9-(2-*C*-Cyano-2-deoxy-*β*-D-*arabino*-pentofuranosyl)guanine, a Potential Antitumor Agent against B-Lymphoma Infected with Kaposi's Sarcoma-Associated Herpesvirus

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Abstract: Several 9-(2-*C*-cyano-2-deoxy-l-*β*-D-*arabino*-pentofuranosyl)purine derivatives were tested against Kaposi's sarcoma-associated herpesvirus (KSHV)-infected primary effusion lymphoma (PEL) cells. The guanine derivative (**3**, CNDAG), as well as the 2-amino-6-substituted-purine derivatives **4**, **5**, and **6**, exhibited cell growth inhibitory activity against KSHV-infected cells, but showed no cytotoxicity against KSHV-negative cells at >15 *μ*M concentrations. Therefore, it was found that compounds **3**, **4**, **5**, and **6** showed selective cytotoxicity against PEL cells infected with KSHV.

Kaposi's sarcoma-associated herpesvirus (KSHV[a]), also known as human herpesvirus 8, was initially discovered in acquired immunodeficiency syndrome (AIDS)-associated Kaposi's sarcoma (KS), the endothelial cell malignancy KS.[1] KSHV is also associated with B-cell malignancies, primary effusion lymphoma (PEL), and plasmablastic variant multicentric Castleman's disease.[2] The neoplastic potentials of KSHV have been established within the context of immunosuppressed patients who are undergoing organ (bone marrow) transplant or AIDS by human immunodeficiency virus (HIV).[3] Currently, cytotoxic chemotherapeutic agents such as doxorubicin are used in established KS, however, serious side effects and only a transient tumor response to any chemotherapeutic regimen render them questionable. Other than in prophylactic antiviral therapy, the use of antiviral drugs as effective treatment against KS is still a matter of debate.[4−9] At the present time, there is no definitive chemotherapy to treat KS and PEL. Therefore, the development of novel antitumor agents against KSHV-infected transformed cells such as PEL is necessary.

We have developed 9-(2-*C*-cyano-2-deoxy-1-*β*-D-*arabino*-pentofuranosyl)cytosine (CNDAC, Figure 1, **1**)[10−17] as an antitumor nucleoside antimetabolite with a novel mechanism-based DNA-strand breaking ability, which is currently being evaluated in clinical trials. The structure−activity relationship of **1** in terms of a nucleobase revealed that the adenine derivative (CNDAA, **2**) was much less cytotoxic than **1**. We anticipated that the guanine analog (CNDAG, **3**) and its derivatives might exhibit cytotoxicity against tumor cells transformed by virus infection without toxicity against uninfected cells. Here we report the synthesis of **3**−**7** and their selective cytotoxicity against PEL caused by infection with KSHV.

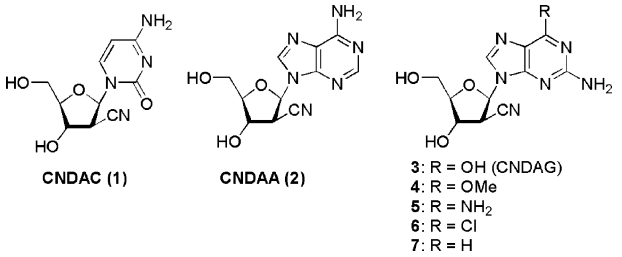
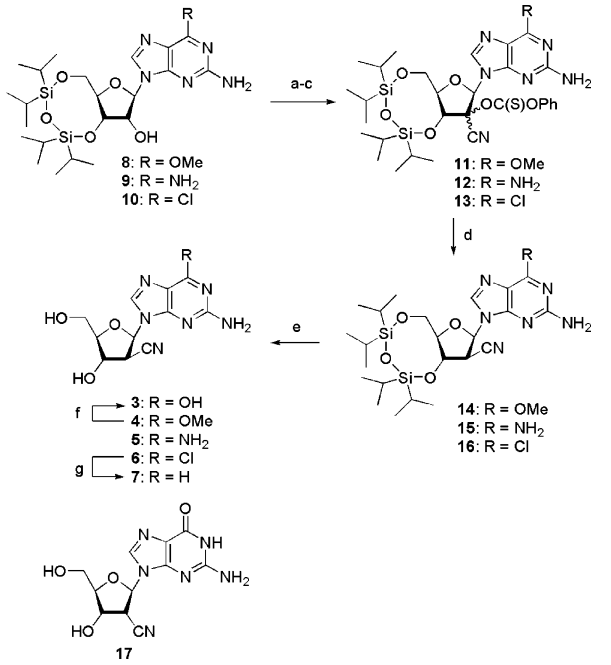


Figure 1. Structures of CNDAC and target compounds.

Scheme 1[a]



[a] Reagents and conditions: (a) CrO_3, Ac_2O, pyridine, MS4A, CH_2Cl_2, 0 °C; (b) Bu_4NCN, 0 °C; (c) PhOC(S)Cl, DMAP, Et_3N, CH_2Cl_2, 0 °C; (d) Bu_3SnH, AIBN, toluene, reflux; (e) TBAF, AcOH, THF, 0 °C; (f) bovine adenosine deaminase, phosphate buffer (pH 7.0), 37 °C; (g) H_2, Pd/C, MeOH, room temperature.

The target compounds **3**−**7** are expected to be prone to degradation under basic conditions, based on the previous study of **1**.[13] Therefore, we planned to synthesize the target nucleosides using the 2-amino-6-substituted purine ribosides **8**,[18] **9**,[19] and **10**[20] and to conduct enzymatic hydrolysis by adenosine deaminase (ADA) in the final step.[21,22] The synthesis of **3**−**7** is shown in Scheme 1. The 2′-hydroxyl groups in the 2-amino-9-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*β*-D-*ribo*-pentofuranosyl]-6-substituted purine derivatives **8**−**10** were oxidized using CrO_3, pyridine, and Ac_2O to afford the corresponding 2′-ketone derivatives, which were treated with Bu_4NCN[23] in CH_2Cl_2 to give cyanohydrins. They were further treated with the phenyl chlorothionoformate in the presence of DMAP in CH_2Cl_2 to give thionocarbonates **11**−**13**, respectively. Compounds **11**−**13** were heated with Bu_3SnH and AIBN in refluxing toluene to furnish in four steps the desired 2′-*β*-cyano-2′-deoxy derivative **14**−**16** in 22−33% yields, respectively. However, the reaction conditions have to be optimized. Deprotection of the TIPDS group of each **14**−**16** was conducted with TBAF in the presence of AcOH in THF at 0 °C to give the desired **4**−**6**, respectively,

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[a] Abbreviations: KSHV, Kaposi's sarcoma-associated herpesvirus; KS, Kaposi's sarcoma; PEL, primary effusion lymphoma; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; ADA, adenosine deaminase; EBV, Epstein−Barr virus; ACV, acyclovir; GCV, ganciclovir; BVDU, 5-bromovinyl-2′-deoxyuridine; TK, thymidine kinase.

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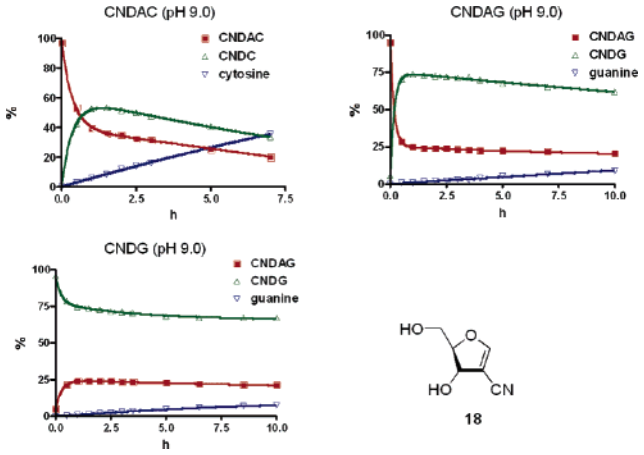

Figure 2. Chemical stability of CNDAC (**1**) and CNDAG (**3**) and the structure of glycal **18**. Compounds were incubated in Tris HCl buffer (pH 9.0) at 37 °C, and time profiles of the decrease of CNDAG (**3**) and formation of CNDG (17) and guanine were analyzed by HPLC.

without any epimerization at the 2′-position. The configuration at the 2′-position of these compounds was confirmed to be 2′-"up" by NOE experiments, in which a correlation with the 4′-proton was observed upon irradiation at the 2′-proton.

Next, enzymatic deamination of **4** was examined. Compound **4** was incubated with bovine ADA in a phosphate buffer (pH 7.0) at 37 °C for 24 h to furnish **3** along with its 2′-epimer, 9-(2-*C*-cyano-2-deoxy-*β*-D-*ribo*-pentofuranosyl)guanine (**17**, CNDG), which was separated by high performance liquid chromatography (HPLC). Compound **3** was also obtained by the treatment of **5** and **6** with ADA. Compound **6** was further treated with Pd/C under a hydrogen atmosphere to give the 2-aminopurine derivative **7**.

Compound **1** is chemically labile in alkaline solution, leading to epimerization and degradation via the E1cB mechanism to give cytosine.[13] Therefore, we examined the chemical stability of **3** in alkaline solution in comparison with **1**. Compounds **3** or **17** were incubated in Tris HCl buffer (pH 9.0) at 37 °C, and time profiles of the decrease of **3** and formation of **17** and guanine were examined. The results are shown in Figure 2. Indeed, the concentration ratio of **3** to **17** is approximately $^1/_3$ in the slow phase, confirming that **17** is thermodynamically more stable than **3**. Compared with **1**, compound **3** is, as expected, more susceptible to the epimerization. However, interestingly, the formation of guanine and 1,4-anhydro-2-*C*-cyano-2-deoxy-D-*erythro*-pent-l-enitol (**18**, glycal), which were *β*-elimination products of **3**, was more suppressed than that of **1**. Both **3** and **17** were stable under neutral conditions (pH = 7.0) and no epimerization or degradation was observed. Using the same experiments, we found that other 6-modified analogs **4**−**7** had similar chemical properties by the same experiments (data not shown).

Cytotoxic effects of the above synthesized compounds were evaluated on B-cell lines isolated from a patient suffering from KSHV infection (BC3 cells), from a patient suffering both KSHV and Epstein−Barr virus (EBV) infections (BC2 cells), and from a patient with no known KSHV infections (DG75 cells).[24] The results are summarized in Table 1. Compound **3** exhibited cell growth inhibitory activity against KSHV-infected BC3 and BC2 cells with IC_{50} values of 1.14 and 7.61 μM, respectively. On the other hand, **3** did not show any cytotoxicity against KSHV-negative DG75 cells at >15 μM concentrations. Compound **1**, which is currently in a phase I clinical trial as an anticancer drug, was toxic against all the cells tested in this assay, and thus, **3** was revealed to be a selective cytotoxic agent

Table 1. Cytotoxic Effects of Nucleosides on B-Lymphoma Cells[a]

cmpd	IC_{50} (μM) BC3	BC2	DG75
CNDAC (**1**)	0.23	0.13	0.21
CNDAG (**3**)	1.14	7.61	>15
4	0.79	1.51	>15
5	0.47	1.56	>15
6	1.48	1.73	>15
7	>15	>15	>15
CNDG (**17**)	1.73	2.61	>15
CNDAA (**2**)	>15	>15	>15
acyclovir (ACV)	>100	>100	>100
ganciclovir (GCV)	70	81	>100
BVDU	>15	>15	>15

[a] Assay was performed according to the procedures described previously for activity against herpesvirus-infected lymphoma cells.[24] KSHV-positive PEL cells (BC2 and BC3 cells) and herpesvirus-negative B-lymphoma cells (DG75 cells) were incubated with nucleoside analogs, varying concentrations, for 120 h and were then subjected to cell viability assay. In each experiment, viability was assessed in six replicate wells. The optical densities at 560 nm in untreated respective cells are defined as 100%. The means ± standard deviations were determined in three separate experiments.

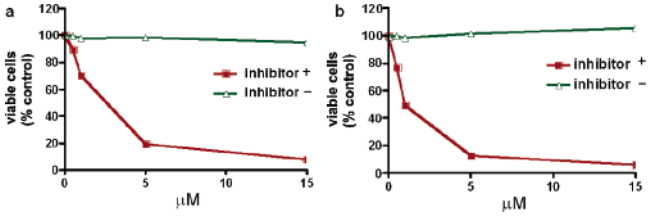

Figure 3. Cytotoxic effect of **4** (a) and **5** (b) on BC3 cells in the presence of adenosine deaminase inhibitor. Assay was performed according to the procedures described previously for BC3 cells in the presence of 10 μM deoxycoformycin.

against tumor cells infected by KSHV. Clinically used anti-herpes drugs such as acyclovir (ACV), ganciclovir (GCV), and 5-bromovinyl-2′-deoxyuridine (BVDU) were inactive against any KSHV-positive PEL cells at 15 μM concentrations. The 2-amino-6-methoxypurine derivative **4**, the 2,6-diaminopurine derivative **5**, and the 2-amino-6-chloropurine derivative **6** were also active against KSHV-infected BC3 cells with IC_{50} values of 0.79, 0.47, and 1.48 μM, respectively. The 2-aminopurine derivative **7** and CNDAA, which is an adenine derivative, showed no activity. Compound **17** is also effective against BC3 cells similar to **3**. The tumor cell growth inhibitory activity of **17** would mainly be related to the epimerized **3**.[13] The IC_{50} values for **3** itself should be much lower than the apparent ones, because **3** should also be epimerized in the cells. It is presumed that **4**, **5**, and **6** would be deaminated by a cellular ADA to produce **3**, which would be the active nucleoside. In fact, BC3 cells pretreated with 10 μM deoxycoformycin escaped from the cytotoxicity of **4**−**6** (Figure 3). These results clearly showed that **4**−**6** themselves exhibited no cytotoxicity and were metabolized to the active **3** by the action of cellular ADA.

The effect of **3** on the synthesis of DNA (incorporation of [^{3}H]thymidine), RNA (incorporation of [^{3}H]guanosine), and protein (incorporation of [^{3}H]leucine) was also examined with BC3 cells (Figure 4). Contrary to **1**, which mainly inhibits DNA synthesis, RNA synthesis was inhibited by 80% at 10 μM concentration of **3**, although **3** has a deoxyribonucleoside structure. It may be suggested that the mode of action of **3** in exhibiting tumor cell growth inhibitory activities might not be the same as the known deoxyribonucleoside antimetabolites.

Most of the nucleoside antimetabolites have to be phosphorylated at the 5′-hydroxyl group by a nucleoside and nucleotide kinases, and its efficiency sometimes plays an important role

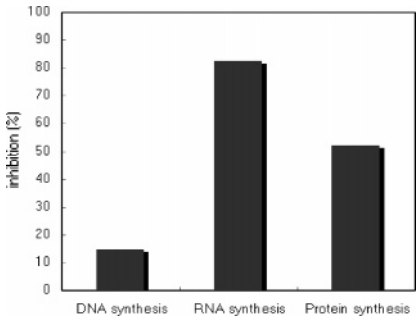

Figure 4. Effect of CNDAG (**3**) on the synthesis of DNA, RNA, and protein. After BC3 cells (2.5×10^5 cells/mL) were incubated for 24 h, **3** was added to the culture and incubated for 1 h. Before cell harvest, cells were pulse-labeled for 30 min with [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]leucin. The radioactivity of the acid-insoluble fractions was measured by a liquid scintillation counter.

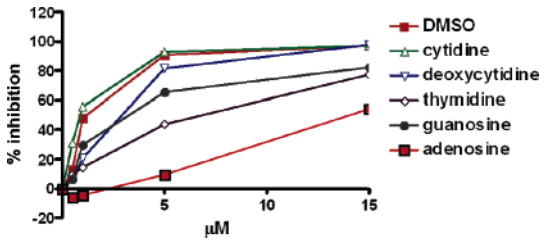

Figure 5. Competitive effects of common nucleosides on the cytotoxicity of CNDAG (**3**) against the growth of BC3 cells. BC3 cells (10^4 cells/mL) were seeded in a 96-well microplate and treated with graded concentrations of **3**, and each of the common nucleosides were simultaneously added in triplicate to each well. The plate was incubated for 3 days at 37 °C in a humidified atmosphere of 5% CO_2. The cytotoxicity of **3** was evaluated by MTT assay.

in exhibiting cytotoxic activity. To estimate the metabolic pathway of **3**, the inhibitory effect of **3** on the growth of BC3 cells was next examined in the presence or absence of common nucleosides. BC3 cells were treated with graded concentrations of **3** and each nucleoside (final concentration; 50 μM) for 72 h. The inhibitory effect of **3** on the growth of BC3 cells was prevented by the addition of adenosine and thymidine, although the effect was moderate (Figure 5). Considering the narrow substrate specificity of cellular thymidine kinase (TK), the cytotoxicity of **3** might require phosphorylation by virus-encoded TK. However, there is a controversy associated with the efficacy and selectivity of phosphorylation of nucleoside analogs by these enzymes.[25−27] Differences in substrate specificity between virus-encoded and cellular TK might be related to the selective activity of **3** and its derivatives against KSHV-positive cells.

While the cellular pharmacological study of antiherpes drug actions has been carried out extensively, very little is known about the details of how these drugs actually lead to the death of virus-infected cells. Therefore, the elucidation of cytotoxic mechanisms of actions of **3** derivatives will be important for the development of novel agents to treat KS and PEL, and these studies are currently underway.

In conclusion, we have synthesized 1-(2-*C*-cyano-2-deoxy-1-β-D-*arabino*-pentofuranosyl)purine derivatives and found that compounds **3**−**6** exhibit selective cytotoxic effects on KSHV-infected PEL. It is significant that they do not inhibit B-lymphoma cells without virus infection, but rather inhibit PEL cells infected with KSHV selectively. This study provides a novel strategy toward the development of virus-associated anticancer chemotherapy.

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Supporting Information Available: Synthetic procedures of compounds **3**−**17** and procedures for cell viability assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jenner, R. G.; Boshoff, C. The molecular pathology of Kaposi's sarcoma-associated herpesvirus. *Biochim. Biophys. Acta* **2002**, *1602*, 1−22.
(2) Ablashi, D. V.; Chatlynne, L. G.; Whitman, J. E.; Cesarman, E. Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. *Clin. Microbiol. Rev.* **2002**, *15*, 439−464.
(3) Chang, Y.; Cesarman, E.; Pessin, M. S.; Lee, F.; Culpepper, J.; Knowles, D. M.; Moore, P. S. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* **1994**, *266*, 1865−1869.
(4) Glesby, M. J.; Hoover, D. R.; Weng, S.; Graham, N. M.; Phair, J. P.; Detels, R.; Ho, M.; Saah, A. J. Use of antiherpes drugs and the risk of Kaposi's sarcoma: Data from a multicenter AIDS cohort study. *J. Infect. Dis.* **1996**, *93*, 14862−14867.
(5) Mocroft, A.; Youle, M.; Gazzard, B.; Morcinek, J.; Halai, R.; Phillips, A. N. Anti-herpesvirus treatment and risk of Kaposi's sarcoma in HIV infection. *AIDS* **1996**, *10*, 1101−1105.
(6) Mazzi, R.; Parisi, S. G.; Sarmati, L.; Uccella, I.; Nicastri, E.; Carolo, G.; Gatti, F.; Concia, E.; Andreoni, M. Efficacy of cidofovir on human ferpesvirus 8 viremia and Kaposi's sarcoma progression in two patients with AIDS. *AIDS* **2001**, *15*, 2061−2063.
(7) Fife, K.; Gill, J.; Bourboulia, D.; Gazzard, B.; Nelson, M.; Bower, M. Cidofovir for the treatment of Kaposi's sarcoma in an HIV-negative homosexual man. *Br. J. Dermatol.* **1999**, *141*, 1148−1150.
(8) Little, R. F.; Merced-Galindez, F.; Staskus, K.; Whitby, D.; Aoki, Y.; Humphrey, R.; Pluda, J. M.; Marshall, V.; Walters, M.; Welles, L.; Rodriguez-Chavez, I. R.; Pittaluga, S.; Tosato, G.; Yarchoan, R. A pilot study of cidofovir in patients with Kaposi sarcoma. *J. Infect. Dis.* **2003**, *187*, 149−153.
(9) Casper, R. Defining a role for antiviral drugs in the treatment of persons with HHV-8 infection. *Herpes* **2006**, *13*, 42−47.
(10) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. Nucleosides and nucleotides. 100. 2′-*C*-Cyano-2′-deoxy-l-β-D-arabinofuranosylcytosine (CNDAC): Design of a potential mechanism-based DNA-strand-breaking antineoplastic nucleoside. *J. Med. Chem.* **1991**, *34*, 2917−2919.
(11) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakawa, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Tanaka, M.; Sasaki, T.; Matsuda, A. Nucleosides and nucleotides. 122. 2′-*C*-Cyano-2′-deoxy-l-β-D-arabinofuranosylcytosine and its derivatives. A new class of nucleoside with a broad antitumor spectrum. *J. Med. Chem.* **1993**, *36*, 4183−4189.
(12) Tanaka, M.; Matsuda, A.; Terao, T.; Sasaki. T. Antitumor activity of a novel nucleoside, 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC) against murine and human tumors. *Cancer Lett.* **1992**, *64*, 67−74.
(13) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. Nucleosides and nucleotides. 141. Chemical stability of a new antitumor nucleoside, 2′-*C*-cyano-2′-deoxy-l-β-D-*arabino*-pentofuranosylcytosine alkaline medium: Formation of 2′-*C*-cyano-2-deoxy-l-β-D-*ribo*-pentofuranosylcytsine and its antitumor activity. *J. Med. Chem.* **1995**, *38*, 3391−3397.
(14) Matsuda, A.; Azuma, A. 2′-*C*-Cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC): A mechanism-based DNA-strand-breaking antitumor nucleoside. *Nucleosides Nucleotides* **1995**, *14*, 461−471.
(15) Azuma, A.; Huang, P.; Matsuda, A.; Plunkett, W. 2′-*C*-Cyano-2′-deoxy-1-β-D-*arabino*-pentofuranosylcytosine (CNDAC): A novel acting anticancer nucleoside analog that causes DNA strand-breaks and G2 arrest. *Mol. Pharmacol.* **2001**, *59*, 725−731.
(16) Azuma, A.; Huang, P.; Matsuda, A.; Plunkett, W. Cellular pharmacokinetics and pharmacodynamics of the deoxycytidine analog 2′-*C*-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylsytosine (CNDAC). *Biochem. Pharmacol.* **2001**, *61*, 1497−1507.

(17) Matsuda, A.; Sasaki, T. Antitumor activity of sugar-modified cytosine nucleosides. *Cancer Sci*. **2004**, *95*, 105−111.
(18) Wunk, S. F.; Lewandowska, E.; Companioni, D. R.; Garcia, P. I., Jr.; Secrist J. A., III. Synthesis and cytotoxicity of 9-(2-deoxy-2-alkyldithio-*β*-D-arabinofuranosyl)purine nucleosides which are stable precursors to potential mechanistic probes of ribonucleoside reductase. *Org. Biomol. Chem*. **2004**, *2*, 120−126.
(19) Beigelman, L.; Haeberli, P.; Sweedler, D.; Karpeisky, A. Improved synthetic approaches toward 2′-*O*-methyl-adenosine and guanosine and their N-acyl derivatives. *Tetrahedron* **2000**, *56*, 1047−1056.
(20) Robins, M. J.; Wilson, J. S. Nucleic acid related compounds. 41. Restricted furanose conformations of 3′,5′-*O*-(1,1,3,3-tetraisopropyldisilox-1,3-diyl)nucleosides provide a convenient evaluation of anomeric configuration. *Can. J. Chem*. **1983**, *61*, 1911−1920.
(21) Nair, V.; Sells, T. B. Enzymic synthesis of 2′,3′-dideoxyguanosine. *Synlett* **1991**, 753−754.
(22) Almond, M. R.; Collins, J. L; Reitter, B. E; Rideout, J. L; Freeman, G. A.; St Clair, M. H. Synthesis of 2-amino-9-(3′-azido-2′,3′-dideoxy-beta-D-*erythro*-pentofuranosyl)-6-methoxy-9*H*-purine (AzddMAP) and AzddGuo. *Tetrahedron Lett.* **1991**, *32*, 5745−5748.
(23) Garegg, P. J.; Hoffman, J.; Lindberg, B.; Samuelsson, B. Synthesis of 6-deoxy-D-*altro*-heptose. *Carbohydr. Res*. **1978**, *67*, 263−266.
(24) Fujimuro, M.; Inoue, H.; Teishikata, Y.; Yokosawa, H. Apoptotic effect of ganciclovir on lymphoblastoid cells stably harboring KSHV and EBV. *Nucleosides Nucleotides Nucleic Acids* **2006**, *25*, 635−645.
(25) Cannon, J. S.; Hamzeh, F.; Moore, S.; Nicholas, J.; Ambinder, R. F. Human herpesvirus 8-encoded thymidine kinase and phosphotransferase homologues confer sensitivity to ganciclovir. *J. Virol*. **1999**, *73*, 4786−4793.
(26) Gustafson, E. A,; Schinazi, R. F,; Fingeroth, J. D. Human herpesvirus 8 open reading frame 21 is a thymidine and thymidylate kinase of narrow substrate specificity that efficiently phosphorylates zidovudine but not ganciclovir. *J. Virol*. **2000**, *74*, 684−692.
(27) Lock, M. J.; Thorley, N.; Teo, J.; Emery, V. C. Azidodeoxythymidine and didehydrodeoxythymidine as inhibitors and substrates of the human herpesvirus 8 thymidine kinase. *J. Antimicrob. Chemother*. **2002**, *49*, 359−366.
(28) Kudoh, T.; Murayama, T.; Matsuda, A.; Shuto, S. Substitution at the 8-position of the highly potent Ca^{2+}-mobilizing agent 3′′-deoxy-cyclic ADP-carbocyclic-ribose provides partial agonists. *Bioorg. Med. Chem*. **2007**, *15*, 3032−3040.

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