

# Synthesis and anti-HIV activity of (–)-β-D-(2*R*,4*R*)-1,3-dioxolane-2,6-diamino purine (DAPD) (amdoxovir) and (–)-β-D-(2*R*,4*R*)-1,3-dioxolane guanosine (DXG) prodrugs

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## Abstract

Prodrugs of (–)-β-D-(2*R*,4*R*)-1,3-dioxolane-2,6-diamino purine (DAPD), organic salts of DAPD, 5′-L-valyl DAPD and *N* – 1 substituted (–)-β-D-(2*R*,4*R*)-1,3-dioxolane guanosine (DXG) have been synthesized with the objective of finding molecules which might be superior to DAPD and DXG in solubility as well as pharmacologic profiles. Synthesized prodrugs were evaluated for anti-HIV activity against HIV-1<sub>LAI</sub> in primary human lymphocytes (PBM cells) as well as their cytotoxicity in PBM, CEM and Vero cells. DAPD prodrugs, modified at the C6 position of the purine ring, demonstrated several folds of enhanced anti-HIV activity in comparison to the parent compound DAPD without increasing the toxicity. The presence of alkyl amino groups at the C6 position of the purine ring increased the antiviral potency several folds, and the most potent compound (–)-β-D-(2*R*,4*R*)-1,3-dioxolane-2-amino-6-aminoethyl purine (**8**) was 17 times more potent than that of DAPD. 5′-L-Valyl DAPD **20** and organic acid salts **21–24** also exhibited enhanced anti-HIV activity in comparison to DAPD, while DXG prodrugs **16** and **17** exhibited lower potency than that of DXG or DAPD.

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## 1. Introduction

Despite the effectiveness of nucleoside reverse transcriptase inhibitors (NRTIs) (De Clercq, 2001) in combination with a protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitor (NNRTI), a long-term use of these drugs result in the selection of viral resistance mutants (Turner et al., 2004). Furthermore, individuals, who have become recently infected with resistant strains of HIV-1, have significantly been increased (Mocroft et al., 2002; Tamalet et al., 2003; Little et al., 2002). Thus, the identification of novel anti-HIV agents with antiviral activity against drug-resistant strains of HIV-1 is critical.

For past years, we have been interested in dioxolane nucleosides as potential anti-HIV agents (Chu et al., 1991a,b; Kim et al., 1992; Kim et al., 1993a,b; Furman et al., 2001; Chu et

al., 2005; Liang et al., 2006), which possess a dioxolane ring in place of a classical carbohydrate moiety in nucleosides. The dioxolane nucleosides, such as (–)-β-D-(2*R*,4*R*)-1,3-dioxolane 2,6-diamino purine (DAPD) and (–)-β-D-(2*R*,4*R*)-dioxolane-tymine (DOT), are interesting in that they demonstrated interesting anti-HIV activity against drug resistant mutants (Chong et al., 2002; Chong and Chu, 2004; Chu et al., 2005; Thompson et al., 2005) (Fig. 1). DAPD (amdoxovir), a reverse transcriptase inhibitor (NRTI), is deaminated in vitro as well as in vivo, yields a metabolite (–)-β-D-1,3-dioxolane guanosine (Gu et al., 1999a,b). Both DAPD and DXG demonstrated potent in vitro anti-HIV activity against wild type as well drug resistant strains (such as AZT, 3TC and abacavir) (Furman et al., 2001). Preliminary in vitro studies of dioxolane nucleosides shown that the lack of cross-resistance to AZT and the reversal of AZT resistance by DXG resistance mutations provide strong rationales for the use of these compounds in combination with other agents (Bazmi et al., 2000; Gallant et al., 2003; Walmsley, 2003). In order to understand the mechanism of this antiviral

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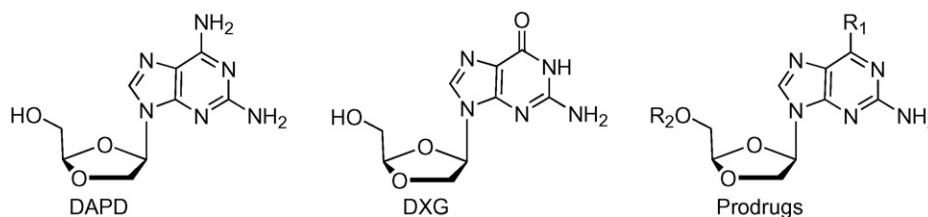


Fig. 1. Chemical structure of DAPD, DXG and its prodrug prototypes.

activity, we have conducted molecular modeling studies, which revealed that the 1,3-dioxolane moiety plays a significant role in stabilizing the binding between the mutant HIV-1 RT and the nucleoside triphosphate (Chong and Chu, 2004; Chu et al., 2005).

DAPD has been undergoing clinical trials as an anti-HIV agent against drug resistant patients. Although the time of exposure of patients to DAPD was relatively short during the phase I trials, the profile of adverse effects of the drug was favorable (Gu et al., 1999a,b; Margolis et al., 2006). In addition, DAPD exhibited a favorable resistant profile in patients in that there were no genetic changes in the HIV reverse transcriptase coding regions, observed between the pretreatment and post treatment HIV genotypes within 15 days of DAPD monotherapy (Deeks et al., 2000). In the Phase IIa clinical trials (ACTG-5165) of amdoxovir (Margolis et al., 2006), highly treatment-experienced individuals failing existing antiretroviral therapy were treated. In a 96-week trial conducted by the AIDS Clinical Trials Group (ACTG), sponsored by the National Institutes of Health (NIH), amdoxovir 500 mg bid or amdoxovir 500 mg bid with mycophenolate mofetil (MMF) 500 mg bid, were administered in addition to patients' current failing treatment regimens. Amdoxovir was effective in subjects with viral strains resistant to nucleosides, including those harboring multiple thymidine analog mutations (TAMS) such as M41L and L210W mutations, as well as the M184V mutation selected by Epivir and Emtriva.

Despite of these positive profiles, optimum anti-HIV activity of DAPD has not been achieved due to its low oral bioavailability (approximately 30% in monkeys and rats) due to its low solubility in water (Chen et al., 1999). It is critical that anti-HIV agents also penetrate to the blood–brain barrier (BBB) and suppress the HIV replication in order to alleviate the CNS dysfunction. The objective of the current study is to prepare DAPD/DXG prodrugs and evaluate their anti-HIV activity in vitro, which may provide the better pharmacokinetic profiles. Accordingly, we have designed and synthesized a series of lipophilic and water-soluble prodrugs of DAPD/DXG, and evaluated for their anti-HIV activity in vitro in order to select potential candidates for additional biological evaluations.

## 2. Materials and methods

### 2.1. Chemistry

All chemicals were obtained from Aldrich Chemical Company. Melting points were determined on a MEL1-TEMP II apparatus and were uncorrected. TLC was performed on

Uniplat (silica gel) purchased from Analtech Co. Column chromatograph was performed using either silica gel-60 (220–240 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. NMR spectra were recorded on Varian Inova 500 spectrometer at 500 MHz with software VNMR6-1 with Me<sub>4</sub>Si as the internal standard. Chemical shifts ( $\delta$ ) are reported as s (single), d (doublet), t (triplet), q (quartet), m (multiplet, or b (broad). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectra were measured on a Micromass Autospec mass spectrometer. Elemental analyses were performed by Atlantic Micro lab, Inc. (Norcross, GA). Five milligram (5 mg) of the compound was dissolved in minimum amount of water with sonication to calculate the solubility of compound in 1 mL of water.

### 2.2. Experimental procedure

#### 2.2.1. Isobutyric acid-4-acetoxy-[1,3]-dioxolan-2-(R)-yl methyl ester (2)

To a well stirred solution of isobutyric acid-4-oxo-[1,3]-dioxolan-2-(R)-yl methyl ester (**1**) (51.5 g, 271.7 mmol) in dry THF (600 mL) at  $-78^{\circ}\text{C}$ , LiAl(OBu<sup>t</sup>)<sub>3</sub>H (330 mL, 329.6 mmol, 1 M solution in THF) was slowly added over a period of 1 h under N<sub>2</sub> atmosphere. The reaction mixture was allowed to stir for 2 h at  $-78^{\circ}\text{C}$  and for additional 2 h at  $-10^{\circ}\text{C}$ . To this solution DMAP (41.4 g, 339.5 mmol) was added in one portion and stirred for 30 min followed by drop wise addition of Ac<sub>2</sub>O (141 mL, 1359 mmol). After stirring the bright yellow solution for 2 h at  $-10^{\circ}\text{C}$ , the temperature of the cold bath was allowed to rise to room temperature and stirred for overnight. The dark brown solution was poured into saturated NH<sub>4</sub>Cl (300 mL) solution, stirred for 30 min, filtered (to remove Li salt), concentrated and extracted with ethyl acetate (3  $\times$  200 mL). The combined organic solutions were washed with saturated NaHCO<sub>3</sub> (300 mL), brine and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure to afford a crude product (red syrup). The crude product was purified by using column chromatography (ethyl acetate:hexanes 1:10–1:4) to afford isobutyric acid-4-acetoxy-[1,3]-dioxolan-2-(R)-yl methyl ester (**2**) (44.14 g, 70%) as yellow syrup. *R*<sub>f</sub>: 0.45 (ethyl acetate:hexanes 1:4). NMR showed 1:1 mixture of  $\alpha$ ,  $\beta$  isomers. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 1.18 and 1.19 (2s, 6H, 2  $\times$  CH<sub>3</sub>); 2.10 (s, 3H, CH<sub>3</sub>); 4.20–4.42 (m, 4H, 2CH<sub>2</sub>); 5.38 and 5.42 (2d, 1H, CH); 5.82 (t, 1H, CH) 5.42; 6.41 and 6.35 (2d, 1H, CH).

### 2.2.2. Isobutyric acid-4-(*R*)-(2-amino-6-chloropurin-9-yl)-[1,3]-dioxolan-2-(*R*)-yl methyl ester (**4a**)

Isobutyric acid-4-acetoxy-[1,3]-dioxolan-2-(*R*)-yl methyl ester (**2**) (40 g, 172.3 mmol) was dissolved in  $\text{CHCl}_3$  (600 mL) and cooled to  $-20^\circ\text{C}$ . Iodotrimethylsilane (41.37 g, 206.8 mmol) was slowly added to this pre-chilled solution by syringe over a period of 1.5 h and stirred for 2 h at  $-20^\circ\text{C}$ . To this solution was added per silylated 2-amino-6-chloropurine, dissolved in  $\text{CHCl}_3$  (600 mL) and stirred at  $-20^\circ\text{C}$  for 2 h. The reaction mixture was allowed to warm up to room temperature, stirred overnight and heated to gentle reflux. Reaction was monitored by TLC and after 4 h mixture was cooled, quenched with water, washed with saturated  $\text{NaHCO}_3$  ( $2 \times 200$  mL), saturated sodium thiosulfate solution (100 mL), water ( $2 \times 200$  mL), brine ( $2 \times 200$  mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and evaporated under reduced pressure to yield a yellow solid of  $\alpha$ ,  $\beta$ -mixture of **4** (39 g, 66%). The crude product was further purified using vacuum silica gel column (hexane:ethyl acetate, 7:3–3:7) to afford **4** as a 1:2,  $\alpha$ : $\beta$  mixture of anomers. TLC:  $R_f$ : 0.55 (ethyl acetate:hexane 7:3,  $\alpha$ -anomer);  $R_f$ : 0.50 (ethyl acetate:hexane 7:3,  $\beta$ -anomer).  $\beta$ -Isomer (**4a**):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.12 and 1.17 (2d,  $J=7.5$  Hz, 6H,  $2 \times \text{CH}_3$ ); 2.59 (m,  $J=7.5$  Hz 1H, CH); 4.28 (m,  $J=5.5$  Hz, 1H,  $\text{CH}_2$ ); 4.37 (m, 2H,  $\text{CH}_2$ ); 4.54 (dd,  $J=1.5$  Hz,  $J=10.5$  Hz, 1H,  $\text{CH}_2$ ); 5.17 (bs, 2H,  $\text{NH}_2$ ); 5.31 (t,  $J=3.5$ , 1H, CH); 6.37 (dd,  $J=1.5$  Hz,  $J=5.0$  Hz, 1H, CH) and 8.12 (s, ArH).  $\alpha$ -Isomer (**4b**):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$ : 1.20 and 1.21 (2s, 6H,  $2 \times \text{CH}_3$ ); 2.63 (m, 1H,  $J=7.0$  Hz CH); 4.18 and 4.20 (2d,  $J=3.5$  Hz,  $J=4$  Hz, 1H,  $\text{CH}_2$ ); 4.26 and 4.28 (2d,  $J=3.5$  Hz,  $J=4$  Hz, 1H,  $\text{CH}_2$ ); 4.48 (m, 2H,  $\text{CH}_2$ ); 5.17 (bs, 2H,  $\text{NH}_2$ ); 5.71 (t,  $J=4$ , 1H, CH); 6.36 (dd,  $J=5$  Hz,  $J=3$  Hz, 1H, CH) and 7.89 (s, ArH).

### 2.2.3. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-6-chloro-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)purine (**5**)

A solution of **4a** (1.0 g, 2.92 mmol) in MeOH (25 mL) was saturated with ammonia at  $0^\circ\text{C}$ . Then the solution was allowed to stir for overnight at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified over flash silica gel chromatography (19:1, EtOAc:MeOH) to give 0.77 g (97%) of the title compound as a white solid which was recrystallized from MeOH/ $\text{CH}_2\text{Cl}_2$ : mp  $192^\circ\text{C}$  (lit<sup>1</sup> mp  $193^\circ\text{C}$ ).  $^1\text{H}$  NMR (MeOH- $d_4$ ):  $\delta$  3.83 (m); 4.30 (dd,  $J=5.5$ ,  $J=9.5$ ); 4.58 (d,  $J=9.5$ ); 5.16 (t,  $J=2.5$ ); 6.44 (d,  $J=5.5$ ); 8.08 (s, H-8); HRMS calcd  $\text{C}_9\text{H}_{10}\text{N}_5\text{O}_3\text{Cl}$  ( $\text{MH}^+$ ) 272.0550; found: 272.0536.

### 2.2.4. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-6-azido-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)purine (**6**)

A solution of **5** (0.15 g, 0.55 mmol) and  $\text{NaN}_3$  (0.10 g, 1.66 mmol) in DMF (7 mL) was heated at  $65^\circ\text{C}$  overnight. After cooling, the solvent was removed under reduced pressure and the crude compound was purified over flash chromatography silica gel (19:1,  $\text{CH}_2\text{Cl}_2$ :MeOH) to provide 0.14 g (91%) of the title compound as a white solid which was recrystallized from MeOH/ $\text{H}_2\text{O}$ : mp  $196^\circ\text{C}$  (decomposed);  $[\alpha]_D^{25} = -68.67$  (c 0.21, MeOH: $\text{H}_2\text{O}$ , 1:1); UV (MeOH)  $\lambda_{\text{max}}$  271 nm ( $\epsilon$  8784),

300 nm ( $\epsilon$  10,584); pH 7, 301 nm ( $\epsilon$  10,698); pH 11, 301 nm ( $\epsilon$  9727); pH 2, 303 nm ( $\epsilon$  1657);  $^1\text{H}$  NMR (MeOH- $d_4$ ):  $\delta$  3.84 (m); 4.35 (dd,  $J=5.5$ ,  $J=10.5$ ); 4.61 (dd,  $J=1.5$ ,  $J=10.5$ ); 5.19 (t,  $J=2.5$ ); 6.56 (dd,  $J=1.0$ ,  $J=5.5$ ); 8.02 (s, H-8). Anal. calcd for  $\text{C}_9\text{H}_{10}\text{N}_8\text{O}_3$ ; C 38.85; H 3.62; N 40.27; found: C 38.92; H 3.68; N 40.03.

### 2.2.5. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-methylaminopurine (**7**)

A solution of **5** (0.10 g, 0.37 mmol) and methylamine (40% solution in  $\text{H}_2\text{O}$ , 5 mL) in MeOH (5 mL) was heated at  $85^\circ\text{C}$  in a steel bomb for 5 h. After cooling the solvent was evaporated under reduced pressure and the crude compound was purified over flash chromatography silica gel (19:1,  $\text{CH}_2\text{Cl}_2$ :MeOH) to give 0.095 g (96%) of the title compound as a white solid which was recrystallized from MeOH/pentane: mp  $181$ – $182^\circ\text{C}$ .  $[\alpha]_D^{27} = -42.08$  (c 0.25, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  281 nm ( $\epsilon$  18,454); pH 7, 279 nm ( $\epsilon$  12,266); pH 11, 279 nm ( $\epsilon$  11,347); pH 2, 259 nm ( $\epsilon$  14,103);  $^1\text{H}$  NMR (MeOH- $d_4$ ):  $\delta$  3.06 (br s, N- $\text{CH}_3$ ); 3.82 (m); 4.28 (dd,  $J=5.5$ ,  $J=10.0$ ); 4.49 (dd,  $J=1.5$ ,  $J=10.0$ ); 5.15 (t,  $J=2.5$ ); 6.32 (dd,  $J=1.5$ ,  $J=5.5$ ); 8.05 (s, H-8); HRMS calcd  $\text{C}_{10}\text{H}_{14}\text{N}_6\text{O}_3$  ( $\text{MH}^+$ ) 267.1205; found: 267.1180. Anal. calcd for  $\text{C}_{10}\text{H}_{14}\text{N}_6\text{O}_3$ ; C 45.11; H 5.30; N 31.56; found: C 44.84; H 5.35; N 31.46.

### 2.2.6. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-ethylaminopurine (**8**)

Compound **5** (0.507 g 1.87 mmol), ethyl amine (2 mL) in MeOH (10 mL) was heated at  $80^\circ\text{C}$  in a steel bomb for 16 h in an oil bath. The reaction mixture was cooled concentrated purified over silica gel column using ethyl acetate:MeOH, 98:2 to yield 0.44 g (84%) of the title compound. mp  $159$ – $160^\circ\text{C}$ ;  $[\alpha]_D^{26} = -31.75$  (c 0.53, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  282 nm ( $\epsilon$  11,440); pH 7, 281 nm ( $\epsilon$  11,767); pH 11, 280 nm ( $\epsilon$  12,185); pH 2, 291 nm ( $\epsilon$  10,018);  $^1\text{H}$  NMR (MeOH- $d_4$ ):  $\delta$  1.29 (t,  $J=7.0$ ,  $\text{CH}_3$ ); 3.58 (br s, N- $\text{CH}_2$ ); 3.81 (m); 4.29 (dd,  $J=5.5$ ,  $J=10.5$ ); 4.48 (d,  $J=10.0$ ); 5.15 (t,  $J=2.5$ ); 6.32 (d,  $J=5.0$ ); 8.06 (s, H-8); HRMS calcd  $\text{C}_{11}\text{H}_{16}\text{N}_6\text{O}_3$  ( $\text{MH}^+$ ) 281.1362; found: 281.1360. Anal. calcd for  $\text{C}_{11}\text{H}_{16}\text{N}_6\text{O}_3$ ; C 47.14; H 5.75; N 29.98; found: C 46.84; H 5.62; N 29.78.

### 2.2.7. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-6-dimethylamino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)purine (**9**)

A solution of **5** (0.10 g, 0.37 mmol) and dimethylamine (40% solution in  $\text{H}_2\text{O}$ , 4 mL) in MeOH (6 mL) was heated at  $85^\circ\text{C}$  in a steel bomb for 5 h. After cooling the solvent was evaporated under reduced pressure and the crude compound was purified over flash chromatography silica gel (19:1,  $\text{CH}_2\text{Cl}_2$ :MeOH) to give 0.095 g (92%) of the title compound as a white solid which was recrystallized from MeOH/pentane: mp  $119$ – $121^\circ\text{C}$ ;  $[\alpha]_D^{27} = -44.16$  (c 0.14, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  284 nm ( $\epsilon$  12,068); pH 7, 284 nm ( $\epsilon$  11,207); pH 11, 284 nm ( $\epsilon$  12,084); pH 2, 295 ( $\epsilon$  12,661);  $^1\text{H}$  NMR (MeOH- $d_4$ ):  $\delta$  3.42 (br s, N- $(\text{CH}_3)_2$ ); 8.02 (s, H-8) 3.81 (m); 4.28 (dd,  $J=5.5$ ,  $J=9.5$ ); 4.81 (dd,  $J=1.5$ ,  $J=9.5$ ); 5.15 (t,  $J=2.5$ ); 6.33 (dd,  $J=1.5$ ,  $J=5.5$ ); HRMS calcd  $\text{C}_{11}\text{H}_{16}\text{N}_6\text{O}_3$  ( $\text{MH}^+$ ) 281.1362; found 281.1360. Anal. calcd

for  $C_{11}H_{16}N_6O_3 \cdot 0.5H_2O$ ; C 45.67; H 5.92; N 29.05; found: C 46.00; H 5.88; N 28.81.

2.2.8. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-isopropylaminopurine (**10**)

Compound **10** was prepared from compound **5** following the procedure given for the compound **8**. Yield: 81%. mp 103–104 °C;  $[\alpha]_D^{27} = -31.48$  (c 0.44, MeOH); UV (MeOH)  $\lambda_{max}$  282 nm ( $\epsilon$  16,389); pH 7, 281 nm ( $\epsilon$  15,967); pH 11, 282 nm ( $\epsilon$  16,180); pH 2, 293 nm ( $\epsilon$  13,239);  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  1.56, 1.40 (2s,  $(CH_3)_2$ ); 2.21 (m), 2.09 (m), 1.62 (m); 3.57 (m); 4.15 (dd,  $J=5.2$ ,  $J=14.8$ ); 4.38 (br s, N–CH); 4.40 (dd,  $J=1.6$ ,  $J=14.8$ ); 5.00 (t,  $J=3.2$ ); 5.14 (t,  $J=7.0$ , OH); 5.84 (br s,  $NH_2$ ); 6.23 (d,  $J=4.0$ ); 6.97 (br s, NH); 7.83 (s, H-8); HRMS calcd  $C_{12}H_{18}N_6O_3$  ( $MH^+$ ) 295.1518; found: 295.1517. Anal. calcd for  $C_{12}H_{18}N_6O_3 \cdot 0.4H_2O$ : C 47.80; H 6.28; N 27.87; found: C 47.56; H 6.24; N 27.63.

2.2.9. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-methoxypurine (**11**)

To a stirring solution of **5** (0.10 g, 0.37 mmol) in MeOH (7 mL), NaOMe (0.5 M soln. in MeOH, 2 mL) was added and allowed to stir at room temperature overnight. After cooling the solvent was evaporated under reduced pressure and the crude compound was purified over flash chromatography silica gel (19:1, EtOAc:MeOH) to give 0.09 g (91%) of the title compound as a white solid which was recrystallized from MeOH/ $CH_2Cl_2$ /pentane: mp 196 °C;  $[\alpha]_D^{27} = -44.88$  (c 0.5, MeOH); UV (MeOH)  $\lambda_{max}$  248 nm ( $\epsilon$  9276), 281 nm ( $\epsilon$  8605); pH 7, 279 nm ( $\epsilon$  8444); pH 11, 279 nm ( $\epsilon$  8645); pH 2, 288 nm ( $\epsilon$  5865);  $^1H$  NMR (MeOH- $d_4$ ):  $\delta$  3.81 (m); 4.07 (s, O– $CH_3$ ); 8.19 (s, H-8); 4.29 (dd,  $J=5.0$ ,  $J=10.0$ ); 4.51 (dd,  $J=1.5$ ,  $J=10.0$ ); 5.15 (t,  $J=2.5$ ); 6.39 (dd,  $J=1.5$ ,  $J=5.0$ ); HRMS calcd  $C_{10}H_{13}N_5O_4$  ( $MH^+$ ) 268.1046; found: 268.1091. Anal. calcd for  $C_{10}H_{13}N_5O_4 \cdot 0.25H_2O$ : C 44.20; H 5.00; N 25.77; found: C 44.46; H 5.01; N 25.66.

2.2.10. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-6-cyclopropylamino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)purine (**12**)

A solution of **5** (0.10 g, 0.37 mmol) and cyclopropylamine (3 mL) in MeOH (7 mL) was heated at 65 °C in a steel bomb for 5 h. After cooling the solvent was evaporated under reduced pressure and the crude compound was purified over flash chromatography silica gel (19:1,  $CH_2Cl_2$ :MeOH) to give 0.10 g (92%) of the title compound as a white solid which was recrystallized from MeOH/pentane: mp 86 °C (decomposed);  $[\alpha]_D^{23} = -34.50$  (c 0.27, MeOH); UV (MeOH)  $\lambda_{max}$  284 nm ( $\epsilon$  11,700); pH 7, 283 nm ( $\epsilon$  11,972); pH 11, 283 nm ( $\epsilon$  12,664); pH 2, 296 nm ( $\epsilon$  13,885);  $^1H$  NMR (MeOH- $d_4$ ):  $\delta$  0.63 (m,  $CH_2$ ), 0.86 (m,  $CH_2$ ), 2.94 (br s, N–CH); 3.81 (m); 4.28 (dd,  $J=5.5$ ,  $J=10.0$ ); 4.48 (d,  $J=10.0$ ); 5.15 (t,  $J=2.5$ ); 6.33 (d,  $J=5.5$ ); 8.07 (s, H-8); HRMS calcd  $C_{12}H_{16}N_6O_3$  ( $MH^+$ ) 293.1292; found: 293.1218. Anal. calcd for  $C_{12}H_{16}N_6O_3 \cdot 0.5H_2O$ : C 47.84; H 5.68; N 27.89; found: C 48.21; H 5.53; N 27.61.

2.2.11. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-cyclobutylaminopurine (**13**)

Compound **13** was prepared from compound **5** following the procedure given for the compound **8**. Yield: 84%. mp 170–171 °C;  $[\alpha]_D^{27} = -30.85$  (c 0.71, MeOH); UV (MeOH)  $\lambda_{max}$  284 nm ( $\epsilon$  9987); pH 7, 283 nm ( $\epsilon$  10,562); pH 11, 283 nm ( $\epsilon$  10,118); pH 2, 294 nm ( $\epsilon$  8656);  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.60 (m); 4.20 (dd,  $J=5.5$ ,  $J=15.0$ ); 4.43 (d,  $J=8.0$ ); 4.68 (br s, N–CH); 5.04 (t,  $J=3.0$ ); 5.18 (t,  $J=6.0$ , OH); 5.91 (br s,  $NH_2$ ); 6.22 (d,  $J=4.5$ ); 7.56 (br s, NH); 7.88 (s, H-8); HRMS calcd  $C_{13}H_{18}N_6O_3$  ( $MH^+$ ) 307.1518; found: 307.1518. Anal. calcd for  $C_{13}H_{18}N_6O_3$ : C 50.97; H 5.92; N 27.44; found: C 50.78; H 5.82; N 27.26.

2.2.12. (–)- $\beta$ -D-(2*R*,4*R*)-2-Amino-9-(2-hydroxymethyl-1,3-dioxalan-4-yl)-6-benzylaminopurine (**14**)

Compound **10** was prepared from compound **5** following the procedure given for the compound **8**. Yield: 90%. mp 160–161 °C;  $[\alpha]_D^{26} = -42.18$  (c 0.112, MeOH); UV (MeOH)  $\lambda_{max}$  283 nm ( $\epsilon$  11,819); pH 7, 281 nm ( $\epsilon$  11,711); pH 11, 282 nm ( $\epsilon$  11,502); pH 2, 286 nm ( $\epsilon$  9790);  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.60 (dd,  $J=3.0$ ,  $J=8.5$ ); 4.20 (dd,  $J=5.5$ ,  $J=10.0$ ); 4.45 (d,  $J=10.0$ ); 4.65 (br s, N–CH); 4.65 (br s, NH– $CH_2$ ); 5.04 (t,  $J=3.0$ ); 5.17 (t,  $J=7.0$ , OH); 5.94 (br s,  $NH_2$ ); 6.23 (d,  $J=5.0$ ); 7.20–7.35 (m, 5ArH); 7.89 (s, H-8); HRMS calcd  $C_{16}H_{18}N_6O_3$  ( $MH^+$ ) 343.1518; found: 343.1508. Anal. calcd for  $C_{16}H_{18}N_6O_3$ : C 56.13; H 5.30; N 24.55; found: C 56.12; H 5.34; N 24.17.

2.2.13. (–)- $\beta$ -D-(2*R*,4*R*)-9-(2-Hydroxymethyl-1,3-dioxalan-4-yl)guanosine (**15**)

A solution of **4a** (1.0 g, 2.92 mmol), mercaptoethanol (2.75 g, 35.25 mmol) and NaOMe (25 wt.% soln., 9.5 mL) in MeOH (30 mL) was heated at reflux for 3.5 h. The solution was cooled to room temperature and neutralized to pH 7.0 with acetic acid. The solution was evaporated to dryness and the solid obtained was washed with  $CHCl_3$  followed by MeOH to give 0.6 g (81%) of the title compound as a white solid: mp 280 °C (decomposed) (lit<sup>1</sup> mp 280 °C); UV ( $H_2O$ )  $\lambda_{max}$  250 nm;  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.56 (dd,  $J=3.2$ ,  $J=5.6$ ); 4.15 (dd,  $J=5.5$ ,  $J=9.5$ ); 4.40 (d,  $J=9.5$ ); 4.99 (t,  $J=3.2$ ); 5.11 (t,  $J=5.6$ ); 6.12 (d,  $J=5.5$ ); 6.54 (br s,  $NH_2$ ), 7.81 (s, H-8), 10.71 (br s, NH).

2.2.14. (–)- $\beta$ -D-(2*R*,4*R*)-9-(2-Hydroxymethyl-1,3-dioxalan-4-yl)- $N_1$ -methylguanosine (**16**)

The title compound was synthesized according to a similar literature procedure (Badet et al., 1984). A solution of **15** (0.10 g, 0.39 mmol) and KOH (33 mg, 0.59 mmol) dissolved in  $H_2O$  (0.2 mL) and DMF (2 mL) in DMF (10 mL) was stirred at room temperature for 30 min. Diphenylmethylsulfoniumtetrafluoroborate (0.11 g, 0.39 mmol) in DMF (2 mL) was slowly added to the reaction mixture and allowed to stir for an additional 2 h. The solution was evaporated to dryness and the solid obtained was purified over flash chromatography silica gel (19:1,  $CH_2Cl_2$ :MeOH) to give 0.08 g (77%) of the title compound which was recrystallized from MeOH/pentane: mp 210–212 °C;  $[\alpha]_D^{27} = -49.18$  (c 0.21, MeOH); UV (MeOH)  $\lambda_{max}$  255 nm ( $\epsilon$

3535); pH 7, 255 nm ( $\epsilon$  12,841); pH 11, 254 nm ( $\epsilon$  15,212); pH 2, 258 nm ( $\epsilon$  12,091);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  3.57 (s, N-CH<sub>3</sub>), 5.14 (t,  $J$  = 5.6); 3.60 (dd,  $J$  = 3.2,  $J$  = 5.6); 4.20 (dd,  $J$  = 5.5,  $J$  = 10.0); 4.46 (dd,  $J$  = 1.5,  $J$  = 10.0); 5.04 (t,  $J$  = 3.2); 6.17 (dd,  $J$  = 1.5,  $J$  = 5.5); 7.10 (br s, NH<sub>2</sub>), 7.87 (s, H-8); HRMS calcd C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub> (MH<sup>+</sup>) 268.1046; found: 268.0938. Anal. calcd for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>·0.5H<sub>2</sub>O; C 43.48; H 5.11; N 25.35; found: C 43.39; H 5.14; N 25.39.

#### 2.2.15. (–)- $\beta$ -D-(2*R*,4*R*)-*N*<sub>1</sub>-Benzyl-9-(2-hydroxymethyl-1,3-dioxolan-4-yl)guanosine (**17**)

A solution of **15** (0.11 g, 0.44 mmol) and NaH (13 mg, 0.53 mmol) in DMF (5 mL) was stirred at 0 °C for 10 min followed by 20 min stirring at room temperature. Then benzyl bromide (0.075 g, 0.44 mmol) was slowly added to the reaction mixture at 0 °C and then continued stirring at room temperature for 2 h. The solution was evaporated to dryness and the solid obtained was purified over flash chromatography silica gel (19:1, CH<sub>2</sub>Cl<sub>2</sub>:MeOH) to give 0.10 g (66%) of the title compound as a white solid which was recrystallized from MeOH/pentane: mp 168–170 °C;  $[\alpha]_D^{26}$  = –38.18 (c 0.21, MeOH); UV (MeOH)  $\lambda_{\text{max}}$  257 nm ( $\epsilon$  3733); pH 7, 255 nm ( $\epsilon$  12,114); pH 11, 254 nm ( $\epsilon$  13,048); pH 2, 261 nm ( $\epsilon$  39,467);  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  3.62 (dd,  $J$  = 3.5,  $J$  = 6.0); 4.20 (dd,  $J$  = 5.5,  $J$  = 10.0); 4.59 (dd,  $J$  = 1.5,  $J$  = 10.0); 5.05 (t,  $J$  = 3.5); 5.14 (t,  $J$  = 6.0); 5.26 (br s, CH<sub>2</sub>-benzylic); 6.18 (d, dd,  $J$  = 1.5,  $J$  = 5.5); 7.09 (br s, NH<sub>2</sub>), 7.20 (d,  $J$  = 7.0, 2Ar-H); 7.26 (dd,  $J$  = 7.0,  $J$  = 7.5, Ar-H); 7.33 (dd,  $J$  = 7.0,  $J$  = 7.5, 2Ar-H); 7.91 (s, H-8); HRMS calcd C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub> (MH<sup>+</sup>) 342.1203; found: 342.1256. Anal. calcd for C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub>·0.5H<sub>2</sub>O; C 54.54; H 5.14; N 19.88; found: C 54.61; H 5.11; N 19.82.

#### 2.2.16. 2-tert-Butoxycarbonylamino-3-methyl-butyric acid 4-(2,6-diamino-purin-9-yl)-[1,3]dioxolan-2-ylmethylester (**18**)

Boc-L-valine (8.61 g, 0.039 mol) and dicyclohexylcarbodiimide (4.1 g, 0.039 mol) were taken in DMF (250 mL) and stirred at room temperature under nitrogen for 1 h. Reaction mixture was cooled to 0–5 °C. Pre-cooled solution (0–5 °C) of DAPD (5 g, 0.0198 mol) and 4-dimethylaminopyridine (2.41 g, 0.0396 mol) dissolved in DMF (750 mL) was slowly added into the reaction mixture. Reaction mixture was stirred for 3 h at 0–5 °C and further stirred at room temperature for overnight. Reaction mixture was concentrated under reduced pressure and crude product was stirred with 500 mL of water at 0–5 °C for 2 h. Solid residue was filtered and washed with water (200 mL) and dichloromethane (200 mL). Aqueous portion was further extracted with 3 × 100 mL of dichloromethane. Organic layers were combined, washed with sat. NaHCO<sub>3</sub>, brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated to provide the crude product and further purified by column chromatography using ethyl acetate and methanol to yield 4 g (45%) of pure white solid.  $^1\text{H}$  NMR (DMSO)  $\delta$ : 0.86–89 (m, 6H, valyl-2CH<sub>3</sub>), 1.40 (s, 9H, 3CH<sub>3</sub>), 1.97–2.01 (m, 1H, valyl-3'' CH), 3.87 (m, 1H, valyl-2'' CH), 4.25–4.31 (m, 3H, 2' CH and 5' CH<sub>2</sub>), 4.56–4.59 (m, 1H, 2' CH), 5.26–5.27 (t,  $J$  = 4.0 Hz, 1H, 4' CH), 5.88 (br s, 2H, NH<sub>2</sub>), 6.25–6.26 (m, 1H, 1' CH), 6.78

(br s, 2H, NH<sub>2</sub>), 7.18–7.20 (d,  $J$  = 8 Hz, 1H, NH), 7.83 (s, 1H, CH-purine).

#### 2.2.17. 2-Amino-3-methyl-butyric acid

##### 4-(2,6-diamino-purin-9-yl)-[1,3]dioxolan-2-ylmethylester. TFA salt (valyl DAPD-TFA salt) (**19**)

Boc-Val-DAPD (**18**) (8.5 g, 0.019 mol) was dissolved in 200 mL dichloromethane and cooled to 0–5 °C. Trifluoroacetic acid (15 mL) was slowly added in portions (3 × 5 mL) with stirring at 0–5 °C for 3 h. Reaction mixture was further stirred at room temperature of 3 h and monitored by TLC, and after completion, reaction mixture was concentrated under reduced pressure. Ether (100 mL) was added into the concentrated residue to get precipitate. White solid obtained was filtered and washed with ether (25 mL) and crystallized with methanol:DCM (1:4) to provide 7.0 g (53.0%) of the title compound. mp 178–180 °C;  $[\alpha]_D^{26}$  = –22.96 (c 0.54, MeOH);  $^1\text{H}$  NMR (DMSO)  $\delta$ : 0.95–1.00 (dd,  $J$  = 7.0 Hz, 6H, valyl-2CH<sub>3</sub>), 2.12–2.18 (m, 1H, valyl-3'' CH), 3.82 (bs, 1H, valyl-2'' CH), 4.31–4.34 (q,  $J$  = 6.0 Hz,  $J$  = 4.5 Hz, 1H, 2' CH), 4.46–4.47 (d,  $J$  = 3.5 Hz, 2H, 5' CH<sub>2</sub>), 4.69–4.72 (dd,  $J$  = 1.5 Hz,  $J$  = 9.0 Hz, 1H, 2' CH), 5.35–5.37 (t,  $J$  = 3.5 Hz, 1H, 4' CH), 6.27–6.28 (m, 1H, 1' CH), 7.25 (br s, 2H, NH<sub>2</sub>), 8.08 (s, 1H, CH-purine), 8.40 (br s, 4H, 2NH<sub>2</sub>).

#### 2.2.18. 2-Amino-3-methyl-butyric acid 4-(2,6-diamino-purin-9-yl)-[1,3]dioxolan-2-ylmethylester (**20**)

Trifluoroacetic acid salt 3.5 g (5.04 mmol) (**19**) was dissolved in isopropanol (1.2 L). PL-HCO<sub>3</sub> resin (9 g, washed with 500 mL of isopropanol) was added into the clear solution of trifluoroacetic acid salt with stirring and further stirred for 1 h at room temperature. Free base (**20**) formation was achieved using PL-HCO<sub>3</sub> resin and it was monitored by TLC. After completion of reaction, isopropanol was removed under reduced pressure at 30 °C. Residue was taken in DCM (200 mL) and concentrated under reduced pressure to remove the traces of isopropanol. DCM (200 mL) was added into the residue and sonicated the reaction mass for 5–10 min. After sonication, 100 mL of pentane was added into the reaction mass and stirred for 15 min at room temperature. Separated white solid was filtered and washed with 100 mL of pentane to yield 1.5 g (85%) of pure title compound. mp 77–80 °C;  $[\alpha]_D^{26}$  = –38.00 (c 0.48, CHCl<sub>3</sub>);  $^1\text{H}$  NMR (DMSO)  $\delta$ : 0.82–0.88 (dd,  $J$  = 6.5 Hz,  $J$  = 19 Hz, 6H, valyl-2CH<sub>3</sub>), 1.64 (bs, 2H, valyl-NH<sub>2</sub>), 1.79–1.83 (m, 1H, valyl-3'' CH), 3.10–3.11 (d,  $J$  = 5.0 Hz, 1H, valyl-2'' CH), 4.24–4.33 (m, 3H, 2' CH, 5' CH<sub>2</sub>), 4.58–4.60 (dd,  $J$  = 2.5 Hz,  $J$  = 7.5 Hz, 1H, 2' CH), 5.27–5.29 (t,  $J$  = 3.0 Hz, 1H, 4' CH), 5.89 (bs, 2H, NH<sub>2</sub>), 6.23–6.25 (dd,  $J$  = 2.0 Hz,  $J$  = 3.5 Hz, 1H, 1' CH), 6.78 (bs, 2H, NH<sub>2</sub>), 7.82 (s, 1H, CH-purine);  $^{13}\text{C}$  NMR (DMSO)  $\delta$ : 17.84, 19.63, 32.32, 59.85, 62.93, 70.46, 79.16, 102.51, 113.21, 134.98, 152.03, 156.62, 160.94, 175.49. Anal. (C<sub>14</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub>) C, 47.86; H, 6.02; N, 27.90; found: C, 47.91; H, 6.08; N, 27.95.

#### 2.3. General procedure for the synthesis of DAPD organic salts

DAPD (0.2 g, 0.79 mmol) and organic acid (0.86 mmol) was stirred in 6 mL of water for 24 h. Reaction mixture was filtered



and washed with 1 mL of water and then 20 mL of acetone. White solid obtained was dried under vacuum to provide title compound.

**2.3.1. [4-(2,6-Diamino-purin-9-yl)-[1,3]dioxolan-2-yl]-methanol maleate (DAPD-maleate) (21)**

mp 161–163 °C; <sup>1</sup>H NMR (DMSO) δ: 3.63–3.64 (d, *J* = 3.0 Hz, 2H, 5' CH<sub>2</sub>), 4.20–4.24 (q, *J* = 5.5 Hz, 1H, 2' CH), 4.49–4.51 (m, 1H, 2' CH), 5.06–5.07 (t, 1H, *J* = 3.5 Hz, 4' CH), 6.17 (s, 2H, maleate-CH), 6.22–6.23 (m, 1H, 1' CH), 6.73 (br s, 2H, NH<sub>2</sub>), 7.86 (bs, 2H, NH<sub>2</sub>), 8.08 (s, 1H, CH-purine). Anal. (C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>·C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>·H<sub>2</sub>O) C, 40.42; H, 4.70; N, 21.75; found: C, 40.65; H, 4.77; N, 21.75.

**2.3.2. [4-(2,6-Diamino-purin-9-yl)-[1,3]dioxolan-2-yl]-methanol tartarate (DAPD-tartarate) (22)**

mp 212–214 °C; <sup>1</sup>H NMR (DMSO) δ: 3.61–3.62 (d, *J* = 3.5 Hz, 2H, 5' CH<sub>2</sub>), 4.19–4.22 (q, *J* = 5.5 Hz, 1H, 2' CH), 4.33 (s, 1H, tartarate-CH), 4.44–4.47 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H, 2' CH), 5.05–5.06 (t, 1H, *J* = 3.5 Hz, 4' CH), 5.22 (br s, 1H, 5' OH), 5.89 (br s, 2H, NH<sub>2</sub>), 6.21–6.23 (m, 1H, 1' CH), 6.79 (bs, 2H, NH<sub>2</sub>), 7.89 (s, 1H, CH-purine). Anal. (C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>·C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>·C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>) C, 40.37; H, 4.62; N, 25.68; found: C, 40.50; H, 4.63; N, 25.74.

**2.3.3. [4-(2,6-Diamino-purin-9-yl)-[1,3]dioxolan-2-yl]-methanol citrate (DAPD-citrate) (23)**

mp 168–170 °C; <sup>1</sup>H NMR (DMSO) δ: 2.66, 2.69 (2s, 2H, citrate-CH<sub>2</sub>), 2.76, 2.79 (2s, 2H, citrate-CH<sub>2</sub>), 3.62–3.63 (d, 2H, 5' CH<sub>2</sub>), 4.19–4.22 (m, 1H, 2' CH), 4.45–4.47 (m, 1H, 2' CH), 5.05–5.06 (t, 1H, *J* = 3.0 Hz, 4' CH), 5.24 (bs, 1H, 5' OH), 5.96 (br s, 2H, NH<sub>2</sub>), 6.21–6.23 (m, 1H, 1' CH), 6.88 (br s, 2H, NH<sub>2</sub>), 7.91 (s, 1H, CH-purine). Anal. (C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>·C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>) C, 41.38; H, 4.63; N, 24.13; found: C, 41.32; H, 4.72; N, 24.19.

**2.3.4. [4-(2,6-Diamino-purin-9-yl)-[1,3]dioxolan-2-yl]-methanol. Fumarate (DAPD-fumarate) (24)**

mp 212–214 °C; <sup>1</sup>H NMR (DMSO) δ: 3.58–3.59 (d, *J* = 3.0 Hz, 2H, 5' CH<sub>2</sub>), 4.16–4.19 (q, *J* = 5.5 Hz, 1H, 2' CH), 4.42–4.44 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H, 2' CH), 5.02–5.03 (t, 1H, *J* = 3.0 Hz, 4' CH), 5.86 (bs, 2H, NH<sub>2</sub>), 6.19–6.20 (m, 1H, 1' CH), 6.63 (s, 1H, fumarate-CH), 6.76 (bs, 2H, NH<sub>2</sub>), 7.86 (s, 1H, CH-purine). Anal. (C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>·C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>·C<sub>9</sub>H<sub>12</sub>N<sub>6</sub>O<sub>3</sub>) C, 42.58; H, 4.55; N, 27.09; found: C, 42.45; H, 4.65; N, 26.17.

### 3. Virology

#### 3.1. Antiviral assays

The procedures for the antiviral assays in human peripheral blood mononuclear (PBM) cells have been published previously (Schinazi et al., 1990, 1998). In summary, PBM cells were isolated by Ficoll–Hypaque discontinuous gradient centrifugation of whole blood samples (obtained from Atlanta Red Cross) from

healthy seronegative donors. Cells were stimulated with phytohemagglutinin A (3 μg/mL; Sigma–Aldrich, St. Louis, MO) for 2–3 days prior to use. HIV-1<sub>LAI</sub> obtained from the Centers for Disease Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. Infections were done in bulk for 1 h, either with 100 TCID<sub>50</sub>/1 × 10<sup>7</sup> cells for a flask (T25) assay or with 200 TCID<sub>50</sub>/2 × 10<sup>6</sup> cells/well for 24 well plate assay. Previous studies indicated that this was the optimum virus concentration in order to obtain good replication on day 5 when the virus is harvested from the supernatant. Cells were added to a flask or plate containing a 10-fold serial dilution of the test compound. Assay medium was RPMI-1640 supplemented with heat inactivated 16% fetal bovine serum, 1.6 mM L-glutamine, 80 IU/mL penicillin, 80 μg/mL streptomycin, 0.0008% DEAE-Dextran, 0.045% sodium bicarbonate, and 26 IU/mL recombinant interleukin-2 (Chiron Corp., Emeryville, CA). AZT was used as the positive control for the assay. Uninfected PBM cells were grown in parallel at equivalent cell concentrations as a control. The cell cultures were maintained in a humidified 5% CO<sub>2</sub>–air at 37 °C for 5 day, and supernatants were collected for reverse transcriptase activity. One milliliter of each supernatant was centrifuged at 9740 × *g* for 2 h to pellet the virus. The pellet was solubilized with vortexing in 100 μL of virus solubilization buffer (0.5% Triton X-100, 0.8 M NaCl, 0.5 mM phenylmethylsulfonyl chloride, 20% glycerol, and 0.05 M Tris, pH 7.8). Each sample (10 μL) was added to 75 μL of RT reaction mixture (0.06 M Tris, pH 7.8, 0.012 M MgCl<sub>2</sub>, 0.006 M dithiothreitol, 0.006 mg/mL poly(rA)<sub>n</sub> oligo (dT)<sub>12–18</sub>, 96 μg/mL dATP, and 1 μM of 0.08 mCi/mL <sup>3</sup>H-thymidine-5'-triphosphate) (Perkin-Elmer, Boston, MA) and incubated at 37 °C for 2 h. The reaction was stopped by the addition of 100 μL of 10% trichloroacetic acid containing 0.05% sodium perophosphate. The acid-insoluble product was harvested onto filter paper using a Packard Harvester (Meriden, CT), and RT activity was read on a Packard Direct Beta Counter (Meriden, CT). RT results were expressed in counts per minute (CPM) per milliliter. The antiviral 50% effective concentration (EC<sub>50</sub>) and 90% effective concentration (EC<sub>90</sub>) was determined from the concentration–response curve using the median effect method (Balzarini, 1994).

#### 3.2. Cytotoxicity assays

The compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cell, CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD) and Vero (African green monkey kidney) cells. Log phase Vero, CEM and PHA-stimulated human PBM cells were seeded at a density of 5 × 10<sup>3</sup>, 2.5 × 10<sup>3</sup>, and 5 × 10<sup>4</sup> cells/well, respectively. All of the cells were plated in 96-well cell culture plates containing 10-fold serial dilutions of the test drug. The cultures were incubated for 2, 3, and 4 days for Vero, CEM, and PBM cells, respectively, in a humidified 5% CO<sub>2</sub>–air at 37 °C. At the end of incubation, MTT tetrazolium dye solution (cell titer 96, Promega, Madison, WI) was added to each well and incubated overnight. The reac-

tion was stopped with stop solubilization solution (Promega, Madison, WI). The plates were incubated for 5 h to ensure that the formazan crystals were dissolved. The plates were read at 570 nm using an ELISA plate reader (Bio-Tek Instruments, Inc., Winooski, VT, Model EL 312e). The 50% inhibition concentration ( $IC_{50}$ ) was determined from the concentration–response curve using the median effect method (Stuyver et al., 2002; Balzarini, 1994).

### 3.3. Adenosine deaminase study

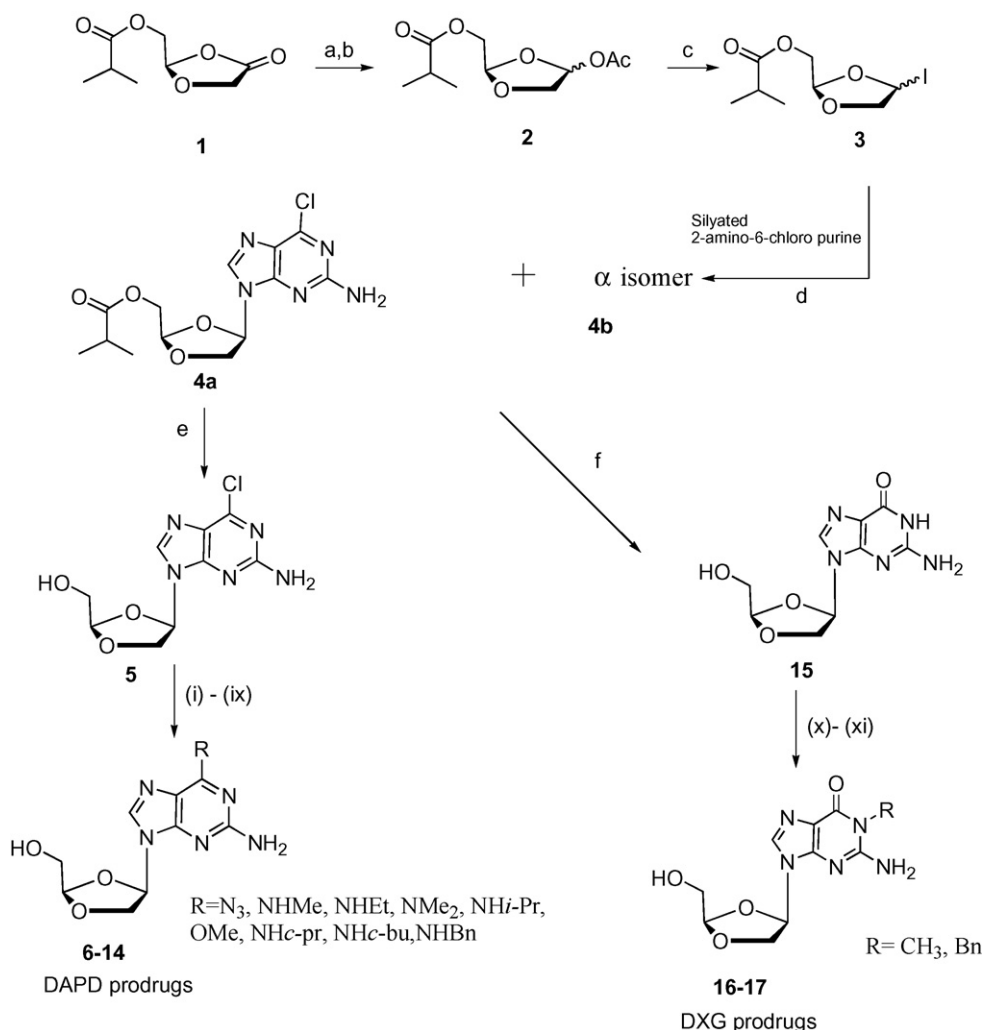
All the enzyme reactions were performed at 37 °C in phosphate buffer solution (pH 7.4). Initially, adenosine deaminase (EC. 3.5.4.4) from calf intestinal mucosa from Sigma–Aldrich (20  $\mu$ g/mL) was used for the qualitative assay with the substrate concentration of 1.5 mM, to determine whether the DXG is the sole product of hydrolytic deamination reaction of DAPD prodrugs (6–14). Adenosine deaminase from bovine spleen from

Sigma–Aldrich (5.0 units/mL) was then used in the quantitative assays, which was monitored with a UV spectrometer at 285 nm with the substrate concentration of 100  $\mu$ M. The change in absorbance ( $\Delta A_t = A_t - A_0$ ) at certain time ( $t$ ) of each substrate was recorded and compared with the total change of absorbance ( $A_{DXG} - A_{\text{substrate}}$ ) to calculate the percentage of substrate conversion in the enzyme reaction.

## 4. Results and discussion

### 4.1. Chemistry

Antiviral activity of nucleosides depends on the cellular uptake as well as the conversion to the corresponding 5'-triphosphate by cellular kinases (Balzarini, 1994). However, their clinical utility is often hampered (Kennedy, 1997) by their unfavorable physiochemical properties. To alleviate these problems, prodrug approaches have been adopted to improve



Scheme 1. Synthesis of DAPD and DXG prodrugs. Reagents and conditions: (a)  $LiAl(OBu^t)_3H$ , THF,  $-78^\circ C$ , (b) DMAP,  $(CH_3CO)_2O$ ,  $-20^\circ C$ , (c) TMSI,  $CHCl_3$ ,  $-20^\circ C$ , 2 h, (d)  $(NH_4)_2SO_4$ ,  $-20^\circ C$ , 2 h, rt, 8 h, gentle reflux, 2 h, (e) saturated methanolic ammonia,  $0^\circ C$ , 2 h, room temperature, overnight, (f)  $HOC_2H_4SH$ , NaOMe, MeOH, 3.5 h reflux, (i)  $NaN_3$ , DMF, (ii) methylamine 40% solution in  $H_2O$ , MeOH, (iii) ethylamine, MeOH, (iv) dimethylamine 40% solution in  $H_2O$ , MeOH, (v) isopropylamine, MeOH, (vi) NaOMe, MeOH, (vii) cyclopropylamine, MeOH, (viii) cyclobutylamine, MeOH, (ix) benzylamine, MeOH, (x) KOH, DMF, diphenylsulfoniumtetrafluoroborate and (xi) NaH,  $C_6H_5CH_2Br$ .

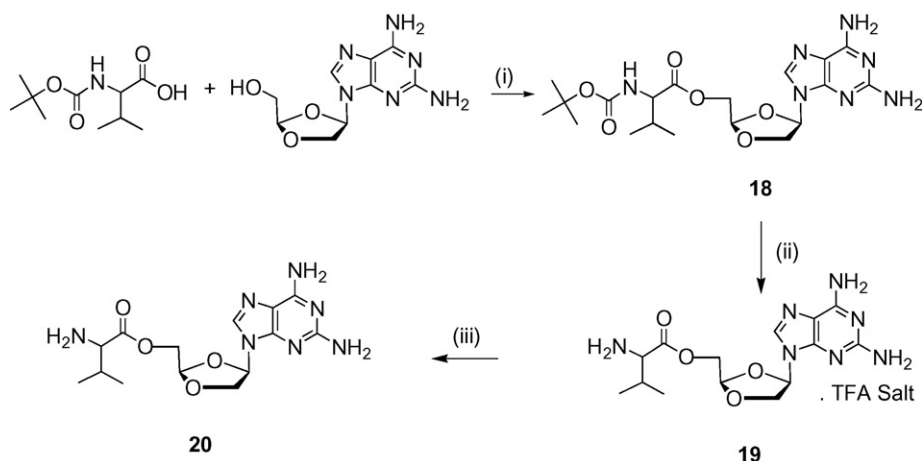
the pharmacokinetic properties of drugs (Fleisher et al., 1996; Han and Amidon, 2000). Several clinically useful nucleoside drugs such as abacavir, tenofovir disoproxil fumarate, adefovir dipivoxil, valaciclovir and famciclovir are the products from these efforts.

Abacavir is a prodrug of carbovir. A number of different C6-aminosubstituents have been explored to improve the oral bioavailability of carbovir, resulting in the discovery of the C6-cyclopropylamine prodrug, abacavir (Vince and Hua, 1990; Daluge et al., 1997). In view of the success of abacavir, we explored modifications of the C6-position of the purine ring of DAPD. Modifications at the C6-position of DAPD were achieved from the key intermediate (–)- $\beta$ -D-(2*R*,4*R*)-2-amino-6-chloro-9-(2-hydroxymethyl-1,3-dioxolan-4-yl) purine (**5**), which was synthesized from isobutyric acid-4-oxo-[1,3]-dioxolan-2-(*R*)-yl methyl ester (**1**) in four steps as depicted in Scheme 1. Literature methods were modified and improved to synthesize the compound **4a**. Compound **1** was reduced by  $\text{LiAl}(\text{O}i\text{Bu})_3\text{H}$  in THF at  $-78^\circ\text{C}$ , and acetylated in situ using acetic anhydride and 4-dimethylaminopyridine, and then purified by a silica gel column to obtain an  $\alpha$ ,  $\beta$ -mixture. The resulting  $\alpha$ ,  $\beta$ -mixture was treated with iodotrimethylsilane to provide the iodo compound **3**. 2-Amino-6-chloro-purine was persilylated using 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and an equivalent amount of ammonium sulfate to form the persilylated base. The compound **3** was then condensed with persilylated 2-amino-6-chloro purine at  $-20^\circ\text{C}$  in  $\text{CHCl}_3$  to provide an  $\alpha$ ,  $\beta$ -mixture (**4a** and **4b**) in the ratio of 1:2 (66%). The reported method (Kim et al., 1993a,b) gave a 1:1 ratio. The resulting anomeric mixture was separated by flash silica gel column chromatography using ethyl acetate–hexanes as the eluent. In this reaction, it is observed that kinetically controlled  $N_7$  isomers were converted into the thermodynamically controlled desired  $N_9$  product after stirring overnight at room temperature. After extensive investigation of this reaction, by employing several solvents, we optimized the reaction conditions and found chloroform to give the best results with an enhanced  $\beta$  isomer ratio ( $\alpha$ : $\beta$  = 1:2) in 66% yield. Another improvement we made in this reaction was the use of one equivalent of ammonium sulfate (Ye et al., 2004) instead of catalytic amounts to enhance the solubility of 2-

amino-6-chloropurine. The key intermediate **5** was then reacted with various amines in steel bomb using methanol as the solvent to yield various prodrugs of DAPD as shown in the Scheme 1. To synthesize DXG prodrugs, compound **4a** was converted into DXG (**15**) using mercaptoethanol and NaOMe in 81% yield, which was then treated with different reagents as shown in the Scheme 1.

A number of nucleosides exhibit only modest oral bioavailability (Deeks et al., 2000). The oral bioavailability can be improved by modification of the free hydroxyl group (Chulay et al., 1999; Lalezari et al., 2002). Amino acids substitution may be suitable for this modification without compromising for the water solubility of a compound. L-Valine was proved to be a good choice for the synthesis of several prodrugs (Benzaria et al., 2003; Ghosh and Miller, 1995; Song et al., 2005). Therefore, L-valine was used to synthesize 5'-valyl-DAPD to improve its anti-HIV activity (Scheme 2). Boc-L-val-DAPD **18** was synthesized by the reaction of Boc-L-val with DAPD in the presence of 4-dimethylaminopyridine and dicyclohexylcarbodiimide in DMF. Deprotection of Boc-L-valine with trifluoroacetic acid at  $0^\circ\text{C}$  in dichloromethane resulted in trifluoroacetic acid salt **19**. Free 5'-L-valyl-DAPD **20** was obtained by treating **19** with PL- $\text{HCO}_3$  resin.

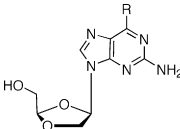
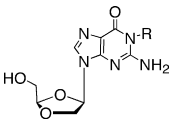
Initial attempts to isolate pure free base **20**, after deprotection of the Boc-group by acidic conditions, were failed which may be attributed to the presence of three free amino groups, leading to hydrolysis of the 5'-L-valyl ester during its chromatographic purification using 10% methanol in the mobile phase. This fact was also supported by NMR and optical rotational analysis, which showed that the compound **20** has a half-life less than 6 h in methanol and water. The trifluoroacetic acid salt **19** was prepared by using an excess of trifluoroacetic acid in dry dichloromethane and isolated as a pure white solid. A pure free-base **20** was obtained as a white powder by treating **19** with quaternary amine- $\text{HCO}_3$  resin (PL- $\text{HCO}_3$  SAX-resin) in isopropanol. The compound **20** was highly soluble in water and was stable at room temperature under closed conditions. However, it decomposed in a mixture of water and methanol with time, which was monitored and confirmed by NMR, optical rotation as well as by TLC.



Scheme 2. Synthesis of 5'-O-valyl-DAPD. Reagents and conditions: (i) DCC, DMAP, DMF, (ii) DCM, TFA, 0–5 °C and (iii) IPA, PL- $\text{HCO}_3$  resin.



Table 1  
Anti-HIV activity and cytotoxicity of DAPD, DXG prodrugs and DAPD salts

	DAPD, #	Prodrugs, R	Anti-HIV activity <sup>a</sup>		Cytotoxicity IC50 (μM)			Calculated log <i>P</i> <sup>b</sup>	Solubility (mg/mL, water)
			EC <sub>50</sub> (μM)	EC <sub>90</sub> (μM)	PBM	CEM	VERO		
	<b>6</b>	N <sub>3</sub>	13.0 ± 8.30	≥70.0	>100	>100	>100	−0.169	1.33
	<b>7</b>	NHMe	1.30 ± 0.071	8.5 ± 1.8	>100	>100	>100	−0.748	3.10
	<b>8</b>	NHEt	0.41 ± 0.25	7.5 ± 4.6	99.5	>100	>100	−0.219	1.64
	<b>9</b>	NMe <sub>2</sub>	0.87 ± 0.33	3.6 ± 0.49	>100	>100	>100	−0.694	1.16
	<b>10</b>	NH <i>i</i> -Pr	5.40 ± 5.20	≥25.0	97.3	>100	>100	0.090	1.48
	<b>11</b>	OMe	3.80±4.10	11.4 ± 12.6	>100	>100	>100	−0.680	3.08
	<b>12</b>	NH <i>c</i> -pr	0.85 ± 0.26	13.0 ± 14.1	>100	>100	>100	−0.394	2.09
	<b>13</b>	NH <i>c</i> -bu	2.20 ± 1.40	16.0 ± 15.7	>100	>100	>100	0.165	1.74
	<b>14</b>	NHBn	44.90 ± 27.9	>100	>100	>100	>100	0.700	0.68
	<b>DAPD</b>	NH <sub>2</sub>	6.90 ± 4.70	12.5 ± 26.3	>100	>100	>100	−1.579	0.40
	<b>16</b>	CH <sub>3</sub>	14.2 ± 4.70	47.3 ± 22.6	>100	>100	>100	−2.498	3.0
	<b>17</b>	Bn	>100	>100	>100	>100	>100	−0.730	1.0
	<b>DXG</b>		0.51 ± 0.44	2.4 ± 1.7	>100	>100	>100	−2.634	0.1
	<b>20</b> L-5'-Val-DAPD		1.7 ± 1.10	11.5 ± 2.0	>100	>100	>100	−0.370	70.0
	<b>21</b> DAPD maleate mono salt		2.2 ± 0.17	11.3 ± 1.3	>100	>100	>100	–	3.0
	<b>22</b> DAPD tartarate hemi salt		0.7 ± 0.03	6.2 ± 4.6	>97.3	>100	>100	–	3.4
	<b>23</b> DAPD citrate hemi salt		1.6 ± 0.14	7.2 ± 3.0	>100	>100	>100	–	7.0
	<b>24</b> DAPD fumarate hemi salt		2.6 ± 2.00	6.1 ± 2.5	>100	>100	>100	–	3.8
	<b>DAPD</b>		6.90 ± 4.70	12.5 ± 26.3		>100	>100	−1.579	0.40
	<b>AZT</b>		0.007 ± 0.0047	0.04 ± 0.02	>100	14.3	50.6	–	–

<sup>a</sup> In human PBM cells unless otherwise indicated. Based on three replicate assays.

<sup>b</sup> Calculated from ChemDraw ultra 7.1 Version.

Changing the salt form of a drug is a recognized means of modifying its solubility without modifying its structure. Different salts of the same active drug are distinct products with their own chemical and biological profiles that may cause differences in their clinical efficacy and safety (Glynis, 2001). Subsequently, different organic acid salts **21–24** were prepared using maleic, tartaric, citric and fumaric acid, and they were evaluated for its anti-HIV activity.

#### 4.2. Anti-HIV activity

Anti-HIV activity of the synthesized prodrugs of DAPD and DXG were evaluated against HIV-1<sub>LAI</sub> in peripheral blood mononuclear (PBM) cells (Schinazi et al., 1990) and cytotoxicity in PBM, CEM and Vero cells, and the results are summarized in Table 1. Compounds **6–17** exhibited varying degrees of anti-HIV activity. As shown in Table 1, most of the synthesized compounds showed good anti-HIV activity without the enhancement of cytotoxicity. Compound **8**, having an ethyl amino group at the C6 position, exhibited a 17-fold increased potency in comparison to the parent compound. Compound **12** with a cyclopropyl amino group also showed an eight-fold increase of anti-HIV activity compared to that of DAPD. Substitution of an azido or a methoxy group at C6 showed the similar potency as that of DAPD. The least potent compound in this series was the compound **14** having benzyl amino group at the C6 position. The descending order of anti-HIV activity of C6 substituted 1,3-dioxonyl-2-aminopurine in comparison to DAPD is as follows: ethylamino (17 times) > cyclopropylamino (eight times) > dimethylamino (eight times) > methylamino (five times) > cyclobutylamino (four times) > methoxy (two times) isopropylamino (same as DAPD) > azido (less than DAPD) > benzylamino (less than DAPD). From the preliminary in vitro studies, it can be concluded that, a small-size substitution with an electron donating group on the C6 position increases the anti-HIV activity (ethylamino EC<sub>50</sub> 0.41 μM), whereas the bulky-substituent at the same position reduces (benzyl EC<sub>50</sub> 44.90 μM) the biological activity, which may be related to hydrolysis by adenosine deaminase. This conclusion may be supported by the earlier work, a hydrolytic deamination of 2'-fluoro-2',3'-dideoxyarabinofuranosyl-adenine nucleosides (Barchi et al., 1991) as well as by 2-amino-6-halo and 6-halo-2',3'-dideoxy purine ribofuronosides by adenosine deaminase (Shirasaka et al., 1990). However, it was not the case in our studies (see below). All the synthesized organic acid salts of DAPD exhibited enhanced anti-HIV activity in comparison to DAPD without increasing the cytotoxicity, in which the DAPD tartarate salt exhibited a 10-fold activity in comparison to that of DAPD (Table 1). Similarly, 5'-L-valyl-DAPD (**20**) showed enhanced water solubility as well as anti-HIV activity in comparison to that of DAPD.

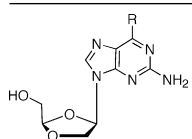
#### 4.3. Lipophilicity, solubility, hydrolysis and stability

Modification at the C6-position of the purine ring of DAPD with C6-alkyl amino groups instead of amino group expected to

change the biochemical and pharmacokinetic properties of the compounds. It can increase the biochemical stability that may leads to potential improvement for intracellular transportation of the compounds. Accordingly, prodrugs of DAPD were synthesized with a series of lipophilic groups at the C6-position, and calculated the log *P* values of these compounds (Table 1) compared to that of DAPD. Table 1, indicates that compound **8** (log *P* −0.219; EC<sub>50</sub> 0.41 μM) showed a 17-fold increase in anti-HIV activity in comparison to DAPD (log *P* 1.579; EC<sub>50</sub> 6.9 μM). Compound **12** (log *P* −0.394; EC<sub>50</sub> 0.85 μM) and compound **7** (log *P* −0.748; EC<sub>50</sub> 1.30 μM) exhibited an eight-fold enhancement in their anti-HIV activity compared to DAPD. If the calculated log *P* is positive compound **10** (log *P* 0.09; EC<sub>50</sub> 5.4 μM), compound **13** (log *P* 0.165; EC<sub>50</sub> 2.2 μM), they showed slightly reduced antiviral activity in comparison to DAPD. Solubility of the synthesized prodrugs were measured in water and observed that all the synthesized prodrugs were more soluble than that of DXG or DAPD, and the anti-HIV activity was, in general, increased with increase of solubility in water. Hydrolytic deamination of the prodrugs is very critical allowing the release of DXG into the intracellular compartment for phosphorylation. It has been documented that a numerous C6-substituted purine nucleosides were converted to their inosine or guanosine counterparts by adenosine deaminase (ADA) (Corey and Suhadolnik, 1966; Frederiksen, 1996; Chassy and Suhadolink, 1967; Baer et al., 1968; Pauwels et al., 1988; Biron et al., 1991; Marquez et al., 1990; Robins and Basom, 1973). In order to determine if there is any relationship between the enzymatic deamination with respect to the anti-HIV activity, enzymatic hydrolysis studies with adenosine deaminase for selective prodrugs (potents **8** and **9**, moderately active **10** and slightly active or inactive **14**) were carried out (Table 2). The preliminary qualitative assay indicates that, except compound **14**, most of the studied compounds (**6–13**) were partially converted to DXG over the time. In quantitative studies, the most active compounds (**8** and **9**), the moderately active compound (**10**) and the slightly active compound **14** were selected for deamination. From the studies, we observed that after 240 min, an 89% of DAPD (used as the positive control) (Furman et al., 2001) was converted into DXG, whereas the conversion was significantly lower in compounds **8–10** (0.7%, 1.0% and 1.1%, respectively) in comparison to that of DAPD. Thus, these results did not show any significant correlation between the rate of hydrolysis and the antiviral activity in cell culture studies.

In summary, we have synthesized various DAPD and DXG prodrugs. Some of the synthesized DAPD prodrugs (**8**, **9** and **12**) exhibited significantly enhanced anti-HIV activity in comparison to that of DAPD. DAPD-organic salts and L-5'-valyl DAPD also showed enhanced antiviral activity in comparison to DAPD, whereas the DXG prodrugs exhibited lower activity than the parent drug. However, there seems no correlation between the rate of hydrolysis and anti-HIV activity in vitro. Additional biological studies, including the anti-HIV activity against drug resistance mutants as well as pharmacokinetic studies, are warranted to assess the full potential of these (**8**, **9** and **12**) promising DAPD prodrugs.

Table 2  
Anti-HIV activity and enzymatic hydrolysis of DAPD prodrugs

	Anti-HIV activity <sup>a</sup>			Enzymatic hydrolysis <sup>b</sup> (%)
	#	R	EC <sub>50</sub> (μM)	
	8	NHEt	0.41 ± 0.25	0.7
	9	NMe <sub>2</sub>	0.87 ± 0.33	1.0
	10	NH <i>i</i> -Pr	5.40 ± 5.20	1.1
	14	NHBn	44.90 ± 27.9	0.0
	DAPD <sup>c</sup>	NH <sub>2</sub>	6.90 ± 4.70	89.0

<sup>a</sup> In human PBM cells unless otherwise indicated. Based on three replicate assays.

<sup>b</sup> Percentage conversion of DAPD prodrugs to DXG by adenosine deaminase (bovine spleen) after 240 min in pH 7.4 buffer solution at 37 ± 0.5 °C.

<sup>c</sup> DAPD was used as the positive control.

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