

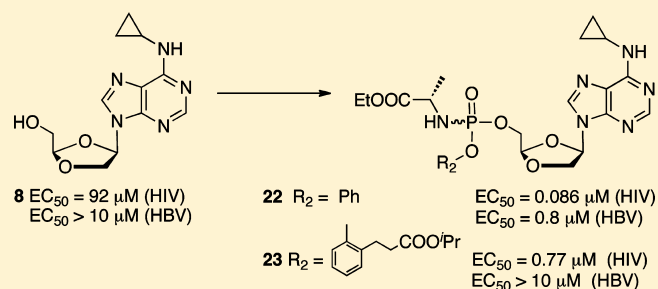
Adenosine Dioxolane Nucleoside Phosphoramidates As Antiviral Agents for Human Immunodeficiency and Hepatitis B Viruses

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S Supporting Information

ABSTRACT: There are currently six nucleoside reverse transcriptase inhibitors (NRTI) that are FDA approved for human clinical use and these remain the backbone of current HIV therapy. In order for these NRTIs to be effective they need to be phosphorylated consecutively by cellular kinases to their triphosphate forms. Herein, we report the synthesis of C-6 modified (–)-β-D-(2R,4R)-1,3-dioxolane adenosine nucleosides and their nucleotides including our novel phosphoramidate prodrug technology. We have introduced a side chain moiety on the phenol portion of the phosphoramidate to reduce the toxicity potential. The synthesized phosphoramidates displayed up to a 3600-fold greater potency versus HIV-1 when compared to their corresponding parent nucleoside and were up to 300-fold more potent versus HBV. No cytotoxicity was observed up to 100 μM in the various cell systems tested, except for compounds 17 and 18, which displayed a CC₅₀ of 7.3 and 12 μM, respectively, in Huh-7 cells. The improved and significant dual antiviral activity of these novel phosphoramidate nucleosides was partially explained by the increased intracellular formation of the adenosine dioxolane triphosphate.

KEYWORDS: Dioxolane adenosine nucleoside, nucleoside reverse transcriptase inhibitor, phosphoramidate prodrug, ProTide, HIV, HBV



Nucleoside reverse transcriptase inhibitors (NRTI) are the backbone of current HIV highly active antiretroviral therapy (HAART).¹ However, resistance and toxicity with chronic use of NRTI are still a major concern, as is true for all classes of HIV drugs.^{2–4} Therefore, exploration of novel nucleosides with the goal of improved efficacy, resistance profile, and safety are warranted. Amdoxovir (DAPD), a prodrug of (–)-β-D-2-amino-6-hydroxy dioxolane guanosine (DXG) is currently in phase 2 clinical development under a U.S. Food and Drug Administration investigational new drug application (Figure 1).^{5,6}

(–)-β-D-(2R,4R)-6-amino dioxolane adenosine (dioxolane-A) is an adenosine analogue that has been previously

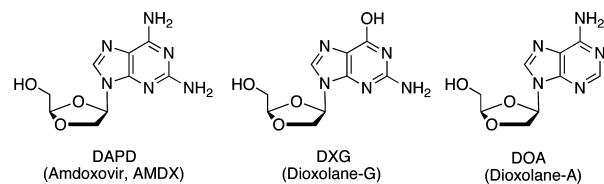


Figure 1. Structure of antiviral dioxolane nucleosides.

synthesized and evaluated by the Chu and Schinazi group.⁷ It has been shown that some nucleoside analogues are poor substrates for phosphorylation kinases that convert the nucleoside analogue to the corresponding nucleoside monophosphate.⁸ In order to bypass this rate-limiting step, nucleoside phosphoramidate derivatives were developed as more lipophilic monophosphate prodrugs. The phosphoramidate strategy bypasses the initial nucleoside kinase step, by intracellular delivery of the monophosphorylated nucleoside analogue.^{9–11} The phosphoramidate strategy has been shown to improve the pharmacological activity of parent nucleosides both in vitro and in vivo.^{12–16}

Recently, several reports have demonstrated that phosphoramidates of dioxolane sugar nucleosides with both purines¹⁷ as well as pyrimidines^{18,19} improved the in vitro antiviral potency. These reports demonstrated that phosphoramidates of these dioxolane analogues displayed enhancement of anti-HIV activity (100 fold) and anti-HBV activity (38 fold) when

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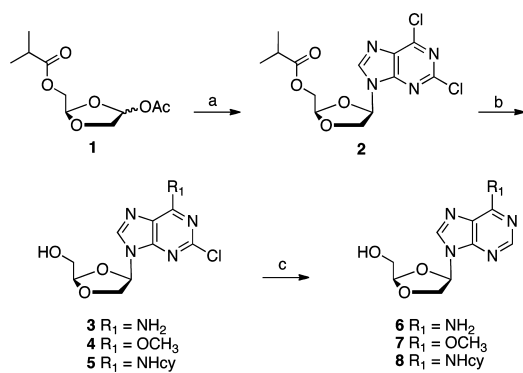
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compared with the parent nucleoside, without an increase in cytotoxicity. These findings prompted us to explore the dioxolane series of 6-substituted-2-*H*-purine nucleosides and their corresponding phosphoramidates.

Surprisingly, adenine substituted dioxolane nucleoside phosphoramidates have not been previously explored. In our study, the objective was to identify analogues with better antiviral potency with no enhancement of cytotoxicity. The 6-modified purine analogues were prepared as more lipophilic adenine precursors, which would require a two step intracellular conversion to the adenosine dioxolane analogue. The (–)-β-D-(2*R*,4*R*)-1,3-dioxolane purine modified nucleosides were synthesized from key intermediate (–)-β-D-1,3-dioxolanyl-2,6-dichloropurine, **2**, which was obtained by the glycosylation reaction of silylated 2,6-dichloropurine with acetoxy dioxolane derivative **1** (Scheme 1).²⁰

Scheme 1a



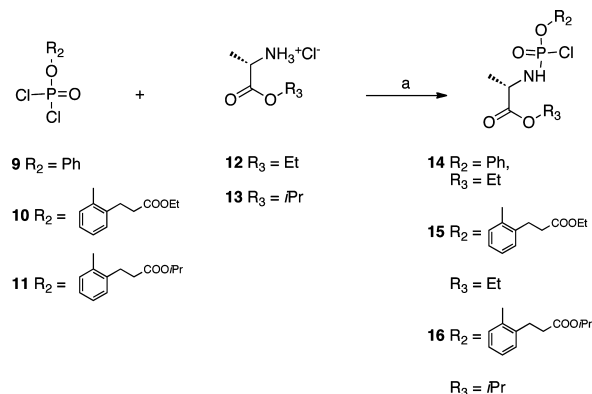
^aReagents and conditions: (a) Ref 20; (i) HMDS, NH₄SO₄, nucleoside base; (ii) TMSI, CHCl₃, –10 °C; (b) (i) nucleophile, THF, reflux; (ii) NH₃/CH₃OH, CH₃OH, rt, 12 h; (c) Pd/C, H₂, NaOH, 20 h.

Compound **3** was synthesized by the reaction of **2** with methanolic ammonia at room temperature for 10 h to give nucleoside **3** (2-chloro-6-amino) in 63% yield and nucleoside **4** (2-chloro-6-methoxy) in 15% yield. Compound **2** was reacted with methanol in the presence of K₂CO₃ at room temperature for 1 h and gave **4** in 90% yield and 2,6-dimethoxynucleoside in 5% yield. Compound **5** was synthesized in 79% yield by the reaction of compound **2** with cyclopropylamine in THF at 80 °C for 4 h, followed by treatment with methanolic ammonia at room temperature to facilitate removal of the 5'-isobutyl ester group.

Catalytic hydrogenation of the 2-chloro derivatives **3**, **4**, and **5** was accomplished by dissolving the nucleoside in methanol in the presence of 2 N NaOH, in an atmosphere of hydrogen in the presence of catalytic palladium on carbon.²¹ 2-Unsubstituted nucleosides **6**, **7**, and **8** were obtained in 82%, 80%, and 79% yields, respectively (Scheme 2).

Phosphoramidates were synthesized by the reaction of dioxolane nucleoside analogues **6**, **7**, and **8** with suitably substituted phosphoryl chlorides **14**, **15**, or **16**, which were prepared following previously described chemistry.⁹ Accordingly, a suitably substituted phenol was reacted with phosphorus oxychloride to afford an aryloxy phosphoryl dichlorides **9**, **10**, or **11**, which were subsequently reacted with an amino acid ester (ethyl **12** or isopropyl **13**) in the presence of Et₃N in THF to afford aryloxy phosphorochloridates **15** and

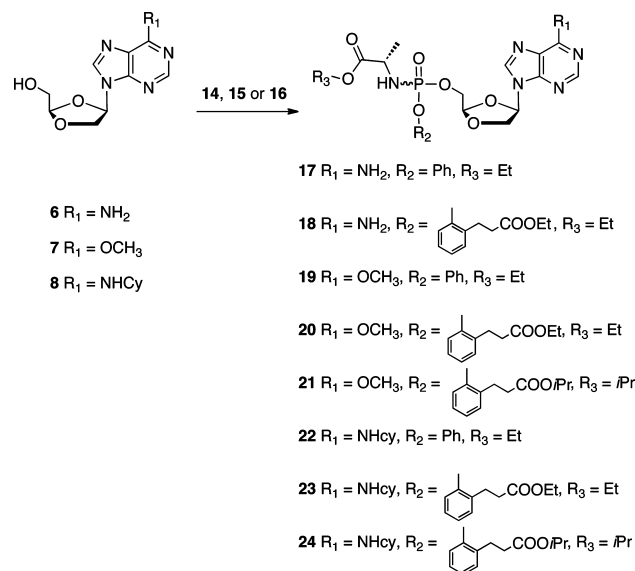
Scheme 2a



^aReagents and conditions: (a) Et₃N, THF, –5 to 20 °C.

16. Subsequent reaction with the dioxolane nucleoside analogues provided the desired 5'-phosphoramidates **17–24** as an approximate 1:1 mixture of phosphorus diastereomers (Scheme 3).


Scheme 3a








^aReagents and conditions: *t*-BuMgCl, THF, rt, 18 h.

The typical utilization of the phosphoramidate prodrug approach involves the intracellular delivery of phenol. Phenol is used in some over-the-counter products as an oral anesthetic/analgesic and commonly used to temporarily treat pharyngitis (inflammation of the throat). Phenol may cause harmful effects on the central nervous system, kidneys, and heart, resulting in dysrhythmia, seizures, and coma.^{22–24} The reported LD₅₀ for phenol is 270 mg/kg in mouse, while chronic exposure in humans may have harmful effects on the liver and kidneys. We sought to overcome these potential liabilities by the introduction of a propyl ester side chain at the ortho position of the phenol portion of our prodrugs. Cellular pharmacology in human peripheral blood mononuclear (PBM) cells showed that unmasking to the monophosphate from this substituted phosphoramidate results in the formation of hydroxyphenyl propanoic acid, which is a known nontoxic

Table 1. Antiviral Activity and Cytotoxicity of C-6 Modified Dioxolane-A Nucleosides and Their Corresponding Phosphoramidates



Compound			Activity EC ₅₀ , μM ± SD		Cytotoxicity, CC ₅₀ , μM					Cellular pharmacology in PBM cells	
Cmpd	R ₁	R ₂	R ₃	Anti-HIV	Anti-HBV	PBM	HepG2	Huh7	Vero	CEM	Dioxolane A-TP (6- $\text{HN} \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$ dioxolane-TP), pmol/10 ⁶ cells
DAPD	NH ₂	NA	NA	0.4 ± 0.1	9.0 / > 10	> 100	> 100	> 100	> 100	> 100	NA
6	NH ₂	NA	NA	2.0 ± 0.86	9.4 / ≥ 10	> 100	> 100	> 100	> 100	28	0.18 ± 0.039
17	NH ₂	Ph	Et	0.00056 ± 0.00034	0.03 ± 0.02	2.0	> 100	7.3	80	18	200 ± 7.5
18	NH ₂		Et	0.0033 ± 0.0006	0.30 ± 0.34	12	> 100	12	36	53	90 ± 6.6
7	OCH ₃	NA	NA	> 100	> 10	> 100	> 100	> 100	> 100	> 100	NA
19	OCH ₃	Ph	Et	0.34 ± 0.59	> 10	> 100	> 100	80	> 100	> 100	NA
20	OCH ₃		Et	0.42 ± 0.38	> 10	> 100	> 100	80	93	> 100	NA
21	OCH ₃		<i>i</i> Pr	7.0	> 10	> 100	> 100	52	> 100	> 100	NA
8	$\text{HN} \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$	NA	NA	92	> 10	> 100	> 100	> 100	> 100	> 100	NA
22	$\text{HN} \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$	Ph	Et	0.086 ± 0.11	0.8 ± 0.2	> 100	> 100	> 100	> 100	> 100	0.11 ± 0.029 (0.13 ± 0.034)
23	$\text{HN} \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$		Et	0.77 ± 0.81	> 10	> 100	> 100	65	> 100	> 100	0.14 ± 0.059 (0.19 ± 0.047)
24	$\text{HN} \begin{smallmatrix} \diagup \\ \diagdown \end{smallmatrix}$		<i>i</i> Pr	11 ± 6.0	> 10	> 100	> 100	57	> 100	84	NA

metabolite²⁵ of dihydrocoumarin, a common flavoring agent widely used in food²⁶ and cosmetics.²⁷

The antiviral activities of the synthesized nucleosides and their phosphoramidates were evaluated versus HIV and HBV. The HIV-1 susceptibility assays were performed using human PBM cells infected with HIV-1_{LAI}.^{28–31} Antiviral activity against HBV was determined using the HepAD38 cell system, as described previously.^{28,32} Cytotoxicity was assessed in human PBM cells, Vero cells (kidney epithelial cells from the African green monkey), and CEM cells (a human-T-cell-derived cell line) as described previously.^{28,31} The antiviral activity and cytotoxicity in five cell systems for the C-6 modified dioxolane-A nucleosides and their phosphoramidates are summarized in Table 1.

The dioxolane-A analogue **6** (2-H, 6-amino) was found to be significantly less active versus HIV when compared to the related clinical compound DAPD; however, their anti-HBV (low micromolar) and cytotoxic profile (no cytotoxicity up to 100 μM) were quite similar. Related analogues **7** (2-H, 6-methoxy) and **8** (2-H, 6-cyclopropylamino) were essentially not active versus HIV and HBV but also did not display any cytotoxicity in any of the five tested cell lines. The corresponding phosphoramidate prodrugs **17**, **18**, **19**, **20**, and **22** were up to 3600-fold more active than their parent nucleosides **6**, **7**, and **8**. Unfortunately, the most interesting

compounds based on their antiviral profile also displayed cytotoxicity in one or more of the five tested cell lines that ranged from a CC₅₀ of 2 to 84 μM. Compound **22** with a 6-cyclopropyl amine group was the exception and was overall the best compound with an EC₅₀ of 0.086 μM toward HIV and an EC₅₀ of 0.8 μM toward HBV and no observed cytotoxicity in the five tested cell systems.

As a first step toward understanding the antiviral data obtained with these compounds, we undertook a study of their cellular pharmacology in PBM cells (Table 1). We determined the levels of nucleoside triphosphates formed and also defined the relationship between intracellular triphosphate levels and antiviral activity. Compounds **6** (dioxolane-A), **17**, **18**, **22**, and **23** were evaluated in PBM cells and all were found to form the dioxolane-A triphosphate. Prodrugs **17** and **18** were found to give the highest levels of dioxolane-A triphosphate, which correlates to their exceptional antiviral potency. Interestingly, the 6-cyclopropylamino prodrugs **22** and **23** were also converted to the 6-cyclopropylamino purine dioxolane triphosphate. The effect of this newly identified triphosphate on antiviral activity is currently under investigation and will be reported elsewhere.

In conclusion, the phosphoramidate prodrug approach alone and in combination with modification at the C-6 position of the purine ring resulted in a significant enhancement of the anti-

HIV and anti-HBV activity compared to the parent nucleoside analogues. While the 6-methoxy and 6-cyclopropylamino purine modifications alone resulted in a significant loss of activity when compared to dioxolane-A, their corresponding phosphoramidates exhibited up to 3600-fold greater potency versus HIV-1 and up to 300-fold greater potency versus HBV when compared with the parent nucleoside dioxolane-A. Although no cytotoxicity was observed up to 100 μM in PBM and HepG2 cells, most of the highly potent compounds displayed μM cytotoxicity versus CEM, Huh7, and Vero cells.

Compound **22** (6-cyclopropylamine phosphoramidate) was the most interesting compound identified as it had no observed cytotoxicity in any of the five cell systems tested and displayed potent submicromolar anti-HIV ($\text{EC}_{50} = 0.086 \mu\text{M}$) and anti-HBV ($\text{EC}_{50} = 0.8 \mu\text{M}$) activity. Further preclinical profiling of compound **22** is currently in progress to determine the potential for clinical application of this dioxolane-A phosphoramidate strategy.

■ ASSOCIATED CONTENT

■ Supporting Information

Biological assays and complete experimental section with full characterization of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare the following competing financial interest(s): RFS is the founder and a major shareholder in RFS Pharma, LLC. Dioxolane nucleosides he invented have been licensed to RFS Pharma, LLC and he may receive future royalties from these products.

■ REFERENCES

- (1) De Clercq, E. New Approaches Toward Anti-HIV Chemotherapy. *J. Med. Chem.* **2005**, *48*, 1297–1313.
- (2) Richman, D. D.; Fischl, M. A.; Grieco, M. H. The Toxicity of Azidothymidine (AZT) in the Treatment of Patients With AIDS and AIDS-Related Complex. A Double-Blind Placebo-Controlled Trial. *N. Engl. J. Med.* **1987**, *317*, 192–197.
- (3) Starnes, M. C.; Cheng, Y. C. Cellular Metabolism of 2',3'-Dideoxycytidine, a Compound Active Against Human Immunodeficiency Virus in Vitro. *J. Biol. Chem.* **1987**, *262*, 988–991.
- (4) Petersen, E. A.; Ramirez-Ronda, C. H.; Hardy, W. D. Dose-Related Activity of Stavudine in Patients Infected With Human Immunodeficiency Virus. *J. Infect. Dis.* **1995**, *171* (suppl 2), S131–S139.
- (5) Gripshover, B. M.; Ribaud, H.; Santana, J.; Gerber, J. G.; Campbell, T. B.; Hogg, E.; Jarocki, B.; Hammer, S. M.; Kuritzkes, D. R. Amdoxovir Versus Placebo With Enfuvirtide Plus Optimized Background Therapy For HIV-1 Infected Subjects Failing Current Therapy (AACTG A5118). *Antiviral Ther.* **2006**, *11*, 619–623.
- (6) Margolis, D. M.; Mukherjee, A. L.; Fletcher, C. V.; Hogg, E.; Ogata-Arakaki, D.; Petersen, T.; Rusin, D.; Martinez, A.; Mellors, J. W. The Use of Beta-D-2,6-Diaminopurine Dioxolane With or Without Mycophenolatemofetil in Drug Resistant HIV Infection. *AIDS* **2007**, *21*, 2025–2032.

- (7) Kim, H. O.; Schinazi, R. F.; Nampalli, S.; Shanmuganathan, K.; Cannon, D. L.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Chu, C. K. 1,3-Dioxolanyl-purine Nucleosides (2R,4R) and (2R,4S) With Selective Anti-HIV-1 Activity in Human Lymphocytes. *J. Med. Chem.* **1993**, *36*, 30–37.

- (8) Deville-Bonne, D.; El Amri, C.; Meyer, P.; Chen, Y.; Agrofoglio, L. A.; Janin, J. Human and Viral Nucleoside/Nucleotide Kinases Involved in Antiviral Drug Activation: Structural And Catalytic Properties. *Antiviral Res.* **2010**, *86*, 101–120.

- (9) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Aryl Phosphoramidate Derivatives of d4t Have Improved Anti-HIV Efficacy In Tissue Culture and May Act by the Generation of a Novel Intracellular Metabolite. *J. Med. Chem.* **1996**, *39*, 1748–1753.

- (10) Cahard, D.; McGuigan, C.; Balzarini, J. Aryloxy Phosphoramidate Triesters As Pro-Tides. *Mini-Rev. Med. Chem.* **2004**, *4*, 371–381.

- (11) Perrone, P.; Daverio, F.; Valente, R.; Rajyaguru, S.; Martin, J. A.; Lévêque, V.; Pogam, S. L.; Najera, I.; Klumpp, K.; Smith, D. B.; McGuigan, C. First Example of Phosphoramidate Approach Applied To a 4'-Substituted Purine Nucleoside (4'-Azidoadenosine): Conversion of an Inactive Nucleoside To A Submicromolar Compound Versus Hepatitis C Virus. *J. Med. Chem.* **2007**, *50*, 5463–5470.

- (12) McGuigan, C.; Sutton, P. W.; Cahard, D. Synthesis, Anti-Human Immunodeficiency Virus Activity and Esterase Liability of Some Novel Carbocyclic Ester Modified Phosphoramidate Derivatives of Stavudine (d4T). *Antiviral Chem. Chemother.* **1998**, *9*, 473–479.

- (13) Bera, S.; Malik, L.; Bhat, B.; Carrol, S. S.; MacCoss, M.; Olsen, D. B.; Tomassini, J. E.; Eldrup, A. B. Synthesis and Evaluation of Optically Pure Dioxolanes as Inhibitors of Hepatitis C Virus RNA Replication. *Bioorg. Med. Chem.* **2003**, *13*, 4455–4458.

- (14) Du, J.; Patterson, S.; Shi, J.; Chun, B.-K.; Stuyver, L. J.; Watanabe, K. A. Synthesis of 5'-C-methyl-1',3'-dioxolan-4'-yl Nucleosides. *Bioorg. Med. Chem.* **2004**, *14*, 1243–1245.

- (15) Storer, R. Method For the Treatment or Prevention of Flaviviridae Viral Infection Using Nucleoside Analogues. PCT Int. Appl. WO2001032153, 2001.

- (16) Sofia, M. J. Nucleoside Prodrugs For HCV Therapy. *Antiviral Chem. Chemother.* **2011**, *22*, 23–49.

- (17) Bondada, L.; Zhou, L.; Detori, M.; Solomon, S.; Bassit, L.; Coats, S. J.; Schinazi, R. F. Synthesis and Biological Evaluation of Purine Dioxolane Nucleosides and Their Phosphoramidates Against Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV). *J. Med. Chem.* **2013**, in preparation.

- (18) Wang, P.; Rachaconda, S.; Zennou, V.; Keilman, M.; Niu, C.; Bao, D.; Ross, B. S.; Furman, P. A.; Otto, M. J.; Sofia, M. J. Phosphoramidate Prodrugs of (–)- β -D-(2R,4R)-Dioxolane-Thymine (DOT) as Potent Anti-HIV Agents. *Antiviral Chem. Chemother.* **2012**, *22*, 217–238.

- (19) Liang, Y.; Narayanasamy, J.; Schinazi, R. F.; Chu, C. K. Phosphoramidate and Phosphate Prodrugs of (–)-Beta-D-(2R,4R)-dioxolane-thymine: Synthesis and Anti-HIV Activity And Stability Studies. *Bioorg. Med. Chem.* **2006**, *14*, 2178–2189.

- (20) Sznajdman, M. L.; Du, J.; Pesyan, A.; Cleary, D. G.; Hurley, P. K.; Waligora, F.; Almond, M. R. Synthesis of (–)-DAPD. *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 1875–1887.

- (21) Salvatori, D.; Volpini, R.; Vincenzetti, S.; Vita, A.; Costanzi, S.; Lambertucci, C.; Cristalli, G.; Vittori, S. Adenine and Deazaadenine Nucleoside and Deoxynucleoside Analogues: Inhibition of Viral Replication of Sheep MVV (In-Vitro Model For HIV) and Bovine BHV-1. *Bioorg. Med. Chem.* **2002**, *10*, 2973–2980.

- (22) Warner, M. A.; Harper, J. V. Cardiac Dysrhythmias Associated with Chemical Peeling with Phenol. *Anesthesiology* **1985**, *62*, 366–367.

- (23) International Program on Chemical Safety (IPCS) INCHEM. <http://www.inchem.org/documents/icsc/icsc/eics0070.htm>.

- (24) U.S. Department of Health and Human Services. How can phenol affect my health? <http://www.atsdr.cdc.gov/toxprofiles/tp115.pdf>.

(25) WHO. *Evaluation of Certain Food Additives and Contaminants: Sixty-First Report of the Joint FAO/WHO Expert Committee on Food Additives*, WHO technical report series #922, 2004.

(26) Fenaroli, G. *Fenaroli's Handbook of Flavor Ingredients*, 6th ed; Burdock, G. A., Ed.; CRC Press: Boca Raton, FL, 2010; p 2159.

(27) Rastogi, S. C.; Johansen, J. D.; Menne, T. Natural Ingredients Based Cosmetics. Content of Selected Fragrance Sensitizers. *Contact Dermatitis* **1996**, *34*, 423–426.

(28) Consult the Supporting Information for more details on the biological assays.

(29) Schinazi, R. F.; Sommadossi, J. P.; Saalman, V.; Cannon, D. L.; Xie, M. Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. Activities of 3'-Azido-3'-deoxythymidine Nucleotide Dimers in Primary Lymphocytes Infected With Human Immunodeficiency Virus Type 1. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061–1067.

(30) Schinazi, R. F.; Cannon, D. L.; Arnold, B. H.; Martino-Saltzman, D. Combinations of Isoprinosine and 3'-Azido-3'-deoxythymidine in Lymphocytes Infected With Human Immunodeficiency Virus Type 1. *Antimicrob. Agents Chemother.* **1988**, *32*, 1784–1787.

(31) Chou, T. C.; Talalay, P. Quantitative Analysis of Dose-Effect Relationships: The Combined Effects of Multiple Drugs or Enzyme Inhibitors. *Adv. Enzyme Regul.* **1984**, *22*, 27–55.

(32) Stuyver, L. J.; Lostia, S.; Adams, M.; Mathew, J. S.; Pai, B. S.; Grier, S.; Tharnish, P. M.; Choi, Y.; Chong, Y.; Choo, H.; Chu, C. K.; Otto, M. J.; Schinazi, R. F. Antiviral Activities and Cellular Toxicities of Modified 2',3'-Dideoxy-2',3'-Didehydrocytidine Analogues. *Antimicrob. Agents Chemother.* **2002**, *46*, 3854–3860.