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Synthesis and antiviral activity of cyclopropyl-spirocarbocyclic adenosine, (4*R*,5*S*,6*R*,7*R*)-4-(6-amino-9*H*-purin-9-yl)-7-(hydroxymethyl)spiro[2.4]heptane-5,6-diol against hepatitis C virus

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ABSTRACT

An efficient method was developed for the synthesis of 6-exocyclic methylene carbocyclic intermediate **4**. The Simmons–Smith cyclopropanation protocol was applied on the 6-exocyclic methylene of intermediate **4** and demonstrated its utility for the synthesis of novel class of a spiro-carbocyclic nucleoside analog **8**. The titled compound **8** demonstrated a significant antiviral activity against HCV with EC_{50} values of 0.273 and 0.368 μM in genotypes 1A and 1B, respectively.

Hepatitis C virus (HCV) belongs to flaviviridae family.[1] It is one of the most clinically important infection affecting liver with high morbidity and mortality worldwide.[2] The world health organization estimates that chronic infection with HCV occurs in approximately 180 million of the world population and 3–4 million new infection occurs each year.[3] According to the Centers for Disease Control and Prevention (CDC) over 3 million people have chronic HCV infection in the US.

HCV is an RNA virus which possesses a single-stranded positive-sense RNA as the viral genome.[4] This viral RNA plays important roles during viral replication, as it serves as an mRNA for viral protein synthesis, a template for RNA replication as well as a nascent RNA genome for a newly formed virus.[5] There is no anti-HCV vaccine available today. Because of the significant undesirable side effects of the current combination therapy of subcutaneous pegylated INF-α and the ribavirin, there is an urgent unmet medical need to develop anti-HCV therapies that are effective, well-tolerated and safe for the treatment of chronic HCV infection.[6] The direct-acting antivirals (DAAs) are the compounds that target a specific viral protein. Presently, four major classes of DAAs are being investigated in phase II/III clinical trials: inhibitors of NS3 protease, allosteric NS5B polymerase, and NS5B polymerase.[6,7] The major challenges for these DAAs include safety, pan-genotypic activity, and/or emergence of resistant strains. An effective antiviral therapy against hepatitis C should encompass a broad spectrum of activity against all HCV genotypes; shorten treatment duration, minimal side effects and a high barrier to resistance. The HCV non-structural protein NS5B RNA-dependent RNA polymerase is a key component of the replicative complex and is responsible for initiating and catalyzing viral RNA synthesis.[3,8] Therefore, the HCV NS5B is an attractive target for the current drug discovery and development of anti-HCV agents. There are two major subclasses of NS5B inhibitors: nucleoside analogs, which are anabolized to their active triphosphates, which act as alternative substrates for the polymerase, and non-nucleoside inhibitors (NNIs), which bind to allosteric regions on the protein. Nucleoside or nucleotide inhibitors mimic natural polymerase substrate and act as chain terminators. They inhibit the initiation of RNA transcription and elongation of a nascent RNA chain.

Several NS5B nucleoside/nucleotide polymerase inhibitors have been in clinical trials as shown in Figure 1.[9–13] 2′-C-Methylcytidine (NM107), the valine ester of 2′-C-methylcytidine(valocitabine, NM283),[10] was the first polymerase inhibitors in clinical trials, which was discontinued due to the GI toxicity. The second nucleoside inhibitor, 4′-C-azido-nucleoside (R1479) as its tri-isobutyl ester prodrug (R1626), have been developed by Roche,[11] however, it was discontinued due to the haematopoetic toxicity. Currently, Roche and Pharmasset are developing R7128 (mericitabine), a prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-C-methylcytidine (PSI6130).[12] Idenix has been developing a purine analogue, 2′-C-methylguanosine monophosphate prodrug (IDX184),[13] however, it is currently

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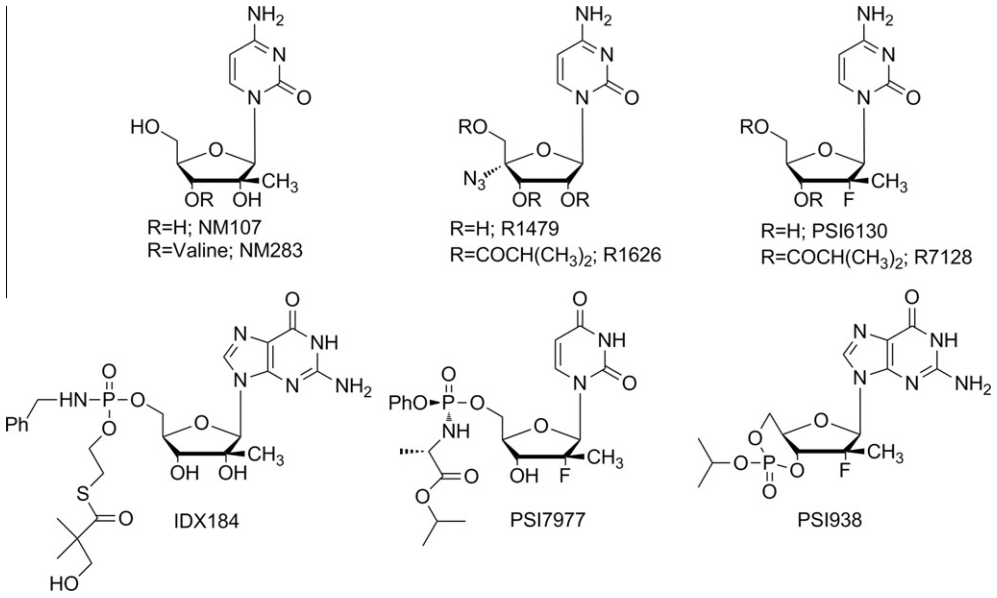
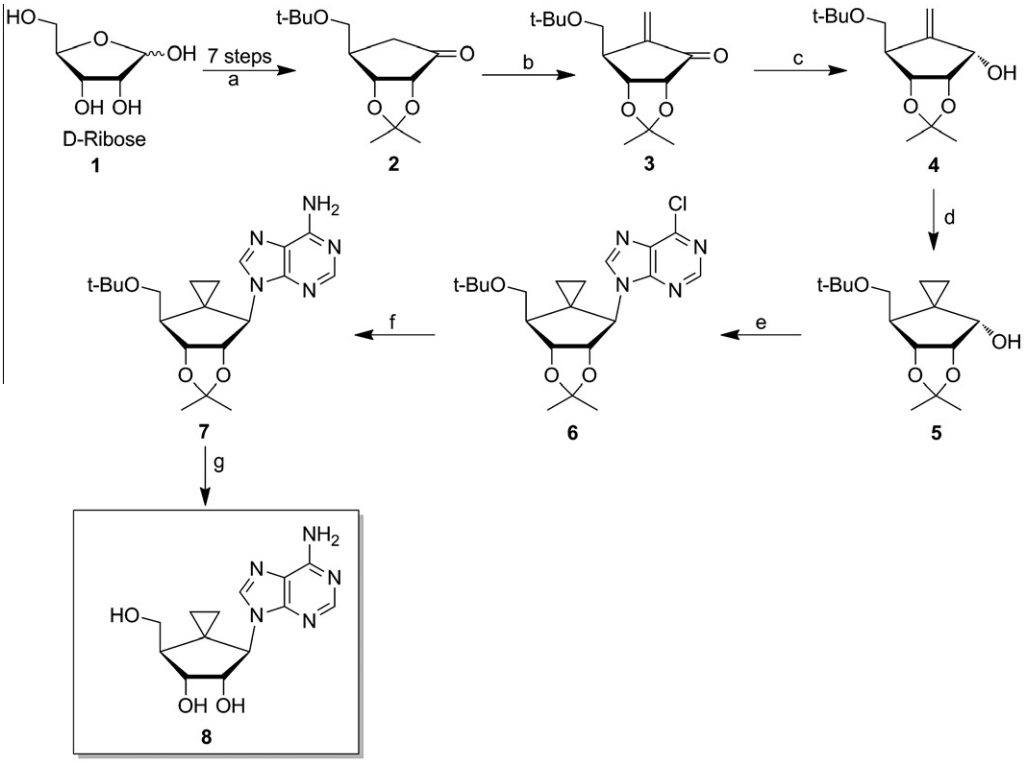


Figure 1. Nucleoside/ nucleotide polymerase inhibitors in clinical trials.



Scheme 1. Synthesis of compound **8**. Reagents and conditions: (a) Refs. 17 and 18; 44% yield; (b) (i) LDA/THF, −78 °C, 3 h; then Eschenmoser's salt, −78 °C, 3 h, rt, 8 h; (ii) CH_3I, 4 h, rt, 10% $NaHCO_3$, 83% yield; (c) $NaBH_4/CeCl_3{\cdot}7H_2O$/MeOH, −78 °C to 0 °C, 90% yield; (d) Et_2Zn/CH_2I_2, ether, reflux, 93% yield; (e) 6-chloropurine, Ph_3P/DIAD, THF, rt, 26.6%; (f) NH_3/MeOH, 100 °C, two steps 48% yield; (g) TFA/H_2O, 80% yield.

under clinical hold due to the potential liver toxicity concern by the FDA. A uridine analogue in a prodrug (PSI7977)[9] form can potently inhibit HCV replication and is considered a second generation of nucleoside inhibitor. Recently, a 3′,5′-cyclic phosphate analogue, PSI-938, has also been reported as a potent anti-HCV agent.[6]

Carbocyclic nucleoside is an interesting class of compounds in which a methylene group replaces the oxygen atom of a furanose ring. As a consequence, the glycosidic bond is resistant to nucleoside phosphorylase as well as nucleoside hydrolase, which renders the carbocyclic nucleosides more stable towards metabolic degradation.[14] Due to these features, carbocyclic nucleosides have received much attention as potential chemotherapeutic agents.[15,16] Carbovir and entecavir are examples of the result of these efforts. As a part of our ongoing antiviral drug discovery program, a number of carbocyclic nucleoside analogues have been synthesized and evaluated for

their potential antiviral activity against HCV, and an interesting novel nucleoside was discovered. Herein, we report the synthesis and anti-HCV activity of hitherto unreported (4*R*,5*S*,6 *R*,7*R*)-4-(6-amino-9*H*-purin-9-yl)-7-(hydroxymethyl)spiro[2.4]heptane-5,6-diol (**8**).

In order to synthesize the target nucleoside **8** (Scheme 1), a ketone **3** was used as the key intermediate, which was synthesized from D-ribose in nine steps. D-Ribose was converted to compound **2** using reported methods.[17,18] The exocyclic methylene group was introduced at the 6-position by quenching the lithium enolate of the ketone **2** with the Eshenmoser's salt[19] (Mannich base), followed by the Hoffmann degradation method,[20] provided an enone **3** in 83% yield. This process is quite convenient and can be generally applied to the chemistry of carbocyclic nucleoside. The resultant enone **3** was treated with sodium borohydride in the presence of cerium(III) chloride heptahydrate, which exclusively provided the allylic alcohol key intermediate **4**[21,22] in 90% yield. For the synthesis of the novel spiro nucleoside, several methods have been tried, however, the Simmon–Smith cyclopropanation[23] of the carbocyclic intermediate **4** using diethyl zinc and diiodomethane under reflux conditions in diethyl ether was found to produce the best results and gave the key spiro-intermediate **5**[22] in 93% yield. The spiro alcohol **5** was coupled with 6-chloropurine under Mitsunobu conditions, which gave an inseparable mixture of compound **6** and the by-product, reduced diisopropylazodicarboxylate (DIAD). The mixture was treated with saturated methanolic ammonia in a steel bomb at 80 °C to give compound **7** in low yield (~48%). The low coupling yield is due to the formation of several unidentified by-products. Finally, the compound **7** was deprotected using a TFA/H_2O (2:1, v/v) solution at 50 °C to give the target nucleoside **8** in 80% yield.[24]

Since the conformation of nucleoside/nucleotide analogues plays an important role in their biological activity as well as to understand the conformation of the novel class of the spiro nucleoside,[25] the conformation of the nucleoside **8** was studied. The conformational properties were studied using a computational empirical calculation as well as X-ray crystallography (Table 1). The conformational studies of compound **8** were performed by the truncated Newton (TCNG) method using OPLS_2001 force field with the GB/SA continuum water solvation model[26] followed by Macromodel energy minimization[27] using the OPLS_2005 force field. The lowest energy conformer was used to estimate the pseudorotational angle and conformational properties. The structure of compound **8** was further confirmed by the single-crystal X-ray diffraction study[28] (CCDC 816703) and the ORTEP diagram is shown in Figure 2.[29] The conformational properties obtained by the empirical calculation, as well as by the crystal structure revealed that the nucleoside carbocyclic ring adopts the 2′-endo (south conformation) with anti-base disposition. The lowest energy conformation found was superposed with the crystallographically determined structure with respect to the carbocyclic sugar ring atoms. The RMSD for the spiro-carbocyclic sugar ring atom positions was 0.058 Å, and the pseudorotational parameters pseudorotational angle (*P*), dihedral angle of O5′–C5′–C4′–C3′ (γ) and puckering amplitude (ν_{max}) were in close agreement (crystal: $P = 173.1$, $\gamma = -71.0$, $\nu_{max} = 44.8$; lowest energy conformer: $P = 187.6$, $\gamma = -65.7$, $\nu_{max} = 38.0$). The orientation of hydroxyl groups and the adenine base was also in between the modeled and experimentally determined structures (Fig. 3).

Table 1
Pseudorotational angle and conformational properties

Compound **8**	γ (Gamma)	ν_{max} (Puckering amplitude)	*P* (Pseudorotational angle)
Computational	−65.7	38.0	187.6
X-ray	−71.0	44.8	173.1

The antiviral activity and cytotoxicity of the synthesized compound **8** was evaluated in the HCV sub-genomic RNA replicon assay system in Huh7 ET cells as previously described.[30] The results are summarized in comparison to the positive control, 2′-C-Me-cytosine (2′-C-Me-C)[31] as shown in Table 2. As the modifications of the sugar portion of 2′-C-methyladenosine (2′-C-Me-A) with the cyclopropyl-spirocarbocyclic sugar exhibited significant anti-HCV activity with EC_{50} values of 0.273 and 0.368 μM in genotype A and B, respectively.

The synthesized cyclopropyl-spirocarbocyclic nucleoside **8** was tested against cowpox, vaccinia, HBV, HSV-1 & 2, VZV, SARS, Flu

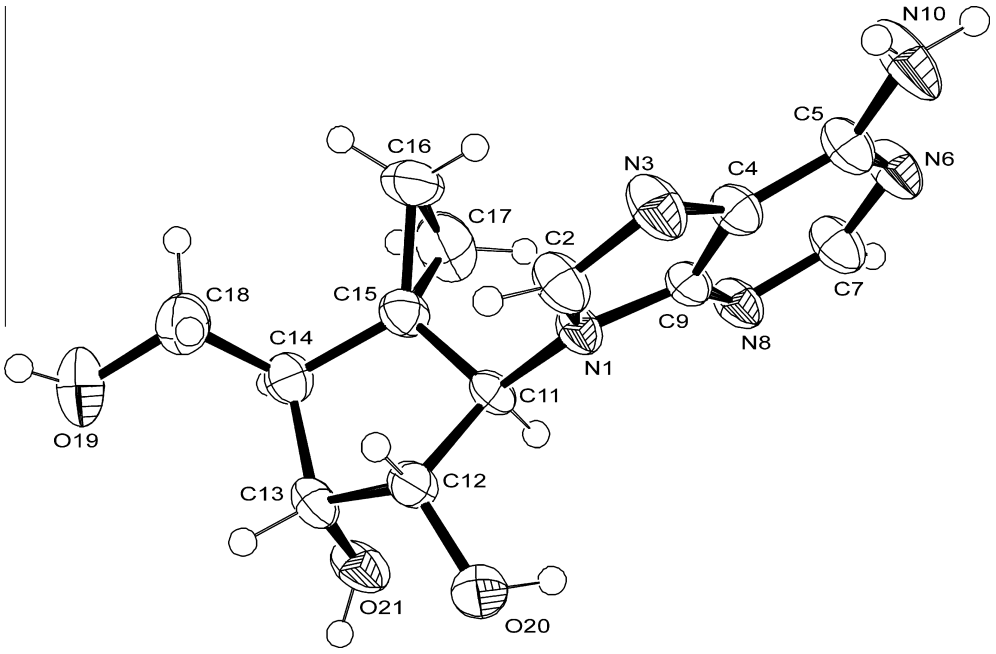


Figure 2. ORTEP diagram showing displacement ellipsoid plot of the X-ray crystal structure of **8**.

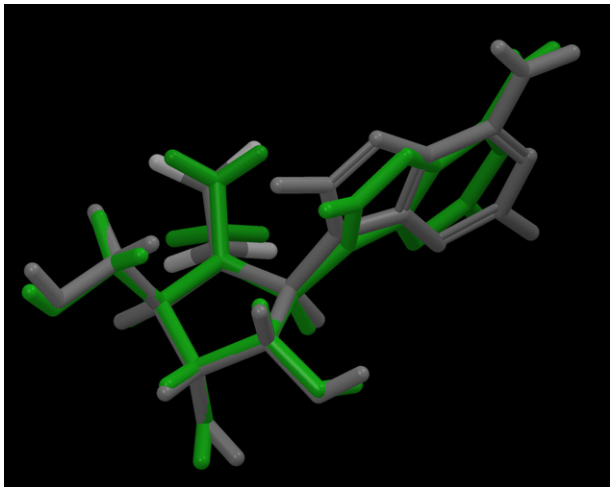


Figure 3. Stereoview of the crystallographically determined structure **8** (green) and its lowest energy conformation (gray) by computational empirical calculation.

Table 2
In vitro anti-HCV activity and cytotoxicity of compound **8** in the HCV RNA replicon Huh7 assay in comparison to the known 2′-C-methylcytidine (2′-C-Me-C)

Compound	Genotype	EC_{50}[a] (μM)	EC_{90}[a] (μM)	CC_{50}[b] (μM)	SI[c]
Compound **8**	1A	0.273	3.0	>100	>366
	1B	0.368	1.2	>100	>272
2′-C-Me-C	1B	1.7	8.2	>300	>167

[a] Effective concentration required for reducing HCV level by 50% and 90% in 5 days.
[b] Cytotoxicity concentration required for reducing the rRNA levels by 50% in 5 days.
[c] Selectivity index (SI) = CC_{50}/EC_{50}.

Table 3
Antiviral activity of compound **8** when tested against various viruses in cell culture

Virus	Assay	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
Vaccinia	CPE	42.7	>300	>7.1
Measles	NR	9	49	5
	Visual	7	15	2
Adeno	NR	3	13	4
	Visual	3	10	3

[a] Effective concentration required for reducing virus level by 50%.
[b] Concentration of compound for 50% cell inhibition without virus.
[c] Selectivity index (SI) = CC_{50}/EC_{50}.

A(H1N1), Flu A (H3N2), Flu A (H5N1), VEE, WNV, rhinovirus, measles, yellow fever, PIV and adeno. The compound was inactive in most of the viruses with the exceptions of weakly active against vaccinia (EC_{50} = 42.7 μM), measles (NR, EC_{50} = 9 μM; visual, EC_{50} = 7 μM) and adeno (EC_{50} = 3 μM in both NR & visual) as shown in (Table 3).

In summary, a novel spiro-carbocyclic adenosine **8** was synthesized and evaluated as a potential antiviral agent. The compound **8** exhibited an interesting anti-HCV activity against both genotype 1A and 1B. The conformation of the novel nucleoside was also studied by X-ray and calculation. In view of this interesting biological activity of the novel nucleoside, the structure–activity relationships as well as further biological studies are warranted.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.012.

References and notes

1. Di Bisceglie, A. M. *Lancet* **1998**, *351*, 351.
2. In World Health Organization fact sheet number 164, October 2000.
3. Lavanchy, D. *Liver Int.* **2009**, *29*, 74.
4. Lindenbach, B.; Rice, C. *Nature* **2005**, *436*, 933.
5. Tan, S.; Pause, A.; Shi, Y.; Sonenberg, N. *Nat. Rev. Drug Disc.* **2002**, *1*, 867.
6. Legrand-Abravanel, F.; Nicot, F.; Izopet, J. *Expert Opin. Invest. Drugs* **2010**, *19*, 963.
7. Kwong, A.; McNair, L.; Jacobson, I.; George, S. *Curr. Opin. Pharmacol.* **2008**, *8*, 522.
8. Ishii, K.; Tanaka, Y.; Yap, C.; Aizaki, H.; Matsuura, Y.; Miyamura, T. *Hepatology* **1999**, *29*, 1227.
9. Brown, N. A. *Expert Opin. Invest. Drugs* **2009**, *18*, 709.
10. Pierra, C.; Benzaria, S.; Amador, A.; Moussa, A.; Mathieu, S.; Storer, R.; Gosselin, G. *Nucleosides, Nucleotides Nucleic Acids* **2005**, *24*, 767.
11. Pockros, P.; Nelson, D.; Godofsky, E.; Rodriguez Torres, M.; Everson, G.; Fried, M.; Ghalib, R.; Harrison, S.; Nyberg, L.; Shiffman, M. *Hepatology* **2008**, *48*, 385.
12. Stuyver, L.; McBrayer, T.; Tharnish, P.; Clark, J.; Hollecker, L.; Lostia, S.; Nachman, T.; Grier, J.; Bennett, M.; Xie, M. *Antiviral Chem. Chemother.* **2006**, *17*, 79.
13. Zhou, X.-J.; Pietropaolo, K.; Chen, J.; Khan, S.; Sullivan-Bolyai, J.; Mayers, D. *Antimicrob. Agents Chemother.* **2011**, *55*, 76.
14. Crimmins, M. *Tetrahedron* **1998**, *54*, 9229.
15. Zhu, X. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 651.
16. Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319.
17. Jin, Y. H.; Liu, P.; Wang, J.; Baker, R.; Huggins, J.; Chu, C. K. *J. Org. Chem.* **2003**, *68*, 9012.
18. Moon, H. R.; Choi, W. J.; Kim, H. O.; Jeong, L. S. *Tetrahedron: Asymmetry* **2002**, *13*, 1189.
19. Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 330.
20. Hofmann, A. W. *Ber* **1881**, *14*, 2725.
21. Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 324.
22. Gaudino, J. J.; Wilcox, C. S. *J. Am. Chem. Soc.* **1990**, *112*, 4374.
23. Simmons, H. E.; Smith, R. D. *J. Am. Chem. Soc.* **1959**, *81*, 4256.
24. (4*R*,5*S*,6*R*,7*R*)-4-(6-Amino-9*H*-purin-9-yl)-7-(hydroxymethyl)spiro[2.4]heptane-5,6-diol (**8**). Compound **7** (100 mg, 0.258 mmol) was dissolved in CF_3CO_2H/H_2O (2:1, v/v) (50 mL) and heated to 50 °C for 3 h. The solvent was removed under the vacuum and the residue was co-evaporated with ethanol (3 × 20 mL) under vacuum. The resultant residue was purified by combiflash chromatography (12% $MeOH/CH_2Cl_2$) to afford **8** (60 mg, 80%) as a white foam. Mp 120–122 °C. $[\alpha]_D^{24}$ 5.01 (*c* 0.45, $CHCl_3$); UV(MeOH) λ_{max} 261 nm (ε 12885, pH 2), 261 nm (ε 13844, pH 7), 261 nm (ε 14038, pH 11); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (s, 1 H, 2-H), 8.58 (s, 1 H, 8-H), 7.73 (br s, 2H, NH_2), 5.58 (d, *J* = 6 Hz, 1H, 1′-H), 5.24–5.30 (m, 3H, 2′-H, 2′& 3′-OH, D_2O exchange), 5.03–5.07 (m, 1H, 5′-OH, D_2O exchange), 4.53 (d, *J* = 2.5 Hz, 1H, 3′-H), 3.91–3.95 (m, 2H, 5′-H), 2.41–2.43 (m, 1H, 4′-H), 1.12–1.16 (m, 1H, -cp), 1.02–1.09 (m, 2 H, -cp), 0.00–0.04 (m, 1 H, -cp); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.9, 151.9, 149.4, 141.4, 118.8, 76.0, 73.8, 66.3, 60.5, 51.6, 22.4, 14.7, 7.0. Anal. Calcd for $C_{13}H_{17}N_5O_3{\cdot}0.3MeOH$: C, 53.09; H, 6.10; N, 23.27. Found: C, 53.48; N, 6.28; H, 22.82.
25. Van Roey, P.; Salerno, J.; Chu, C.; Schinazi, R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3929.
26. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
27. MacroModel, V 9.1 Schrodinger Suit 2006; LLC, New York, NY2005.
28. Crystal data of **8**: $C_{30}H_{42}N_{10}O_8$, M = 670.74, monoclinic, space group *P*2(1), *a* = 14.573(3), *b* = 7.951(2), *c* = 14.888(3) Å, β = 107.93(3)°, *V* = 1641.2(6) Å^3, *T* = 293 K, *Z* = 2, R_1 = 0.0329 for 2300 $F_o > 4sig(F_o)$ and 0.0373 for all 2517 data. Crystallographic data has been deposited with Cambridge Crystallographic Data Centre as supplementary publication number CCDC 816703. These data can be obtained free of charge from www.ccdc.cam.uk/conts/retrieving.html or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; E-mail: deposit@ccdc.cam.ac.uk. Programs: HKL Scalepack (Otwinowski & Minor, 1997), Denzo and Scalepak (Otwinowski & Minor, 1997), SHELXS-97 (Sheldrick, 1990) and SHELXL-97 (Sheldrick, 1997).
29. Farrugia, L. J. J. *Appl. Crystallogr.* **1997**, *30*, 565.
30. Korba, B. E.; Montero, A. B.; Farrar, K.; Gaye, K.; Mukerjee, S.; Ayers, M. S.; Rossignol, J.-F. *Antiviral Res.* **2008**, *77*, 56.
31. Pierra, C.; Amador, A.; Benzaria, S.; Cretton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. N.; Sommadossi, J.-P.; Storer, R.; Gosselin, G. *J. Med. Chem.* **2006**, *49*, 6614.