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Citation/Publisher Attribution
Chhikara, B. S., Rao, M. S., Rao, V. K., Kumar, A., Buckeit, K. W., Buckheit, Jr., R. W., & Parang, K. (2014). Carbocyclodipeptides as modified nucleosides: synthesis and anti-HIV activities. *Canadian Journal of Chemistry*, *92*(12), 1145-1149. doi: 10.1139/cjc-2014-0356
Available at: https://doi.org/10.1139/cjc-2014-0356

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Recommended Citation

Chhikara, B. S., Rao, M. S., Rao, V. K., Kumar, A., Buckheit, K. W., Buckheit Jr. R. W., Parang, K. Carbocyclodipeptides as modified nucleosides: Synthesis and anti-HIV activities. *Can. J. Chem.* (2014) 10.1139/cjc-2014-0356.
DOI:10.1139/cjc-2014-0356

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Carbocyclodipeptides as Modified Nucleosides: Synthesis and Anti-HIV Activities

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TITLE RUNNING HEAD. Synthesis and Biological Evaluation of Carbocyclodipeptides

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Abstract

A new class of nucleoside analogues were synthesized using cyclic dipeptides and modified 2'-deoxyfuranoribose sugars to introduce flexibility by peptides in place of common nucleoside bases and to determine their biological properties. The synthesis was carried out by coupling of a protected ribose sugar with synthesized dipeptides in the presence of hexamethyldisilazane and trimethylsilyltriflate. The final products were characterized by NMR and high resolution MS-TOF spectroscopy. The compounds were evaluated for anti-HIV activities. 1-(4-Azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6-diisopropylpiperazine-2,5-dione (compound **14)** containing 3- and 6-isopropyl groups in the base and 3'-azide (EC_{50} = 1.96 µM) was the most potent compound among all the synthesized analogs.

Keywords: Anti-HIV, Carbopeptides, Cyclodipeptide, Nucleoside analogues, 2'-Deoxyribofuranosyl

Abbreviations: DCM (Dichloromethane), HMDS (Hexamethyldisilazane), TMSOTf (Trimethylsilyl triflate).

Introduction

Nucleoside drugs are modified analogues of naturally occurring nucleosides, pyrimidines and purines[1-3] that have found wide applications in anti-cancer and anti-HIV therapy.[4-7] As an example, cytarabine (ara-C, Figure 1) is a pyrimidine analogue containing an arabinose sugar and has been used in the treatment of different cancers, such as acute myelogenous leukemia (AML) and non-Hodgkin's lymphoma (NHL), chronic myelocytic leukemia (blast phase), acute lymphoblastic leukemia(ALL) and erythroleukemia.[8-9] Other modified pyrimidine analogues drugs such as lamivudine (2′,3′-dideoxy-3′-thiacytidine,3TC) and emtricitabine (5-fluoro-2′,3′-dideoxy-3′-thiacytidine, FTC) (Figure 1) have also found extensive use in anti-HIV therapy.[10-11]

The modified nucleoside analogues have been designed to introduce functional groups or alterations in either sugar, base, or both parts.[12] For example, zidovudine (AZT) (Figure 1), a modified thymidine analogue commonly used in anti-HIV therapy, has an azide ($-N_3$) in place of 3′-hydroxyl (-OH) group of thymidine sugar.[4] Similarly, 3TC and FTC derivatives have sulfur at 3′-position of sugar while FTC also has a fluorine at C5 position of the base.[13] Mechanism of activity of modified nucleoside generally involves binding on growing DNA/RNA, inhibition of DNA/RNA synthesis by chain termination, and/or interacting with enzymes in the process.[14]

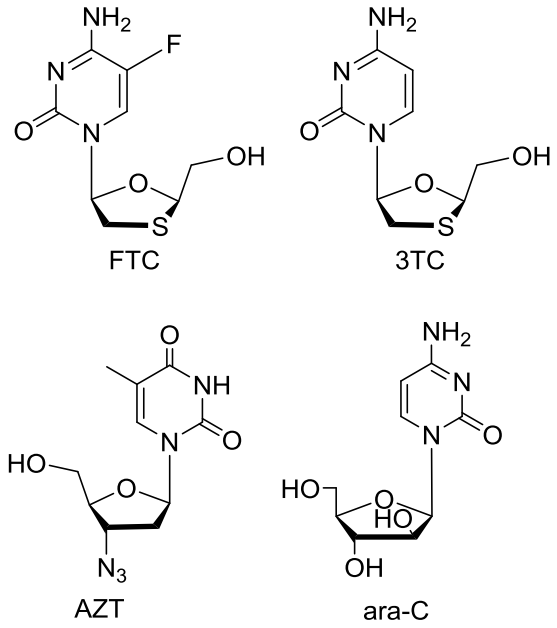


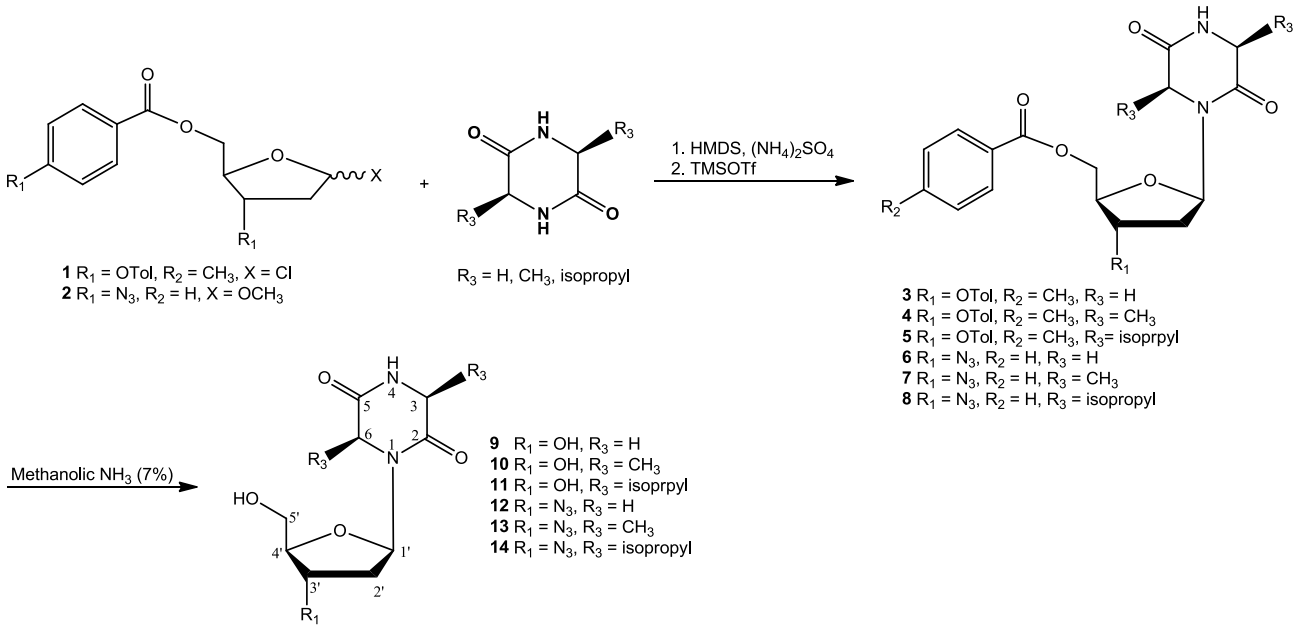
Figure 1. Chemical structures of AZT, FTC, 3TC and Cytarabine.

Herein, we report the synthesis of a new class of modified nucleosides by introducing a cyclic-dipeptide[15-16] at the C-1 position of 2'-deoxyfuranoribose . The cyclic dipeptides containing two amide groups were designed as a six membered ring similar to a pyrimidine base. The double bond of the pyrimidine base[17] was removed to generate more flexibility in the base structure.

Results and Discussion

Three commercial available cyclic dipeptides,[18] diketopiperazine, dimethyldiketopiperazine, and diisopropyldiketopiperazine were used as building blocks for conjugation with protected carbohydrates. The protected 2'-deoxyfuranoribose methoxide or halides were synthesized according to the previously reported procedures.[19] The ditoluoyl2'-deoxyfuranoribose chloride (**1**) was synthesized by

protection of 2'-deoxyfuranoribosemethoxide followed by chlorination with dry HCl. The benzyloyl protected azido-2′-deoxyribosemethoxide(**2**) was synthesized from D-xylose (Supporting information).



Scheme 1. Chemical synthesis of 2'-deoxyribofuranosylcyclodipeptides.

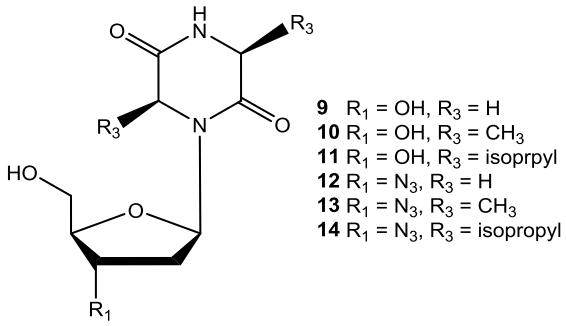
Previous reports have shown that cyclodipeptide can react with alkyl halides using various mild to strong bases.[20-21] Similar coupling/substitution reactions were used with protected sugar chloride using different bases viz. sodium hydride (NaH), 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU),[22] butyl lithium, sodium methoxide, sodium hydroxide, and potassium hydroxide, but these methods were unsuccessful for the synthesis of desired products. In most of the cases, degraded sticky mixture of undesired products was obtained. In the case of reaction performed using NaH showed the presence of a negligible amount of desired product along with a number of different

undesired products as shown by mass spectrometry of crude reaction mixture but we were unable to isolate the product. The reaction performed using DBU as a base led to conjugation of DBU to sugar (DBU-sugar product) instead of formation of desired peptide-sugar product (data not shown).

Alternatively, the cyclodipeptide was reacted with hexamethyldisilane[23] in dioxane with refluxing to form bis-trimethanesilanyl cyclopeptide, which was subsequently coupled with carbohydrate chloride (**1**) and carbohydrate methoxide (**2**).[24-25] Subsequent complex was cleaved/deprotected using trimethylsilyl(TMS)-triflate to the desired protected carbohydrate-cyclopeptide products (**3-8**) in a mixture of α,β- isomers. Finally, the sugar protecting groups (toluolyl or benzoyl) were removed in the presence of methanolic ammonia solution (7% w/v) to generate 2′-deoxyribofuranose-cyclodipeptide products **9-14**. The final products were purified by HPLC using the protocol described in the experimental section. The final product was identified by 1D, 2D NMR spectroscopy and high resolution MS-TOF spectroscopy.

All the synthesized compounds were evaluated for their inhibitory activity of HIV–1 (subtype B, US/92/727) replication in human peripheral blood mononuclear (PBMC) cells.[26] Table 1 illustrates the anti-HIV-1 activity (EC_{50}) and cytotoxicity (TC_{50}) of the nucleoside ester conjugates compared with AZT. No cytotoxicity was observed up to the highest tested concentration for the synthesized conjugates 9-14 (TC_{50}>100 μM) (**1-7**).

Table 1. Inhibition of HIV-1 subtype B (US/92/727) replication in Human PBMC cells.



Compound	EC_{50} (µM)[a]	TC_{50} (µM)[b]	TI (µM)[c]
9	72.3	>100.0	>1.38
10	27.6	>100.0	>3.62
11	74.5	>100.0	>1.34
12	89.5	>100.0	>1.12
13	13.7	>100.0	>7.30
14	1.96	>100.0	>51.02
AZT	0.001	>1.0	>1000.0

[a]EC_{50} (50% effective concentration), All the assays were carried out in triplicate (n = 3);

[b]TC_{50} (50% toxic concentration), All the assays were carried out in triplicate (n = 3);

[c]Therapeutic index (TC_{50}/EC_{50}).

All the conjugates (**9-14**, EC_{50} = 1.96-89.5µM) exhibited consistently less anti-HIV activity than that of AZT (EC_{50} = 0.001µM), indicating that the planar structure of the base is critical for maximum anti-HIV activity. Compounds **9-14** contain a flexible CHR_3 groups at positions **3** and **6** while AZT has a rigid 5-6 alkene and an amide group at

positions 3-4. Rigidity and aromaticity of the base appear to be important in generating optimal anti-HIV activity.

Compound **14** containing 3- and 6-isopropyl groups in the base and 3′-azide (EC_{50} = 1.96 µM) was the most potent compound among all the synthesized analogs. 3′-Azido analog **13** with a 3,6-methyl substituent at the base also showed modest anti-HIV activity (EC_{50} -= 13.7 µM).

The anti-proliferative activity of the synthesized derivatives was evaluated in human leukemia (CCRF-CEM), ovarian adenocarcinoma (SK-OV-3), colorectal carcinoma (HT-29), and breast carcinoma (MDA-MB-468) cells. Doxorubicin was used as the positive control.[27-28] The 2′-deoxyribofuranose-cyclodipeptide products did not exhibit any significant anti-proliferative activity in all cancer cell lines at the concentration of 50 µM and up to the incubation period of 96 h. Compound **14** showed nearly 20-25% anti-proliferative activity after 120h of incubation with MDA-MB-468 cells. (detailed activity in supporting information).

Conclusions

In conclusion, novel six 2'-deoxyribofuranosyl cyclodipeptides were synthesized and characterized by using NMR and mass spectroscopy. The synthesized derivatives were evaluated for their anti-HIV activity. The compounds exhibited inferior anti-HIV activity than AZT. The data indicate that the loss of planarity (aromaticity) of base moiety in the structures is detrimental in biological activity of these compounds. On the

other hand, incorporation of more lipophilic alkyl groups at positions 3 and 6 of the base in 3′-azido analogs generated compounds with modest anti-HIV activity.

Experimental Protocols

Materials and methods. Cyclodipeptides were purchased from Chem-Impex International, Wood dale, IL, USA. Anhydrous solvents and other chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). The chemical structures of final products were characterized by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) obtained on a Bruker NMR spectrometer (400 MHz) or a Varian NMR spectrometer (500 MHz). ^{13}C NMR spectra are fully decoupled. Chemical shifts were reported in parts per millions (ppm) using deuterated solvent peak or tetramethylsilane (internal) as a standard. The chemical structures of final products were confirmed by a high-resolution Biosystems QStar Elite time-of-flight electrospray mass spectrometer. Details of synthetic procedures and spectroscopic data of the respective compounds are presented below. Final compounds were purified on a Phenomenex Prodigy 10 μm ODS reversed-phase column (2.1 cm × 25 cm) with a Hitachi HPLC system using a gradient system of acetonitrile or methanol and water ($CH_3CN/CH_3OH/H_2O$, 0−100%, pH 7.0, 60 min).

5-(2,5-Doxopiperazin-1-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate (3). Diketopiperazine (5 mg, 0.04 mmol), hexamethyldisilane(HMDS) (12 mg, 0.08 mmol), and $(NH_4)_2SO_4$ (2 mg) were mixed in dry dioxane (7 mL) under nitrogen atmosphere and refluxed for 4 h. The reaction mixture was brought to room temperature. 2'-deoxyribofuranosylchloride(**1**, 16 mg, 0.04 mmol) was added and the solution was stirred at room temperature for 10 min followed by heating at 80°C for 6h. The reaction mixture was ice cooled. TMSOTf (18 mg, 0.08 mmol) was added dropwise to the solution, and the reaction mixture was allowed to stir at room temperature for 10 h. The reaction mixture was neutralized with excess of a dilute solution of $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 30 mL). The crude product was concentrated and purified on silica flash chromatography using hexane and ethyl acetate (0-10%, *v/v*) as the eluent solvent. The product was further purified on reverse phase HPLC eluted with water and acetonitrile as the eluents. Yield: 11 mg, 55%. (α:β: 2:3 from NMR), ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.88-7.97 (m, 4 H, Ar-o-H), 7.21-7.30 (m, 4 H, Ar-m-H), 7.06 (s, 1 H, NH), 6.48-6.57 (m, 1 H, C1′H), 5.50-5.60 (m, 1 H, C4′H), 4.72 (s, 1/2 H, C3′H), 4.55-4.65 (m, 1 H, C5′H), 4.49-4.53 (m, 1 H, C5′H), 4.42 (s, 1/2 H, C3′H), 4.20-4.30 (m, 1/2 H, C6-H), 4.11-4.18 (m, 1 H, C6-H), 4.00-4.07 (m, 2 H, C3-H),3.90-3.92 (m, 1/2 H, C6-H), 2.87-2.97 (m, 1/2 H, C2′H), 2.36-2.44 (m, 6 H, 2 × CH_3), 2.29-2.35 (m, 1 H, C2′H), 2.18-2.25 (m, 1/2 H, C2′H);^{13}CNMR (125MHz, $CDCl_3$) δ ppm: 166.30, 166.23 (CO), 166.02, 165.96 (CO), 164.32, 163.85 (CO), 144.47, 144.24 (CO-C-Ar), 129.82, 129.70 (Ar-o-C), 129.44, 129.35 (Ar-m-C), 129.32, 129.26 (Ar′-m-C),84.41 (α3′), 83.56 (β3′) 82.79 (α1′), 81.57 (β1′), 74.98 (α4′), 74.58 (β4′), 64.54 (α5′), 64.03 (β5′), 45.43 (3$COCH_2$), 43.46 (α

6$COCH_2$), 42.95 (β 6$COCH_2$), 36.47 (α2′), 33.68 (β2′), 21.75, 21.73 (Ar-CH_3). HRMS (ESI-TOF): calcd: 466.1740 for $C_{25}H_{26}N_2O_7$, found: 467.1662 $[M+H]^+$.

1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-piperazine-2,5-dione (9). Compound **3** (10 mg, 0.02 mmol) was stirred with methanolic ammonium solution (7%, 10 mL) at room temperature for 30 min. The solvent was evaporated under reduced pressure, and the crude product was purified on flash chromatography using $CHCl_3$/methanol (0-100%, *v/v*) eluent. The product was further purified on a reverse phase HPLC using water:methanol eluents (3 mg, 62% yield). ^{1}H NMR (500 MHz, Methanol-*d3*) δ ppm 6.06-6.17 (m, 1 H, C1′H), 5.69 - 5.79 (m, 1 H, C4′H), 4.00-4.27 (m, 3H, C5′H, C3′H), 3.88-3.94 (m, 1 H, C6-H), 3.35-3.66 (m, 3 H, C3-H, C6-H), 2.59-2.78 (m, 1/2 H, C2′H), 1.92-2.51 (m, 1/2 H, C2′H), 1.73-1.89 (m, 1 H, C2′H).HRMS (ESI-TOF): calcd.: 230.0903 for $C_9H_{14}N_2O_5$, found: 231.1032 $[M+H]^+$.

5-(2,5-Dimethyl-3,6-dioxopiperazin-1-yl)-2-(((4-methylbenzoyl)oxy)methyl) tetrahydrofuran-3-yl 4-methylbenzoate (4). Compound **4** was synthesized from dimethyldiketopiperazine(6 mg, 0.04 mmol) and sugar chloride **1**(16 mg, 0.04 mmol) by following the procedure similar to the synthesis of **3** (10 mg, 48% yield). HRMS (ESI-TOF): cacld: 494.2053 for $C_{27}H_{30}N_2O_7$, found: 495.2198 $[M+H]^+$, 517.2039 $[M+Na]^+$.

1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6-dimethylpiperazine-2,5-dione (10). Compound **4**(8 mg, 0.016 mmol) was hydrolyzed with methanolic ammonia solution and purified by flash chromatography and reverse phase HPLC (2.5 mg, 61% yield). HRMS (ESI-TOF): calcd: 258.1216 for $C_{11}H_{18}N_2O_5$, found: 259.1095 $[M+H]^+$.

5-(2,5-Diisopropyl-3,6-dioxopiperazin-1-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate(5). Compound **5** was synthesized from the reaction of diisopropyldiketopiperazine(8 mg, 0.04 mmol) with sugar chloride**1** (16 mg, 0.04 mmol) by following the procedure similar to the synthesis of **3** (14 mg, 63% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 7.82-7.96 (m, 4 H, Ar-o-H), 7.23-7.32 (m, 4 H, Ar-m-H), 6.51-6.58 (m, 1 H, C1′H), 5.49-5.57 (m, 1 H, C4′H), 4.70-4.76 (m, 1 H, C4′H), 4.57-4.66 (m, 2 H, C5′H), 4.50-4.54 (m, 1 H, C3′H), 4.20-4.30 (m, 1 H, C6-H), 4.09-4.18 (m, 2 H, C3-H), 4.00-4.07 (m, 2 H, isopr C3″-H), 2.87-2.97 (m, 1/2 H, C2′H), 2.36-2.44 (m, 6 H, 2 × CH_3), 2.29-2.35 (m, 1 H, C2′H), 2.18-2.25 (m, 1/2 H, C2′H), 0.92-1.09 (m, 12H, 4 × CH_3).^{13}CNMR (125MHz, $CDCl_3$) δppm:166.30, 166.23 (CO), 166.02, 165.96 (CO), 164.32, 163.85 (CO), 144.47, 144.24 (CO-C-Ar), 129.82, 129.70 (Ar-o-C), 129.44, 129.35 (Ar-m-C), 129.32, 129.26 (Ar′-m-C), 84.41 (α3′), 83.56 (β3′), 82.79 (α1′), 81.57 (β1′), 74.98 (4′), 64.54 (5′), 45.43 (3COCH), 43.46 (6COCH), 30.99 (2′), 21.70 (Ar-CH_3), 18.85 (isopr C3″),16.37 (isopr CH_3).HRMS (ESI-TOF): calcd: 550.2679 for $C_{31}H_{38}N_2O_7$, found: 573.0976 $[M+Na]^+$.

1-(4-Hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6-diisopropylpiperazine-2,5-dione (11). Compound **5** (10 mg, 0.018 mmol) was hydrolyzed with methanolic ammonia solution (2 mL of 7% sol) and was purified by flash chromatography and reverse phase HPLC (yield 2.5 mg, 45%). MS (ESI-TOF): calcd: 314.1842 for $C_{15}H_{26}N_2O_5$, found: 315.5 $[M+H]^+$.

(3-Azido-5-(2,5-dioxopiperazin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (6). Compound **6** was synthesized from the reaction of diketopiperazine(5 mg, 0.04 mmol) with azido-sugar methoxide **2** (11 mg, 0.04 mmol) by following the procedure similar to the synthesis of **3** (9 mg, 58 % yield)(α:β: 35:65 from ^{13}C NMR). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.04 (d, *J* = 8Hz, 2H, Ar-o-H), 7.60 (t, *J* = 8Hz, 1H, Ar-p-H), 7.49 (t, *J* = 8Hz, 2H, Ar-m-H), 7.11 (s, 1 H, NH), 6.33 - 6.42 (m, 1 H, C1′H), 4.52 (br s, 1 H, C4′H), 4.34-4.47 (m, 2 H, C5′H), 3.97-4.12 (m, 4H, C6-H, C3-H), 2.65-2.77 (m, 1 H, C3′H), 2.23-2.31 (m, 1 H, C2′H), 2.00-2.11 (m, 1H, C2′H).^{13}CNMR (100MHz, $CDCl_3$) δ ppm: 166.76, 166.36 (CO), 164.35, 163.16 (CO), 133.61 (Ar-C4), 129.75 (Ar-C2), 128.72 (ArC1), 128.68 (Ar-C3), 83.72 (3′), 82.41 (α1′), 82.33 (β1′), 80.79 (4′), 64.23 (5′), 45.46 (C3$\underline{C}H_2$), 43.62 (α C6$\underline{C}H_2$), 43.49 (β C6$\underline{C}H_2$), 35.61 (β2′), 33.90 (α2′). HRMS (ESI-TOF): calcd: 359.123 for $C_{16}H_{17}N_5O_5$, found: 360.0913 $[M+H]^+$.

1-(4-Azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-piperazine-2,5-dione (12). Compound **4**(7 mg, 0.019 mmol) was hydrolyzed with methanolic ammonia solution (3 mL of 7% sol) and purified by flash chromatography and reverse phase HPLC (yield 2.6

mg, 52%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.99 (s, 1H, NH), 6.24-6.31 (m, 1 H, C1′H), 4.24-4.32 (m, 1/2 H, C4′H), 4.09-4.14 (m, 1/2 H, C4′H),3.92-3.99 (m, 1 H, C3′H), 3.55-3.69 (m, 2 H, C5′H), 3.01 (s, 2H, C6-H), 2.88 (s, 2H, C3-H), 2.61-2.74 (m, 1 H, C2′H), 1.99-2.09(m, 1 H, C2′H). ^{13}CNMR (100MHz, CD_3OD) δ ppm: 165.67 (CO), 85.30 (1′), 83.76 (3′), 82.33 (4′), 61.82 (5′), 44.69 (C3 $\underline{C}H_2$), 43.09(C6 $\underline{C}H_2$), 34.93 (2′). HRMS (ESI-TOF): cacld: 255.0968 for $C_9H_{13}N_5O_4$, found: 278.0526 $[M+Na]^+$.

(3-Azido-5-(2,5-dimethyl-3,6-dioxopiperazin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (7). Compound **7** was synthesized from the reaction of dimethyldiketopiperazine (6 mg, 0.04 mmol) with azido-sugar methoxide**2**(11 mg, 0.04 mmol) by following the procedure similar to the synthesis of **3** (8 mg, 48 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.01 (d, *J* = 4Hz, 2H, Ar-o-H),7.82 (s, 1 H, NH), 7.58 (t, *J* = 4Hz, 1H, Ar-p-H), 7.46 (t, *J* = 4Hz, 2H, Ar-m-H), 6.03 - 6.12 (m, 1 H, C1′H), 4.35-4.45 (m, 3 H, C4′H, C5′H), 4.03-4.22 (m, 3H, C6-H, C3-H, C3′H), 2.67-2.78 (m, 1 H, C2′H), 2.20-2.34 (m, 1H, C2′H), 1.61 (d, *J* = 8Hz, 3H, C*H*$_3$), 1.54 (d, J = 8Hz, 3H, C*H*$_3$). ^{13}CNMR (100MHz, $CDCl_3$) δ ppm: 170.29, 167.90, 166.19 (CO), 133.57 (Ar-C4), 129.70 (Ar-C2), 129.30 (ArC1), 128.66 (Ar-C3), 86.28 (1′), 82.63 (4′), 64.21 (5′), 61.18, 52.59 (C3 $\underline{C}H$), 51.95 (C6 $\underline{C}H$), 37.04 (2′), 22.56 (C3 $\underline{C}H_3$), 21.71 (C6 $\underline{C}H_3$). HRMS (ESI-TOF): calcd: 387.1543 for $C_{18}H_{21}N_5O_5$, found: 410.1029 $[M+Na]^+$.

1-(4-Azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6-dimethylpiperazine-2,5-dione (13). Compound **7**(5 mg, 0.013 mmol) was hydrolyzed with methanolic ammonia

solution (3 mL of 7% sol) and purified by flash chromatography and reverse phase HPLC (1.5 mg, 42%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.87 (s, 1 H, NH), 5.93-6.14 (m, 1 H, C1′H), 3.94-4.33 (m, 4 H, C4′H, C3′H, C6-H, C3-H), 3.61 (s, 2H, C5′H), 2.60-2.74 (m, 1 H, C2′H), 2.02-2.27 (m, 1H, C2′H), 1.60 (d, *J* = 4Hz, 3H, C*H*$_3$), 1.51 (d, *J* = 4Hz, 3H, C*H*$_3$). ^{13}CNMR (100MHz, CD_3OD) δ ppm: 168.86, 168.52, (CO), 86.71 (1′), 86.21 (4′), 85.96 (3′), 61.88 (5′), 61.01, 52.46 (C3 CH), 51.55 (C6 CH), 36.62 (2′), 21.43, 20.32 (C3 CH_3), 17.73, 15.64 (C6 CH_3). HRMS (ESI-TOF): calcd: 283.1281 for $C_{11}H_{17}N_5O_4$, found: 284.0849 $[M+H]^+$.

(3-Azido-5-(2,5-diisopropyl-3,6-dioxopiperazin-1-yl)tetrahydrofuran-2-yl)methyl benzoate (8). Compound **8** was synthesized from the reaction of diisopropyldiketopiperazine(8 mg, 0.04 mmol) with azido-sugar methoxide**2**(11 mg, 0.04 mmol) by following the procedure similar to the synthesis of **3**(9.5 mg, 54% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.98-8.17 (m, 2H, Ar-o-H),7.38-7.66(m, 3H, Ar-p-H, Ar-m-H), 6.39-6.49 (m, 1 H, C1′H), 5.31 (s, 1H, C4′H), 4.35-4.58 (m, 2 H, C5′H), 4.07-4.32 (m, 1H, C6-H), 3.58-3.88 (m, 1H, C3-H), 3.26-3.88 (br s, 1H,C3′H), 2.49-2.86 (m, 1 H, C2′H), 1.78-2.48 (m, 3H, C2′H, 2 × isopr CH), 0.94-1.24 (br s,12H, 4 × isopr C*H*$_3$). ^{13}CNMR (100MHz, $CDCl_3$) δ ppm: 168.96, 166.17 (CO), 133.51 (Ar-C4), 129.81 (Ar-C2), 129.75 (ArC1), 128.61 (Ar-C3), 88.86 (1′), 82.90 (4′), 64.13 (5′), 62.95, 62.25 (C3 CH), 60.97 (C6 CH), 37.17 (2′), 34.59, 32.79 (isopr CH),20.10, 19.93, 19.46, 19.31 (isopr 4 × CH_3). HRMS (ESI-TOF): calcd: 443.2169 for $C_{22}H_{29}N_5O_5$ found: 444.1338 $[M+H]^+$.

1-(4-Azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,6-diisopropylpiperazine-2,5-dione (14). Compound **4**(5 mg, 0.011 mmol) was hydrolyzed with methanolic ammonia solution (2 mL of 7% sol) and purified by flash chromatography and reverse phase HPLC. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 5.69–5.77 (m, 1 H, C1′H), 4.15-4.28 (m, 2H, C4′H, C3′H), 3.76 (d, *J* = 8 Hz, 1H, C6H), 3.60 (s, 2H, C5′H), 3.46 (d, *J* = 8Hz, 1H, C3H), 2.57-2.68 (m, 1 H, C2′H), 2.35-2.45 (m, 1H, C2′H), 1.97-2.12 (br s, 2H, 2 × isopr C*H*), 0.97-1.17 (m, 12H, 4 × isopr C*H*$_3$). ^{13}CNMR (100MHz, CD_3OD) δ ppm: 170.25, 167.85(CO), 88.99 (C1′), 86.64 (C4′), 62.40 (C5′), 62.29, 61.86 (C3 CH), 60.84 (C6 CH), 36.64 (C2′), 34.61 (isopr CH), 33.35 (isopr CH),19.12, 18.99, 18.78 (isoprCH_3). HRMS (ESI-TOF): calcd: 339.1907 for $C_{15}H_{25}N_5O_4$, found: 340.1554 $[M+H]^+$.

Anti-HIV-1 Evaluation in PMBC Assay. PBMC based anti-HIV assays were performed as previously described.[26] Briefly, PHA-stimulated PBMCs cultured in the presence of IL-2 were suspended at 1 × 10^6 cells/mL and were added to a 96-well round-bottom plate. Serially diluted test materials were added to the plate in triplicate followed by the appropriate pre-titered strain of HIV. The culture was incubated for 7 days at 37 °C/5% CO_2. Following the incubation, supernatants were collected for analysis of virus replication by supernatant RT activity and cells analyzed for viability by XTT dye reduction. AZT was used as an internal assay standard. All the assays were carried out in triplicate.

Acknowledgement

We thank National Center for Research Resources, NIH, and Grant Number 1 P20 RR16457 for sponsoring the core facility and University Grant Commission, New Delhi, India.

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