O*H*), 5.10 (s, 2H), 7.74 (d, $^3J_{H-F}$ 6.8, 1H), 11.82 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 23.95, 29.55, 50.01, 62.07, 67.88, 128.46 (d, $^2J_{C-F}$ 33.0), 139.58 (d, $^1J_{C-F}$ 228.8), 149.60, 157.38 (d, $^2J_{C-F}$ 25.4), 178.03. HRMS *m/z* calcd for $C_{11}H_{14}N_3O_5FNa$ (M+Na)$^+$ 310.0810, found 310.0824.

4.11.4. 4-Amino-1-{[5′,5′-bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*-pyrimidin-2-one (9C). According to the general procedure for the deprotection, **9C** was synthesized from **7C^{Bz}**. Chromatographic purification (chloroform/acetone, 8:2, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9C** (41 mg, 43%) as a white, amorphous powder; mp 222–224 °C (from diethyl ether/methanol). ν_{max}/cm^{-1} 3385s (OH), 3308s (NH_2), 3087m (NH_2), 1662s (C═O), 1614s (C═O), 1502m, 1409m, 1280m. δ_H (200 MHz; DMSO-d_6) 1.86–1.94 (m, 2H), 2.23–2.32 (m, 2H), 3.30–3.50 (m, 4H), 4.93 (t, $^3J_{H-H}$ 5.2, 2H, O*H*), 5.09 (s, 2H), 5.69 (d, $^3J_{H-H}$ 7.4, 1H), 7.07 (br s, 1H, N*H*), 7.23 (br s, 1H, N*H*), 7.57 (d, $^3J_{H-H}$ 7.4, 1H). δ_C (50 MHz; DMSO-d_6) 24.48, 30.23, 51.80, 62.93, 68.82, 94.88, 145.99, 156.58, 166.57, 178.49. HRMS *m/z* calcd for $C_{11}H_{16}N_4O_4Na$ (M+Na)$^+$ 291.1064, found 291.1074.

4.11.5. 6-Amino-9-{[5′,5′-bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-9*H*-purine [9A(*N*^{9})]. According to the general procedure for the deprotection, **9A(*N*^{9})** was synthesized from **7A(*N*^{9})**. Chromatographic purification (chloroform/methanol, 9:1, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9A(*N*^{9})** (52 mg, 69%) as a white, amorphous powder; mp >190 °C (dec) (from diethyl ether/methanol). ν_{max}/cm^{-1} 3153br (OH), 1662s, 1610m, 1407m, 1348m, 1217 m. δ_H (200 MHz; DMSO-d_6) 1.85–1.93 (m, 2H, H-4′), 2.25–2.33 (m, 2H, H-3′), 3.27–3.47 [m, 4H, 2×–CH_2OH], 5.12 (br s, 2H, 2×O*H*), 5.51 (s, 2H, –N–CH_2–N), 7.30 (br s, 2H, NH_2), 8.07 (s, 1H, H-8), 8.16 (s, 1H, H-2). δ_C (50 MHz; DMSO-d_6) 23.98 (C-4′), 29.33 (C-3′), 47.04 (–N–CH_2–N–), 62.42 (2×–CH_2OH), 67.82 (C-5′), 118.00 (C-5), 141.17 (C-8), 148.83 (C-4), 152.48 (C-2), 156.07 (C-6), 177.30 (C-2′). HRMS *m/z* calcd for $C_{12}H_{17}N_6O_3$ (M+H)$^+$ 293.1357, found 293.1369.

4.11.6. 6-Amino-7-{[5′,5′-bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-7*H*–purine [9A(*N*^{7})]. According to the general procedure for the deprotection, **9A(*N*^{7})** was synthesized from **7A(*N*^{7})**. Chromatographic purification (chloroform/methanol, 9:1, v/v) followed by precipitation with diethyl ether from a methanolic solution afforded **9A(*N*^{7})** (20 mg, 52%) as a white, amorphous powder; mp >180 °C (dec) (from diethyl ether/methanol). ν_{max}/cm^{-1} 3160br (OH), 1646s, 1615m, 1422m, 1337m, 1222m. δ_H (200 MHz; DMSO-d_6) 1.84–1.93 (m, 2H, H-4′), 2.33–2.41 (m, 2H, H-3′), 3.16–3.44 (m, 4H, 2×–CH_2OH), 5.02 (br s, 2H, 2×O*H*), 5.76 (s, 2H, –N–CH_2–N–), 7.21 (br s, 2H, NH_2), 8.16 (s, 1H, H-2), 8.42 (s, 1H, H-8). δ_C (50 MHz; DMSO-d_6) 24.39 (C-4′), 29.41 (C-3′), 51.08 (–N–CH_2–N–), 62.58 (2×–CH_2OH), 68.82 (C-5′), 110.71 (C-5), 146.52 (C-8), 151.79 (C-6), 152.36 (C-2), 159.80 (C-4), 178.05 (C-2′). HRMS *m/z* calcd for $C_{12}H_{17}N_6O_3$ (M+H)$^+$ 293.1357, found 293.1370.

4.12. 2-Amino-9-{[5′,5′-bis(hydroxymethyl)pyrrolidin-2′-on-1′-yl]methyl}-1*H*,9*H*-dihydropurin-6-one (9G)

A mixture of *N*^{2}-acetyl-*O*^{6}-(diphenylcarbamoyl)guanine (**G^{Pac}**) (780 mg, 2.0 mmol) and BSA (815 mg, 4.0 mmol, 1.0 mL) in dry 1,2-dichloroethane (10 mL) was heated at 80 °C in a sealed tube under an argon atmosphere for 15 min. The solvent was distilled off and the residue was dissolved in dry toluene (20 mL). A solution of **6** (428 mg, 1.0 mmol) in dry toluene (1 mL) and then TMSOTf (370 mg, 1.7 mmol, 0.3 mL) were added to this solution. The reaction mixture was heated at 80 °C for 1 h and cooled to room temperature. Ethyl acetate (50 mL) and a saturated aqueous solution of sodium bicarbonate (1 mL) were added to the reaction mixture. The resulting mixture was stirred for 1 h at room temperature. The mixture was filtered through a Celite pad. The organic phase was separated and washed with water, brine, and dried. The solvent was distilled off. The residue was passed through a short column (chloroform/acetone, 9:1, v/v) to afford the crude **7G^{Pac}** (288 mg, 40%) as a foam; an analytical sample was purified by column chromatography (chloroform/acetone, 9:1, v/v). ν_{max}/cm^{-1} 1732s, 1690s, 1648s, 1497m, 1330m, 1292m. δ_H (200 MHz; $CDCl_3$) 1.07 (s, 18H), 2.00–2.09 (m, 2H), 2.43–2.51 (m, 2H), 4.25 and 4.40 (AB quartet, $^2J_{A-B}$ 12.1,

4H, 2×–CH_2OPiv), 5.56 (s, 2H), 7.21–7.43 (m, 10H), 8.38 (m, 1H), 8.47 (br s, 1H, N*H*). δ_C (50 MHz; $CDCl_3$) 25.16, 25.83, 27.12, 29.13, 38.92, 47.34, 64.57, 65.01, 120.11, 127.06, 129.27, 141.86, 145.85, 150.32, 152.28, 154.47, 156.34, 169.79, 177.14, 177.86. HRMS *m*/*z* calcd for $C_{37}H_{43}N_7O_8Na$ $(M+Na)^+$ 736.3065, found 293.3100.

A mixture of the crude **7G^{Pac}** (290 mg), concentrated ammonium hydroxide (2 mL), and methanol (5 mL) was heated in a sealed tube at 70 °C for 24 h. The volatiles were evaporated to dryness under reduced pressure. The residue was purified by column chromatography (chloroform/methanol, 8:2, v/v). After removal of the solvent from the combined fractions, the residue was dissolved in a minimal amount of methanol and azanucleoside **9G** was precipitated with diethyl ether as a white, amorphous powder (101 mg, 81%); mp >155 °C (subl.) (from diethyl ether/methanol). ν_{max}/cm^{-1} 3358m, 3122m, 1715s, 1658s, 1614m, 1405m, 1339m, 1185m. δ_H (400 MHz; DMSO-d_6) 1.88–1.92 (m, 2H, H-4′), 2.27–2.31 (m, 2H, H-3′), 3.28 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{A-X}$ 5.0, 2H, 2×–C*H*HOH), 3.40 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{B-X}$ 4.4, 2H, 2×–CH*H*OH), 4.89 (ABX triplet, 2H, 2×O*H*), 5.28 (s, 2H, –N–CH_2–N–), 6.47 (br s, 2H, NH_2), 7.58 (s, 1H, H-8), 10.13 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 23.89 (C-4′), 29.30 (C-3′), 46.51 (–N–*C*H_2–N–), 62.31 (2×–*C*H_2OH), 67.77 (C-5′), 115.78 (C-5), 137.24 (C-8), 150.60 (C-4), 153.68 [C-2(6)], 156.82 [C-6(2)], 177.06 (C-2′). HRMS *m*/*z* calcd for $C_{12}H_{16}N_6O_4Na$ $(M+Na)^+$ 331.1125, found 331.1131.

4.13. 2-Amino-9-{[5′-(hydroxymethyl)-5′-(pivaloyloxymethyl)pyrrolidin-2′on-1′-yl]methyl}-1*H*,9*H*-dihydropurin-6-one (9G-Piv)

A mixture of the crude **7G^{Pac}** (291 mg), concentrated ammonium hydroxide (2 mL), and methanol (5 mL) was kept at room temperature for 24 h. The volatiles were evaporated to dryness under reduced pressure. The residue was purified by column chromatography (chloroform/methanol, 9:1, v/v). After removal of the solvent from the combined fractions, the residue was dissolved in a minimal amount of methanol and azanucleoside **9G-Piv** was precipitated with diethyl ether as a white, amorphous powder (108 mg, 68%); mp >212 °C (dec) (from diethyl ether/methanol). ν_{max}/cm^{-1} 3329m, 3166m, 1693s, 1636m, 1606m, 1540m, 1482m, 1374m, 1344m. δ_H (400 MHz; DMSO-d_6) 0.92 (s, 9H), 1.76–1.90 (m, 1H), 1.98–2.10 (m, 1H), 2.32–2.37 (m, 2H), 3.45 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{A-X}$ 4.4, 1H, –C*H*HOH), 3.62 (ABX, X=O*H*, $^2J_{A-B}$ 11.6, $^3J_{B-X}$ 5.6, 1H, –CH*H*OH), 3.90 and 4.10 (AB quartet, $^2J_{A-B}$ 11.7, 4H, 2×–CH_2OPiv), 5.16 and 5.43 (AB quartet, $^2J_{A-B}$ 14.4, 2H, –N–CH_2–N–), 5.22 (AB triplet, 1H, O*H*), 6.43 (br s, 2H, NH_2), 7.60 (s, 1H), 10.59 (br s, 1H, N*H*). δ_C (50 MHz; DMSO-d_6) 24.56, 26.63, 29.16, 38.12, 46.00, 62.01, 65.11, 65.68, 115.66, 137.27, 150.49, 153.64, 156.74, 176.66, 176.90. HRMS *m*/*z* calcd for $C_{17}H_{24}N_6O_5Na$ $(M+Na)^+$ 415.1700, found 415.1688.

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22. The antiviral activity was expressed as the minimum inhibitory concentration (IC_{50}) required to reduce virus-induced cytopathogenicity by 50%.
23. The cytotoxicity was expressed as the minimum cytotoxic concentration (MCC) required to cause a microscopically detectable alteration of normal cell morphology.
24. In the same test *ribavirin* displayed IC_{50} of 150 μM.
25. In the same test *ganciclovir* displayed IC_{50} of 0.0064 μM.
26. The reference compounds displayed the following MCC values: *brivudin* and *ribavirin* (Vero, HEL or HeLa cells, >250 μM); (*S*)-DHPA (HeLa cells, >250 μM); *acyclovir* (HEL cells, >250 μM); *ganciclovir* (HEL cells, >100 μM).
27. The antiviral activity was expressed as the effective concentration (EC_{50}) required to reduce virus plaque formation by 50%. Virus input was 100 plaque-forming units (PFU) for cytomegalovirus, or 20 PFU for varicella-zoster virus.
28. The cytotoxicity was expressed as MCC and, in parallel, the cytotoxic concentration (CC_{50}) required to reduce cell growth by 50%.
29. The reference compounds displayed the following MCC and CC_{50} values: *ganciclovir*, MCC>394 μM, CC_{50}=63 μM; *cidofovir*, MCC>1270 μM, CC_{50}=40 μM; *acyclovir*, MCC>1778 μM, CC_{50}=150 μM; *brivudin*, MCC>1201 μM, CC_{50}=257 μM.
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