Published in final edited form as:
Bioorg Med Chem. 2014 December 1; 22(23): 6665–6671. doi:10.1016/j.bmc.2014.10.003.

Synthesis and antiviral evaluation of 2-amino-6-carbamoylpurine dioxolane nucleoside derivatives and their phosphoramidates prodrugs

Jong Hyun Cho[a], **Lavanya Bondana**[a], **Mervi A. Detorio**[a], **Cathy Montero**[a], **Leda C. Bassit**[a], **Franck Amblard**[a], **Steven J. Coats**[b], and **Raymond F. Schinazi**[a]

[a]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, 1670 Haygood Drive, NE, Atlanta,, GA 30322, USA

[b]RFS Pharma, LLC, 1860 Montreal Road, Tucker, GA 30084, USA

Abstract

The synthesis of 9-(β-D-1,3-dioxolan-4-yl)2,6-diaminopurine nucleoside phosphoramidate prodrugs as well as various 2-amino-6-carbamoylpurine dioxolane derivatives and their phosphoramidates prodrugs is reported. Their ability to block HIV and HBV replication along with their cytotoxicity toward HepG2, human lymphocyte, CEM and Vero cells was also assessed.

Keywords

β-D-1; 3-dioxolan-4-yl nucleosides; DAPD; Antiviral agent; Anti-HIV; Anti-HBV

1. Introduction

Nucleoside reverse transcriptase inhibitors (NTRI) are the backbone in fixed dose combinations now called highly active antiretroviral therapy (HAART) for human immunodeficiency virus type 1 (HIV-1).[1] Despite the effectiveness of these drugs, resistance can result from their long-term use and latent toxicity remains an issue.[2] Therefore, studies on novel nucleoside analogs with improved efficacy, resistance profile and safety are continuously needed to improve clinical outcome. Among all the modified nucleosides prepared over the years, β-D-1,3-dioxolan-4-yl nucleosides appeared to be a very promising family. Therefore, 9-(β-D-1,3-dioxolan-4-yl)2,6-diaminopurine (DAPD) **1**, a prodrug of 9-(β-D-1,3-dioxolan-4-yl)guanine (DXG, **2**), was evaluated for the treatment of HIV-1 infected persons in phase 2 clinical studies. However, the study was abandoned due to slow enrollment and high dosing regimens (500 mg twice a day). Over the years of nucleoside analog development, two main approaches have been utilized to improve the potency of a compound and potentially decrease the administered dose: A) Formation of a monophosphate prodrug to bypass the rate limiting first phosphorylation step.[3] For instance, (−)-β-D-(2*R*,4*R*)-1,3-dioxolane-2-amino-6-aminopropyl purine nucleoside phosphoramidate

prodrug **4** displayed submicromolar activities against HBV and HIV while its corresponding nucleoside **3** was devoid of activity (Figure 1).[4] B) Conversion of hydrophilic functional groups (such as amino or hydroxy) into corresponding lipophilic groups (such as amides or esters) in order to improve cell penetration. Thus, L-1,3-dixolane-cytidine (L-OddC) bearing a fatty acid group at its N^4-position demonstrated significantly improved antitumor activity (170-fold) *in vitro* when compared to the parent nucleoside.[5] Fatty acyl derivatives of (−)-2′, 3′-dideoxy-3′-thiacytidine (3TC) and (−)-2′,3′-dideoxy-5-fluoro-3′-thiacytidine [(−)-FTC] were 36- and 24-fold, respectively, more potent against HIV when compared to 3TC and (−)-FTC.[6]

Herein, we combine both approaches to potentially improve the antiviral potency of DAPD. Thus, we report the synthesis and antiviral evaluation of DAPD phosphoramidate prodrugs as well as their 2-amino-6-carbamoylpurine derivatives as a potential strategy to increase intracellular delivery of DXG-TP.

2. Results and discussion

We first envisaged to prepare the desired 2-amino-6-carbamoylpurine dioxolane nucleoside phosphoramidate prodrugs **10a-i** from DAPD in a 2 steps sequence by 1) formation of the phosphoramidate prodrug **5**; 2) introduction of the carbamoyl moiety. However, reaction of DAPD **1** with phosphoramidate chloride **6**[7] in the presence of *t*-BuMgCl afforded DAPD prodrug **5** in only 45% yield (Scheme 1). To further complicate this approach, acylation reactions with **5** would either proceed to only about 50% or when forced gave a substantial amount of the N^2,N^6-diacylated product. These low yields combined with the difficulty of purifying **5** using repeated tedious silica gel column chromatography lead us to design an alternative sequence which also allowed us to prepare and evaluate the 2-amino-6-carbamoylpurine dioxolanes **9a-i** (Scheme 2).

Thus, DAPD **1** was reacted with *t*-butyldimethylsilyl chloride (TBSCl) in the presence of imidazole in pyridine to give 5′-*O*-TBS-2,6-diaminopurine dioxolane **7** in 89% yield. With **7** in hand, its chemoselective N^6-acylation was investigated using oleoyl chloride as the model system for optimization (Table 1). Interestingly, the reaction of **7** with oleoyl chloride (1.1 eq) in the presence of pyridine (entry 2), DMAP (entry 4), Et_3N (entry 6), imidazole (entry 7) or their combination (entries 3 and 5) lead to the formation of 5′-*O*-TBS-2-amino-6-oleoylpurine dioxolane **8i** in poor to moderate yields along with N^2,N^6-diacylated DAPD in ca. 5–25% yields. On the other hand, treatment of **7** with oleic acid chloride (1.1 eq) in the presence of *N*-methylimidazole (entry 1),[8] provided the desired compound **8i** in 80% yield with less than 5 % of the N^2,N^6-diacylated byproduct. Using these optimized conditions, compound **7** was reacted with a variety of acyl chlorides to give the corresponding 5′-*O*-TBS-2-amino-6-carbamoylpurine dioxolane derivatives **8a-i** in 78–86% isolated yields. The final phenyloxy phosphoramidate dioxolane derivatives (**10a-i**) were obtained after removal of the 5′-silyl group with Et_3N-3HF and subsequent coupling with phosphoramidate chloride reagent **6**.

Both anti-HIV and anti-HBV potency of DAPD monophosphate prodrug **5**, as well as 2-amino-6-carbamoylpurine dioxolanes (**9a-i**) and their phosphoramidate dioxolane prodrugs

(**10a-i**) were evaluated in primary human lymphocytes and HepG2 cells, respectively. In addition, cytotoxicity was determined in human peripheral blood mononuclear (PBM) cells, human lymphoblastoid CEM, African Green monkey Vero cells and HepG2 cells.[9] The antiviral activity and cytotoxicity are summarized in Table 2. Generating phosphoramidate prodrug of DAPD **5** barely increased DAPD's potency against HIV (EC_{50} of 0.45 vs 0.25 μM) but, more interestingly, boosted its potency against HBV by a factor of 40 (EC_{50} of 0.3 vs 12 μM). C_1 to C_{11} N^6-carbamoyl derivative **9a-f**, **9i** and their corresponding prodrugs **10a-f**, **10i** displayed either equal or less potency than DAPD against HIV and HBV. On the other hand, phosphoramidate prodrugs **10g** and **10h,** with EC_{50}'s of 0.041 and 0.083 μM respectively, were 3 to 11 times more potent than DAPD **1** or DAPD prodrug **5** against HIV while their anti-HBV potency was in the same range as DAPD prodrug **5**. Unfortunately, these compounds also displayed cytotoxicity in various cell lines that ranged from a CC_{50} of 7.5 to 71.0 μM.

In conclusion, this work confirms the potential of the phosphoramidate prodrug approach to reveal or enhance the antiviral activity of nucleosides. In our hands, DAPD phosphoramidate prodrug **5** was 40 times more potent than DAPD against HBV. Combination of this approach with the introduction of long fatty ester chains known to mask polar groups allowed us to even further increase their potency against HIV. However, this augmentation of potency came, in this case, with an increase of cellular toxicity.

V. Experimentals

General

Nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C and ^{31}P) were recorded on a Varian Unity Plus 400 MHz and a Bruker Ascend™ 400 MHz Fourier transform spectrometer at rt, with tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), dd (doublet of doublets), or ddd (doublet of doublets of doublets). The phosphoramidates are an approximate 50:50 mixture of diastereomers (R_P/S_P) and the ^{13}C NMR data is reported as observed, that is, some carbon signals overlap. High-resolution mass spectra (HRMS) were recorded on a Micromass Autospec high-resolution mass spectrometer with electrospray ionization (ESI). Thin-layer chromatography (TLC) was performed on 0.25 mm silica gel. Purifications were carried out on silica gel column chromatography (60 Å, 63–200 μm, or 40–75 μm).

(2*S*)-ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-hydroxy-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (5)—To a solution of DAPD (0.10 g, 0.40 mmol) in THF (10 mL) was added *t*-butylmagnesium chloride (1.19 mL, 1.0 M in THF, 1.19 mmol) at −78 °C under argon atmosphere. After stirred for 30 min, the reaction mixture was additionally stirred at rt for 30 min. To the solution was added phosphoramide chloride **6** (0.24 g, 0.80 mmol) at −78 °C under argon atmosphere. The reaction temperature was warmed to rt over 1 h and then stirred for 6 h. The mixture was quenched with saturated ammonium chloride (1.0 mL) at 0 °C, and then stirred for 10 min. The resulting solution was adsorbed on silica gel and purified by silica gel column chromatography

(CH_2Cl_2:MeOH = 20:1 to 10:1 v/v) to give compound **5** in 45% yield (0.09 g, 0.18 mmol). ^{1}H NMR (400 MHz, CD_3OD): δ 7.99-7.98 (s, 1H), 7.37-7.27 (m, 2H), 7.23-7.13 (m, 3H), 6.37-6.33 (m, 1H), 5.36-5.32 (m, 1H), 4.63-4.60 (m, 1H), 4.38-4.30 (m, 3H), 4.14-4.06 (m, 2H), 3.76-3.71 (m, 1H), 1.35-1.19 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.6, 160.6, 156.2, 151.3, 150.6, 135.8, 129.2, 124.6, 119.9, 112.6, 103.3, 79.6, 70.6, 64.6, 60.9, 50.1, 18.9, 13.0; ^{31}P NMR (162 MHz, CD_3OD) δ 3.91, 3.64; MS-ESI$^+$ *m/z* 508 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{20}H_{27}N_7O_7P$ ($M+H^+$) 508.1708, found 508.1704.

9-((2*R*,4*R*)-2-(((*tert*-Butyldimethylsilyl)oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purine-2,6-diamine (7)—To a solution of DAPD (2.0 g, 7.93 mmol) in 25 mL of anhydrous pyridine was added imidazole (0.65 g, 9.52 mmol) and TBDMSCl (1.20 g, 7.93 mmol) at 0 °C under N_2 atmosphere. After stirring for 12 h at rt, the reaction mixture was treated with 10 mL of MeOH, stirred for 30 min at rt and then concentrated under reduced pressure. The residue was purified on silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2: MeOH = 10:1 v/v) to give compound **7** (2.58 g, 7.06 mmol) in 89% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (s, 1H), 6.98 (br, 2H), 6.20 (dd, *J* = 1.6, 5.6 Hz, 1H), 6.05 (br, 2H), 5.04 (t, *J* = 3.6 Hz, 1H), 4.45 (dd, *J* = 1.6, 10.0 Hz, 1H), 4.19 (dd, *J* = 1.6, 5.6 Hz, 1H), 3.79 (d, *J* = 3.2 Hz, 2H), 0.84 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.8, 155.7, 151.5, 134.9, 112.6, 104.7, 78.6, 70.5, 63.1, 25.8, 18.2, −5.37, −5.39; MS-ESI$^+$ *m/z* 367 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{15}H_{31}N_6O_3Si$ ($M+H^+$) 367.1907, found 367.1908.

(*Z*)-*N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)octadec-8-enamide (8i)—To a solution of compound **7** (2.70 g, 7.37 mmol) in 50 mL of anhydrous CH_2Cl_2 was added *N*-methylimidazole (2.0 eq, 1.21 g, 14.7 mmol) and oleoyl chloride (1.1 eq, 3.05 g, 8.11 mmol) at 0 °C under N_2 atmosphere. After stirring for 12 h at rt, the reaction mixture was treated with 10 mL of MeOH, stirred for 30 min at rt and then concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane: EtOAc = 4:1 to 1:4 v/v) to give compound **8i** (3.72 g, 5.90 mmol) in 80 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (br, 1H), 8.07 (s, 1H), 6.34 (dd, *J* = 1.6, 5.2 Hz, 1H), 5.36-5.32 (m, 2H), 5.16 (br, 2H), 5.12 (t, *J* = 3.2 Hz, 1H), 4.42 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.22 (dd, *J* = 5.2, 9.6 Hz, 1H), 3.95-3.88 (m, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 2.20-1.98 (m, 4H), 1.78-1.70 (m, 2H), 1.40-1.24 (m, 20H), 0.91-0.85 (m, 12H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 159.7, 153.1, 149.9, 138.0, 130.1, 129.9, 129.95, 116.0, 106.0, 79.4, 71.7, 63.5, 38.0, 32.1, 29.93, 29.90, 29.7, 29.54, 29.48,, 29.3, 27.39, 27.36, 26.1, 25.0, 22.8, 18.7, 14.3, −5.17, −5.19; MS-ESI$^+$ *m/z* 631($M+H^+$); HRMS-ESI$^+$ calcd for $C_{33}H_{58}N_6O_4NaSi$ ($M+Na^+$) 653.4188, found 653.4181.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)acetamide (8a)—**Compound **8a** was prepared using the same procedure as for compound **8i**; yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.07 (s, 1H), 6.34 (dd, *J* = 1.6, 5.2 Hz, 1H), 5.12 (t, *J* = 3.2 Hz, 1H), 5.03 (br, 2H), 4.42 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.23 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.94-3.86 (m, 2H), 2.56 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 159.6, 153.0, 149.8, 138.2, 115.9,

106.0, 79.5, 71.8, 63.5, 39.3, 26.1, 25.9, 18.8, −5.13, −5.16; MS-ESI$^+$ *m/z* 409 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{17}H_{29}N_6O_4Si$ (M+H$^+$) 409.2008, found 409.2014.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)butyramide (8b)—**Compound **8b** was prepared using the same procedure as for compound **8i**; yield: 84%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 6.45 (d, *J* = 4.0 Hz, 1H), 5.07 (t, *J* = 2.4 Hz, 1H), 4.42 (d, *J* = 10.0 Hz, 1H), 4.20 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.92-3.84 (m, 2H), 2.41 (t, *J* = 5.2 Hz, 2H), 1.71-1.65 (m, 2H), 0.96 (t, *J* = 7.2 Hz, 3H), 0.87 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 157.7, 154.6, 151.4, 140.2, 116.8, 107.0, 81.5, 73.0, 64.1, 40.2, 26.6, 20.1, 19.6, 14.2, −5.1, −5.2; MS-ESI$^+$ *m/z* 437 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{19}H_{33}N_6O_4Si$ (M+H$^+$) 437.2327, found 437.2327.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)hexanamide (8c)—**Compound **8c** was prepared using the same procedure as for compound **8i**; yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.09 (s, 1H), 6.35 (dd, *J* = 1.2, 5.2 Hz, 1H), 5.20 (br, 2H), 5.12 (t, *J* = 3.2 Hz, 1H), 4.41 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.22 (dd, *J* = 5.2, 9.6 Hz, 1H), 3.94-3.87 (m, 2H), 2.77 (t, *J* = 7.6 Hz, 2H), 1.78-1.70 (m, 2H), 1.40-1.32 (m, 4H), 0.95-0.88 (m, 12H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 159.8, 152.9, 150.0, 138.0, 115.8, 106.0, 79.5, 71.8, 63.4, 37.9, 31.6, 26.1, 24.8, 22.7, 18.8, 14.2, −5.1, −5.2; MS-ESI$^+$ *m/z* 465 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{21}H_{37}N_6O_4Si$ (M+H$^+$) 465.2461, found 465.2460.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)octanamide (8d)—**Compound **8d** was prepared using the same procedure as for compound **8i**; yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 8.09 (s, 1H), 6.35 (dd, *J* = 1.2, 4.8 Hz, 1H), 5.33 (br, 2H), 5.12 (t, *J* = 3.2 Hz, 1H), 4.42 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.22 (dd, *J* = 4.8, 10.0 Hz, 1H), 3.92-3.86 (m, 2H), 2.77 (t, *J* = 7.6 Hz, 2H), 1.76-1.69 (m, 2H), 1.42-1.28 (m, 8H), 0.92-0.84 (m, 12H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 159.9, 153.0, 150.0, 138.0, 115.9, 105.9, 79.4, 71.7, 63.5, 37.9, 31.8, 29.3, 29.2, 26.0, 25.1, 22.7, 18.7, 14.2, −5.21, −5.24; MS-ESI$^+$ *m/z* 493(M+H$^+$); HRMS-ESI$^+$ calcd for $C_{23}H_{41}N_6O_4Si$ (M+H$^+$) 493.2946, found 493.2953.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)decanamide (8e)—**Compound **8e** was prepared using the same procedure as for compound **8i**; yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.06 (s, 1H), 6.32 (dd, *J* = 1.2, 5.2 Hz, 1H), 5.32 (br, 2H), 5.09 (t, *J* = 3.2 Hz, 1H), 4.40 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.18 (dd, *J* = 5.2, 9.6 Hz, 1H), 3.90-3.82 (m, 2H), 2.75 (t, *J* = 7.6 Hz, 2H), 1.74-1.66 (m, 2H), 1.36-1.16 (m, 12H), 0.88-0.82 (m, 12H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 159.8, 153.1, 150.0, 138.0, 116.0, 105.9, 79.3, 71.7, 63.5, 37.9, 32.0, 29.6, 29.4, 26.0, 25.0, 22.8, 18.7, 14.3, −5.22, −5.25; MS-ESI$^+$ *m/z* 521(M+H$^+$); HRMS-ESI$^+$ calcd for $C_{25}H_{45}N_6O_4Si$ (M+H$^+$) 521.3267, found 521.3266.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)dodecanamide (8f)—**Compound **8f** was prepared using the same

procedure as for compound **8i**; yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.08 (s, 1H), 6.32 (d, *J* = 4.4 Hz, 1H), 5.34 (br, 2H), 5.10 (t, *J* = 3.2 Hz, 1H), 4.39 (d, *J* = 10.0 Hz, 1H), 4.20 (dd, *J* = 5.6, 10.0 Hz, 1H), 3.92-3.84 (m, 2H), 2.70 (t, *J* = 7.6 Hz, 2H), 1.74-1.66 (m, 2H), 1.38-1.18 (m, 16H), 0.90-0.82 (m, 12H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 159.9, 152.9, 150.0, 138.0, 116.0, 105.9, 79.4, 71.8, 63.4, 37.9, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 26.0, 25.1, 22.8, 18.7, 14.3, −5.22, −5.25; MS-ESI$^+$ *m/z* 549(M+H$^+$); HRMS-ESI$^+$ calcd for $C_{27}H_{49}N_6O_4Si$ (M+H$^+$) 549.3583, found 549.3579.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)tetradecanamide (8g)—**Compound **8g** was prepared using the same procedure as for compound **8i**; yield: 82%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (br, 1H), 8.08 (s, 1H), 6.34 (dd, *J* = 1.2, 4.8 Hz, 1H), 5.14-5.11 (m, 3H), 4.42 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.22 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.93-3.86 (m, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 1.75-1.69 (m, 2H), 1.40-1.20 (m, 20H), 0.92-0.85 (m, 12H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 159.7, 153.0, 150.0, 138.0, 116.0, 106.0, 79.4, 71.8, 63.5, 38.0, 32.1, 29.84, 29.71, 29.65, 29.55, 29.51, 26.1, 25.1, 22.9, 18.8, 14.3, −5.15, −5.18; MS-ESI$^+$ *m/z* 577 (M +H$^+$); HRMS-ESI$^+$ calcd for $C_{29}H_{53}N_6O_4Si$ (M+H$^+$) 577.3889, found 577.3892.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(((*tert*-butyldimethylsilyl) oxy)methyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)palmitamide (8h)—**Compound **8h** was prepared using the same procedure as for compound **8i**; yield: 86%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.30 (br, 1H), 8.11 (s, 1H), 8.07 (s, 1H), 7.20-6.80 (br, 1H), 6.35 (d, *J* = 4.8 Hz, 1H), 5.13 (t, *J* = 3.2 Hz, 1H), 4.46 (dd, *J* = 0.8, 9.6 Hz, 1H), 4.23 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.94-3.86 (m, 2H), 2.97 (br, 2H), 1.77-1.70 (m, 2H), 1.44-1.20 (m, 24H), 0.92-0.84 (m, 12H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 157.0, 153.6, 149.9, 138.0, 116.3, 106.0, 79.7, 71.9, 63.5, 37.4, 32.1, 29.9, 29.8, 29.78, 29.5, 26.1, 25.3, 22.8, 18.7, 14.3, −5.18, −5.20; MS-ESI$^+$ *m/z* 605 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{31}H_{57}N_6O_4Si$ (M+H$^+$) 605.4210, found 605.4205.

(*Z*)-*N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)octadec-8-enamide (9i)—To a solution of compound **8i** (2.20 g, 3.49 mmol) in 50 mL of anhydrous THF was added Et_3N-3HF (5.0 eq, 0.36 g, 4.60 mmol) at 0 °C under N_2 atmosphere. After stirring for 12 h at rt, the reaction mixture was concentrated under reduced pressure. The residue was purified on silica gel column chromatography (CH_2Cl_2 to CH_2Cl_2: MeOH = 20:1 v/v) to give compound **9i** (1.71 g, 3.32 mmol) in 95% yield. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 10.10 (s, 1H), 8.08 (s, 1H), 6.43 (br, 2H), 6.27 (dd, *J* = 1.2, 5.6 Hz, 1H), 5.38-5.29 (m, 2H), 5.14 (t, *J* = 6.4 Hz, 1H), 5.04 (t, *J* = 3.2 Hz, 1H), 4.49 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.20 (dd, *J* = 5.6, 10.0 Hz, 1H), 3.59 (dd, *J* = 3.2, 6.0 Hz, 1H), 2.53-2.48 (m, 2H), 2.04-1.92 (m, 4H), 1.60-1.50 (m, 2H), 1.36-1.18 (m, 22H), 0.86-0.81 (m, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 171.6, 159.9, 153.7, 150.0, 137.7, 129.6, 116.8, 105.5, 78.7, 70.4, 61.1, 36.1, 31.3, 29.13, 29.09, 28.83, 28.73, 28.69, 28.62, 28.58, 26.62, 26.57, 24.81, 22.09, 13.95; MS-ESI$^+$ *m/z* 517 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{27}H_{45}N_6O_4$ (M+H$^+$) 517.3501, found 517.3497.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)acetamide (9a)—**Compound **9a** was prepared using the same procedure as for

compound **9i**; yield: 93%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 8.16 (s, 1H), 7.27 (br, 2H), 6.30 (d, *J* = 3.6 Hz, 1H), 5.14 (t, *J* = 5.4 Hz, 1H), 5.05 (t, *J* = 3.2 Hz, 1H), 4.53 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.22 (dd, *J* = 4.8, 9.6 Hz, 1H), 3.60 (m, 2H), 2.20 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 156.1, 153.1, 150.0, 137.8, 115.5, 105.5, 79.0, 70.5, 61.1, 24.7; MS-ESI$^+$ *m/z* 295 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{11}H_{15}N_6O_4$ (M+H$^+$) 295.1148, found 295.1149.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)butyramide (9b)—**Compound **9b** was prepared using the same procedure as for compound **9i**; yield: 94%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.09 (s, 1H), 6.45 (br, 2H), 6.27 (d, *J* = 4.4 Hz, 1H), 5.14 (t, *J* = 6.0 Hz, 1H), 5.04 (t, *J* = 3.2 Hz, 1H), 4.51 (d, *J* = 9.6 Hz, 1H), 4.20 (dd, *J* = 5.2, 9.6 Hz, 1H), 3.59 (dd, *J* = 3.2, 5.2 Hz, 2H), 1.62-1.56 (m, 2H), 0.91 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 156.0, 153.7, 150.0, 137.7, 116.9, 105.5, 78.7, 70.4, 38.0, 18.3, 13.6; MS-ESI$^+$ *m/z* 323 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{13}H_{19}N_6O_4$ (M+H$^+$) 323.1463, found 323.1462.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)hexanamide (9c)—**Compound **9c** was prepared using the same procedure as for compound **9i**; yield: 96%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H), 6.34 (dd, *J* = 1.2, 4.8 Hz, 1H), 5.16 (t, *J* = 2.8 Hz, 1H), 4.52 (dd, *J* = 1.2, 9.6 Hz, 1H), 4.25 (dd, *J* = 4.8, 10.0 Hz, 1H), 3.82-3.74 (m, 2H), 2.56 (t, *J* = 7.6 Hz, 2H), 1.75-1.67 (m, 2H), 1.40-1.30 (m, 4H), 0.92 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.6, 161.8, 154.6, 150.9, 140.0, 116.5, 107.1, 81.3, 72.5, 62.4, 38.3, 32.6, 26.1, 23.6, 14.1; MS-ESI$^+$ *m/z* 351 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{15}H_{23}N_6O_4$ (M+H$^+$) 351.1781, found 351.1775.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)octanamide (9d)—**Compound **9d** was prepared using the same procedure as for compound **9i**; yield: 95%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H), 6.36 (dd, *J* = 1.2, 5.2 Hz, 1H), 5.11 (t, *J* = 2.4 Hz, 1H), 4.51 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.25 (dd, *J* = 5.6, 10.0 Hz, 1H), 3.82-3.74 (m, 2H), 2.57 (t, *J* = 7.2 Hz, 2H), 1.75-1.66 (m, 2H), 1.44-1.26 (m, 8H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.7, 161.9, 154.7, 151.1, 140.1, 116.6, 107.2, 81.3, 72.5, 62.4, 38.4, 33.0, 30.42, 30.35, 26.4, 23.8, 14.6; MS-ESI$^+$ *m/z* 379 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{17}H_{27}N_6O_4$ (M+H$^+$) 379.2091, found 379.2088.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)decanamide (9e)—**Compound **9e** was prepared using the same procedure as for compound **9i**; yield: 97%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (s, 1H), 6.34 (dd, *J* = 1.2, 5.2 Hz, 1H), 5.11 (t, *J* = 2.4 Hz, 1H), 4.51 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.25 (dd, *J* = 5.6, 10.0 Hz, 1H), 3.82-3.74 (m, 2H), 2.56 (t, *J* = 7.6 Hz, 2H), 1.74-1.68 (m, 2H), 1.42-1.24 (m, 12H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.6, 161.9, 154.6, 151.0, 140.0, 116.5, 107.2, 81.3, 72.5, 62.4, 38.4, 33.2, 30.75, 30.68, 30.56, 30.45, 26.4, 23.9, 14.6; MS-ESI$^+$ *m/z* 407 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{19}H_{31}N_6O_4$ (M+H$^+$) 407.2398, found 407.2401.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)dodecanamide (9f)—**Compound **9f** was prepared using the same procedure as for compound **9i**; yield: 93%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (s, 1H), 6.52 (d, *J* = 4.0 Hz, 1H), 5.12 (t, *J* = 2.4 Hz, 1H), 4.49 (dd, *J* = 1.2, 10.0 Hz, 1H), 4.26 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.84-3.76 (m, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 1.76-1.68 (m, 2H), 1.46-1.22 (m, 16H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 174.5, 157.7, 154.6, 152.5, 140.2, 116.9, 107.2, 81.6, 72.9, 62.3, 38.3, 33.2, 30.9, 30.8, 30.7, 30.6, 30.5, 26.8, 23.9, 14.6; MS-ESI$^+$ *m/z* 435 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{21}H_{34}N_6O_4Na$ (M+Na$^+$) 457.2541, found 457.2538.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)tetradecanamide (9g)—**Compound **9g** was prepared using the same procedure as for compound **9i**; yield: 93%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (br, 1H), 8.01 (s, 1H), 6.28 (d, *J* = 4.8 Hz, 1H), 5.45 (br, 2H), 5.21 (s, 1H), 4.49 (d, *J* = 9.6 Hz, 1H), 4.25 (dd, *J* = 5.2, 10.0 Hz, 1H), 3.97-3.88 (m, 2H), 2.68 (t, *J* = 7.2 Hz, 2H), 1.74-1.66 (m, 2H), 1.40-1.20 (m, 22H), 0.89 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 159.8, 152.6, 149.7, 138.5, 115.4, 106.0, 79.8, 71.3, 61.6, 37.9, 33.9, 32.1, 29.88, 29.77, 29.72, 29.55, 25.1, 22.9, 14.3; MS-ESI$^+$ *m/z* 463 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{23}H_{39}N_6O_4$ (M+H$^+$) 463.3021, found 463.3027.

***N*-(2-Amino-9-((2*R*,4*R*)-2-(hydroxymethyl)-1,3-dioxolan-4-yl)-9*H*-purin-6-yl)palmitamide (9h)—**Compound **9h** was prepared using the same procedure as for compound **9i**; yield: 96%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.16 (s, 1H), 7.26 (br, 1H), 6.29 (d, *J* = 4.4 Hz, 1H), 5.15 (t, *J* = 6.4 Hz, 1H), 5.05 (t, *J* = 3.2 Hz, 1H), 4.52 (d, *J* = 9.6 Hz, 1H), 4.21 (dd, *J* = 5.6, 9.6 Hz, 1H), 3.61-3.59 (m, 2H), 2.47 (t, *J* = 7.2 Hz, 2H), 1.53 (t, *J* = 5.8 Hz, 2H), 1.30-1.17 (m, 28H), 0.84 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.5, 156.1, 153.0, 150.0, 137.7, 115.5, 105.5, 78.9, 70.5, 61.1, 47.3, 36.2, 33.9, 31.3, 29.1, 29.0, 28.9, 28.7, 24.9, 22.1, 14.0; MS-ESI$^+$ *m/z* 491 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{25}H_{43}N_6O_4$ (M+H$^+$) 491.3340, found 491.3340.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-((*Z*)-octadec-8-enamido)-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10i)—To a solution of compound **9i** (0.052 g, 0.100 mmol) in 10 mL of anhydrous THF was added phosphoramidate chloride **6** (2.0 eq, 0.058 g, 0.20 mmol) and *N*-methylimidazole (4.0 eq, 0.033 g, 0.40 mmol) at −78 °C under argon atmosphere. After stirring for 12 h at rt, the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL) and washed with aqueous 0.5 M HCl solution (5 mL × 2) and brine (10 mL). The solution was dried over Na_2SO_4 for 3 h at rt and filtered. The filtrate was concentrated under reduced pressure and purified on silica gel column chromatography (CH_2Cl_2: MeOH = 40:1 to 20:1 v/v) to give compound **10i** (0.062 g, 0.081 mmol) in 81 % yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 0.5H), 8.34 (s, 0.5H), 7.93 (s, 1H), 7.33-7.10 (m, 5H), 6.35-6.33 (m, 1H), 5.39-5.30 (m, 3H), 5.07 (br, 2H), 4.60-4.55 (m, 1H), 4.47-4.25 (m, 3H), 4.18-4.10 (m, 2H), 4.03-3.91 (m, 1H), 3.84-3.65 (m, 1H), 2.84-2.77 (m, 2H), 2.02-1.98 (m, 4H), 1.78-1.71 (m, 2H), 1.44-1.20 (m, 27H), 0.89-0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 173.2, 159.8, 153.1, 150.7, 150.0, 138.0, 130.2, 129.8, 125.2, 120.3, 120.2, 116.1, 103.8,

103.7, 80.0, 70.8, 65.2, 64.9, 61.8, 50.4, 38.1, 32.1, 29.9, 29.7, 29.6, 29.5, 29.4, 27.4, 25.0, 22.9, 21.3, 21.2, 14.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.41, 3.15; MS-ESI$^+$ *m/z* 772 (M +H$^+$); HRMS-ESI$^+$ calcd for $C_{38}H_{59}N_7O_8P$ (M+H$^+$) 772.4183, found 772.4157.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(6-acetamido-2-amino-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10a)—Compound **10a** was prepared using the same procedure as for compound **10i**; yield: 68%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (s, 0.5H), 8.19 (s, 0.5H), 7.33-7.24 (m, 2H), 7.20-7.17 (m, 1H), 7.15-7.12 (m, 2H), 6.54-6.50 (m, 1H), 5.35-5.30 (m, 1H), 4.65-4.61 (m, 1H), 4.38-4.29 (m, 3H), 4.13-4.03 (m, 2H), 3.92-3.68 (m, 1H), 2.26 (br, 3H), 1.32-1.30 (m, 2H), 1.22-1.16 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 186.0, 175.1, 157.8, 154.7, 152.1, 151.4, 141.5, 139.9, 130.9, 126.3, 121.5, 121.4, 117.0, 105.5, 104.9, 81.5, 72.3, 66.0, 62.5, 51.6, 24.8, 20.5, 14.6; ^{31}P NMR (162 MHz, CD_3OD) δ 5.19, 4.91; MS-ESI$^+$ *m/z* 550 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{22}H_{29}N_7O_8P$ (M+H$^+$) 550.1823, found 550.1810.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-butyramido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10b)—Compound **10b** was synthesized using the same procedure as **10i**; yield: 79%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 0.5H), 8.19 (s, 0.5H), 7.34-7.21 (m, 2H), 7.20-7.18 (m, 1H), 7.16-7.10 (m, 2H), 6.56-6.53 (m, 1H), 5.35-5.30 (m, 1H), 4.64-4.60 (m, 1H), 4.39-4.30 (m, 3H), 4.14-4.03 (m, 2H), 3.92-3.69 (m, 1H), 2.47 (t, *J* = 7.2 Hz, 2H), 1.78 (m, 2H), 1.32-1.31 (m, 2H), 1.23-1.19 (m, 3H), 1.04-0.99 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 175.1, 157.8, 154.7, 152.2, 151.7, 141.5, 139.9, 130.8, 126.3, 121.5, 121.3, 117.0, 104.9, 81.5, 72.4, 66.0, 62.4, 51.6, 40.2, 30.9, 20.5, 14.6; ^{31}P NMR (162 MHz, CD_3OD) δ 5.23, 4.96; MS-ESI$^+$ *m/z* 578 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{24}H_{33}N_7O_8P$ (M+H$^+$) 578.2130, found 578.2123.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-hexanamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10c)—Compound **10c** was prepared using the same procedure as for compound **10i**; yield: 75%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 0.5H), 8.08 (s, 0.5H), 7.32-7.07 (m, 5H), 6.39-6.36 (m, 1H), 5.32-5.30 (m, 1H), 4.66-4.64 (m, 1H), 4.36-4.27 (m, 3H), 4.14-4.00 (m, 2H), 3.91-3.66 (m, 1H), 2.58-2.53 (m, 2H), 1.76-1.66 (m, 2H), 1.40-1.26 (m, 6H), 1.23-1.14 (m, 4H), 0.94-0.90 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.5, 161.9, 154.8, 152.1, 151.0, 139.6, 130.8, 126.2, 121.5, 121.2, 116.5, 104.8, 81.1, 72.0, 66.0, 62.4, 51.5, 38.4, 32.7, 26.1, 23.7, 20.6, 20.5, 14.6, 14.5; ^{31}P NMR (162 MHz, CD_3OD) δ 5.07, 4.82; MS-ESI$^+$ *m/z* 606 (M+H$^+$); HRMS-ESI$^+$ calcd for $C_{26}H_{37}N_7O_8P$ (M+H$^+$) 606.2447, found 606.2436.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-octanamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10d)—Compound **10d** was prepared using the same procedure as for compound **10i**; yield: 72%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 0.5H), 8.09 (s, 0.5H), 7.32-7.07 (m, 5H), 6.40-6.37 (m, 1H), 5.34-5.31 (m, 1H), 4.66-4.64 (s, 1H), 4.34-4.28 (m, 3H), 4.14-4.00 (m, 2H), 3.91-3.66 (m, 1H), 2.58-2.52 (m, 2H), 1.75-1.67 (m, 2H), 1.44-1.26 (m, 9H), 1.23-1.15 (m, 5H), 0.91-0.88 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.5, 161.9, 154.8, 152.1, 151.0, 139.6,

130.8, 126.2, 121.5, 121.2, 116.5, 104.8, 81.1, 72.0, 66.0, 62.4, 51.5, 38.4, 32.7, 26.1, 23.7, 20.6, 20.5, 14.6; ^{31}P NMR (162 MHz, CD_3OD) δ 5.10, 4.85; MS-ESI$^+$ *m/z* 634 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{28}H_{41}N_7O_8P$ ($M+H^+$) 634.2758, found 634.2749.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-decanamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10e)— Compound **10e** was prepared using the same procedure as for compound **10i**; yield: 76%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.09 (s, 0.5H), 8.08 (s, 0.5H), 7.31-7.07 (m, 5H), 6.39-6.35 (m, 1H), 5.33-5.30 (m, 1H), 4.66-4.63 (m, 1H), 4.35-4.27 (m, 3H), 4.14-4.00 (m, 2H), 3.92-3.65 (m, 1H), 2.58-2.53 (m, 2H), 1.76-1.66 (m, 2H), 1.42-1.26 (m, 13H), 1.23-1.15 (m, 5H), 0.91-0.88 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 174.5, 161.9, 154.7, 152.1, 151.0, 139.6, 130.8, 126.2, 121.5, 121.2, 116.4, 104.8, 81.1, 72.0, 66.0, 62.4, 51.5, 38.5, 33.2, 30.7, 30.5, 26.4, 23.9, 20.6, 20.5, 14.6; ^{31}P NMR (162 MHz, CD_3OD) δ 5.04, 4.79; MS-ESI$^+$ *m/z* 662 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{30}H_{45}N_7O_8P$ ($M+H^+$) 662.3067, found 662.3062.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-dodecanamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10f)— Compound **10f** was prepared using the same procedure as for compound **10i**; yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (br, 1H), 8.05 (s, 0.5H), 8.04 (s, 0.5H), 7.30-7.08 (m, 5H), 6.89 (br, 2H), 6.38 (m, 1H), 5.34-5.31 (m, 1H), 4.58-4.60 (s, 1H), 4.43-4.26 (m, 3H), 4.19-3.90 (m, 3H), 2.88 (br, 2H), 1.78-1.70 (m, 2H), 1.43-1.18 (m, 23H), 0.89-0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 156.8, 153.7, 150.7, 150.2, 137.9, 129.8, 125.2, 120.3, 120.2, 116.3, 103.8, 80.0, 71.3, 65.2, 61.8, 50.4, 47.9, 37.5, 32.1, 29.9, 29.8, 29.6, 25.3, 22.9, 21.1, 21.0, 14.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.40, 3.16; MS-ESI$^+$ *m/z* 690 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{32}H_{49}N_7O_8P$ ($M+H^+$) 690.3379, found 690.3375.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-tetradecanamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10g)— Compound **10g** was prepared using the same procedure as for compound **10i**; yield: 72%; ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (s, 0.5H), 8.09 (s, 0.5H), 7.32-7.07 (m, 5H), 6.41-6.37 (m, 1H), 5.34-5.31 (m, 1H), 4.66-4.63 (m, 1H), 4.36-4.28 (m, 3H), 4.14-4.00 (m, 2H), 3.91-3.66 (m, 1H), 2.58-2.53 (m, 2H), 1.78-1.68 (m, 2H), 1.43-1.24 (m, 21H), 1.23-1.15 (m, 5H), 0.91-0.88 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 174.6, 162.0, 154.8, 152.1, 151.0, 139.7, 130.8, 126.2, 121.5, 121.3, 116.4, 104.9, 81.1, 72.0, 66.0, 62.4, 51.6, 38.5, 33.2, 30.95, 30.92, 30.89, 30.8, 30.6, 30.4, 26.4, 23.9, 20.6, 20.5, 14.6; ^{31}P NMR (162 MHz, CD_3OD) δ 5.14, 4.88; MS-ESI$^+$ *m/z* 718 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{34}H_{53}N_7O_8P$ ($M+H^+$) 718.3703, found 718.3690.

(2*S*)-Ethyl 2-(((((2*R*,4*R*)-4-(2-amino-6-palmitamido-9*H*-purin-9-yl)-1,3-dioxolan-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (10h)— Compound **10h** was prepared using the same procedure as for compound **10i**; yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (br, 1H), 8.08 (s, 0.5H), 8.07 (s, 0.5H), 7.30-7.08 (m, 5H), 6.92 (br, 2H), 6.40-6.38 (m, 1H), 5.34-5.30 (m, 1H), 4.58-4.56 (s, 1H), 4.42-4.24 (m, 3H), 4.22-4.07 (m, 2H), 4.03-3.90 (m, 1H), 2.88 (br, 2H), 1.77-1.70 (m, 2H), 1.44-1.17

(m, 31H), 0.89-0.86 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 156.8, 153.7, 150.7, 150.1, 137.9, 129.8, 125.1, 120.3, 120.2, 116.3, 103.7, 80.0, 71.3, 65.2, 61.7, 50.4, 39.3, 37.5, 32.1, 29.9, 29.84, 29.81, 29.76, 29.5, 25.3, 22.9, 21.1, 21.0, 14.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.42, 3.19; MS-ESI$^+$ *m/z* 746 ($M+H^+$); HRMS-ESI$^+$ calcd for $C_{36}H_{57}N_7O_8P$ ($M+H^+$) 746.4012, found 746.4001.

Biological testing

Compounds were evaluated against HIV-1 and HBV as previously reported.[8] Cytotoxicity was also determined in various cell systems as described previously.[8]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Tyana Singletary for excellent technical assistance. This work was supported in part by NIH grant 5P30-AI-50409 (CFAR), and by the Department of Veterans Affairs. Dr. Schinazi is the founder and a major shareholder of RFS Pharma, LLC. Emory received no funding from RFS Pharma, LLC to perform this work and *vice versa*.

References and notes

1. a) Huryn DM, Okabe M. Chem Rev. 1992; 92:1745–1768.b) Garg R, Gupta SP, Gao H, Babu MS, Debnath AK, Hansch C. Chem Rev. 1999; 99:3525–3601. [PubMed: 11849030] c) De Clercq E. J Clin Virol. 2001; 22:73–78. [PubMed: 11418355] d) Mehellou Y, De Clercq E. J Med Chem. 2010; 53:521–538. [PubMed: 19785437]
2. Related reviews: Miller V. Antiviral Ther. 2001; 6(suppl 2):1–9.Wainberg MA, White AJ. Antiviral Ther. 2001; 6(suppl 2):11–19.Shafer RW. Clin Microbiol Rev. 2002; 15:247–277. [PubMed: 11932232] Gallant JE, Gerondelis PZ, Wainberg MA, Shulman NS, Haubrich RH, Clair MS, Lanier ER, Hellmann NS, Richman DD. Antiviral Ther. 2003; 8:489–506.Deeks SG. Lancet. 2003; 362:2002–2011. [PubMed: 14683662] Wainberg MA, Turner DJ. Acquir Immune Defic Syndr. 2004; 37(suppl 1):S36–43.Rezende LF, Prasad VR. Int J Biochem Cell Biol. 2004; 36:1716–1734. [PubMed: 15183340]
3. a) Richman DD, Fischl MA, Grieco MH. N Engl J Med. 1987; 317:192–197. [PubMed: 3299090] b) Starnes MC, Cheng YC. J Biol Chem. 1987; 262:988–991. [PubMed: 2433280] c) Larder B, Darby G, Richman D. Science. 1989; 243:1731–1734. [PubMed: 2467383] d) Petersen EA, Ramirez-Ronda CH, Hardy WD. J Infect Dis. 1995; 171(suppl 2):S131–S139. [PubMed: 7861018] e) Yahi N, Tamalet C, Tourres C, Tivoli N, Fantini J. J Biomed Sci. 2000; 7:507–513. [PubMed: 11060499] f) Mocroft A, Phillips AN, Friis-Moller N, Colebunders R, Johnson AM, Hirschel B, Marc-Saint T, Staub T, Clotet B, Lundgren JD. Antiviral Ther. 2002; 7:21–30.g) Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC. N Engl J Med. 2002; 347:385–394. [PubMed: 12167680] h) Tamalet C, Fantini J, Tourres C, Yahi N. AIDS. 2003; 17:2383–2388. [PubMed: 14571191] i) Turner D, Brenner B, Wainberg MA. J Antimicrob Chemother. 2004; 53:53–57. [PubMed: 14645322] j) Marcelin AG, Delaugerre C, Wirden M, Viegas P, Simon A, Katlama C, Calvez V. J Med Virol. 2004; 72:162–165. [PubMed: 14635026] k) Imamichi T. Curr Pharm Des. 2004; 10:4039–4053. [PubMed: 15579086] l) Martinez-Picado J, Martinez MA. Virus Res. 2008; 134:104–123. [PubMed: 18289713] m) Menéndez-Arias L. Virus Res. 2008; 134:124–146. [PubMed: 18272247]
3. For a recent review on the synthesis of nucleoside phosphate and phosphonate prodrugs: Pradere U, Garnier-Amblard EC, Coats SJ, Amblard F, Schinazi RF. Chem Rev. 2014 Article asap.
4. Bondana L, Detorio M, Bassit L, Tao S, Montero CM, Singletary TM, Zhang H, Zhou L, Cho JH, Coats SJ, Schinazi RF. ACS Med Chem Lett. 2013; 4:747–751. [PubMed: 24015326]
5. Radi M, Adema AD, Daft JR, Cho JH, Hoebe EK, Alexander LEMM, Peters GJ, Chu CK. J Med Chem. 2007; 50:2249–2253. [PubMed: 17419604]

6. a) Agarwal HK, Chhikara BS, Hanley MJ, Ye G, Doncel GF, Parang K. J Med Chem. 2012; 55:4861–4871. [PubMed: 22533850] b) Agarwal HK, Chhikara BS, Bhavaraju S, Mandal D, Doncel GF, Parang K. Mol Pharma. 2013; 10:467–476.

7. McGuigan C, Thiery JC, Daverio F, Jiang WG, Davies G, Mason M. Bioorg Med Chem. 2005; 13:3219–3227. [PubMed: 15809157]

8. Cho JH, Amblard F, Coats SJ, Schinazi RF. Tetrahedron. 2011; 67:5487–5493. [PubMed: 22993455]

9. (a) Schinazi RF, Sommadossi JP, Saalmann V, Cannon DL, Xie MW, Hart GC, Smith GA, Hahn EF. Antimicrob Agents Chemother. 1990; 34:1061. [PubMed: 2393266] (b) Stuyver LJ, Lostia S, Adams M, Mathew J, Pai BS, Grier J, Tharnish P, Choi Y, Chong Y, Choo H, Chu CK, Otto MJ, Schinazi RF. Antimicrob Agents Chemother. 2002; 46:3854. [PubMed: 12435688]

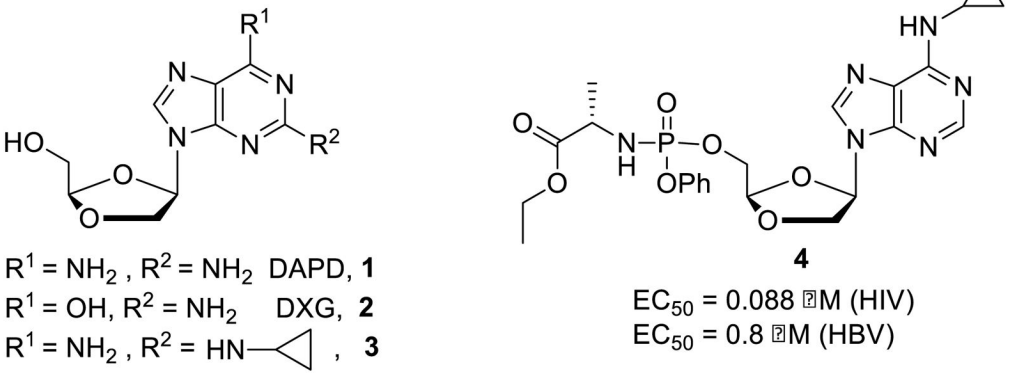
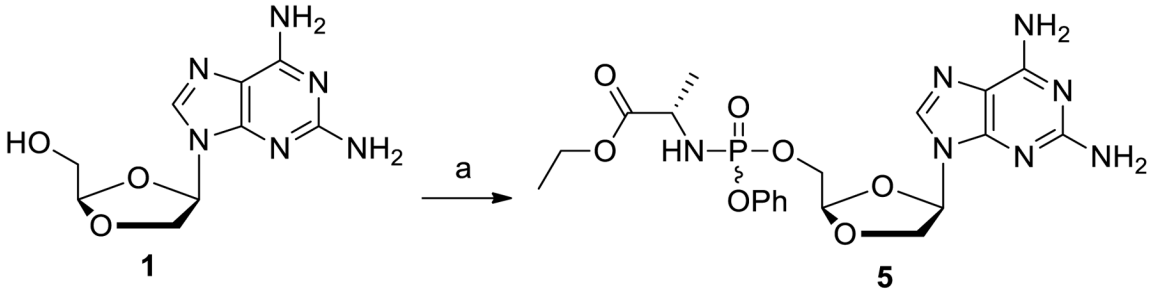
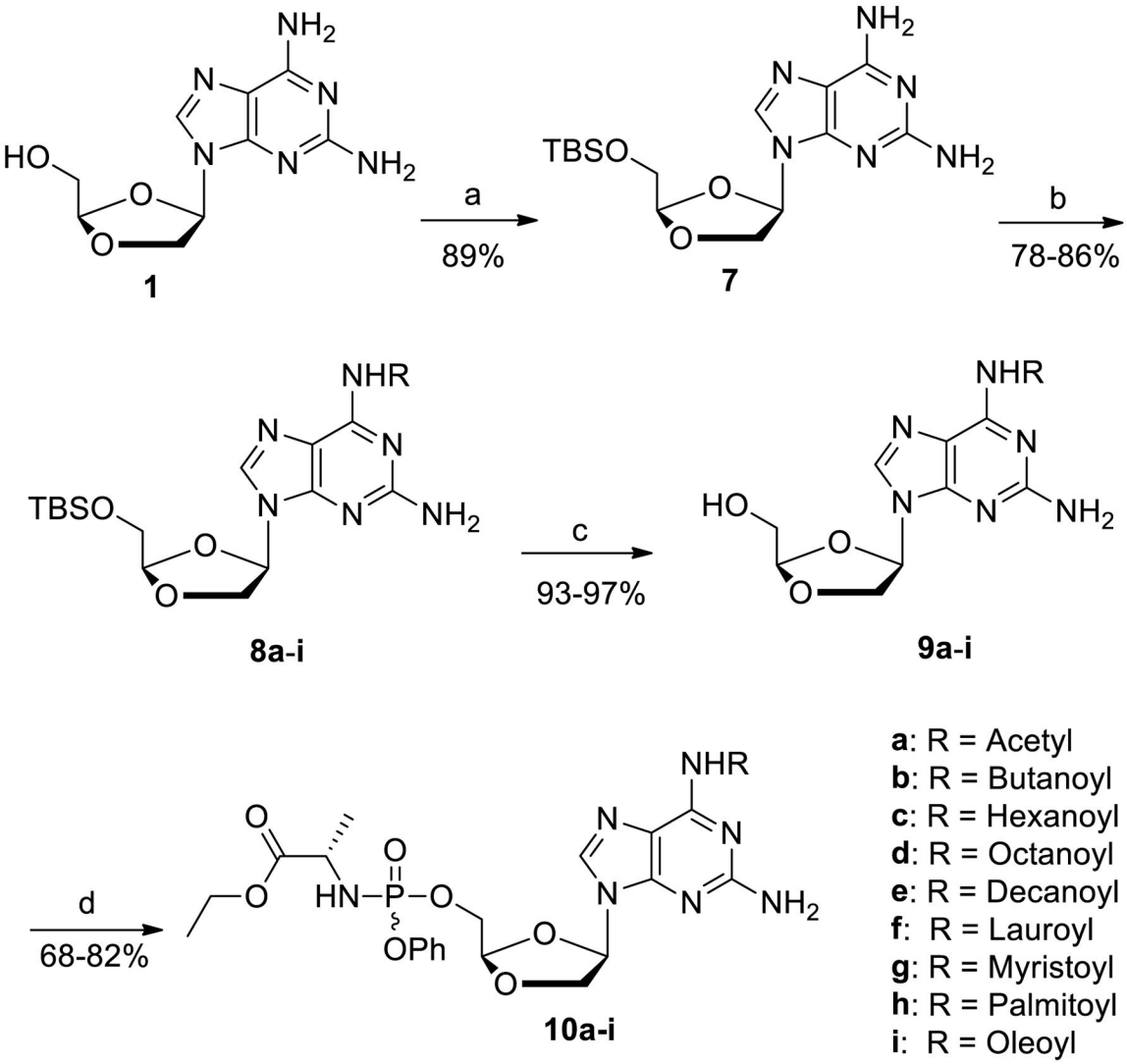


Figure 1.



Scheme 1.

Reagents and reaction conditions: a) (EtOAlaNH)P(=O)(OPh)Cl **6**, *t*-BuMgCl, THF, −78 °C then rt, 8 h.

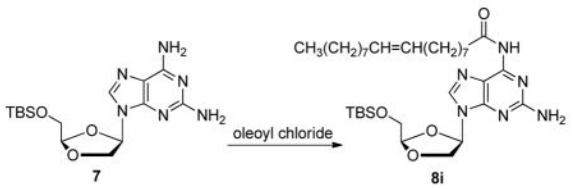


Scheme 2.

Reagents and reaction conditions: a) TBSCl, imidazole, pyridine, 0 °C then rt, 12 h; b) RCl, NMI, CH_2Cl_2, 0 °C then rt, 12 h; c) Et_3N-3HF, THF, rt, 12 h; d) (NHAlaOEt)P(=O)(OPh)Cl **6**, NMI, −78 °C then rt, 8–12 h.

Table 1

Condition for optimization of the N^6-acylation of **7**



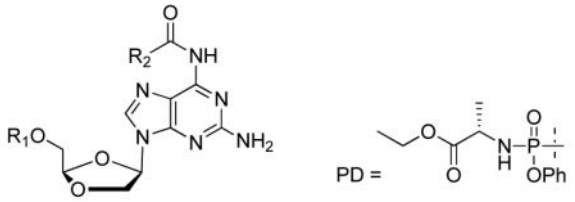
Entry	Conditions	Yield (%)
1	NMI, CH_2Cl_2, 12 h	80
2	pyridine, 12 h	Trace[a]
3	DMAP (0.1 eq), pyridine, 12 h	5[a]
4	DMAP (2.0 eq), CH_2Cl_2, 12 h	43[a]
5	DMAP (0.1 eq), Et_3N (2.0 eq), CH_2Cl_2, 12 h	18[a]
6	Et_3N (2.0 eq), CH_2Cl_2, 12 h	21[a]
7	imidazole (2.0 eq), CH_2Cl_2, 12 h	8[b]

[a] Reactions provided N^2,N^6-diacylated compounds in 20–30% yields and ca. 5–15 % of starting material (**7**) was recovered;

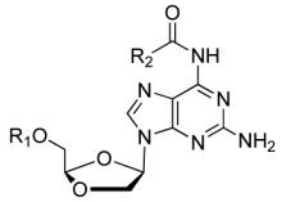
[b] Most of **7** was recovered.

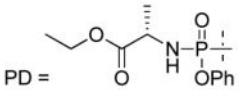
Table 2

In vitro antiviral activity and cytotoxicity of compounds **9a-i** and phosphoramidate prodrugs **5**, **10a-i**.[a]



Cmpd	R_1	R_2	Anti-HIV-1 activity (μM)		Anti-HBV activity (μM)		Cytotoxicity, CC_{50} (μM)			
			EC_{50}	EC_{90}	EC_{50}	EC_{90}	PBM	CEM	Vero	HepG2
AZT	NA	NA	0.0037	0.047	> 10	> 10	> 100	14.0	56.0	> 100
1	H	NA	0.45	7.6	12.0	> 100	> 100	> 100	> 100	> 100
5	PD	NA	0.25	1.5	0.30	2.8	67.0	> 100	> 100	> 100
9a	H	CH_3	0.56	12.0	> 10	> 10	> 100	> 100	> 100	> 100
10a	PD	CH_3	19.0	93.0	9.3	> 10	> 100	> 100	> 100	> 100
9b	H	$CH_3(CH_2)_2$	9.2	38.0	> 10	> 10	> 100	> 100	> 100	> 100
10b	PD	$CH_3(CH_2)_2$	1.2	15.0	3.3	> 10	> 100	> 100	> 100	> 100
9c	H	$CH_3(CH_2)_4$	34.0	> 100	> 10	> 10	> 100	> 100	> 100	> 100
10c	PD	$CH_3(CH_2)_4$	2.2	33.0	1.3	> 10	> 100	89	> 100	> 100
9d	H	$CH_3(CH_2)_6$	21	56.0	> 10	> 10	> 100	> 100	> 100	> 100
10d	PD	$CH_3(CH_2)_6$	0.42	6.6	0.55	≥ 10	> 100	16.0	87.0	9.5
9e	H	$CH_3(CH_2)_8$	26.0	65.0	> 10	> 10	> 100	65.0	> 100	> 100
10e	PD	$CH_3(CH_2)_8$	1.5	9.4	0.43	≥ 10	67.0	13.0	13.0	9.5
9f	H	$CH_3(CH_2)_{10}$	3.6	18.0	> 10	> 10	60.0	13	53.0	> 100
10f	PD	$CH_3(CH_2)_{10}$	0.98	2.9	0.34	≥ 10	14.0	12.0	45.0	71.0
9g	H	$CH_3(CH_2)_{12}$	6.5	27.0	> 10	> 10	> 100	4.6	7.7	56.0
10g	PD	$CH_3(CH_2)_{12}$	0.041	0.89	0.39	8.8	9.9	7.5	13.0	57.0
9h	H	$CH_3(CH_2)_{14}$	1.2	6.0	> 10	> 10	> 100	> 100	90	> 100
10h	PD	$CH_3(CH_2)_{14}$	0.083	0.50	0.33	7.9	7.9	12	> 100	ND





Cmpd	R_1	R_2	Anti-HIV-1 activity (μM)		Anti-HBV activity (μM)		Cytotoxicity, CC_{50} (μM)			
			EC_{50}	EC_{90}	EC_{50}	EC_{90}	PBM	CEM	Vero	HepG2
9i	H	$CH_3(CH_2)_7CH=CH(CH_2)_7$	3.5	13.0	> 10	> 10	76	13	19	11
10i	PD	$CH_3(CH_2)_7CH=CH(CH_2)_7$	0.77	2.1	0.1	5.8	10	10	13	ND